Faecal Contamination of Oyster Growing Areas in the Kerikeri Inlet: Data Analysis and Recommendations for a Faecal Source Tracking Programme

Brenda Hay[1] & Kelly Roberts[2]

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[1]AquaBio Consultants Ltd
P.O. Box 560
Shortland St
Auckland 1140

[2]School of Biological Sciences
University of Auckland
Private Bag 92019
Auckland 1142

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Cover photo: Oyster farm in Kerikeri Inlet. (Source: NRC).

EXECUTIVE SUMMARY

Oysters concentrate contaminants and human pathogens from their growing waters as part of their feeding process. Thus only shellfish grown in unpolluted waters can be safely consumed. Due to increasing and unpredictable faecal contamination, the commercial oyster growing area in the Hauparua Inlet of the Kerikeri Inlet no longer meets the regulatory criteria for classification as "Conditionally Approved", which means that oysters from that growing area may not be harvested directly for sale. In addition, the other oyster farming area on the southern side of the Kerikeri Inlet no longer meets the growing area standard required by European Union markets. Consequently, University of Auckland, in association with AquaBio Consultants Ltd, were asked by Northland Regional Council (NRC) to:

- *Undertake data analysis of available bacterial and environmental data from Kerikeri Inlet; and*
- *Design a sampling programme to identify the sources of contamination to allow effective management.*

Analysis of regular oyster samples collected by Northland District Health Board (NDHB) in oyster growing areas in the Kerikeri Inlet at times when the areas were open for oyster harvest from 2008-February 2012 suggests that the frequency of *E. coli* levels elevated above 230 MPN/100g in oysters has increased, as have the levels of *E. coli* within samples that exhibit unpredicted contamination (i.e. the degree of contamination in non-compliant monitoring results has increased as well as the frequency of contamination events). The comprehensive sanitary surveys and annual updates undertaken by NDHB over the years that oyster farming has been present in Kerikeri Inlet have not identified any increase in agricultural stock numbers or any other increasing potential sources of faecal contamination other than increasing human activity in the catchment e.g. increasing settlement of the coastline and in Kerikeri township itself. The possibility that the deterioration in shellfish quality might be attributable to human faecal contamination therefore needs to be seriously considered.

Available data from monitoring undertaken by NDHB, NRC and the oyster industry were analysed to identify any spatial or temporal patterns that could assist in focussing faecal source tracking sampling to appropriate times and locations. Analysis of data has shown that there is an underlying trend of increasing coliform contamination of water from the mouth of the Kerikeri Inlet to the head. The influence of tide on water quality (based on enterococci levels) is greater at the head of the Kerikeri Inlet than at the mouth. State of the Environment monitoring data show a negative correlation between salinity and faecal coliforms in water samples from the Kerikeri Inlet, and there is a trend of increasing correlation between ammonia and faecal coliforms in water samples from the head of the Kerikeri Inlet to the mouth. There is no relationship between turbidity and faecal coliform levels in water in the same dataset.

On occasion, localised contamination of water and oysters overlies the trend of increasing faecal contamination levels from the mouth of the Kerikeri Inlet to its head. Generally this is related to rainfall-associated run-off in the immediate catchment of each growing area. However, as evidenced by the failure of harvest criteria to ensure the required oyster quality, this relationship has become less predictable in recent years at Site 11 (Hauparua Inlet) and, to a lesser extent, at Site 10 (oyster farms south of Taranaki Island). There is evidence that some high level contamination events at Site 11 during summer may not be related to rainfall-induced run-off. Exfiltration of poorly treated effluent from overloaded on-site sewage systems (e.g. septic tanks), or discharge of effluent from boats are examples of potential faecal contamination sources associated with

increased visitor numbers to the area over summer months. A tenuous association of these sporadic events with winds with an easterly component could be consistent with the implication of effluent derived from boats moored near Windsor Landing, which would provide a sheltered mooring place under such circumstances. The movement of water from Windsor Landing into Hauparua Inlet has been observed by oyster farmers at low tide before the turn of the tide. This might also transport water from further up Kerikeri Inlet into Hauparua Inlet.

Rainfall intensity may impact on the relationship between the level of rainfall and faecal contamination in oyster growing areas. While this could be attributable to a "first flush" effect, the possibility of contamination sources that are induced by heavy rainfall should be investigated further, particularly as intense rainfall events are not new events in the history of oyster growing areas, but levels of contamination associated with such events do appear to have increased. Investigation into the performance of on-site sewage systems, sewage reticulation systems and wastewater treatment plants during intense rainfall events is suggested.

Analysis of rainfall 30 days prior to oyster samples exhibiting elevated coliform levels suggests soil saturation may also impact significantly on the relationship between the level of rainfall and faecal contamination levels. Under these conditions, the possibility of persistent delivery of faecal contamination or delayed contamination arising from very high rainfall events cannot be excluded. Preliminary sampling of oysters adjacent to freshwater springs entering Hauparua Inlet suggests possible faecal contamination of groundwater entering the marine environment. The contamination of groundwater by on-site sewage management systems (e.g. septic tanks), and by STP effluent discharged to the Waitangi wetlands, and the potential for the entry of this groundwater into oyster growing areas at Sites 11 and 10 are issues that require further investigation.

It is still uncertain whether the observed unexpected oyster contamination relates to:

- An increase in the underlying contamination related to rainfall (which means the consequent higher levels of contamination in oysters are slower to depurate out), plus very occasional sporadic contamination unrelated to run-off; or
- More frequent sporadic contamination unrelated to run-off, masked by coincident rainfall events.

Faecal source tracking tools commercially available in New Zealand include:

- *Testing for faecal sterols in water and sediment*. (Provider: ESR, Christchurch) Faecal sterol analysis relies on the analysis of the relative proportions of various sterols and stanols in the environment to gauge the source of faecal contamination, as the profile of sterols and stanols from different animals is different. The interpretation of sterol data is complex. Little is known about the relative persistence of these compounds in the environment, and their low prevalence makes sensitivity of test methods a significant issue. The impact of dilution is a limitation in the detection of faecal sterols in water samples taken at a distance from the source of contamination. ESR considers that only medium certainty can be attributed to the results of their faecal sterol analysis. ESR is trialling the use of "sediment bags" as a means of concentrating *E. coli* and faecal sterols out of water, and this is offered as a service to the NRC without charge at present. However, this technique is still in the very preliminary stages of development, and although positive results could be regarded as significant, negative results cannot be regarded as reliable.

- *Testing water for fluorescent whiteners.* Fluorescent whiteners are used in washing powders etc and can provide an indication contamination from domestic or industrial effluent. Their presence is not directly linked to human faecal contamination. Dilution with distance from source and the rapid degradation of fluorescent whiteners by sunlight can reduce the sensitivity of this test, but the test is relatively cheap and it can be very useful when investigating a specific potential source.

- *Quantitative PCR (q-PCR) to test for Bacteroidales and other bacterial markers in water and shellfish*. *Bacteriodales* bacteria are very prevalent in faeces of many warm-blooded animals and can be host-specific. A universal *Bacteroidales* marker (UBac) is available to detect the presence of faecal contamination in general (this is not sensitive to faecal contamination from gulls, which appear not to have *Bacteriodales* bacteria in their gut). Markers for ruminant (RBac) and human *Bacteroidales* (HBac) are also available. The low sensitivity of the human *Bacteroidales* marker significantly limits the usefulness of this test. There is also significant cross-reactivity with possum faecal material and lesser cross-reactivity with some other animals. These analyses are provided by ESR and Cawthron for water samples, and Cawthron Insititue for shellfish samples. ESR have recently developed a marker for human contamination based on the bacteria *Bifidobacterium adolescentis* that has a sensitivity similar to that of the RBac ruminant marker (>90%), and they also have bacterial markers for wildfowl.

- *End-point PCR for bacterial markers in water and shellfish* (Provider: Cawthron Institute). This technique provides a positive/negative result for the presence of human contamination (not a quantitative result as in q-PCR). Human markers are available for *Bacteroidales* and *Methanobrevibacter* bacteria. The human *Bacteroidales* marker is based on a different set of primers from those targeted in the q-PCR technique, and it is more sensitive than the q-PCR test for HBac. The test for human *Methanobrevibacter* marker is less sensitive than that for the human *Bacteroidales* marker but the combination of these tests with the q-PCR test for the HBac marker significantly improves the level of certainty of result with respect to the detection of human contamination. End-point PCR markers are also available for cattle, wild-fowl (duck etc.) and gulls. A universal marker is also used to check the extraction efficiency of these markers in the test process.

- *RT-PCR assays for human/animal noroviruses and adenoviruses in water and shellfish samples* (Provider: ESR, Wellington). Enteric viruses released with the faeces of humans and animals are accumulated by oysters from their growing waters and retained for several weeks. Multiplex real time RT-PCR assays for enteric viruses in water and shellfish samples can distinguish between human, pig, sheep and cattle faecal contamination. The ESR "Virus Toolbox" of host-specific virus assays includes: human adenovirus species F, norovirus GI and GII (and human polyomavirus (see below)); plus markers for animal faecal contamination including: porcine adenovirus type 3 (pigs), ovine adenovirus (sheep), norovirus GIII (sheep & cows) (and bovine polyomavirus (cows) – see below). These assays rely on the presence of illness in the source population. Human noroviruses and adenoviruses tend to be prevalent in effluent from large sewage treatment plants, but may only be sporadically present if the source of contamination is on-site sewage systems (such as septic tanks). This technique is thus more reliable in detecting human contamination from sources emanating from communities rather than from individual households.

- *End-point or q-PCR for human and bovine polyomavirus* (Providers: Cawthron or ESR, Wellington). Human polyomaviruses, which are excreted with urine, are more prevalent in

the human population than noroviruses and adenoviruses, and reliably detected in most human effluent. End-point PCR markers for human polyomavirus have been trialled in water and shellfish by Cawthron Institute and human polyomavirus can be detected by real time RT-PCR in water and shellfish by ESR in Wellington. However, dilution in the environment and low recovery rates of the markers from shellfish were cited as potential disadvantages of this method, and neither provider would currently recommend this source tracking technique for application in the Kerikeri Inlet.

There is no single faecal source tracking method that provides comprehensive information about potential sources of faecal contamination with a high degree of certainty attached to the result. We therefore propose the use of a suite of source tracking tools to increase the reliability of outcomes.

Detailed investigation of the environmental conditions associated with each of the sample times at which unexpectedly elevated levels of *E. coli* were detected in oyster samples was undertaken to identify the best times to sample for microbial source tracking. Based on this, a sampling programme as outlined in Table (i) (see following page) is suggested. The rationale for this project design is:

- An initial study of the impact of tidal stage on faecal coliform levels in water will inform the timing of water sampling. Analysis for Enterococci and an additional sampling site (Windsor Landing) are included in this sampling to link in with and elucidate the results of an earlier study by the NRC;
- The use of several different human bacterial markers and other indicators of human sewage is proposed to overcome the problem of the comparatively low sensitivity of human markers and reduce the likelihood of false negative results with respect to the presence of human sewage contamination;
- The analysis of historic monitoring data showed elevated coliform levels within several different rainfall regimes, and these are incorporated into the sample design;
- Elevated coliform levels apparently not associated with rainfall appear to occur during the summer holiday period, and thus intensive sampling through the month of January is recommended;
- Local concern about the possibility of human contamination and the observation of higher levels of faecal contamination at the head of the Kerikeri Inlet has prompted the inclusion of two sample sites (near Pah Rd in Kerikeri R entrance and Waipapa R entrance) under one of the rainfall scenarios.

The programme may be modified and extended to investigate local sources of contamination more specifically as results of sampling become available.

It is recommended that this sampling programme is complemented by a study of the reduction of *E. coli* in oysters from Sites 10 and 11 after a rainfall event on three occasions, and by an intensive sanitary survey and investigation of potential faecal contamination sources, including completion of dye testing of onsite sewage systems on the shore around oyster growing areas at Sites 10 and 11 under adverse conditions (high load/saturated ground).

Table (i): Summary of suggested sampling regime. FC = faecal coliforms; FWA = Fluorescent Whitening Agents; qPCR = quantitative polymerase chain reaction; PCR = end-point polymerase chain reaction; UBac = universal Bacteroidales marker; RBac = ruminant Bacteroidales marker; HBac = human Bacteriodales marker; HBif = human Bifidobacterium marker; HMet = human Methanobrevibacter marker. Analysis of bacterial markers in water samples, fluorescent whitening agents in water samples, and faecal sterols in sediment bags will be undertaken by ESR in Christchurch. Analysis of oyster samples for bacterial markers will be undertaken by Cawthron Institute, Nelson. Analysis of oyster samples for enteric viruses will be undertaken by ESR in Wellington.

Sampling Times	**Analysis Required at each Sampling Event**				
	Site 10	**Site 11**	**Windsor Landing**	**Kerikeri R. Entrance**	**Waipapa R. Entrance**
Every 90 minutes across a tidal cycle at spring tide and neap tides 24-48 hours after low-moderate rainfall event	*Three replicate water samples:* FC and Enterococci	*Three replicate water samples:* FC and Enterococci	*Three replicate water samples:* FC and Enterococci		
One sampling event immediately following a high rainfall event (>50 mm)	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols			

One sampling event immediately following each of 3 low rainfall events (15-25 mm rainfall 24-48 hrs) when groundwater levels are high	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols		*Water sample:* FC, then if elevated, FWA. *Pooled Oyster sample:* qPCR for human norovirus (GI, GII) and human adenovirus species F; *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers.	*Water sample:* FC, then if elevated, FWA. *Pooled Oyster sample:* qPCR for human norovirus (GI, GII) and human adenovirus species F; *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers.
One sampling event immediately following each of 3 low rainfall events (10-20 mm in 24 hrs) when groundwater levels are low (no significant rainfall events in previous 30 days)	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols	*Water sample:* FC, then if elevated FWA, & qPCR for UBac, RBac, HBif *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols			

Through January, sampling twice a week at least 3 days apart regardless of rainfall	*Water sample:* FC *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols	*Water sample:* FC *Pooled Oyster sample:* *E. coli*, then if elevated: qPCR for UBac, RBac, HBac; PCR for UBac, HMet, bovine, wildfowl and gull markers. *Sediment Bags (3):* Faecal sterols			

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SECTION 1: INTRODUCTION

1.1 Background and Scope

As required by the Animal Products (Regulated Control Scheme – Bivalve Molluscan Shellfish) Regulations 2006 and the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006, a 12-yearly sanitary survey was undertaken for the oyster growing areas in the Kerikeri and Te Puna Inlets in 2011 (Brandt et al., 2011). All oyster growing areas in these inlets are currently classified as "Conditionally Approved", which means that oysters may be harvested directly for sale at times when specified harvest criteria (in this case, based on rainfall) are met. In order to maintain a "Conditionally Approved" status, certain bacteriological standards must be met in water and shellfish sampled monthly from representative sites in "open" status (i.e. when the criteria for harvesting are met) throughout the shellfish growing area. These standards are:

- A median value of 14 faecal coliform MPN/100 ml in water samples; and
- Not more than 10% of water samples may exceed a value of 43 faecal coliform MPN/100 ml; and
- A median value of 230 *E. coli* MPN/100g in shellfish flesh samples; and
- Not more than 10% of shellfish samples may exceed 700 *E. coli* MPN /100 g.

In addition, for European Union (EU) market designated sites, not more than 10% of shellfish flesh samples may exceed 230 *E. coli* MPN/100 g.

The 2011 Sanitary Survey found that based on the required analysis of the bacteriological data for the last three years, Sector D of the growing area (the oyster farms in the Hauparua Inlet, represented by Sample Site 11) no longer complies with the standard for Conditionally Approved growing areas, and Sector C (the oyster farms at a bay south of Taranaki Island, represented by Sample Site 10) no longer complies with the EU standard for growing areas. (See Figure 1 for the location of these sites). Northland District Health Board (NDHB) and Ministry of Primary Industries (MPI) recommended that with the agreement of the oyster farmers, farms in both these areas be placed under voluntary closure until 1st May 2012 or until such times as further sampling shows a reduction in elevated results sufficient to meet the required standard and the sources of pollution are known, and a suitable management strategy can be implemented to allow re-opening under the Conditionally Approved status (Brandt, 2011).

A working group (the Kerikeri Inlet Water Quality Committee) has been formed from representatives from Northland District Health Board (NDHB), Ministry for Primary Industries (MPI), Northland Regional Council (NRC), Far North District Council (FNDC) and oyster farmers to address the issue of identifying and managing the sources of bacteriological contamination in the Kerikeri Inlet, and specifically in sectors C and D of the growing areas. University of Auckland, in association with AquaBio Consultants Ltd, have been asked to

- *Undertake data analysis of available bacterial and environmental data from Kerikeri Inlet; and*
- *Design a sampling programme to identify the sources of contamination to allow effective management.*

The following report outlines the findings of this work.

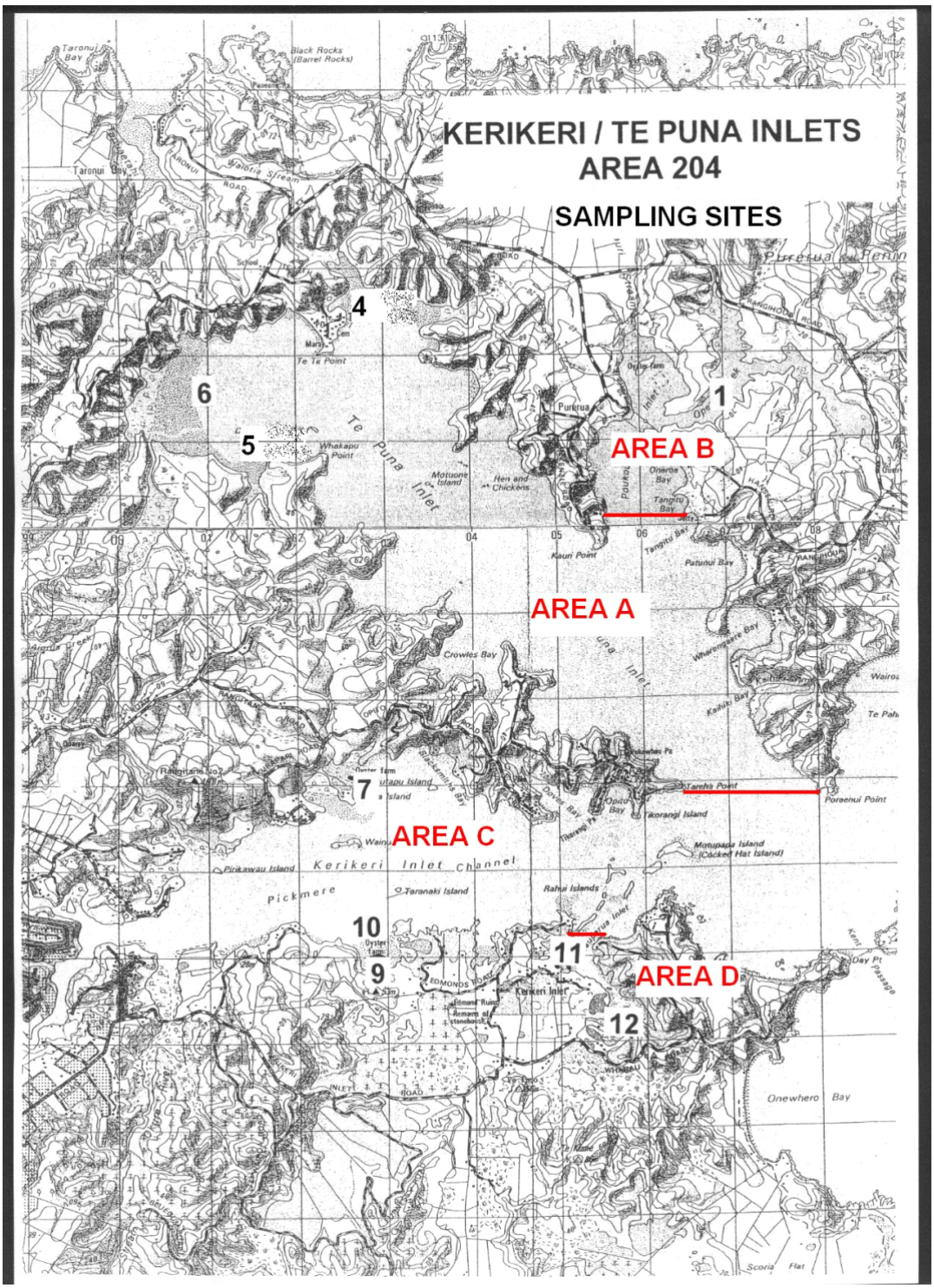

Figure 1: Map showing sectors and sample sites in Kerikeri and Te Puna Inlets. (Source: Northland District Health Board)

SECTION 2: METHODOLOGY

2.1 Overall Approach

The sporadic and unpredictable occurrence of high levels of *E. coli* in oysters that are of concern in the management of shellfish harvest times also presents a challenge when designing a sampling programme to identify sources of contamination. The following steps were identified as a pathway to overcoming this problem:

- Data analysis to determine the characteristics of the oyster contamination problem (e.g. scale, where, when, etc);
- Define the required outcomes to maintain shellfish quality at harvest;
- Identify the information required to achieve the required outcomes;
- Identify how that information could be gathered (e.g. available test methods);
- Match the available methods and opportunities for gathering the required information against the characteristics and patterns of the contamination;
- Identify information gaps and alternative ways of overcoming them.

2.2 Data Analysis

Analysis of water, shellfish and environmental data was undertaken to determine the characteristics of the oyster contamination problem, and to try to identify any patterns that might assist in predicting when the risk of high levels of faecal contamination in oysters in the Kerikeri Inlet occurs. Available datasets included:

- Data from October 2008- February 2012 provided by Northland District Health Board (NDHB) from monitoring of faecal coliforms in water and *E. coli* in oysters at sample sites on oyster farms throughout the Kerikeri and Te Puna Inlets. Generally samples were taken monthly, mostly when the growing area was open for harvest based on the contemporaneous harvest criteria, but following an "Adverse Pollution Condition" event (such as rainfall, spring tides and/or winds over 15 knots). These samples were analysed by AsureQuality Ltd using the standard methods to meet regulatory requirements, and are reported in MPN/100g (shellfish) and MPN/100ml (water).

- Results of post-harvest analysis of *E. coli* in oysters harvested for processing from two oyster farms at Site 11. This sampling was undertaken by shellfish processors, and sample analysis was undertaken at an accredited laboratory using analytical methods that meet the shellfish regulatory requirements. Data cover a period from July 2008 – November 2011. These samples were all taken when the growing area met the criteria for harvesting (i.e. was open for harvest). The temporal distribution of samples is based on occasions when these processors purchased oysters from these farms.

- Data from monitoring of *E. coli* in oysters at a range of sites in Hauparua Inlet in early 2012, provided by Garth Richards, oyster farmer. These samples were analysed by Northland Food Testing Laboratory Services using the Petrifilm method, and are reported in colony forming units (cfu)/100g. The results are not directly comparable with results in the other datasets in which the regulatory test method has been used, but provide useful data for analysis of trends.

- Daily rainfall data collected from a rain gauge at Kerikeri Airport: NDHB provided rainfall data for the period November 2007 to October 2011. A larger dataset from the same rainfall gauge was provided by Emmanuel Malpot of Sanford Ltd. These data continued to mid-March 2012. We observed that there was some discrepancy in these two datasets (mostly, but not limited to, rainfall data offset by one day). This issue was not able to be resolved within the required timeframe for this report. However, where these differences are significant in the interpretation of results this has been noted in our report. In addition, daily rainfall data downloaded from www.kerikeriweather.co.nz were used to assist in the resolution of discrepancies between the two datasets. The data from this website was collected from a rainfall station closer to Kerikeri township. A comparison of the different datasets is provided in Figure 2.

- Kerikeri weather data downloaded from www.kerikeriweather.co.nz including daily maximum rainfall rate, average and maximum wind speed, average and maximum wind gust speed, average wind direction.

- Results of water quality monitoring undertaken by Northland Regional Council as part of the State of the Environment monitoring in Kerikeri and Te Puna Inlets. These data included (amongst other data not relevant to this project): sample depth, dissolved oxygen, dissolved reactive phosphorus, total phosphorus, *Enterococci*, presumptive faecal coliforms, ammoniacal nitrogen, nitrite/nitrate nitrogen, salinity, Secchi depth, total suspended solids, temperature, and turbidity.

- Results of water samples taken by NRC from Kerikeri River in 2011 and analysed by faecal source tracking methods. These data are part of a larger dataset collected by the Council from throughout Northland since 2008. Specific details of analytical methods were not provided with the dataset received.

Data were analysed to determine:

- Temporal trends in the occurrence of contamination of oysters by *E. coli*.
- Spatial trends in the occurrence of contamination of oysters by *E. coli*.
- The relationship between the occurrence of high levels of *E. coli* contamination in oysters and potential environmental indicators such as faecal coliforms in oyster growing waters, rainfall, salinity, wind etc.

In addition, data from two reports on previous faecal source tracking undertaken in the Kerikeri Inlet (Reed, 2011; Cornelisen et al., 2012) were briefly reviewed.

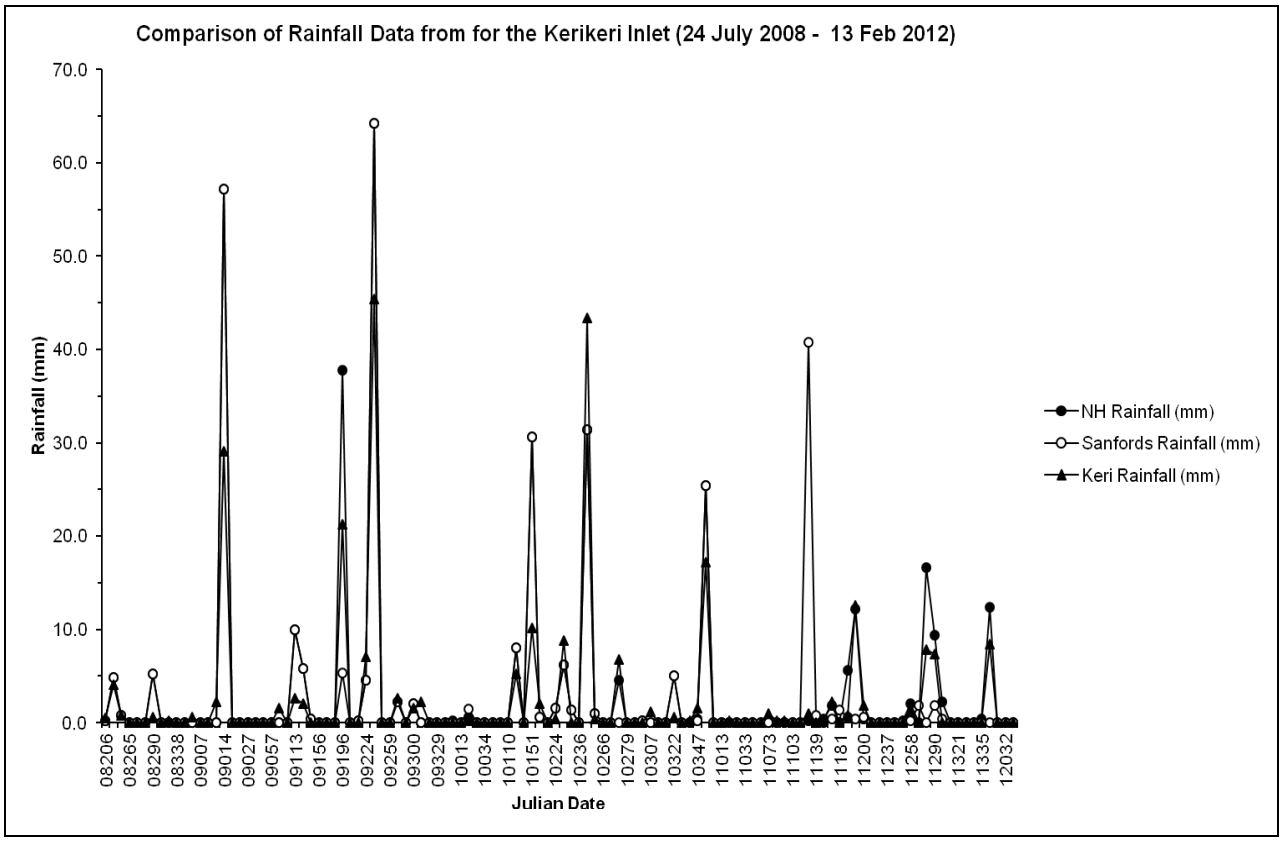

Figure 2: Comparison of rainfall data from three datasets: two datasets of rainfall recorded at Kerikeri Airport, one sourced from NDHB and the other from records kept by Sanford Ltd; and rainfall data sourced from www.Kerikeriweather.co.nz from a rain gauge close to Kerikeri. Correlations between Data Sets: Keri:NDHB r^2 = 0.670; Keri:Sanford r^2 = 0.500; NDHB:Sanford: r^2 = 0.533. (See Appendix I for table of Julian dates).

2.3 Development of Recommendations

- Once the pattern of occurrence of unacceptably high bacterial results in shellfish had been elucidated as much as possible from existing data, the required outcome from microbial source tracking was identified within the context of the requirements of the shellfish quality assurance programme as described in Section 1.
- These required outcomes were matched against the information able to be delivered by available source tracking methods, which were determined through a very brief review of available literature and through discussion with New Zealand microbial source tracking service providers in New Zealand and Australia (Cawthron Institute, ESR in Wellington and Christchurch, University of Newcastle). The advantages and disadvantages of different methods (including cost) were assessed within the context of Kerikeri Inlet.
- This information was synthesized to develop a sampling programme, including recommendations on source tracking methods, sampling sites and timing.

SECTION 3: RESULTS OF DATA ANALYSIS

3.1 Temporal Trends

Results from analysis of *E. coli* in oysters sampled from Kerikeri Inlet and from Te Puna Inlet at times when the growing areas were open for harvest, including samples taken as part of the regulatory programme and data from post-harvest sampling at Site 11 by processors, were plotted against time to investigate changes in the incidence of high levels of contamination over time. These results are presented in Figures 3 and 4 below (Note the difference in scales between the two plots, with the results for Kerikeri Inlet (Figure 3) presented as a log scale).

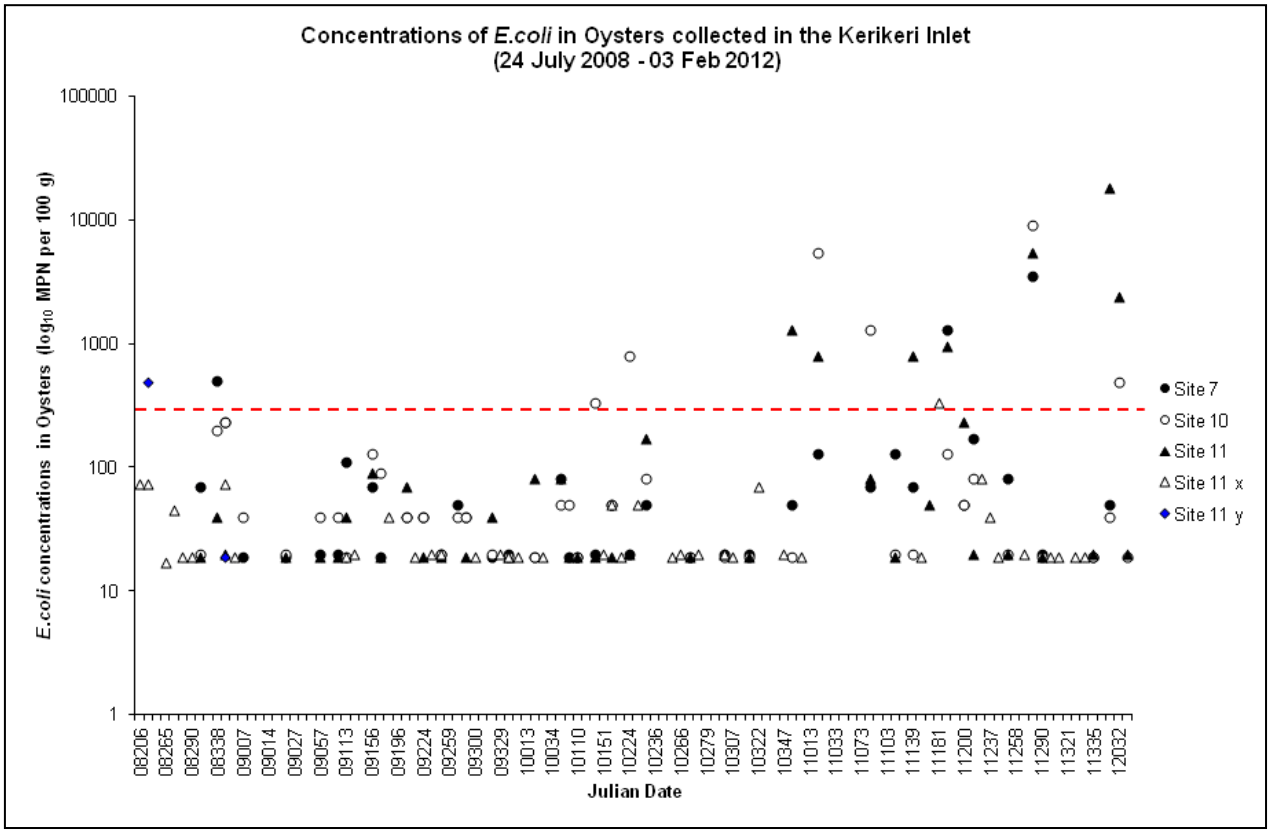

Figure 3: Concentration (presented as MPN/100g on a log scale) of *E. coli* in oysters collected from various sites in the Kerikeri Inlet from 24 July 2008 – 3 February 2012. Samples were collected at times when harvest criteria were met. The red dashed line represents the regulatory level of 230 MPN/100g. Levels below the level of detection are shown as 19 MPN/100g. Sites 11x and 11y represent specific oyster farms in the Hauparua Inlet (see Figure 1 for location of other sample sites). Table of Julian dates is provided in Appendix I.

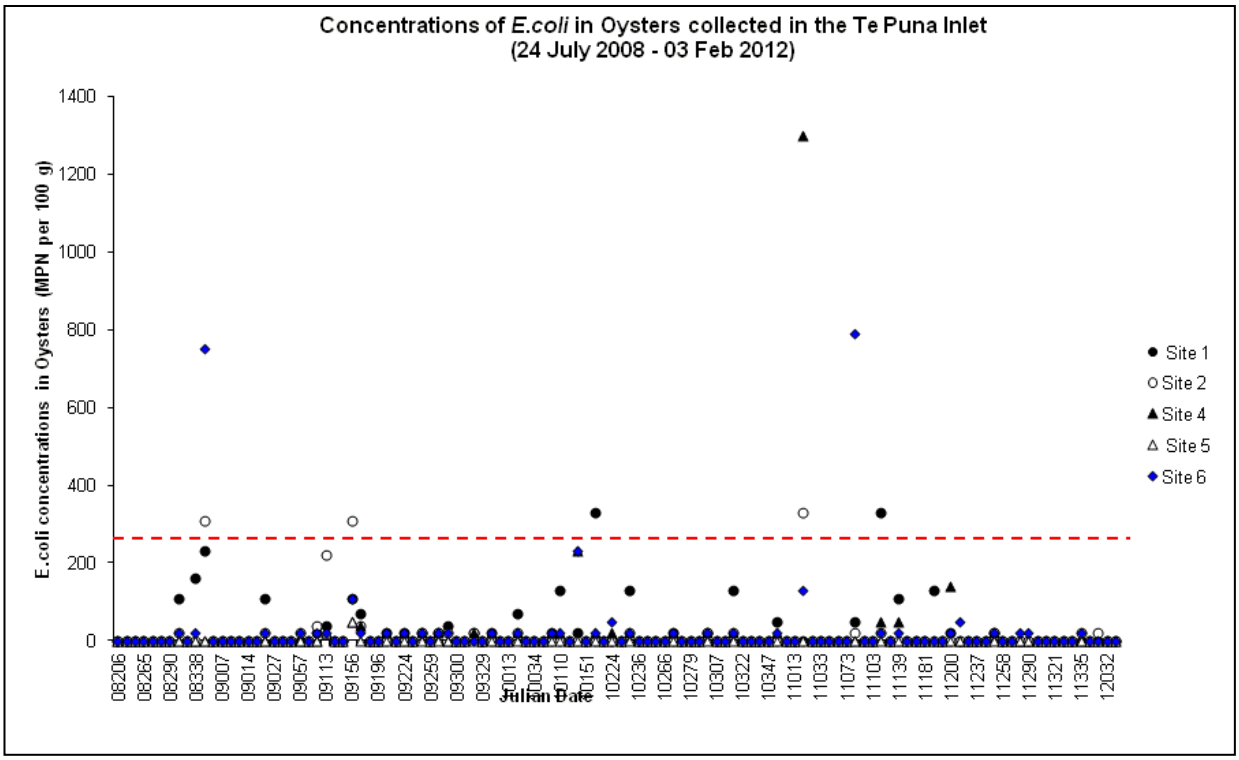

Figure 4: Concentration (MPN/100g) of *E. coli* in oysters collected from various sites in the Te Puna Inlet from 24 July 2008 – 3 February 2012. (See Figure 1 for the location of sites). Samples were collected at times when harvest criteria were met. The red dashed line represents the regulatory level of 230 MPN/100g. Levels below the level of detection are shown as 19 MPN/100g. Table of Julian dates is provided in Appendix I.

Figure 3 shows that the number of samples taken from Kerikeri Inlet in which *E. coli* levels have been greater than the regulatory level of 230 MPN/100g has increased over the last 18 months when compared to the two previous years. Elevated results were predominantly, but not exclusively from Sites 10 and 11. This temporal trend is not evident in samples taken from Te Puna Inlet (Figure 4). Unlike the results from Te Puna Inlet, it is also noticeable that some results from Kerikeri Inlet showed levels of *E. coli* very much higher than the regulatory level.

Figure 5, which is based on the same data for Site 11 only, shows the percentage of oyster samples in which different ranges of *E. coli* levels were found in each year from July 2008 to February 2012 (Note that the final time period does not represent data from a full 12-month period). For the purposes of this analysis, in cases when several samples were taken on the same day, the results have been averaged for the day. These data show increasing percentages of *E. coli* levels in the higher ranges (>230 MPN/100g) in oyster samples over the last two years.

It can be concluded from these data that unpredicted faecal contamination in oysters in the Kerikeri Inlet is increasing in severity, i.e. on the occasions when contamination not predicted by the Shellfish Quality Assurance Programme does occur, the contamination levels are now significantly higher than background coliform levels.

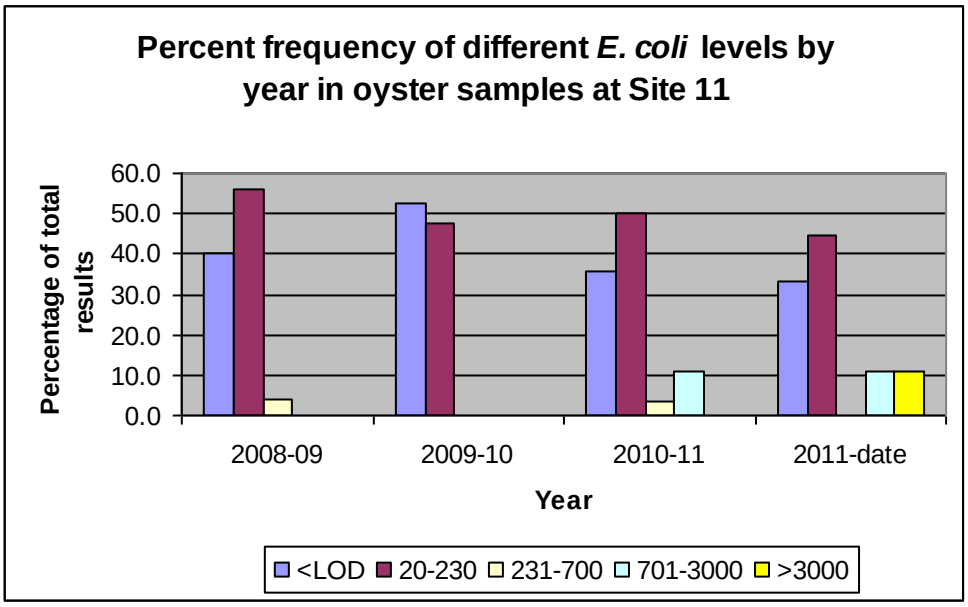

Figure 5: **Percentage frequency of different *E. coli* levels by year in oyster samples taken from Site 11. 12-month periods run from July-June. (Note that the final period runs only from July 2011-February 2012 and thus does not encompass a full 12 months). *E. coli* levels are presented in MPN/100g oyster tissue; LOD = Level of detection.**

3.2 Spatial Patterns

3.2.1 NRC Data

Box and whisker plots of the results from NRC monitoring of water quality at five sites in the Kerikeri Inlet from November 2008-January 2012 are provided in Appendix II. These data were collected roughly bi-monthly as part of the Council's State of the Environment (SoE) monitoring and include: faecal coliforms, enterococci, dissolved oxygen, salinity, turbidity, dissolved reactive phosphorus, total phosphorus, ammonia and total nitrogen. There is a general trend of decreasing turbidity from the Kerikeri River to the mouth of the Kerikeri Inlet, and an increase in salinity levels from the sites at the head of the Inlet to the sites at the mouth. The highest concentrations of ammonia tended to be at the head of the inlet (Waipapa River mouth).

The SoE results of monitoring for faecal coliforms show that there is a general trend of decreasing water quality from the mouth of the Kerikeri Inlet to its head. (See Box plot in Appendix II, and Table 1 on the next page, which summarizes all results for which faecal coliform data are available at each site). However, monitoring by Northland Regional Council at three of these sites (Waipapa River entrance, Wainui Island and Windsor Landing) over a tidal cycle (from 5.15 am to 4.45 pm) on 10th March 2011 illustrates an interesting exception to this general trend. This monitoring was undertaken as part of an investigation into the impact of tidal conditions on water quality monitoring results (Cornelisen et al., 2011), and included analysis of enterococci levels in water samples taken every 90 minutes over this time period. These results in relation to tidal height are shown graphically in Figure 6. Cornelisen et al. (2011) depicts the same data with the addition of the predicted tide heights.

Sample date	Faecal Coliform Levels in Water Samples (cfu/100ml)				
	Jetty Kerikeri R. entrance	Jetty Waipapa R. entrance	Wainui Is - Sth side	Windsor Landing moorings	Doves Bay Marina
12-Nov-08	116	290	26	2	<2
14-Jan-09	80	64	2	<2	<2
19-Mar-09	132	128	10	<2	2
13-May-09	390	240	24	2	6
15-Jul-09	-	280	86	80	56
16-Sep-09	200	270	22	2	10
18-Nov-09	88	72	<2	<2	<2
13-Jan-10	16	18	2	<2	<2
3-Feb-10	64	54	<2	4	<2
18-Mar-10	38	30	2	<2	<2
18-May-10	78	164	4	2	2
15-Jul-10	44	40	<2	2	<2
15-Oct-10	128	92	<2	<2	<2
18-Nov-10	60	28	14	<2	<2
13-Jan-11	16	24	<2	<2	<2
25-Jan-11	400	440	220	88	18
2-Feb-11	32	310	28	6	4
29-Mar-11	190	260	180	56	76
19-May-11	220	176	6	<2	<2
14-Jul-11	82	58	10	<2	8
15-Sep-11	64	6	<2	<2	<2
17-Nov-11	6	4	<2	<2	2
12-Jan-12	220	8	166	<2	2
Mean FC Level	**121.1**	**132.9**	**35.2**	**11.2**	**8.6**
Geometric Mean	**78.7**	**69.8**	**7.2**	**2.3**	**2.6**
Median	**81**	**72**	**6**	**1**	**1**

Table 1: Faecal coliform levels in water samples taken as part of the Northland Regional Council State of the Environment monitoring from five sites in the Kerikeri Inlet. For the purposes of calculation, results <2 have been given a value of 1.

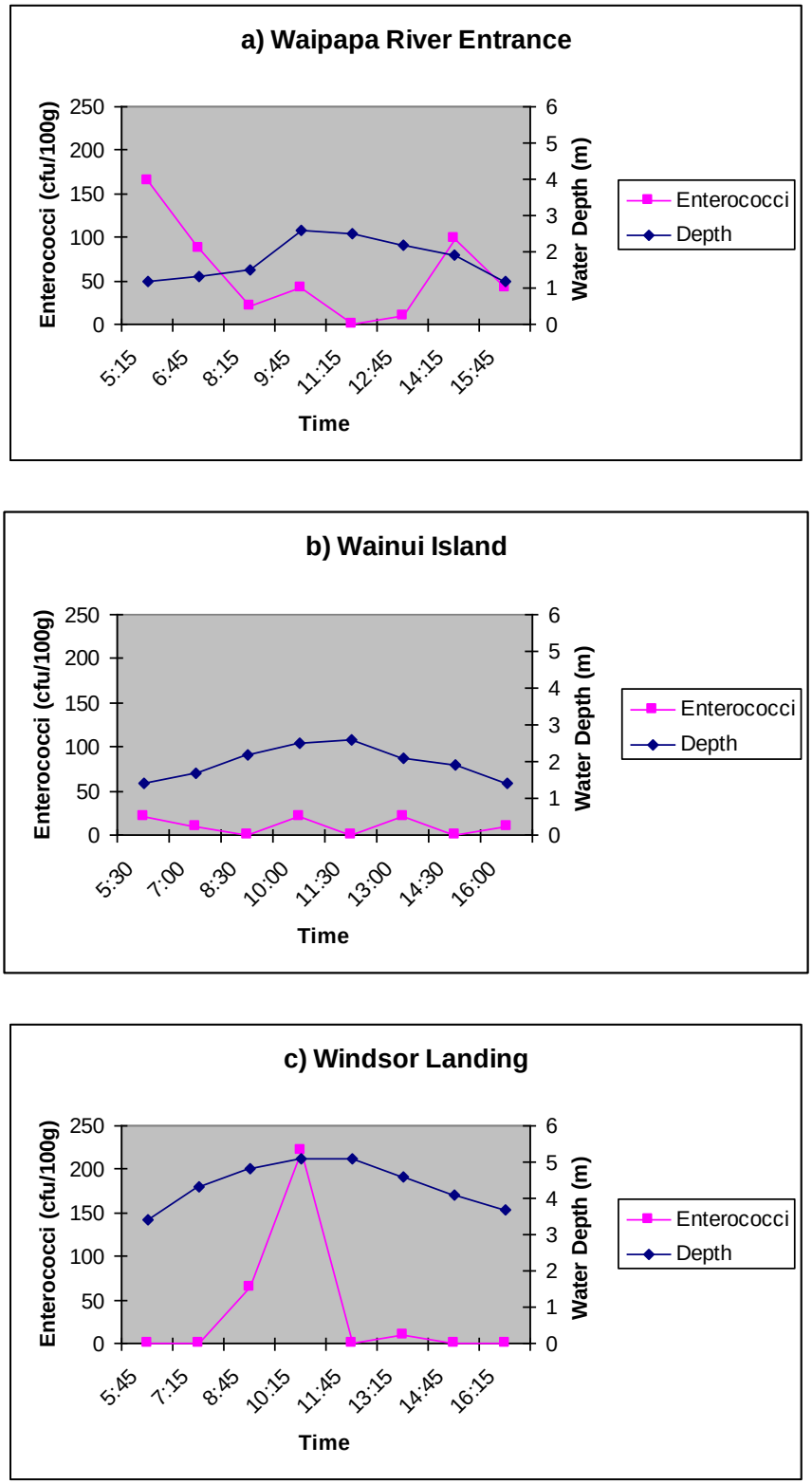

Figure 6: **Enterococci levels in water samples taken on 10th March 2011 at three sites ((a) Waipapa River entrance; (b) Wainui Island; and (c) Windsor Landing) in relation to tidal changes in water depth. The water depth depicted is that measured under the sample boat at each sample site and is thus a relative measure not a tidal height.**

Briefly: enterococci levels in water samples taken from Waipapa River entrance showed a general trend of decreasing levels over the incoming tide (presumably as a result of dilution), followed by increasing levels on the outgoing tide. At Wainui Island there appeared to be no variation in enterococci levels with the tide, showing consistently low levels varying between below the level of detection to 20 MPN/100ml. Based on the general trend arising from the long-term State of the Environment monitoring data, it would have been expected that enterococci levels at Windsor Landing would have been similar or lower than at Wainui Island. In this case however, although the majority of samples had enterococci levels below the level of detection, two consecutive samples taken on the rising tide at 8.45 am and 10.15 am contained enterococci levels of 60 and 222 MPN/100ml respectively. The sample with 222 MPN/100ml was taken within approximately 45 minutes of high water. There were no detectable levels of enterococci in the first of the samples taken on the outgoing tide. These results suggest a source of faecal contamination at Windsor Landing on this occasion that was independent of contamination sources further up the Kerikeri Inlet.

3.2.2 Northland District Health Board Data

The following Figures 7-10 show the results of analysis for *E. coli* in oyster samples taken by NDHB at Sites 7, 10 and 11 by 12-month reporting period (1 Oct- 30 Sept) (see Figure 1 for location of sampling sites). These figures illustrate that in some, but not all sampling events, elevated levels of *E. coli* in oysters sampled from one site are reflected at the other two sites. In some cases, significantly elevated *E. coli* levels at one site occur in the absence of, or at significantly higher levels of *E. coli* in oysters than at other Kerikeri Inlet sites (e.g. at Site 10 in January and June 2009, and March 2010; at Site 11 in January 2012).

These graphs illustrate that generally there is some coincidence in the detection of *E. coli* in oysters among sites. This is not surprising since it might be assumed that the presence of *E. coli* in growing waters (and consequently shellfish) in the Kerikeri Inlet is predominantly associated with run-off from the land which is prompted by a common environmental factor i.e. rainfall. However, there are instances in which levels from one site are substantially higher than at other sites.

The underlying trend of decreasing contamination from the head of the Kerikeri Inlet to the mouth shown in Table 1 is not consistently demonstrated in the NDHB monitoring data in Figures 7-10. This possibly indicates localised sources of contamination at sites 7, 10 and 11 impacting variably at different sample times.

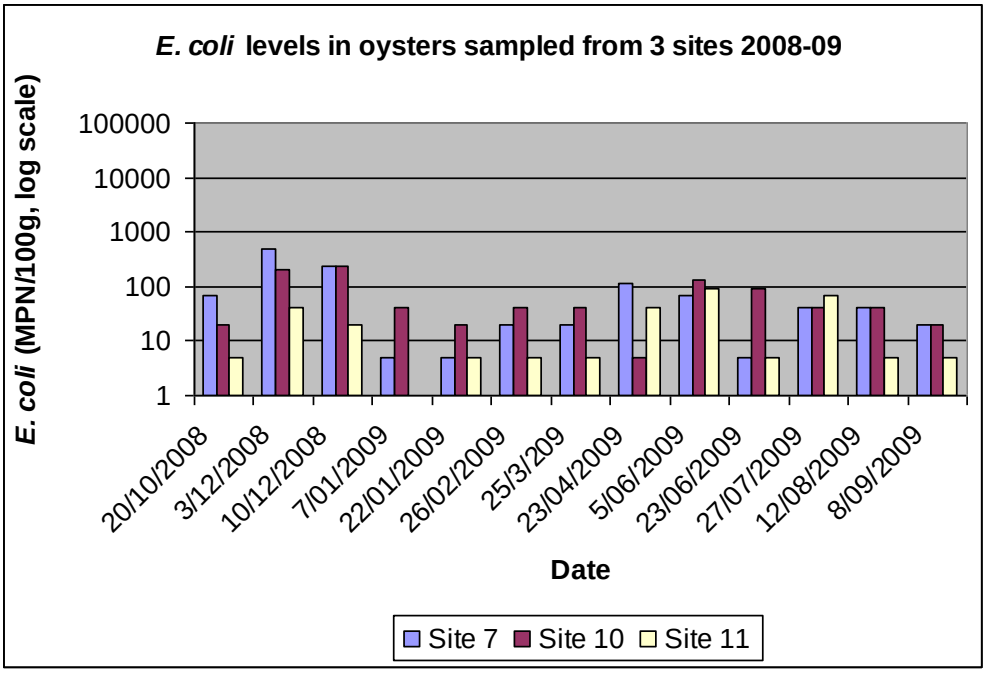

Figure 7: **Comparison of the *E. coli* levels (log scale) in concurrent oyster samples taken from Sites 7, 10 and 11 in the Kerikeri Inlet from October 2008-September 2009. Levels below the level of detection (<20 MPN/100g) are represented as 5 to distinguish the result from an absence of sample.**

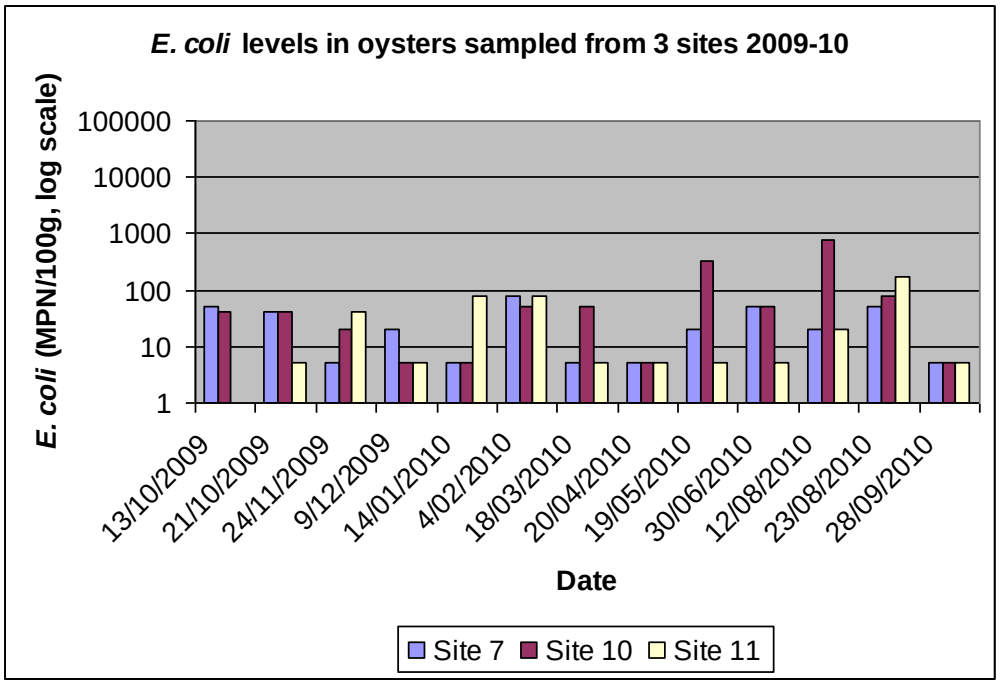

Figure 8: **Comparison of the *E. coli* levels (log scale) in concurrent oyster samples taken from Sites 7, 10 and 11 in the Kerikeri Inlet from October 2009-September 2010. Levels below the level of detection (<20 MPN/100g) are represented as 5 to distinguish the result from an absence of sample.**

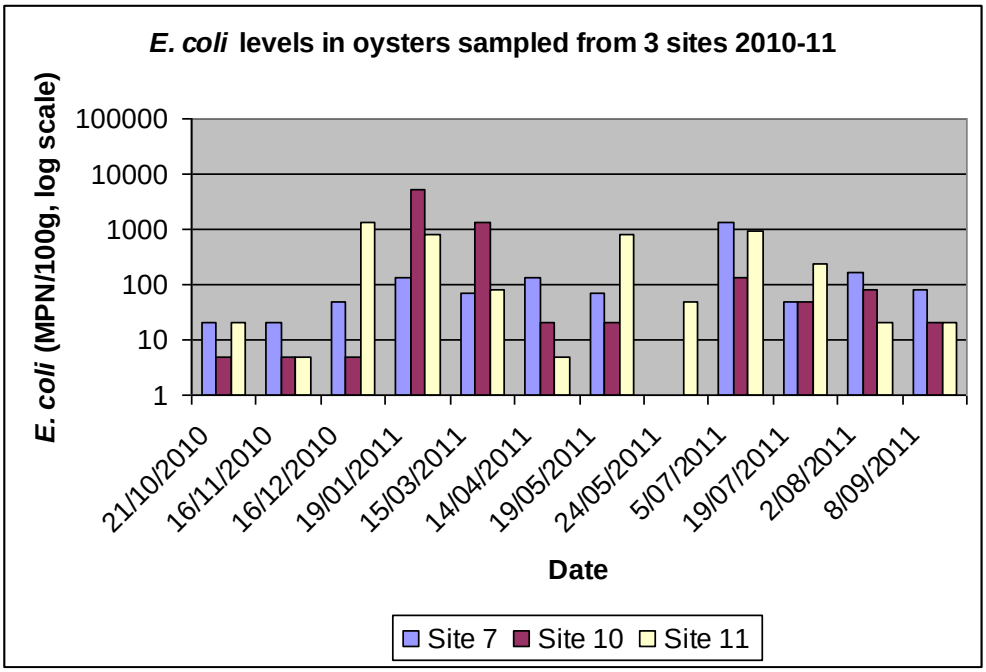

Figure 9: **Comparison of the *E. coli* levels (log scale) in concurrent oyster samples taken from Sites 7, 10 and 11 in the Kerikeri Inlet from October 2010-September 2011. Levels below the level of detection (<20 MPN/100g) are represented as 5 to distinguish the result from an absence of sample.**

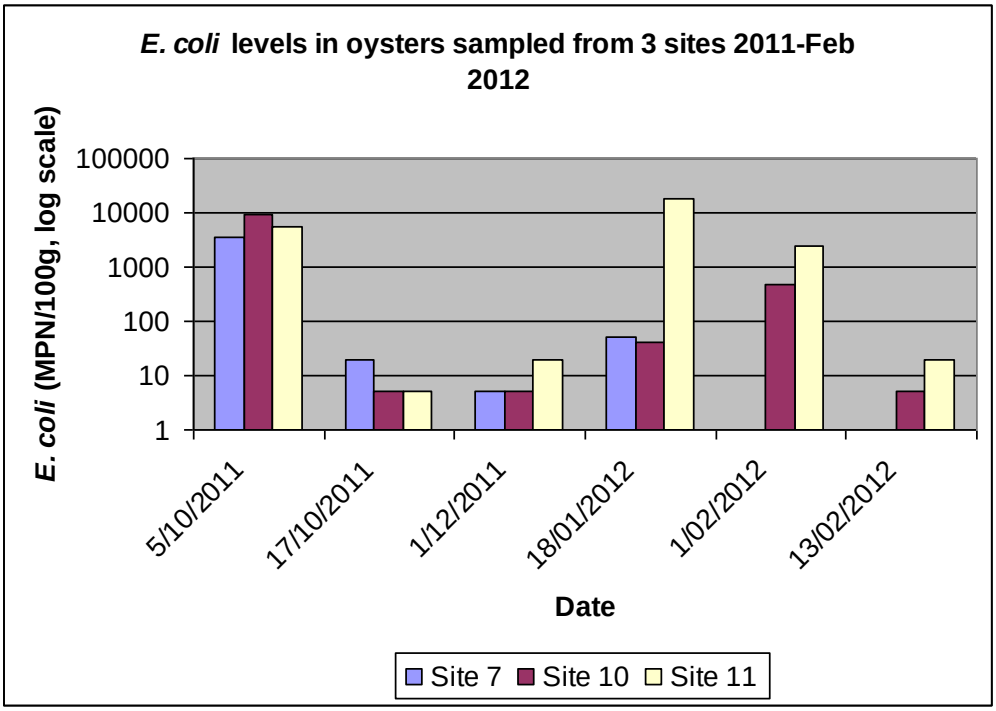

Figure 10: **Comparison of the *E. coli* levels (log scale) in concurrent oyster samples taken from Sites 7, 10 and 11 in the Kerikeri Inlet from October 2011-January 2012. Levels below the level of detection (<20 MPN/100g) are represented as 5 to distinguish the result from an absence of sample.**

3.2.3 Industry Data

More intensive oyster sampling in Hauparua Inlet was undertaken by Garth Richards (Kerikeri Delivery Centre) on 21/2/2012 and 7/3/2012. The location of sample sites is shown in Figure 11. Sample sites included four sites on the oyster farms (Sites 11, 11a, 11b, and 11c), a site in the narrows between the inner and middle inlet (Site 11e), sites where springs enter the Inlet (Sites 11f, g and h), a shoreline site in a small sheltered bay near the farms (Site 11i), and four sites from the shoreline close to the entrance (Sites 11d, and 11j1, 11j2 & 11j3). Oyster samples were tested for *E. coli* by Northland Food Testing Laboratory Services using the Petrifilm method.

The results of the sampling by Garth Richards are shown in Figure 12. These results show a general trend of elevated *E. coli* levels in oysters sampled from the shoreline (including sites close to springs and those not associated with springs) compared to oysters sampled from the oyster farm block. (We note that the result from Site 11c at the second sample time is an exception to this).

It was initially proposed that the elevated *E. coli* levels from sites close to the shore could be a result of oyster ingestion of *E. coli* re-suspended from the fine shallow sediment by wave action or wind. An alternative explanation could be the presence of *E. coli* associated with the seepage of groundwater into the Inlet at the shore. However these do not seem to be likely explanations for the elevated levels of *E. coli* observed in oysters sampled from Sites 11j1, 11j2 or 11j3 located on the rocks that lie between the mouth of the Hauparua Inlet and Windsor Landing, where there is little sediment associated with the shore.

Figure 11: Location of sampling sites within the Hauparua Inlet.

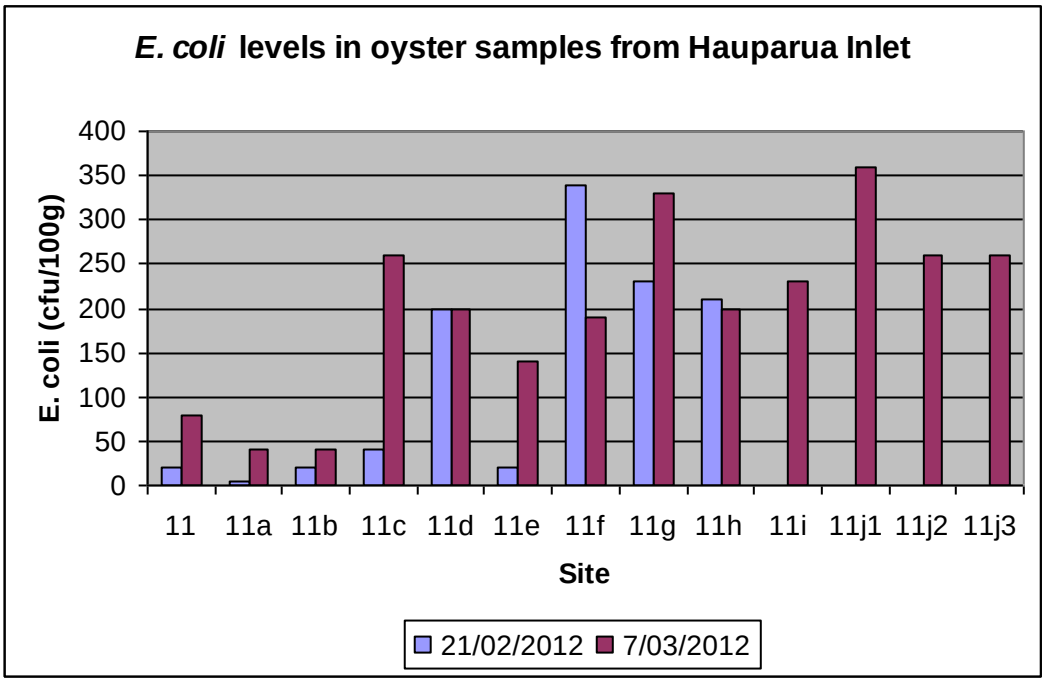

Figure 12: ***E. coli*** **levels in oysters sampled from several sites in Hauparua Inlet on 21st February 2012 and 7th March 2012.** ***E. coli*** **levels below the level of detection (<5 cfu/100g) have been represented as 4 cfu/100g so that they are able to be distinguished from instances when no samples were taken.**

Further preliminary observations by Garth Richards on the movement of water in the Hauparua Inlet may be pertinent to the interpretation of these results[1]. Following significant rainfall (approx. 147 mm) the previous day, on 20/3/2012 Garth tracked the movement of discoloured water from its entry into the head of the Hauparua Inlet to the mouth of the inlet on the ebb tide. The results of his observations are shown in Figure 13. The Kerikeri Weather Station recorded the average wind direction for that day as south-westerly. Garth noted that the discoloured water bypassed the sites close to shore (sites 11a, b, c, e, f, g, h, and i), but impacted on sites 11j1, 2, and 3, and to a lesser extent site 11d (email G. Richards to B. Hay, 20/3/2012).

A drogue-tracking study was undertaken by Garth Richards on 20/3/2012 to investigate the movement of water on the outgoing and turn of the tide in Hauparua Inlet, using oranges as drogues. Drogues were deployed in the Narrows (site 11e) on the outgoing tide, approximately 90 minutes before the predicted low tide on a still, calm day. They followed a course very similar to that previously observed, and reached Site 11 within 47 minutes. In the same study, water movement was tracked through the gaps between the rocky islets at 11j from the west into the channel up the Hauparua Inlet at slack water soon before the low tide. One explanation for this is that at slack water in Hauparua Inlet, the tide in Kerikeri Inlet is still ebbing, resulting in water from the direction of Windsor Landing being carried into the Hauparua Inlet. Any sources of contamination at Windsor Landing (which is an area of boat moorings and provides a sheltered anchorage for boats in easterly winds), or further up the Kerikeri Inlet could thus impact on water quality in the Hauparua Inlet at the turn of low tide.

[1] The Sanitary Survey report by NDHB (Brandt et al., 2011) summarises available information about tidal exchange etc. in the Kerikeri Inlet, and the Oceans 2020 study undertaken by NIWA investigated movement of water in the Bay of Islands in general, but there is a dearth of detailed published information about water movement in the Inlet or associated bays such as the Hauparua Inlet.

Figure 13: Movement of discoloured water out of Hauparua Inlet during ebb tide on 20/3/2012 following a significant rainfall event.

3.3 Relationship between Oyster Coliform Levels and Environmental Factors

A summary of the correlation of each of salinity and ammonia levels to faecal coliforms in water samples taken bi-monthly from 5 sites in the Kerikeri Inlet from 2008-2011 is shown in Table 2 (data from NRC SoE monitoring). The sample sites are distributed from the head of the inlet (Kerikeri River Entrance and Waipapa River Entrance), the middle of the inlet (Wainui Island) to the mouth of the inlet (Doves Bay Marina and Windsor Landing). Wainui Island lies in the inlet roughly between sample sites 7 and 10, and Windsor Landing is just west of Hauparua Inlet (Site 11).

The data show a significant negative correlation between salinity and faecal coliform levels at all sites. This is not unexpected as rainfall, which impacts on salinity levels, results in run-off from the land, transporting faecal coliforms into the estuary.

Sample Site	Correlation with faecal coliform levels in water samples	
	Salinity	**Ammonia[2]**
Kerikeri River Entrance	Significant negative correlation $r = -0.65$; $\alpha = 0.005$	No significant correlation ($r = 0.30$)
Waipapa River Entrance	Significant negative correlation $r = -0.45$; $\alpha = 0.025$	No significant correlation ($r = 0.36$)
Wainui Island	Significant negative correlation $r = -0.69$; $\alpha = 0.005$	Significant positive correlation $r = 0.51$; $\alpha = 0.025$
Doves Bay Marina	Significant negative correlation $r = -0.71$; $\alpha = 0.005$	Significant positive correlation $r = 0.82$; $\alpha = 0.005$
Windsor Landing	Significant negative correlation $r = -0.49$; $\alpha = 0.025$	Significant positive correlation $r = 0.74$; $\alpha = 0.005$

Table 2: Summary of the relationship between faecal coliforms in water samples and concurrent salinity and ammonia levels at 5 sites in the Kerikeri Inlet. Data from State of the Environment monitoring undertaken by NRC 2008-2011.

Table 2 shows that the strength of the relationship between ammonia and faecal coliform levels in water increases from the head of the inlet to the mouth. Ammonia is generated from organic matter by anaerobic microbial denitrification, and is usually utilised by other micro-organisms or plants. However, ammonia can be present in the environment if the supply exceeds biological demand. It can be transported into the aquatic environment with run-off from terrestrial sources (ANZECC 2000; Sigee 2005). We note that the majority of the ammonia readings taken in the Kerikeri Inlet were relatively high, being above the trigger values documented in the ANZECC guidelines (3.910 $g.L^{-1}$ or 0.004 g. m^{-3} at pH 7; ANZECC, 2000; Batley and Simpson, 2009). Several potential sources could account for the observed ammonia levels including runoff from terrestrial sources such as domestic sewage (on-site systems) and wetland discharge, or from marine sources such as the re-suspension anaerobic sediments (Sigee 2005).

The relationships between turbidity and ammonia levels and between turbidity and faecal coliform levels were also investigated in the same dataset, since re-suspension of sediment could be a source of both faecal coliforms and ammonia in the water column. A weak but significant positive correlation was found between turbidity and ammonia levels at Wainui Island ($r = 0.33$, $\alpha = 0.05$, $n = 29$), but not at any of the other sites. No significant correlation was found between turbidity and faecal coliforms in water at any site. We note that this does not necessarily rule out re-suspension of

[2] Ammonia levels in samples taken from all monitored sites on 14th July 2011 were in the range 0.32-0.40 g/m^3, which was roughly 3-6 times greater than the highest of any other results at the same sites. We cannot identify any outstanding environmental conditions (e.g. rainfall, wind) at the time that might be linked to these results. These outliers have been excluded from our analysis in Table 2.

sediment as a source of faecal coliforms, as it is possible that other factors that impact on turbidity (such as phytoplankton levels) could mask the relationship.

Figures 14 to 17 on the following pages show the relationship between the cumulative rainfall in days prior to sampling to the levels of *E. coli* in oyster samples taken from Te Puna Inlet (Figures 14 and 16) and Kerikeri Inlet (Figures 15 and 17) at times when the growing area management plan deemed that the shellfish were safe to consume. Note the differences in scale on the y-axis between plots of data from Kerikeri Inlet and Te Puna Inlet. The plots illustrate that elevated levels of *E. coli* in oysters are predicted less well from rainfall in the preceding 10 days in the Kerikeri Inlet than in the Te Puna Inlet. While this could be a reflection of more persistent input of faecal contamination into the Kerikeri Inlet after rainfall events, this could also suggest that the risk of a source of faecal contamination independent of rain-induced run-off is higher in Kerikeri Inlet than in Te Puna Inlet.

The relationship between the results of the concurrent water sampling for faecal coliforms and oyster sampling for *E. coli* at Sites 7, 10 and 11 is explored by scatter plot in Figure 18. These data are all from the NDHB regulatory sampling programme, and samples were taken at times when it was expected that the coliforms would have cleared from both the water and shellfish. Sampling is normally undertaken by NDHB on the incoming tide. Of the 19 *E. coli* results $\geq$230 MPN/100g, only 1 would have been predicted by a water result $\geq$14 MPN/100 ml, but low levels of faecal coliforms were detected in water in 10 out of 19 instances. In eight instances there were no faecal coliforms in water samples taken concurrently with oyster samples in which *E. coli* levels >230MPN/100g were observed. In four such cases the *E.* coli levels in oyster samples exceeded 2,000 MPN/100g.

Filter-feeding shellfish like oysters can concentrate bacteria out of their growing waters, and in highly contaminated water, oysters can accumulate many thousands of *E. coli* within one hour (this is a personal experimental observation made in spiking oysters and other bivalves with *E. coli* for commercial depuration trials). Once oysters are placed in uncontaminated water, they depurate rapidly, with most bacteria depurated out within the first 12-24 hours. The length of time taken to reduce bacterial numbers to below 230 MPN/100g varies with the initial contamination level: at summer water temperatures oysters containing initial *E. coli* concentrations below 10,000 MPN/100g will depurate within 24 hours, but with initial levels above 20,000 MPN/100g they may take two days to depurate (Buisson et al., 1981). These dynamics can assist in elucidating patterns in faecal contamination of growing waters.

As a comparison with the data from Kerikeri Inlet, a dataset of water faecal coliform and oyster *E. coli* monitoring results over 80 sampling events from an environmentally similar growing area overseas was examined (data not shown). In this dataset *E. coli* levels above the background level of 230 MPN/100g were rarely unaccompanied by faecal coliform levels in water elevated above 14 MPN/100ml (B. Hay, unpublished data). In Kerikeri Inlet, elevated levels of *E. coli* in oyster samples coupled with low or undetectable levels of faecal coliforms in water samples, particularly in samples taken following rainfall events, could indicate that the growing waters had very recently cleared of faecal contamination following a rainfall event, but that the shellfish had not yet had time to fully depurate. This would suggest that the level of pollution entering the water with run-off associated with rainfall has increased, and that the criteria in the shellfish management plan for reopening growing areas to harvest after rainfall should be modified accordingly to provide longer closures to harvest after rainfall events. Alternatively, particularly when the *E. coli* levels in oysters are very high in the absence of significant rainfall, the data may be indicative of very localised and temporally sporadic faecal contamination that was not present in the water at the time of sampling

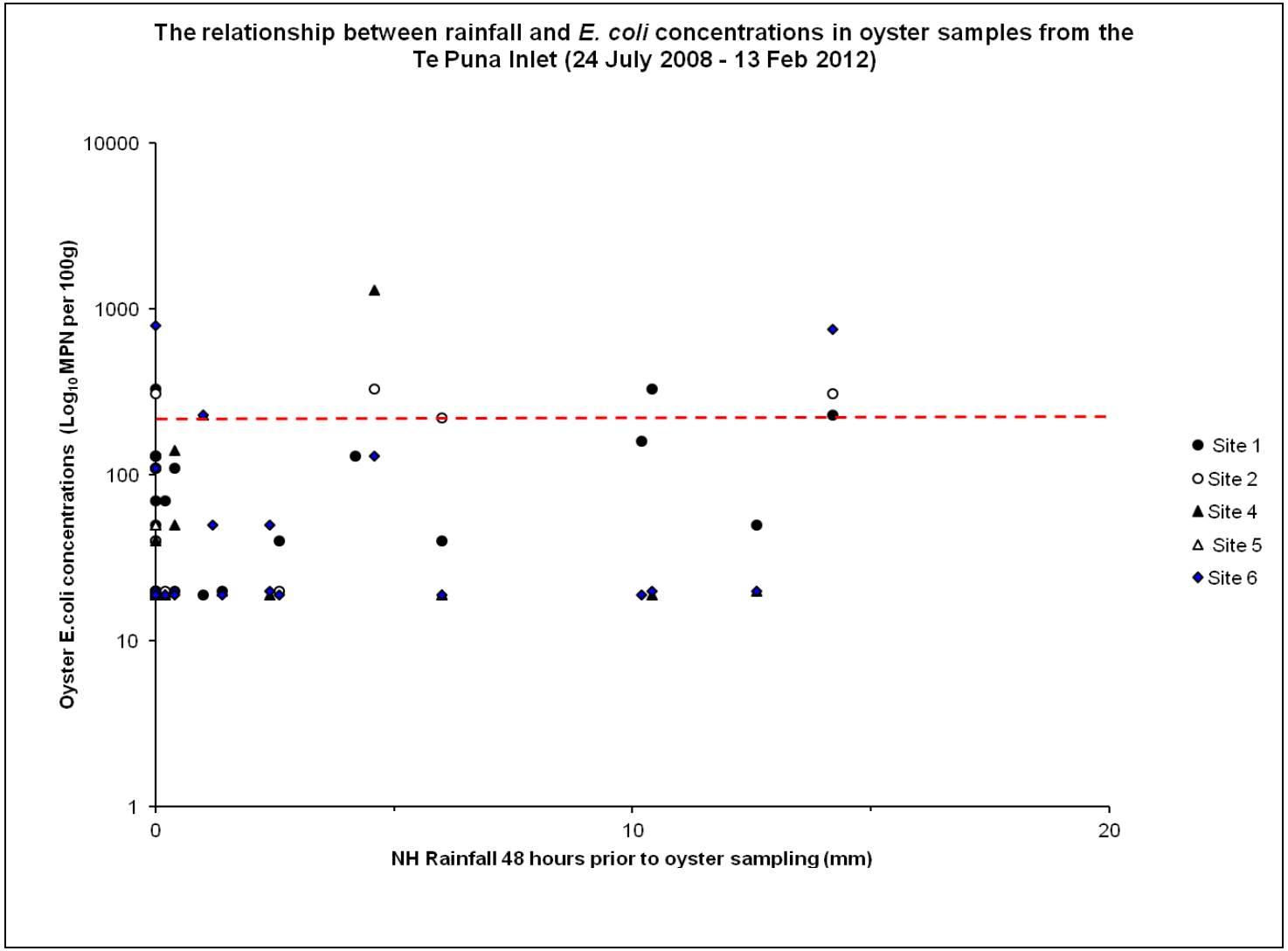

Figure 14: Relationship between *E. coli* levels in oysters sampled from five sites in Te Puna Inlet and cumulative rainfall measured at Kerikeri Airport in the 48 hours prior to sampling. Note log scale. Data from NDHB regulatory sampling from October 2008- January 2012. The red dashed line represents an *E. coli* level of 230 MPN/100g.

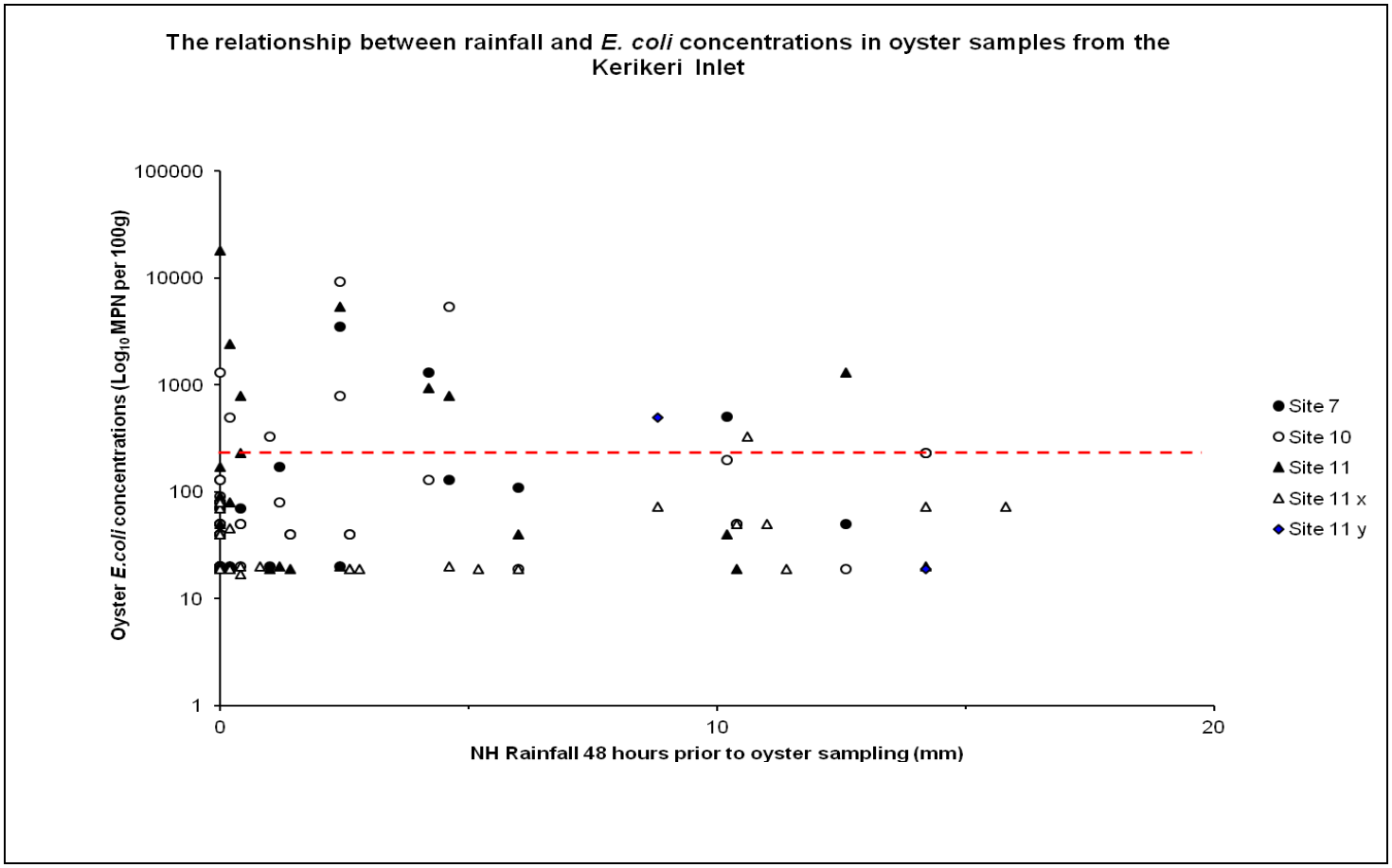

Figure 15: Relationship between *E. coli* levels in oysters sampled from five sites in Kerikeri Inlet and cumulative rainfall measured at Kerikeri Airport in the 48 hours prior to sampling. Note log scale. Data includes NDHB regulatory sampling from October 2008- January 2012 from Sites 7, 10, and 11, and post harvest data from processors for 2 sites within Hauparua Inlet (sites 11x and 11y). The red dashed line represents an *E. coli* level of 230 MPN/100g.

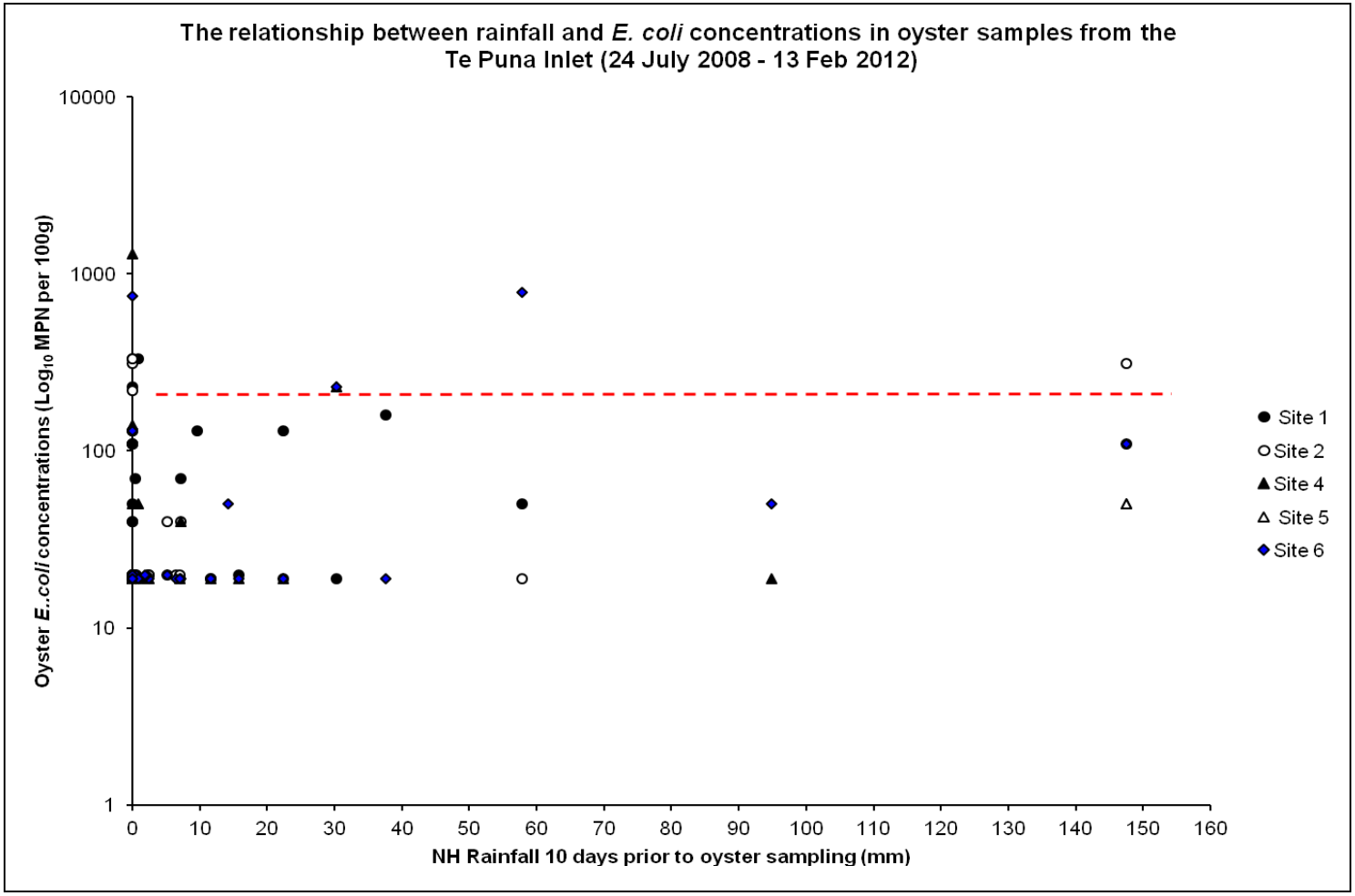

Figure 16: Relationship between *E. coli* levels in oysters sampled from five sites in Te Puna Inlet and cumulative rainfall measured at Kerikeri Airport in the 10 days prior to sampling. Note log scale. Data from NDHB regulatory sampling from October 2008- January 2012. The red dashed line represents an *E. coli* level of 230 MPN/100g.

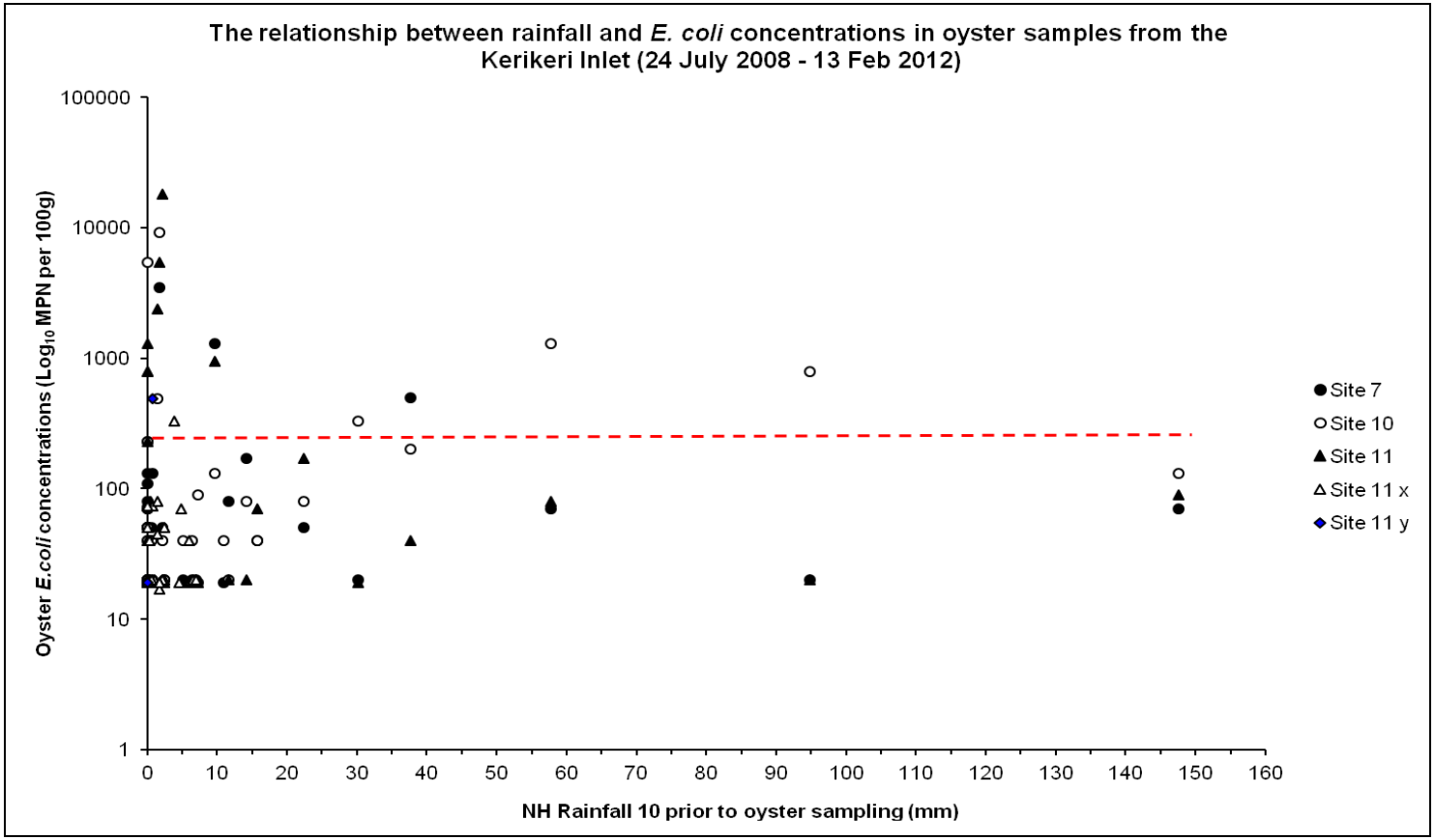

Figure 17: Relationship between *E. coli* levels in oysters sampled from five sites in Kerikeri Inlet and cumulative rainfall measured at Kerikeri Airport in the 10 days prior to sampling. Note log scale. Dataset includes data from NDHB regulatory sampling from October 2008- January 2012 from Sites 7, 10, and 11, and post harvest data from processors for 2 sites within Hauparua Inlet (sites 11x and 11y). The red dashed line represents an *E. coli* level of 230 MPN/100g.

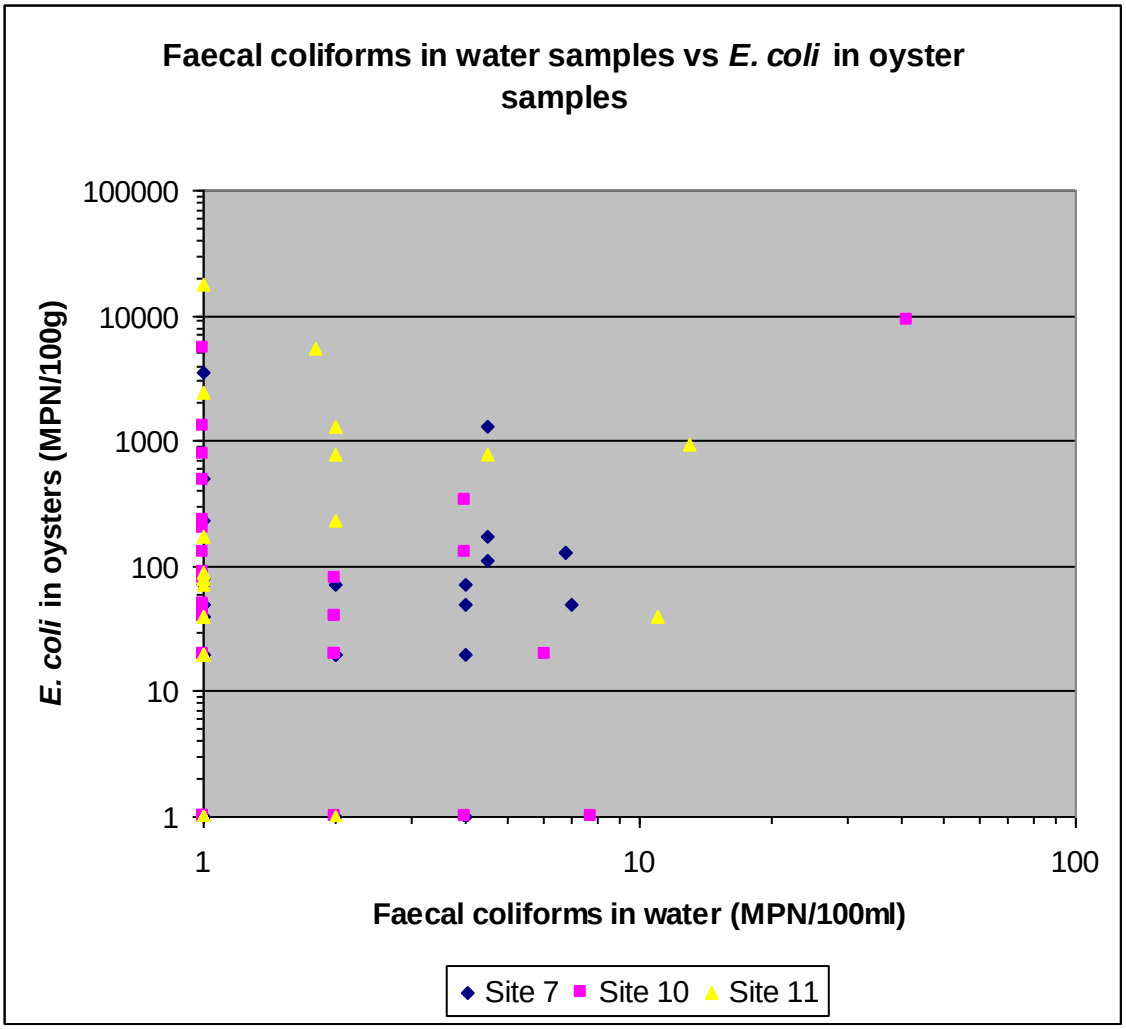

Figure 18: Scatter plot (log scale) showing the relationship between faecal coliform levels in water samples and concurrent *E. coli* levels in oyster samples. Water and oyster samples were taken at the same location at three sample sites in the Kerikeri Inlet. Samples were collected roughly monthly by NDHB from October 2008-February 2012. Levels recorded as less than the level of detection have been assigned a value of 1.

In order to investigate the circumstances surrounding the occurrence of significantly elevated *E. coli* levels in oysters from the Kerikeri Inlet in more detail, data from the regulatory monitoring by NDHB were tabulated with a variety of environmental data. Table 3 shows the results of each regulatory sample event in which *E. coli* levels ≥230 MPN/100g were found in oysters sampled at any of the three NDHB sample sites in Kerikeri Inlet. Sampling events undertaken when the area was closed to harvest have been excluded. A summary of the oyster sample results from sites at Te Puna Inlet have been included to provide background information.

Date	Sample Time	Site 7	Site 10	Site 11	Rainfall 24 hrs previous	Rainfall 48 hrs previous	Rainfall 10 days previous	Max rainfall/minute 24hrs previous	*Te Puna oyster E. coli levels elevated (≥230 MPN/100g)?*
10/12/2008	13:09	230	230	20	0	14.2	58.4	0.2mm/min at 2.51, 9/12	Yes - Sites 1, 2 & 6 Max = 750
19/05/2010	10:10	20	330	0	8	9	116.7	No data	Yes - Sites 4 & 6, Max = 230
16/12/2010	12:00	50	0	1300	2.2	14.8	15.4	0.2mm/min at 22.51, 15/12 0.6 mm/min at 07.02, 16/12	No - 50, 20, 20 at Sites 1, 4, & 6
19/01/2011	11:15	130	5400	790	7.4	12.8	13.8	0.2mm/min at 23.46, 18/1 0.2mm/min at 00.56, 19/1	Yes - Max = 1300 at Site 4 Faecal coliforms in water samples significantly elevated
15/03/2011	12:00	70	1300	80	0	0	61.8	0.2mm/min at 08.25, 14/3 0.2mm/min at 09.10, 15/3	Yes - Max = 790 at Site 6
19/05/2011	11:00	70	20	790	0.4	2.4	33.4	0.2mm/min at 11.37, 18/5	No – Max = 110 at Site 1 (others 50, 20)
5/07/2011	11:00	1300	130	940	6.0	17.4	63.8	0.2mm/min at 13.56, 4/7	No - 130 at Site 1 - no other sites sampled
19/07/2011	10:30	50	50	230	0.4	1.4	36.6	0.2mm/min at 18.15, 18/7 0.4mm/min at 6.16, 19/7	No - Max = 140 at Site 4; 20 & 0 at Sites 1 and 6; Water results significantly elevated at 2 out of 4 sites (79, 13, 2, 2 MPN/100ml)
5/10/2011	12:35	3500	9200	5400	2.4	22.8	41.4	No rain 4/10; 0.8 mm/min at 11.37, 5/10	No - 20 at Site 6 - no other samples
18/01/2012	12:15	50	40	>18000	0	0	15.6	No rain	No - 0 at Site 2, no other samples
1/02/2012	10:30		490	2400	0	0.2	4.2	0.2mm/min at 03.15, 31/1	Not sampled

Table 3: Sample and rainfall data for all samples in which the *E. coli* level in oysters ≥ 230 MPN/100g at any of the three sample sites in Kerikeri Inlet. Only results for samples taken when the area was open for harvest based on concurrent harvest criteria have been included. Data for rainfall levels are from NDHB dataset, and rainfall intensity data are from Kerikeri Weather site (www.kerikeriweather.co.nz). Shading highlights groups of sample events discussed in text below.

Unfortunately the circumstances under which the majority of regulatory samples were taken (i.e. on re-opening growing areas following closures to harvest after rainfall) are not conducive to elucidating whether contamination events are unrelated to rainfall-induced run-off. As an initial step in resolving potential contamination sources, the sample results that appear potentially unrelated to rainfall in the previous 10 days[3] were identified. These sample events occurred in January and February 2012, and are shaded in mauve in Table 3. Although rainfall was not absent in the 30 days prior to sampling on 18/01/2012 (see Figure 19), the extraordinarily high *E. coli* level in the oyster sample taken from Site 11 is not reflected in any other samples from Kerikeri Inlet or Te Puna Inlet. It thus appears to be a result of localised, high level contamination event at the Hauparua Inlet that was not related to rainfall. The elevated levels at Sites 10 and 11 on 1/02/2012 also appear unrelated to rainfall (see Figure 20 on next page), as the total rainfall in the two weeks subsequent to the previous samples from the same sites (on 18/01/2012) was only 4.2 mm (comprised of 0.2 mm on 30/1, 1.8 mm on 27/1, 0.8 mm on 26/1 and 1.4 mm on 22/1). No faecal coliforms were detected at any of the Kerikeri Inlet sites in water samples from either of these sampling events. In both cases, the sampler recorded the samples as having been taken on the low incoming tide. The wind directions on the day before each sample was taken each contained an easterly component, averaging SE, with the maximum gusts ENE on 17/1/12; and NE on 31/1/12 (data from www.kerikeriweather.co.nz).

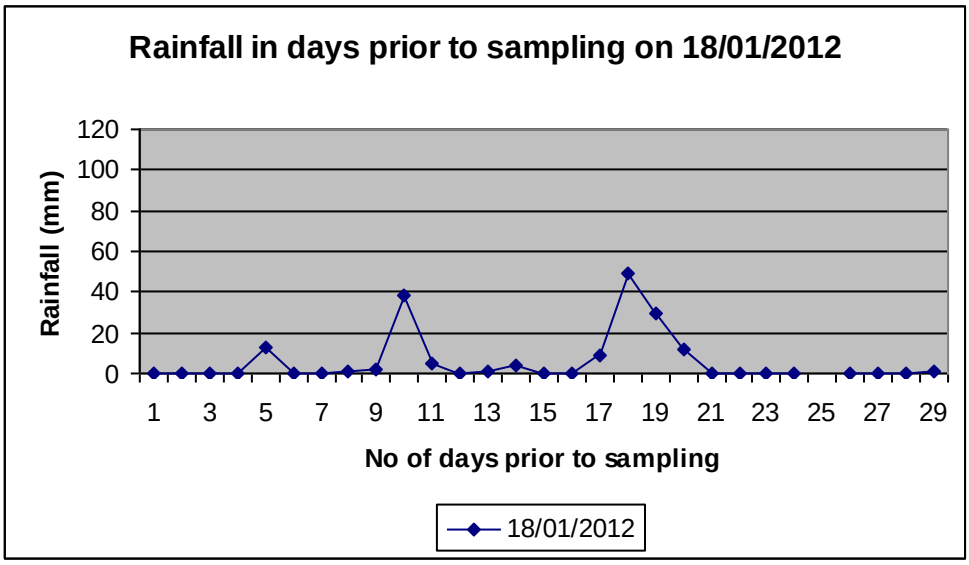

Figure 19: Graph of daily rainfall in the 30 days prior to sampling on 18/01/2012.

[3] Note that the NDHB rainfall dataset has been used in this and following analyses, and that there are discrepancies between these data and those in the rainfall dataset provided by Sanford Ltd.

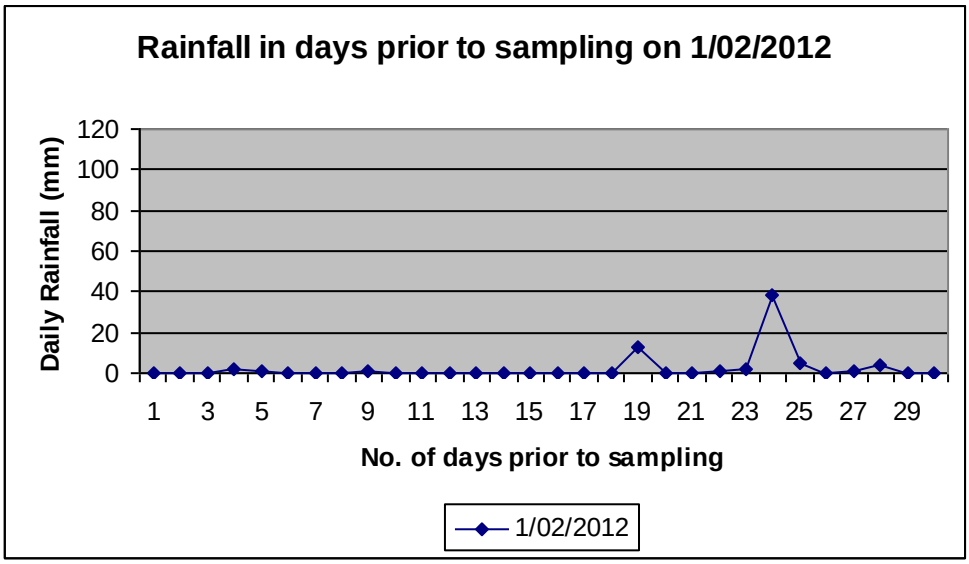

Figure 20: Graph of daily rainfall in the 30 days prior to sampling on 01/02/2012.

Of the remaining sampling events, those with very significantly elevated results at any Kerikeri Inlet site (indicated in red font in Table 3) were examined in more detail. With the exception of results on 19/5/2011 and January/February 2012, these results are also discussed by Brandt et al. (2011). Where our analysis provided potentially useful insight into contamination patterns, these data are discussed below.

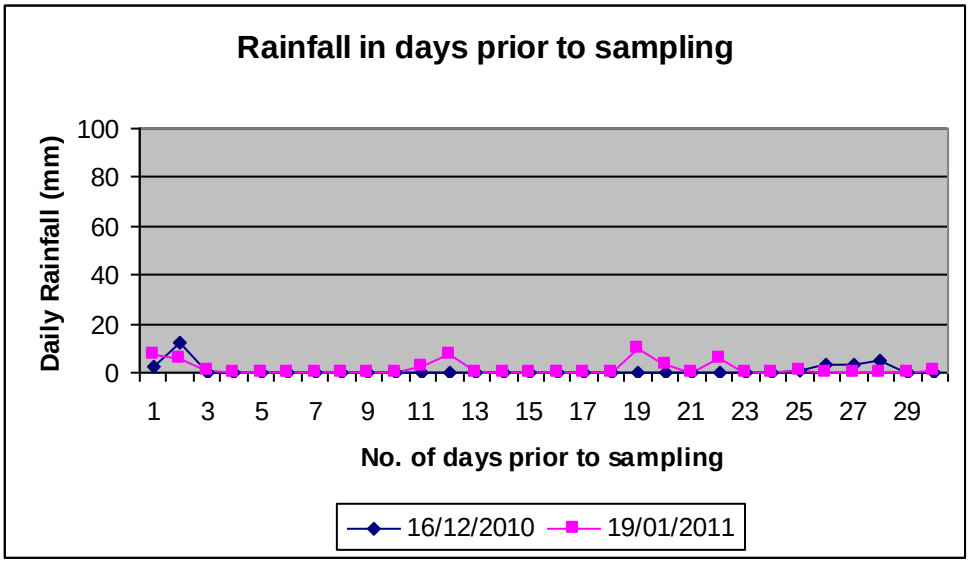

Figure 21: Graph of daily rainfall in the 30 days prior to sampling events on 16/12/2010 and 19/01/2011.

The rainfall conditions prior to sampling on 16/12/10 and 19/1/11 (shaded yellow in Table 3) are similar in that low levels of rainfall occurred in the two days prior to sampling, but there was no rainfall for the week prior to that. In both cases there was little rainfall for the 30 days prior to sampling (see Figure 21). Northland District Health Board reports that the December sample was taken at low incoming tide and the January sample at the latter part of the incoming tide.

Very low levels of faecal coliforms were recorded in all Kerikeri Inlet water samples on 16/12/2010 (range 2 - 4 MPN/100ml), and in Te Puna Inlet (range 2 - 9 MPN/100ml). Early in the morning before sampling on 16/12/2010, a high intensity rainfall event was observed. On 19/1/2011, low levels of faecal coliforms were recorded in 2 out of 3 Kerikeri Inlet water samples (range 4.5 - 6.8 MPN/100ml), but significantly elevated levels were observed in water samples from Te Puna Inlet (33, 70, 130 and 240 MPN/100ml). Rainfall intensity on the two days prior to sampling on 19/1/2011 was observed as low at the Kerikeri Weather Station, but the NDHB sampling officer noted localised heavy showers and high turbidity in the water on the day of sampling (Brandt et al., 2011). Results from both these sampling events could be linked to rainfall run-off, but clearly at these times the impact of rainfall on faecal contamination levels at Site 11 on both occasions and at Site 10 on 19/01/2011 was much greater than expected based on the amount of precipitation.

The sampling event on 5/10/2011 is another instance in which the high intensity of rainfall might possibly have been implicated in the highly elevated *E. coli* levels in oyster samples (data shaded in orange in Table 3). Under the former rainfall criteria for harvest, the growing area would have been closed on this day, but was open under the new rainfall/tidal index criteria (Brandt et al., 2011). Faecal coliform levels in water samples were significantly elevated at one site in Kerikeri Inlet (41 MPN/100ml at Site 10), but just at the level of detection at Site 11 (1.8 MPN/100ml) and not detected at Site 7. Faecal coliforms were observed at the level of detection in a water sample from one site in Te Puna Inlet, but not detected water samples from four other sites. *E. coli* levels in oyster samples from Te Puna Inlet were not elevated above background levels (20 MPN/100g at Site 6). The Kerikeri weather station recorded an extremely high intensity rainfall event early in the morning on which the samples were taken. The wide disparity between the results of oyster samples taken at the Kerikeri Inlet sites and that in Te Puna is remarkable in this sampling event. Whether the sampling results on 5/10/2011 represent the outcome of a localised, high intensity rainfall event, differences in the length of time taken for the respective inlets to clear following an earlier contamination event, or a contamination event that impacted on the Kerikeri Inlet but not at Te Puna Inlet (for example, a spill from the Kerikeri reticulation system as a result of extraordinarily high rainfall intensity) is unknown at present. It is suggested that information from the Far North District Council regarding the performance of the Kerikeri reticulation system (including pump stations) and wastewater treatment plants during this rainfall event should be sought.

The only rainfall in the 10 days prior to sampling on 15/3/2011 (data shaded pink in Table 3) was a total of 57.8 mm over the 9-10 days before sampling, with no subsequent rain prior to the sample event. No other significant rainfall events occurred in the 30 days prior to sampling. Faecal coliforms in water samples were not elevated above background levels at the time of sampling. Sea surges arising from the tsunami in Japan were experienced on the Northland coast on 11/3/2011 and subsequent days. As noted by NDHB (Brandt et al., 2011), it is possible that re-suspension of sediment arising from this may have impacted significantly on water quality at the time. In terms of interpreting the sampling results, these would have to be regarded as exceptional circumstances.

Figure 22 (following page) shows a graph of the daily rainfall in the 30 days prior to sampling on 5/07/2011 and in the 14 days prior to the consecutive sample event on 19/07/2011 (together these lines provide a record of rainfall from 5/06/2011 to 18/07/2011). Associated sample and environmental data are shaded in turquoise in Table 3. An extreme rainfall event occurred 18 days before sampling on 5/07/2011, and subsequent frequent low levels of rainfall suggest that soil conditions are likely to have remained wet throughout this time. The levels of *E. coli* in oyster samples taken from Kerikeri Inlet on 19/7/2011 were generally lower than on 5/7/2011 (see Table

3). The faecal coliform levels in water samples also differed between the two sample times, being significantly elevated at Kerikeri Inlet and Te Puna Inlet sites on 5/7/2011 (ranges 4 – 13 MPN/100ml and 2 – 79 MPN/100ml respectively), and at or below the level of detection at all sites on 19/7/2011. Rainfall levels followed a similar pattern in the 4-5 days prior to sampling on both 5/7/2011 and 19/7/2011, except that the sample on 19/07/2011 was taken on the first day with virtually no rainfall (0.4 mm) following the minor rainfall event, while the sample on 5/7/2011 was taken after a day on which a very low level of rainfall (6mm) occurred (i.e. effectively 2 days earlier in the pattern of rainfall). It is likely that these differences account for the difference in water and shellfish sample results between the two sample events. At both sample times shellfish results suggest that Site 11 is likely to be impacted by low levels of rainfall during wet soil conditions.

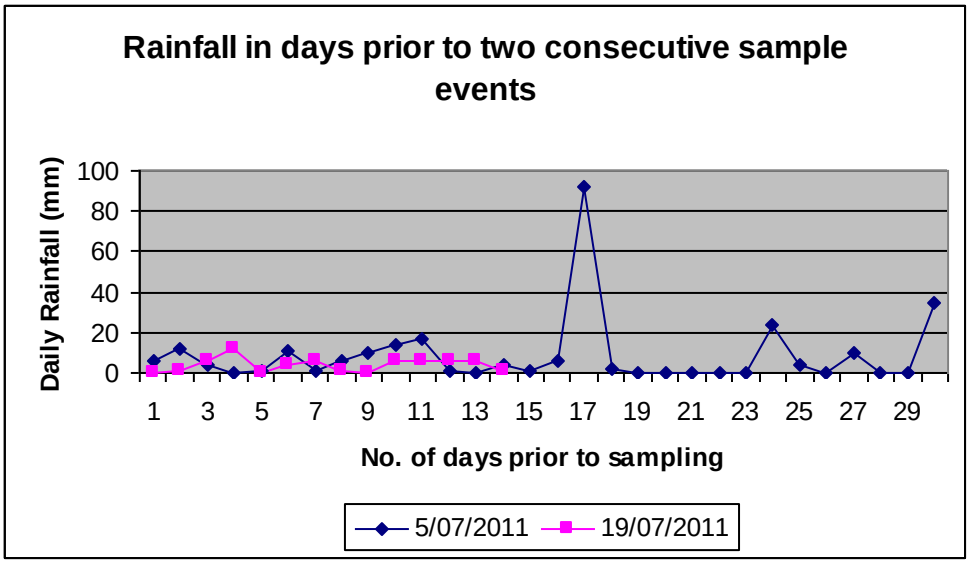

Figure 22: Graph of daily rainfall in the 30 days prior to sampling on 5/07/2011 and the 14 days prior to the consecutive sample taken on 19/7/2011.

An alternative explanation for the comparatively elevated coliform levels in oysters at Site 11 in July 2011 and on 19/05/2011 (see Table 3 and Figure 23 on the following page) could be the relative persistence of contamination in Hauparua Inlet following extreme rainfall events when the ground is wet. Note that an oyster sample taken on 24/05/2011, five days after the elevated sample result, contained only 50 MPN/100g. The inter-tidal nature of the substrate of much of the Hauparua Inlet suggests that it is well-flushed, but continuing faecal input following large rainfall events (for example, from groundwater springs) could be a possibility.

The rainfall history and results of samples taken on 19/05/2010 (see Table 3, and Figure 24) provide an interesting comparison to the situation observed on 19/05/2011. In this sample round no *E. coli* were detected in the oyster sample taken from Site 11. There were significant differences in the rainfall patterns in the 30 days before the sampling events. Of the entire sampling events presented in Table 3, this sampling round had by far the highest level of rainfall in the 10 days prior to sampling (116.7mm), but the lowest recorded *E. coli* level in oysters sampled from Site 11 (i.e. not detected). The day of highest rainfall occurred 22 days prior to sampling (*c.f.* 18 days prior to the other sampling events on 19/05/2011 and 5/07/2011), and the rainfall on this day was not as high as the maximum rainfall that fell in the other winter events discussed above. The remaining rainfall was spread over the 11 days prior to sampling, with no rainfall on other days. The summer and

autumn of 2010 was characterised by drought conditions in Northland, with the only other significant rainfall in the two months prior to sampling being 21 mm on 13/4/2010 and 32 mm on 1-2/3/2010. Possibly the differences in shellfish contamination at Site 11 between the events in 2010 and 2011 may relate to differences in groundwater levels between the years, or alternatively, perhaps the current sources of contamination at Hauparua Inlet were not present or as significant at the earlier time.

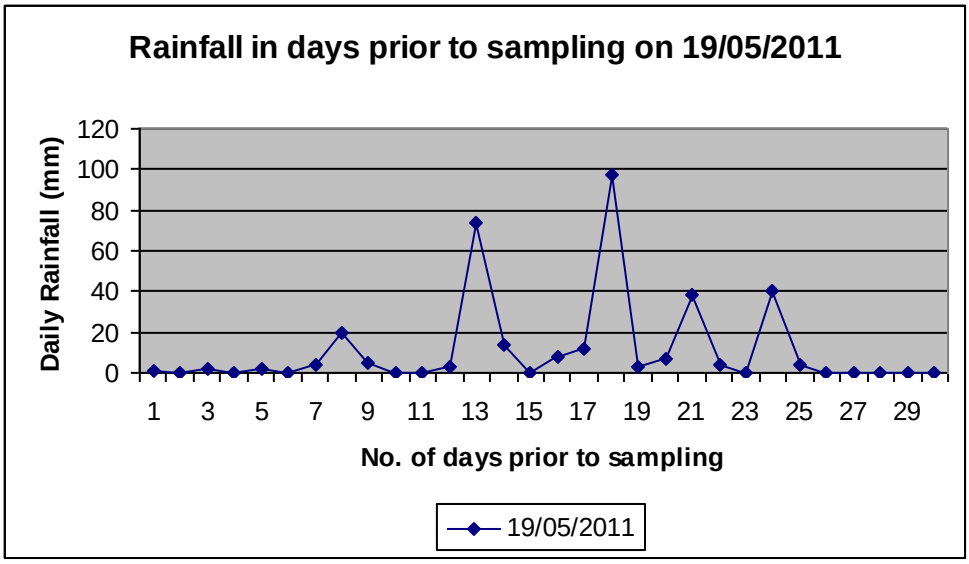

Figure 23: Graph of daily rainfall in the 30 days prior to sampling on 19/05/2011.

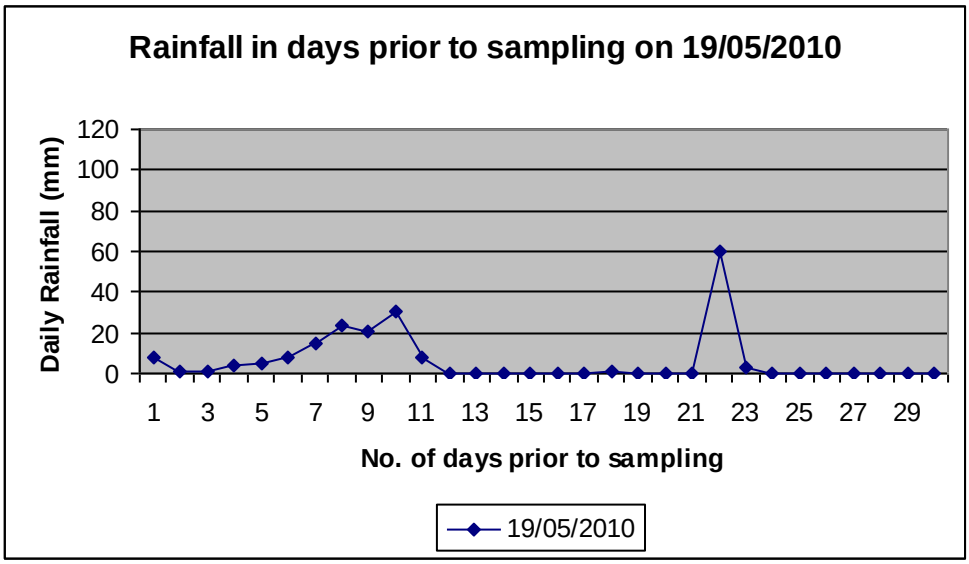

Figure 24: Graph of daily rainfall in the 30 days prior to sampling on 19/05/2010.

In summary therefore, the results presented in Table 3 illustrate that in some instances (e.g. mauve-shaded data), high *E. coli* levels in oysters do not appear to be related to rainfall events (e.g. Sites 10 and 11 on 1/02/2012)), and extremely localised contamination unrelated to rainfall can occur (e.g. Site 11 on 18/01/2012). Both these sampling events occurred during the summer season when

visitor and boat numbers in the region increase. The impact on faecal contamination of growing waters by relatively low levels of rainfall over 2 days following a dry period in summer can be unexpectedly high, and may be linked to rainfall intensity (yellow-shaded data in Table 3). However, we note that these observations occurred during a season in which contamination unrelated to rainfall has also been observed. The possibility that these observations may in part be related to contamination events not directly impacted by rainfall cannot be definitively excluded with the available data. Another sampling event (5/10/2011, shaded orange in Table 3) also provides possible support for the impact of rainfall intensity on faecal contamination of shellfish, although in this case the high level contamination was limited to the Kerikeri Inlet, with no evidence of contamination above background level in the Te Puna Inlet. Whether this is because the rainfall and consequent land run-off was localised to the Kerikeri Inlet catchment, or because the high intensity rainfall caused some sort of spill or contamination event in excess to the "normal" run-off in the Kerikeri Inlet but not Te Puna Inlet is unknown but should be followed up with the Far North District Council. The relationship between rainfall and unexpectedly high *E. coli* results some time after high rainfall events when frequent low rainfall events have kept the ground wet has not been resolved: it may relate to persistent contamination of the Hauparua Inlet following high rainfall events, or increased contamination associated with the subsequent low rainfall events under these conditions. These unexpectedly high levels of contamination were not observed at Site 11 under conditions of high rainfall in the 10 days prior to sampling in May 2010. This may have been a result of unusually dry conditions over much of the early part of 2010, or alternatively because the current source of contamination was not present at that time.

3.4 Discussion of Results

Analysis of monitoring data by NDHB has identified that the frequency of unacceptably high *E. coli* levels in oysters has increased at oyster farming areas on the southern side of the Kerikeri Inlet. At Site 11 the levels of contamination above background level appear to have increased over time (i.e. the degree of contamination in non-compliant monitoring results has increased as well as the frequency of contamination events). This trend, observed over a short time series in the analysis presented here, is consistent with the fact that since 1987 NDHB has had to impose increasingly stringent harvest criteria in the Kerikeri oyster growing areas – these criteria are founded on the observed shellfish contamination levels and the length of time it takes shellfish contamination to clear after rainfall events (Brandt et al., 2011). The comprehensive sanitary surveys and annual updates undertaken by NDHB over the years that oyster farming has been present in Kerikeri Inlet have not identified any increase in agricultural stock numbers or any other increasing potential sources of faecal contamination other than increasing human activity in the catchment e.g. increasing settlement of the coastline and in Kerikeri township itself (Brandt et al., 2011). The possibility that the deterioration in shellfish quality might be attributable to human faecal contamination therefore needs to be seriously considered.

The data analysis presented in this report attempted to determine any patterns in faecal contamination or associated environmental drivers that might elucidate the potential contamination sources or assist in focussing faecal source tracking work. Analysis of data has shown that there is an underlying trend of increasing coliform contamination of water from the mouth of the Kerikeri Inlet to the head. The influence of tide on water quality (based on enterococci levels) is greater at the head of the Kerikeri Inlet than at the mouth. State of the Environment monitoring data show a negative correlation between salinity and faecal coliforms in water samples from the Kerikeri Inlet, and there is a trend of increasing correlation between ammonia and faecal coliforms in water

samples from the head of the Kerikeri Inlet to the mouth. There is no relationship between turbidity and faecal coliform levels in water in the same dataset.

On occasion, localised contamination of water and oysters overlies the trend of increasing faecal contamination levels from the mouth of the Kerikeri Inlet to its head. Generally this is related to rainfall-associated run-off in the immediate catchment of each growing area. However, as evidenced by the failure of harvest criteria to ensure the required oyster quality, this relationship has become less predictable in recent years at Site 11 and, to a lesser extent, at Site 10. There is evidence that some high level contamination events at Site 11 during summer may not be related to rainfall-induced run-off. Exfiltration of poorly treated effluent from overloaded on-site sewage systems (e.g. septic tanks), or discharge of effluent from boats are examples of potential faecal contamination sources associated with increased visitor numbers to the area over summer months. The tenuous association of these sporadic events with winds with an easterly component could be consistent with the implication of effluent derived from boats moored near Windsor Landing, which would provide a sheltered mooring place under such circumstances. The movement of water from Windsor Landing into Hauparua Inlet has been observed at low tide before the turn of the tide. This might also transport water from further up Kerikeri Inlet into Hauparua Inlet.

Rainfall intensity may impact on the relationship between the level of rainfall and faecal contamination in oyster growing areas. While this could be attributable to a “first flush” effect, the possibility of contamination sources that are induced by heavy rainfall should be investigated further, particularly as intense rainfall events are not new events in the history of oyster growing areas, but levels of contamination associated with such events do appear to have increased. Investigation into the performance of on-site sewage systems, sewage reticulation systems and wastewater treatment plants during intense rainfall events is suggested.

Soil saturation may also impact significantly on the relationship between the level of rainfall and faecal contamination levels. Under these conditions, the possibility of persistent delivery of faecal contamination or delayed contamination arising from very high rainfall events cannot be excluded. The contamination of groundwater by on-site sewage management systems (e.g. septic tanks), and by STP effluent discharged to the Waitangi wetlands, and the potential for the entry of this groundwater into oyster growing areas at Sites 11 and 10 are issues that require further investigation.

SECTION 4: RESULTS OF PREVIOUS SOURCE TRACKING STUDIES

4.1 Introduction

To date, two faecal source tracking studies have been undertaken in the Kerikeri Inlet. Both studies have incorporated an element of field trialling of new source tracking tools. In addition, the NRC has undertaken some sampling and faecal source tracking from one site in the Kerikeri River as part of a wider source tracking programme to track trends in microbial contamination at a range of sites throughout Northland. The results of these studies are outlined in this section.

4.2 NRC Study 2009-10

In 2009-2010 the Northland Regional Council undertook a faecal source tracking project in conjunction with ESR to identify sources of faecal contamination in various Northland harbours in which oyster farms are situated. This work is reported in Reed (2011).

As part of the study, sampling was undertaken at 15 sites in Kerikeri Inlet on 27-28th August 2009 and subsequently at some of the same sites on 28th April 2010. As well as sites at the head and upstream of the inlet, all three oyster farming areas in the Kerikeri Inlet (at Sites 7, 10 and 11) were included in the sampling plan. Sampling of surface water and sediment for faecal sterol analysis was undertaken concurrently with sampling of water and shellfish for analysis of *E. coli* levels. All samples for *E. coli* testing were analysed initially, and if elevated *E. coli* levels were detected, water and sediment samples were analysed for faecal sterols. In addition, *E. coli* from oyster samples were analysed by Rep-PCR to determine their source by comparison with a library of *E. coli* arising from various sources (i.e. different kinds of animals). Had this latter experimental technique been successful the results could have provided useful complementary data for the faecal sterol analysis, but unfortunately these results were not considered reliable enough for publication now and are excluded from the project report (Reed, 2011).

None of the water samples taken from the oyster growing areas represented by the NDHB Sites 7, 10 or 11 on 27th August 2009 contained sufficient *E. coli* for further work to be undertaken using faecal sterol analysis (a minimum of 100 cfu/100ml is required). However, sediment samples taken from Site 7 and Site 10 on the same day were analysed for faecal sterols. The results provided no indication of human faecal contamination and were suggestive of faecal contamination by herbivores/wildfowl. Shellfish *E. coli* levels at these sites were significantly elevated despite the low water coliform results: 8,300 cfu/100g at Site 7, and 2,200 cfu/100g and 3,600 cfu/100g at two sites at Site 10. Water samples taken from all other sites, including from: No. 9 Buoy; the mouth of the Rangitane River; in the stream adjacent to Pagoda Lodge; at the Kerikeri Stone Store; and at Waipapa Landing (Waipapa R.) contained sufficient *E. coli* for faecal sterol analysis to be undertaken. Faecal sterol analysis suggested a ruminant source of faecal contamination at all sites. Further water samples were taken from these same 5 sites on 28th April 2010. The results in this second sample round were as follows: No. 9 Buoy: wildfowl; Rangitane River mouth: wildfowl; Pagoda Lodge: Wildfowl (dominant result) and ruminant (weak result); Kerikeri Stone Store: wildfowl (dominant result); Waipapa Landing: Ruminant and wildfowl. Shellfish *E. coli* levels at

No. 9 Buoy, Pagoda Lodge and Waipapa Landing were 4,453 cfu/100g, 21,463 cfu/100g and 7,018 cfu/100g respectively.

The results of this study provided no indication of human faecal contamination. However the possibility of human contamination cannot be excluded on the basis of these results. Faecal sterol analysis relies on the analysis of the relative proportions of various sterols and stanols in the environment to gauge the source of faecal contamination, as the profile of sterols and stanols from different animals is different. The interpretation of sterol data is complex. Little is known about the relative persistence of these compounds in the environment, and their low prevalence makes sensitivity of test methods a significant issue (Bitton, 2005; Scott et al., 2002). ESR considers that only medium certainty can be attributed to the results of their faecal sterol analysis (ESR Decision-Analysis Tree on www.waterquality.org.nz).

4.3 Cawthron Project 2011

In collaboration with ESR, Cawthron Institute undertook a project to evaluate the use of quantitative-PCR-based microbial source tracking markers within Regional Council monitoring programmes for identifying the presence and relative contributions of human and ruminant sources of faecal pollution in coastal waterways. This study is reported in Cornelisen et al. (2012).

Bacteria of the order Bacteroidales are very abundant in the gut of warm-blooded animals and can be highly host specific. Four quantitative PCR-based markers targeting the Order Bacteroidales were investigated and trialled, including: a universal Bacteroidales marker (UBac) that serves as an overall measure of faecal contamination but is not host-specific; a Bacteroidales marker specific to humans (HBac); a Bacteroidales marker associated with ruminant animals (RBac) such as sheep and cows; and a bovine Bacteroidales marker (BBac) specific to cows. These microbial source tracking tools were trialled in coastal areas throughout New Zealand, including at one site in the Kerikeri Inlet. Water samples were taken from the NRC sampling site at the mouth of the Waipapa River on 28/2/11, 14/3/11, 13/4/11 and 26/4/11. Samples were analysed for enterococci levels, and tested for each of the four Bacteroidales markers.

On two sampling occasions (28/02/2011 and 14/03/2011) Enterococci were below the level of detection but the universal marker (UBac) was nevertheless detected in water samples. On these occasions none of the other markers were detected, although the report notes that had ruminants been a major source they would have been detected in the samples. The report also notes that the contamination level detected by the UBac marker was too low for the absence of bovine and human sources to be confirmed.

Enterococci were detected in water samples taken on 13/04/2011 (just at the level of detection, 10 MPN/100 ml) and 26/04/2011 (364 MPN/100ml), and there was a strongly positive result for the UBac marker in both cases. The ruminant markers were also positive in both samples, and it was estimated from the results that on each occasion ruminants could have comprised up to 10% of the faecal contamination source.[4] Neither the bovine nor human markers produced a positive result. The contamination measured by the UBac marker was too low to confirm the absence of bovine

[4] We note that in the "Report Comment" field of the table in Appendix 2 of Cornelisen et al. (2012) it states that 10%-50% of faecal contamination could arise from ruminant sources. This does not appear to be consistent with the information in the "Results" field of the same table.

contamination, but the report notes that the human marker would have been detected in both samples if human contamination were a major contributor to the faecal contamination.

The source of most of the faecal contamination at the mouth of the Waipapa River remained unidentified in this study because the markers used did not cover all the likely sources. Birds are one potentially significant source that was not investigated, and the results from the earlier study using faecal sterols suggest that this source could potentially account for some of the unidentified contamination in these samples.

These results do not rule out the presence of human contamination. The HBac marker was found to have a low sensitivity (reporting positive results from only 62% of human faecal samples and 71% from WWTP effluent), and exhibited significant cross-reactivity with possum faecal samples. The low sensitivity could be a limiting factor in its usefulness, particularly if the source of human contamination is a few septic tanks rather than effluent from a more diverse human population discharged from a sewage treatment plant.

The researchers also concluded that the bovine marker BBac was unlikely to be a useful tool in source tracking because of its low sensitivity (reporting positive results in 72% of bovine faecal samples) and relatively high limit of detection on the PCR (<100 gene copies *c.f.* <10 gene copies for the other three markers).

4.4 Results of NRC Monitoring

Northland Regional Council has undertaken faecal source tracking at a number of sites throughout Northland, including at a sample site located in the Kerikeri River adjacent to the Stone Store. Water samples were taken from this site on three occasions in January 2011 and once in December 2011, and were analysed for fluorescent whitening agents, faecal sterols, and by PCR for bacterial markers. The results are presented in Table 4 below.

Date collected	FWA	PCR	Sterols
5/01/2011	ND	ND	Wildfowl
17/01/2011	ND	ND	Wildfowl
24/01/2011	ND	Ruminant	Wildfowl
12/12/2011	ND	Ruminant (up to 100%)	Ruminant

Table 4: Results of faecal source tracking undertaken in water samples collected from Kerikeri River adjacent to the Stone Store. (FWA = Fluorescent Whitening Agents; PCR = Polymerase Chain Reaction for bacterial markers; Sterols = Analysis for faecal sterols; ND = Not detected). (Source: NRC).

The results of this work indicate faecal contamination of the Kerikeri River by ruminants (e.g. cows, sheep etc) and wildfowl. None of the results indicate the presence of human faecal contamination at the time of sampling. However we note that because of limitations in the analysis methods (e.g. the low sensitivity of the human bacterial markers employed in the analysis), the rapid dilution (and degradation due to UV) of fluorescent whitening agents away from their source, and the potential for low levels of human contamination to be masked by much more dominant sources of faecal sterols (Brent Gilpin, ESR, pers. comm.), the absence of human contamination cannot be definitively confirmed.

4.5 Conclusion

None of the faecal source tracking studies undertaken in the Kerikeri Inlet to date has provided any evidence of human faecal contamination. However, none of the source tracking methods could be regarded as providing much assurance of the absence of human contamination, so this important issue remains to be resolved using improved techniques such as the bacterial markers for human contamination that have been developed more recently. We note that human faecal contamination does not need to be the major contributor to a high contamination load to be of human health significance, and in an area like the Kerikeri Inlet which includes pastoral and lifestyle farming in its catchment, rainfall-induced run-off is always likely to contain a component of ruminant faecal contamination as well as contamination from other domestic and feral animals and birds. The potential risk from human faecal contamination in the Kerikeri Inlet requires further investigation.

SECTION 5: RECOMMENDATIONS FOR SOURCE TRACKING

5.1 Introduction

Analysis of the existing data from Kerikeri Inlet undertaken in this report has attempted to clarify the nature of the contamination problem in impacted oyster growing areas, and to identify any patterns in contamination events that might assist in focussing future investigations. This has not been particularly fruitful. There is evidence of increasing levels and frequency of contamination in the southern oyster growing areas in the Inlet, but the nature of the contamination problem still remains somewhat unclear. It is still uncertain whether the observed unexpected oyster contamination relates to:

- An increase in the underlying contamination related to rainfall (which means the consequent higher levels of contamination in oysters are slower to depurate out), plus very occasional sporadic contamination unrelated to run-off; or
- More frequent sporadic contamination unrelated to run-off, masked by coincident rainfall events.

There is some evidence to suggest the former, but further investigation is required. The danger in assuming the first scenario when in fact the second applies is that increasingly more restrictive harvest criteria fail to prevent unexpected high levels of contamination when oyster growing areas are open for harvest.

The following outcomes are required by the Shellfish Quality Assurance Programme in the Kerikeri Inlet to ensure the sustainability of oyster farms:

- Background levels of faecal contamination need to be within an acceptable range;
- Pollution sources need to be manageable and predictable.

As part of the Shellfish Quality Assurance Programme, it must be possible to demonstrate these outcomes. Whether or not the farms are allowed to continue under their current "Conditionally Approved" classification right now, it is strongly suggested that action be taken to improve and prevent further deterioration of water quality in the Kerikeri Inlet.

Answers to the following questions are required regarding the unexpectedly high levels of contamination in shellfish samples taken from Sites 10 and 11:

- What is the source of contamination (bovine, avian, human etc)?
- What is the route of contamination (i.e. how does it enter the growing area e.g. exfiltration from septic tank systems, groundwater from STP wetland, boats etc.)?
- When does it happen?
- How can it be managed to achieve the required outcome?

The scope of this report requires us to make recommendations regarding the first point. However an investigation to determine the source of contamination must also address the third point, since knowledge of when contamination occurs is a precursor to determining its source (i.e. one must be able to focus investigatory sampling events to times at which contamination is likely to occur). At this stage some contamination events remain unpredictable.

5.2 Timing of Sampling

5.2.1 Timing of Investigations

In designing an investigation to determine the source of problematic contamination levels, we first need to define the environmental context of the contamination events to be investigated, as this may reflect their source. It would be convenient if the increase in unexpected high levels of contamination in Kerikeri Inlet could be discovered to be attributable to one source via one route of contamination, but this may not be the case. The data supporting the occurrence of high levels of contamination in any particular contexts is relatively weak, but it is suggested that it would be worthwhile investigating sources of contamination under the following conditions:

a) Immediately following a high rainfall event (>50 mm in one day);

b) Immediately following a low rainfall event (15-25 mm in 24-48 hours) that occurs 17-19 days after a high rainfall event (>60mm in one day) when groundwater levels are high;

c) Immediately following a low rainfall event (10-20 mm in one day) after a prolonged dry spell in summer when groundwater levels are low;

d) In January when visitor numbers are high in the area.

Ideally, contamination under each set of environmental conditions should be investigated several times.

5.2.2 Timing of Sampling Events

Further investigation is required so that water sampling in the source tracking investigation can be targeted to times at which there is the greatest risk of faecal contamination in the water. With the exception of the unexpected results at Windsor Landing, the NRC study of water quality across a tidal cycle suggested that the tidal influence on water quality in the main body of the Kerikeri Inlet decreased from the head of the Inlet to the mouth, with minimal differences in water quality across a tide near the mouth. However the oyster growing areas (particularly Site 11, Hauparua Inlet) are in more enclosed areas that could be more significantly influenced by their immediate catchment area after rainfall. It is suggested that these dynamics are investigated by sampling water for faecal coliforms and enterococci at 90 minute intervals across a tidal cycle. This should be undertaken on a tide about 24 hours after a low-moderate rainfall event. The same sampling should also be undertaken concurrently at Windsor Landing at the location of the NRC sampling site used in their previous study, to investigate whether the previously-observed pattern in water quality across a tide is repeated. Testing for both faecal coliforms and Enterococci will allow the results to be interpreted both within the context of the previous NRC study, and with respect to the regular monitoring that is undertaken at Sites 10 and 11 by NDHB. Ideally this study should be repeated several times under slightly different environmental conditions to ascertain the patterns in water quality. In addition to informing the best time to sample water in the source tracking investigation, this could also provide some preliminary spatial information about whether contamination sources are located within or outside the immediate catchment of the growing areas.

5.3 Potential Source Tracking Methods

There is no single faecal source tracking method that provides comprehensive information about potential sources of faecal contamination with a high degree of certainty attached to the result (Scott et al., 2002; Bitton, 2005; ESR Decision Analysis Tree at www.waterquality.org.nz). We therefore propose that each sampling event utilises a suite of source tracking tools to increase the reliability of the outcomes (this is the foundation of the "Toolbox" approach recommended by ESR[5]).

Discussion with the New Zealand providers of validated faecal source tracking services (ESR, Cawthron Institute) indicates that a range of tools are available based on analysis of water, sediment and shellfish samples (see Appendix III for more detailed information, including costs, from each provider). These include:

- *Testing for faecal sterols in water and sediment*. Faecal sterol analysis is undertaken by ESR in Christchurch (refer back to the final paragraph of Section 4.2 for an outline of this test method). This analysis forms one part of ESR's faecal source tracking "Toolbox", but the results of this test alone are accorded only medium certainty (www.waterquality.org.nz). The impact of dilution is a limitation in the detection of faecal sterols in water samples taken at a distance from the source of contamination. Sediment, in which faecal sterols may become concentrated, requires careful sampling to ensure that the results represent recent contamination (Brent Gilpin, ESR, Christchurch, pers. comm.). ESR is also trialling the use of sediment bags as a means of concentrating *E. coli* and faecal sterols out of water, and this is offered as a service to the NRC without charge at present. The potential advantage of this method is that it provides a temporal component to the information gleaned from the test results, in that any contamination observed from the bags must have occurred during the time between their deployment and retrieval. (This differs from the interpretation of results from *in situ* sediment sampling, from which the timing of contamination is not able to be deduced because *E. coli* and faecal sterols can persist in sediment for some time). However, this technique is still in the very preliminary stages of development, and although positive results could be regarded as significant, negative results cannot be regarded as reliable (Brent Gilpin, ESR, Christchurch, pers. comm.).

- *Testing water for fluorescent whiteners.* Fluorescent whiteners are used in washing powders etc and can provide an indication contamination from domestic or industrial effluent. Their presence is not directly linked to human faecal contamination. Dilution with distance from source and the rapid degradation of fluorescent whiteners by sunlight can reduce the sensitivity of this test, but the test is relatively cheap and it can be very useful when investigating a specific potential source.

- *Quantitative PCR (q-PCR) to test for Bacteroidales and other bacterial markers in water and shellfish*. *Bacteriodales* bacteria are very prevalent in faeces of many warm-blooded animals and can be host-specific. As discussed previously, a universal *Bacteroidales* marker (UBac) is available to detect the presence of faecal contamination in general (this is not sensitive to faecal contamination from gulls, which appear not to have *Bacteriodales* bacteria in their gut (Brent Gilpin, ESR, Christchurch, pers. comm.)), and for markers for ruminant (RBac) and human *Bacteroidales* (HBac). A bovine marker is also available, but this has low sensitivity and a relatively high level of detection by PCR, and is therefore of limited usefulness (Cornelisen et al., 2012). The low sensitivity of the human *Bacteroidales*

[5] www.waterquality.org.nz

marker (62-71% detection in samples known to contain human faeces; Cornelisen et al., 2012) significantly limits the usefulness of this test if, as in this case, it is important that human contamination be identified. There is also significant cross-reactivity with possum faecal material and lesser cross-reactivity with some other animals (Cornelisen et al., 2012). Based on the results of blind testing of the same samples by two laboratories, the q-PCR testing at ESR achieves more sensitive results for all *Bacteroidales* markers in water samples than those achieved by Cawthron Institute (see the differences in scale on the axes in plots in Figure 5 of Cornelisen et al., 2012). ESR have subsequently developed markers for human contamination based on the bacteria *Bifidobacterium adolescentis* that have a sensitivity similar to that of ruminant markers (>90%), which represents a very significant improvement. They also have bacterial markers for wildfowl. At this stage however, testing for these bacterial markers by ESR has only been validated for water samples, not shellfish samples (Brent Gilpin, ESR, pers. comm.). Cawthron Institute has a validated method for analysis of bacterial and other markers in shellfish (Kirs & Cornelisen, 2011).

- *End-point PCR for bacterial markers (Cawthron Institute).* This technique provides a positive/negative result for the presence of human contamination (not a quantitative result as in q-PCR) and can be applied to water and shellfish samples by Cawthron Institute. Human markers are available for *Bacteroidales* and *Methanobrevibacter* bacteria. The human *Bacteroidales* marker is based on a different set of primers from those targeted in the q-PCR technique, and it is more sensitive than the q-PCR test for HBac (Jonathan Banks, Cawthron Institute, pers. comm.). The combination of this test with the q-PCR test for the HBac marker significantly improves the level of certainty of result with respect to the potential for human contamination (Chris Cornelisen, Cawthron Institute, pers. comm.). The test for human *Methanobrevibacter* marker is less sensitive than that for the human *Bacteroidales* marker (Jonathan Banks, Cawthron Institute, pers. comm.). End-point PCR markers are also available for cattle, wild-fowl (duck etc.) and gulls. A universal marker is also used to check the extraction efficiency of these markers in the test process.

- *RT-PCR assays for human/animal noroviruses and adenoviruses in water and shellfish samples (ESR, Wellington).* Enteric viruses released with the faeces of humans and animals are accumulated by oysters from their growing waters and retained for several weeks. Multiplex real time RT-PCR assays for enteric viruses in water and shellfish samples can distinguish between human, pig, sheep and cattle faecal contamination (e.g. Wolf et al., 2007; Wolf et al., 2009). The ESR "Virus Toolbox" of host-specific virus assays includes: human adenovirus species F, norovirus GI and GII (and human polyomavirus (see below)); plus markers for animal faecal contamination including: porcine adenovirus type 3 (pigs), ovine adenovirus (sheep), norovirus GIII (sheep & cows) (and bovine polyomavirus (cows) – see below). These assays rely on the presence of illness in the source population. Human noroviruses and adenoviruses tend to be prevalent in effluent from large sewage treatment plants, but may only be sporadically present if the source of contamination is on-site sewage systems (such as septic tanks). This technique is thus more reliable in detecting human contamination from sources emanating from communities rather than from individual households.

- *End-point or q-PCR for human and bovine polyomavirus (Cawthron or ESR, Wellington).* The use of bovine polyomavirus markers is under development by ESR but currently requires further validation. Human polyomaviruses, which are excreted with urine, are more prevalent in the human population than noroviruses and adenoviruses, and reliably detected in most human effluent. End-point PCR markers for human polyomavirus have been

trialled in water and shellfish by Cawthron Institute and human polyomavirus can be detected by real time RT-PCR in water and shellfish by ESR in Wellington. Concentrations of human polyomavirus in estuarine water were found to be comparable with human adenovirus species F and norovirus GII (ESR presentation to Industry Advisory Group, March 2012). The Cawthron end-point PCR for the human polyomavirus in shellfish is less sensitive than their end-point PCR for the human *Bacteroidales* or *Methanobrevibacter* markers (Kirs & Cornelisen, 2011). Kirs & Cornelisen (2011) suggested that in the absence of improved recovery methods (i.e. the method of extraction of virus from shellfish tissue), the human polyomavirus marker is not very useful in shellfish samples due to its low abundance (high dilution) in the marine environment and the possible increased likelihood of a false negative result.

5.4 Sample Matrix

The sample matrix used in source tracking potentially impacts on the likelihood of detection of contamination. While a water sample provides a "snapshot" of the contamination in a shellfish growing area at the time of sampling, the tendency for shellfish to concentrate and retain contaminants out of the water column results in samples that provide information about contamination over a longer time period. In the testing undertaken by NDHB, there have usually been only low levels or no faecal coliforms detected in water samples compared to the concurrent oyster samples in which elevated levels of *E. coli* were detected. While this may be a function of the choice of timing of sampling events, it is suggested that where possible, faecal source tracking work should utilise shellfish samples rather than water samples. This will maximise the chance of capturing the results of a contamination event.

5.5 Sampling Sites

The two areas of most concern with respect to oyster quality are represented by Sites 10 and 11, and it is suggested that these sites should be the focus of the source tracking investigation. However, contamination from the head of the estuary is also of potential significance at Site 10, and therefore a limited investigation is suggested at the head of the Inlet, where two shellfish sampling sites are proposed: one in the mouth of the Waipapa River, and another further up the Kerikeri Inlet, but downstream of the entrance of the small stream at the end of Pah Road. Care will need to be taken to locate sampling sites in areas where the salinity is suitable for the normal functioning of oysters.

5.6 Sampling Regime

In consideration of the limited budget and low reliability of individual source tracking methods, we propose a sampling regime that provides intensive investigation of relatively few events rather than scant investigation of a greater number of events. The following sampling regime is proposed for the four environmental scenarios outlined in Section 5.2:

a) Immediately following a high rainfall event (>50 mm in one day).

Sampling at each of Site 10 and Site 11:

- One water sample to be tested for faecal coliforms;

- One water sample to be tested for fluorescent whitening agents (ESR, Christchurch);
- Three experimental sediment bags to be tested for faecal sterols (and *E. coli*) after 2 days in the water (ESR, Christchurch – only undertake if test continues to be free of charge).
- One water sample to be tested by q-PCR for universal and ruminant *Bacteroidales* markers and human *Bifidobacterium* marker (ESR, Christchurch).
- One pooled oyster sample, to be analysed by Cawthron Institute as follows:
    - Extraction of DNA for analysis of bacterial markers;
    - Test for *E. coli;*
    - If elevated *E. coli* levels are detected, test extraction by q-PCR for universal, human and ruminant *Bacteroidales* markers, and by end-point PCR for the universal marker, and for human *Bacteroidales*, human *Methanobrevibacter*, bovine, wildfowl and gull markers.

b) Immediately following a low rainfall event (15-25 mm in 24-48 hours) that occurs when groundwater levels are high (preferably approximately 17-19 days after a high rainfall event (>60mm in one day)).

Sampling at each of Site 10 and Site 11 as outlined in Scenario a) above, plus sampling at each of Kerikeri River and Waipapa River sites as follows:

- One water sample to be tested for faecal coliforms;
- One pooled oyster sample, to be analysed by Cawthron Institute as follows:
    - Extraction of DNA for analysis of bacterial markers;
    - Test for *E. coli;*
    - If elevated *E. coli* levels are detected, test extraction by q-PCR for universal, human and ruminant *Bacteroidales* markers, and by end-point PCR for the universal marker, and for human *Bacteroidales*, human *Methanobrevibacter*, bovine, wildfowl and gull markers.
- One pooled oyster sample to be tested by ESR in Wellington for human norovirus (GI, GII) and human adenovirus species F.
- One water sample to be tested for fluorescent whitening agents (ESR, Christchurch);
- Three experimental sediment bags to be tested for faecal sterols (and *E. coli*) after 2 days in the water (ESR, Christchurch – only undertake if test continues to be free of charge).

c) Immediately following a low rainfall event (10-20 mm in one day) when groundwater levels are low after a prolonged dry spell in summer.

Sampling at each of Site 10 and Site 11 as outlined in Scenario a) above.

d) In January:

Sample twice a week at least 3 days apart for 4 weeks (regardless of the rainfall) at each of Sites 10 and 11 and test as follows:

- One water sample to be tested for faecal coliforms;

- One pooled oyster sample, to be analysed by Cawthron Institute as follows:
    - Extraction of DNA for analysis of bacterial markers;
    - Test for *E. coli;*
    - If elevated *E. coli* levels are detected, test extraction by q-PCR for universal, human and ruminant *Bacteroidales* markers, and by end-point PCR for the universal marker, and for human *Bacteroidales*, human *Methanobrevibacter*, bovine, wildfowl and gull markers.
- Three experimental sediment bags to be tested for faecal sterols (and *E. coli*) after 2 days in the water (ESR, Christchurch – only undertake if test continues to be free of charge).

(This proposed regime could be revised if results from earlier components of the study indicate that alternative source tracking methods are more effective).

The total analysis cost to complete this part of the project is estimated as approximately $13,700. Ideally the sampling at Sites 10 and 11 during environmental scenarios a), b) and c) should be repeated twice. The cost of this additional analysis would be approximately $13,600. An additional $7,800 is required to repeat the sampling at Kerikeri and Waipapa Rivers on two more occasions. Assuming the January testing results in two *E. coli* levels high enough to prompt further testing by PCR, the cost of the January component would be approximately $3,000, giving a total cost for three sampling rounds under scenarios a), b) and c) plus January sampling as approximately $35,200.[6]

As results of this work become available, more intensive investigation of potential sources will be required to identify the route(s) of contamination. The sampling programme and analysis methods will need to be extended or modified to accomplish this.

Implementation of this sampling regime will require good advance planning, active monitoring and prompt responses to environmental conditions. The Kerikeri Inlet Water Quality Committee may be able to assist in this.

5.7 Recommendations for Supporting Investigatory Work

Additional sampling to investigate the patterns of *E. coli* contamination after rainfall events is also suggested to provide further information about the patterns of unexpected contamination that have been observed in oysters in the regulatory monitoring programme. For each of the first three environmental scenarios outlined above (i.e. a), b) and c) in Section 5.2.1), it is suggested that shellfish samples to be tested for *E. coli* should be taken daily from each of Sites 10 and 11 from the conclusion of significant rainfall until the growing area would be deemed open for harvest under the current harvest criteria. Three replicate samples of shellfish should be taken each day. This should provide some indication of whether unexpectedly high levels of contamination when the area should be open results from slow depuration of very high contamination levels or from unexpected contamination events. The Petrifilm method of *E. coli* analysis could be used in this study as it provides prompt results and has a significant cost advantage. However if resources are available, the use of the regulatory MPN analysis method would link the results to the Shellfish Quality Assurance Programme and inform harvest criteria.

[6] Note that this excludes the cost of the initial study of water quality across different stages of the tide described earlier.

The work outlined above will not provide all the information required by the Shellfish Quality Assurance Programme. An intensive investigation of the potential sources of contamination (i.e. a detailed sanitary survey, and in-depth investigation of Council records to determine whether any contamination events could be linked to recorded spills, or malfunctioning/overloading of systems) is recommended to complement the source tracking investigation. As part of this, some dye studies have already been undertaken to ascertain whether on-site sewage management systems are a source of contamination at Hauparua Inlet. It is suggested that this work should be completed, with a clear focus on the required outcome of the investigation. Contamination of water from on-site sewage systems may occur as a result of malfunctioning or poor maintenance of systems. Alternatively, systems may be well-maintained and functioning within their design specifications, but their design may be inappropriate for environmental conditions at their location (e.g. soil type, slope etc). This may not be readily visible if contamination is sporadically linked to exfiltration into groundwater. In Hauparua Inlet there must be serious doubt about the design of some on-site systems with respect to substrate/soil type, and the dye study investigation should therefore be designed to investigate this as well as any malfunction in individual systems. It is also suggested that the dye study should be undertaken under adverse conditions (for example, when the ground is saturated, when occupancy is high etc.). If this cannot be done, adverse conditions should be simulated (for example, by undertaking the study in conjunction with a high flow though the system).

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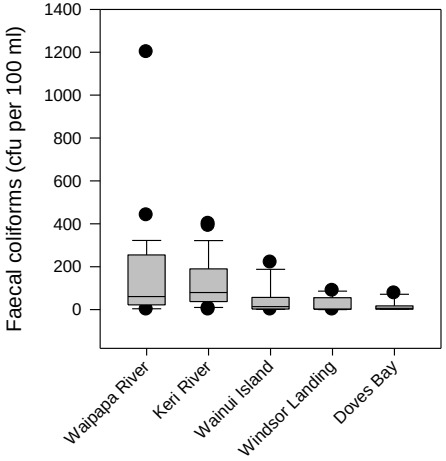
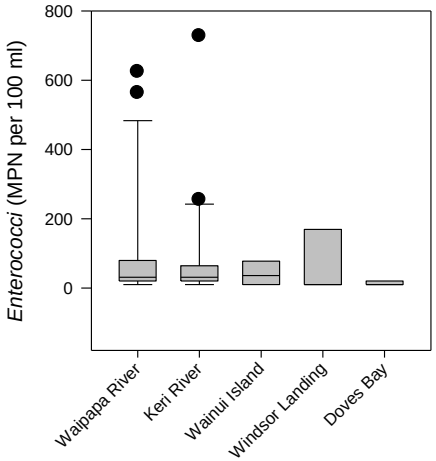
APPENDIX I: TABLE OF JULIAN DATES

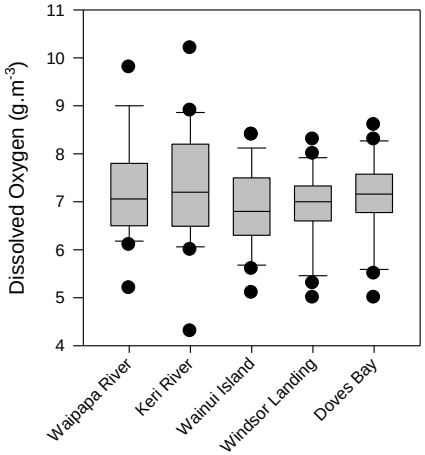
Sampling Date	Julian Day	Sampling Date	Julian Day	Sampling Date	Julian Day	Sampling Date	Julian Day
24-Jul-08	08206	15-Jul-09	09196	24-Aug-10	10236	19-May-11	11139
14-Aug-08	08227	27-Jul-09	09208	25-Aug-10	10237	23-May-11	11143
18-Sep-08	08262	30-Jul-09	09211	3-Sep-10	10246	24-May-11	11144
21-Sep-08	08265	12-Aug-09	09224	23-Sep-10	10266	30-Jun-11	11181
22-Sep-08	08266	14-Aug-09	09226	28-Sep-10	10271	5-Jul-11	11186
25-Sep-08	08269	8-Sep-09	09251	30-Sep-10	10273	14-Jul-11	11195
16-Oct-08	08290	16-Sep-09	09259	6-Oct-10	10279	19-Jul-11	11200
20-Oct-08	08294	13-Oct-09	09286	15-Oct-10	10288	2-Aug-11	11214
12-Nov-08	08317	21-Oct-09	09294	21-Oct-10	10294	3-Aug-11	11215
3-Dec-08	08338	27-Oct-09	09300	3-Nov-10	10307	25-Aug-11	11237
10-Dec-08	08345	18-Nov-09	09322	15-Nov-10	10319	7-Sep-11	11250
3-Jan-09	09003	24-Nov-09	09328	16-Nov-10	10320	8-Sep-11	11251
7-Jan-09	09007	25-Nov-09	09329	18-Nov-10	10322	15-Sep-11	11258
8-Jan-09	09008	9-Dec-09	09343	25-Nov-10	10329	26-Sep-11	11269
12-Jan-09	09012	22-Dec-09	09356	30-Nov-10	10334	5-Oct-11	11278
14-Jan-09	09014	13-Jan-10	10013	13-Dec-10	10347	17-Oct-11	11290
21-Jan-09	09021	14-Jan-10	10014	16-Dec-10	10350	31-Oct-11	11304
22-Jan-09	09022	27-Jan-10	10027	4-Jan-11	11004	16-Nov-11	11320
27-Jan-09	09027	3-Feb-10	10034	13-Jan-11	11013	17-Nov-11	11321
30-Jan-09	09030	4-Feb-10	10035	19-Jan-11	11019	29-Nov-11	11333
4-Feb-09	09035	18-Mar-10	10077	25-Jan-11	11025	30-Nov-11	11334
26-Feb-09	09057	20-Apr-10	10110	2-Feb-11	11033	1-Dec-11	11335
19-Mar-09	09078	18-May-10	10138	28-Feb-11	11059	12-Jan-12	12012
25-Mar-09	09084	19-May-10	10139	10-Mar-11	11069	18-Jan-12	12018
23-Apr-09	09113	31-May-10	10151	14-Mar-11	11073	1-Feb-12	12032
7-May-09	09127	30-Jun-10	10181	15-Mar-11	11074	13-Feb-12	12044
13-May-09	09133	15-Jul-10	10196	29-Mar-11	11088		
5-Jun-09	09156	12-Aug-10	10224	13-Apr-11	11103		
23-Jun-09	09174	20-Aug-10	10232	14-Apr-11	11104		
25-Jun-09	09176	23-Aug-10	10235	26-Apr-11	11116		

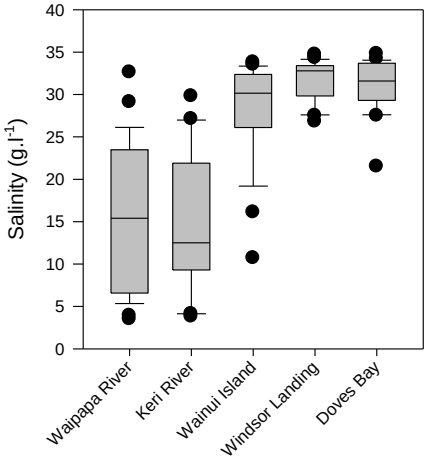
The dates for the oyster data presented in Figures 3 & 4 in the text have been converted to Julian dates to enable the graphed data to be spread out more evenly. These dates have the year as the first two numbers followed by the number of the day in the year (out of 365 days).

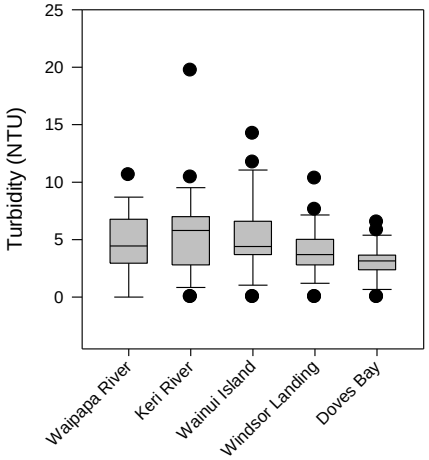
APPENDIX II: NRC SoE MONITORING RESULTS

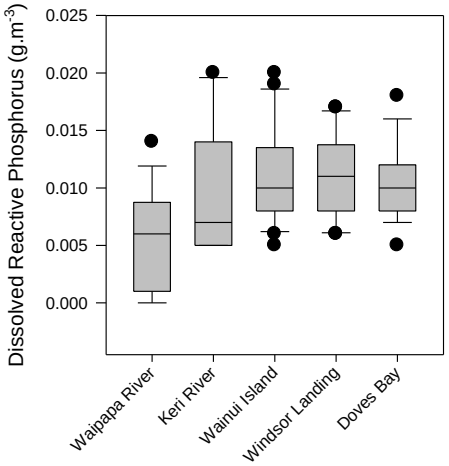


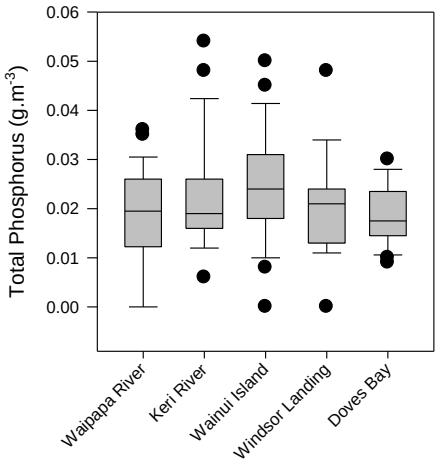


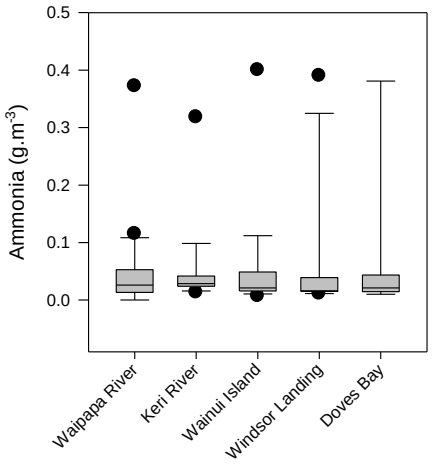


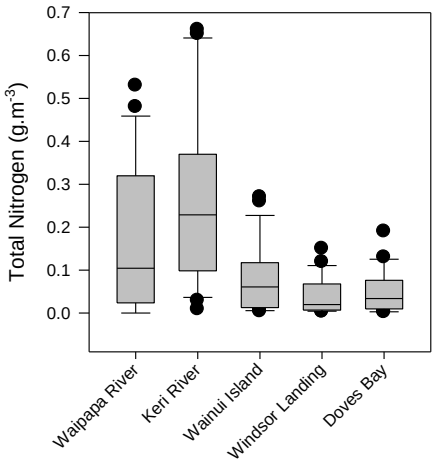


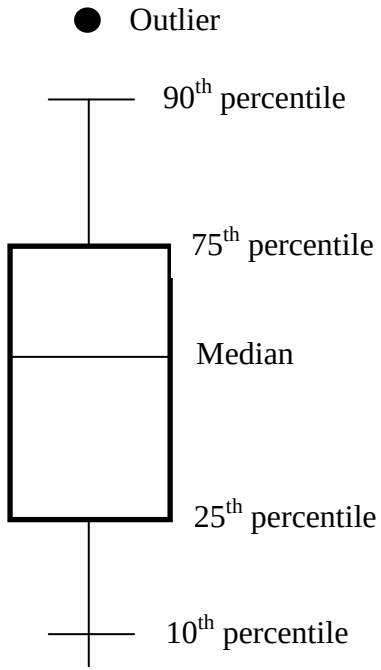












50 % of the data is bounded within the box with the line indicating the median. 90 % of the data is located between the whiskers of the plot. The final 10 % of data is data outside these parameters

APPENDIX III INFORMATION FROM MST SERVICE PROVIDERS

1. Services provided by Cawthron Institute

Details of the analytical tests in a shellfish matrix provided by Cawthron Institute, and their costs are provided in the copy of an email below from Jonathan Banks (Cawthron Institute) to Elizabeth Watts (NDHB), Richie Griffiths (NRC) and Brenda Hay (AquaBio Consultants Ltd) on 5/3/2012:

Hi Brenda, Elizabeth and Richard

Just to update everyone with the discussion so far regarding MST for the Kerikeri inlet oysters.

We suggest sending 10 oysters for MST at 4oC by overnight courier. We can then extract the microbe DNA from the shellfish and store it in buffer (this stabilises the DNA preventing degradation and loss of signal). Once the DNA is stabilised we can wait until the faecal indicator bacteria (FIB) test results are available before running the MST tests or we can run the MST tests immediately. The advantage of waiting for FIB is that if the faecal indicator bacteria concentrations are not elevated it is not particularly useful to run the MST tests.

There are some options for the MST markers.

There are quantitative tests that will provide information on the relative contribution of hosts to the microbial contamination and/or there are tests that identify the hosts contributing to the contamination through presence or absence of the hosts' faecal bacteria.

Quantitative markers are available for human, ruminant (cows, sheep, goats etc), and bovine (cow) specific markers. A universal marker is also run to check on extraction efficiency. These markers are compared with standards of known concentration to provide information on the concentration of the bacteria in the sample.

Presence/absence markers that are available are three human markers (Bacteroidales, Methanobrevibacter and Polyoma virus), bovine, wildfowl (duck etc), and gull. A universal marker is also run to check on extraction efficiency. The three human markers differ in their sensitivity and provide some quantitative information on human faecal contamination as the Bacteroidales marker amplifies from low to high concentrations of human contamination whereas the Methanobrevibacter and Polyomavirus markers amplify only at higher concentrations.

The costs

Extraction and storage would be $100 per sample (excluding GST).

Three markers and the universal marker would be $150 per sample (excluding GST and extraction cost).

Six markers and the universal marker would be $250 per sample (excluding GST and extraction cost).

You can choose a mixture of quantitative and presence/absence markers if that suits your needs.

Chris Cornelisen, who is the lead author on the attached MST report, suggested that it might be useful to treat this work as a pilot study and to look for funding such as Envirolink tools funding to do a detailed study of the whole catchment to improve water quality.

Please phone or email me if you would like more information.

Kind regards

Jonathan

Jonathan Banks
Aquaculture and Biotechnologies

CAWTHRON INSTITUTE. 98 Halifax Street East, Nelson 7010**.** Private Bag 2, Nelson 7042**. NEW ZEALAND**
Tel +64 3 548 2319 Ext. 259**.** Fax + 64 3 546 9464**. www.cawthron.org.nz**

2. Services provided by ESR

The faecal source tracking services provided by ESR in Christchurch, and their cost, are outlined on the website at: www.waterquality.org.nz . This covers testing for fluorescent whiteners, faecal sterols and the use of PCR in the detection of bacterial markers.

The virus testing service provided by ESR in Wellington is outlined in the pamphlet that can be downloaded at:

www.esr.cri.nz/competencies/Health/Pages/Norovirus%20PCR.aspx