Identifying Sources of Faecal Contamination in Coastal Waters and Shellfish in Northland's Harbours.

Putting Northland first

NORTHLAND REGIONAL COUNCIL

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CONTENTS

1. INTRODUCTION

The Northland Regional Council (NRC) undertakes monitoring of coastal and estuarine water at various sites in our harbours to assess the overall quality of Northland's waters as part of the Council's 'State of the Environment' monitoring. This monitoring is undertaken to check that the appropriate water quality standards are being met, such as Coastal Water Quality Standards as described by the Regional Coastal Plan or the Ministry for the Environment's (MfE) Microbiological Water Quality Guidelines (MfE, 2003) .

A recent review of faecal coliform and *Escherichia coli* (*E. coli)* bacteria levels in Whangaroa Harbour and the Kerikeri Inlet over the last few years (2007-2009) showed waters were not meeting NRC's water quality standards at some sites. In addition, Sanford Limited tested marine waters in the Whangaroa Harbour and Kerikeri Inlet, as part of their routine monitoring of their marine farms, and found *E. coli* levels were periodically above the guidelines (Sanford, pers. comm., 2009). Results of water and shellfish sampling undertaken by Northland District Health Board (NDHB) in the Houhora Harbour showed faecal coliform bacteria concentrations were occasionally above the guidelines for the collection of shellfish in 2008-2009. In light of these recent results, NRC and NDHB agreed that there was unacceptably high levels of bacterial indictors in these three estuaries and microbial source tracking was required to determine the sources of *E. coli* entering waters and shellfish in these estuarine areas.

Potential sources of the unacceptably high levels of *E. coli* in the three estuaries may be from:

(1) Human contamination from either wastewater treatment plant (WWTP) effluent (in Whangaroa harbour and Kerikeri Inlet), leaking from septic tanks and/or liveaboard vessels and other marine craft;
(2) Ruminants (cattle, sheep);
(3) Avian (wildfowl) in the Houhora harbour, there is concern that faecal matter from wildfowl is causing localised high levels of *E. coli*.
(4) Others (e.g. deer, pig, possum, goat, horse, dog).

Tracking the sources of faecal contamination is commonly undertaken by identifying sterol compounds in the environmental sample. Sterol compounds are found in different proportions in faecal contamination from different animals and they are persistent in the environment. Identifying the sterol can help identify the source but it cannot identify its exact discharge point.

Genetic fingerprinting, which is the matching of DNA profiles from a sample measured in the environment with known animal sources, is also commonly used (this is called Rep-PCR analysis). Rep-PCR analysis is undertaken on *E. coli* bacteria which are found in human and animal faeces This was not undertaken in this study but could be used in future investigations as a weight of evidence approach to help identify sources of faecal contamination entering these estuaries.

1.1. Aim and Objectives

The overall aim is to identify the source or sources of faecal contamination in water and sediment from three estuaries in Northland.

The objectives are to:

- Collect water and sediment samples at sites in each of the following areas; Kerikeri Inlet, Whangaroa Harbour and Houhora Harbour.
- Undertake *E. coli* testing and faecal sterol analysis of selected water samples.
- Undertake *E. coli* testing and faecal sterol analysis of sediments sampled near the oyster farms in the selected estuaries.
- Undertake *E. coli* testing of shellfish to determine the level of bacteria in the oyster meat.

2. METHODOLOGY

2.1. Sampling Sites

Figures 1-3 show the sampling sites at Whangaroa Harbour, Kerikeri Inlet and Houhora Harbour, respectively. Water sampling sites were located to provide representation of the longitudinal movement of water through the estuary, and sediment samples were located in areas where oyster harvesting occurs. There were no replicate samples at these sites due to budget constraints.

Sampling was conducted after rain when faecal contaminants such as E. coli can run-off from land adjacent to the harbours. Whangaroa Harbour and Kerikeri Inlet sampling was undertaken on 27-28 August 2009 (after rain and before the beginning of the drought) and then again (sampling water and shellfish only) on 28 April 2010, after the first storm after the drought. In the Houhora Harbour, sampling was undertaken on 11 March 2010 (after the drought).

Note that during and after rain events oyster farms are normally closed for harvesting under strict guidelines from Northland District Health Board (NDHB).

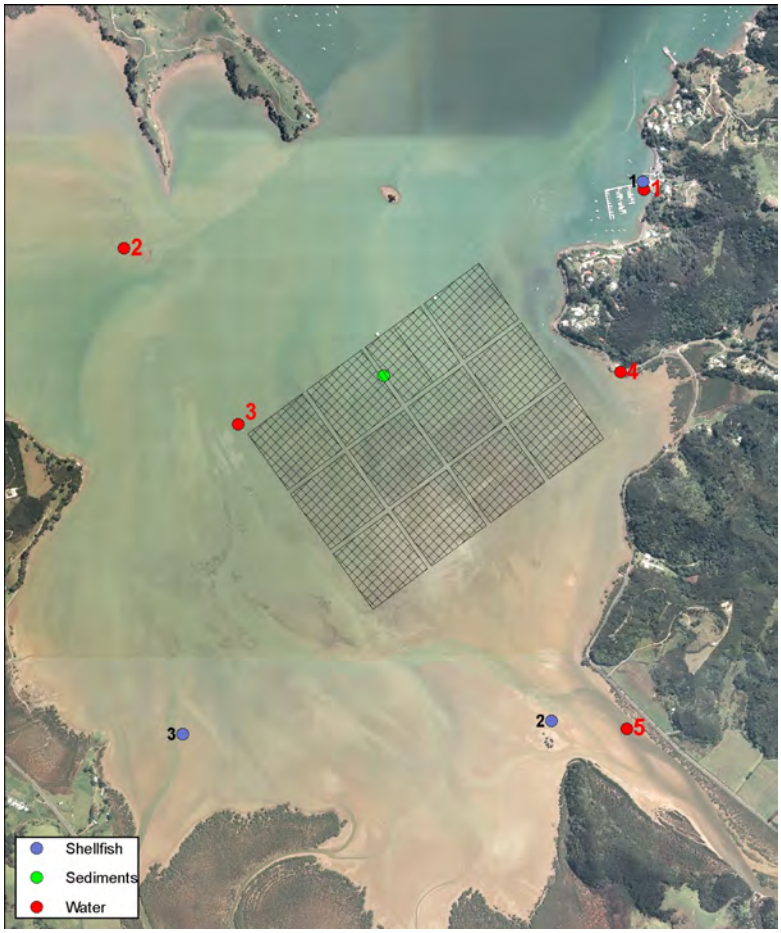

Figure 1: Water, sediment and shellfish sampling sites in Whangaroa Harbour. (Red dots are water samples, green dots are sediment samples and blue dots are shellfish samples). Hatched area is the marine farm.

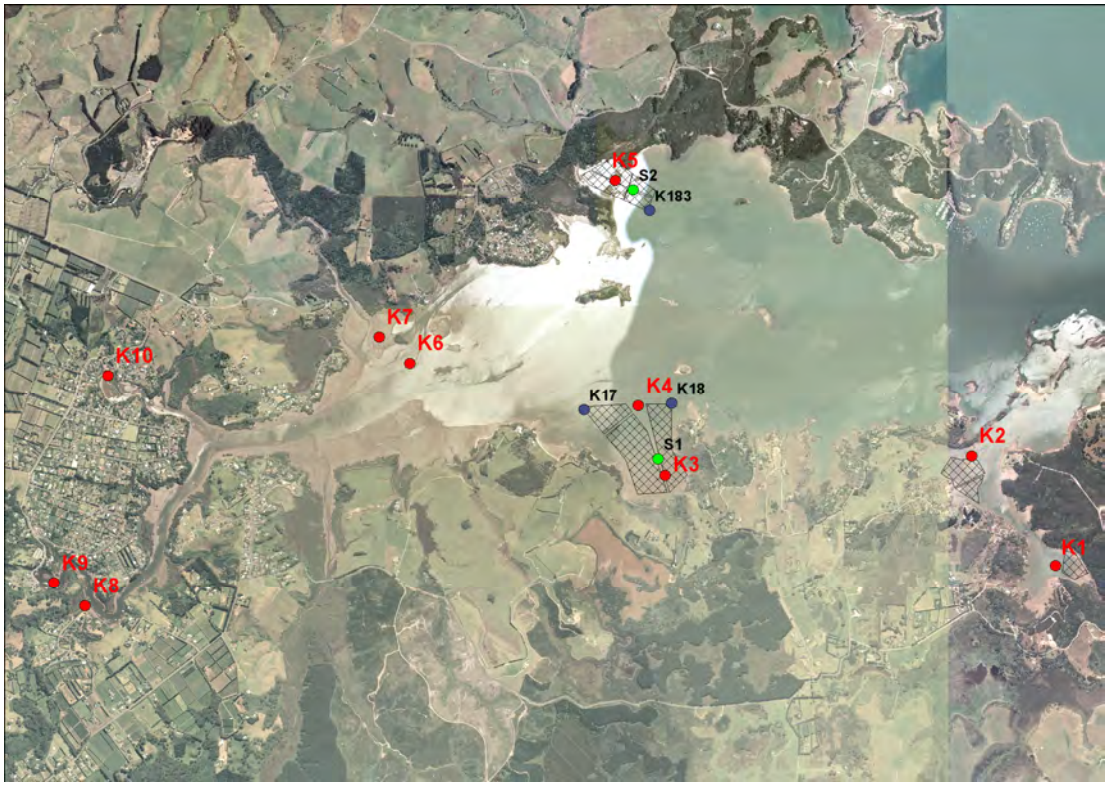

Figure 2: Water, sediment and shellfish sampling sites in Kerikeri Inlet. (Red dots are water samples, green dots are sediment samples and blue dots are shellfish samples). Hatched area is the marine farm.

Figure 3: Water, sediment and shellfish sampling sites in Houhora Harbour. (Red dots are water samples, green dots are sediment samples and blue dots are shellfish samples). Hatched area is the marine farm.

2.2. Rainfall

Rainfall was measured at NRC's gauging stations in Kaeo and Touwai (Whangaroa Harbour), Maungaparerua (Kerikeri) and Kaitaia (nearest gauge to Houhora Harbour). Data were retrieved for the sampling period to assess the rain inputs (and therefore likely runoff sources) prior to sampling.

2.3. Water, Sediment and Shellfish

Surface water was sampled at a depth of 0.5m using a sampling pole. A 10L water sample was collected in a pre-cleaned plastic bottle for faecal sterol analysis. A 250mL water sample was collected in a sterile glass bottles for *E. coli* testing. Water samples were processed within 10 hours of sampling.

A 200g surface sediment sample (taken from the top 2cm surface) was collected using a hand-held van veen grab. Each sediment sample was stored in a plastic bag on ice and was sent overnight to Institute of Environmental Science & Research (ESR) for analysis.

Adult oysters (*n*=24) were collected from sites in each harbour. Each batch of oyster samples was stored in a plastic bag on ice and was sent overnight to ESR for analysis.

2.4. Analytical Methods

All samples were initially analysed for levels of *E. coli* and if elevated results were recorded in water and sediment samples they were analysed for faecal sterols.

Faecal sterols were not able to be analysed in shellfish flesh due to problems with the analytical methodology at ESR.

The faecal sterol results were classified against a known library of human, ruminant and wildfowl types to determine the local source of *E. coli* at the time of sampling.

2.4.1. E. coli Analysis of Water, Sediment and Oyster Samples

E. coli levels in water were determined using the Standard Methods for the Examination of Water and Wastewater 21st Ed APHA AWWA WEF Method No 9.223B. Colilert®-18 determines the presence of total coliforms and E. coli using Defined Substrate Technology®.

E. coli levels in shellfish were determined by taking 100 grams of shellfish that was homogenised in an equal weight/volume of buffered peptone water in whirlpak bags in a stomacher. The supernatant was diluted down to 10-5 in peptone water. A 1ml of each dilution was plated onto chromocult agar and incubated overnight at 35℃.

E. coli levels in Sediments were determined by analysing 1 gram of sediment using Colilert® assays.

2.4.2. Faecal Sterol Analysis for Water and Sediment Samples

Faecal sterols are a group of C27-, C28- and C29- cholestane-based sterols found mainly in animal faeces. The sterol profile of faeces depends on the interaction of three factors. Firstly, the animal's diet determines the relative quantities of sterol precursors (cholesterol, 24-ethylcholesterol, 24-methylcholesterol, and/or stigmasterol) entering the digestive system. Secondly, animals differ in their endogenous biosynthesis of sterols (for example, human beings on a low cholesterol diet synthesise cholesterol). Thirdly and perhaps most importantly, is that the anaerobic bacteria in the animal gut biohydrogenate sterols to stanols of various isomeric configurations.

The sterol, cholesterol, can be hydrogenated to one or more of four possible stanols. In human beings, cholesterol is preferentially reduced to coprostanol, whereas in the environment cholesterol is predominately reduced to cholestanol. Similarly, plant-derived 24-ethylcholesterol is reduced to 24-ethylcoprostanol and 24-ethylepicoprostanol in the gut of herbivores, whereas in the environment it is primarily reduced to 24-ethylcholestanol. As a consequence, analysis of the sterol composition of animal faeces can generate a sterol fingerprint, which can be quite distinctive from one species to another. Coprostanol is the principal human biomarker. High relative amounts indicate fresh human faecal material. Coprostanol constitutes 60% of the total sterols found in human faeces, while dogs and birds have either no coprostanol or only trace amounts, present in their faeces.

Faecal sterols analysis was performed, by filtering up to 4 litres of estuary water onto glass fibre filters. The quantity of water filtered was recorded. Filters were stored frozen (-20℃) until they were analysed at ESR using the extraction procedure described by Devane et al. (2006). Each sterol and stanol detected is expressed as parts per trillion (ppt).

Interpretation of the sterol is based on comparisons of ratios of key sterols. The ratios used in this study include two indicators of faecal pollution, three indicators of human faecal source, two of herbivore source, and one suggesting plant decay.

Table 1. Sterols used in the identification of sources of faecal contamination in environmental samples (Gilpin, pers. comm., 2010).

Ratio	Stanols/Sterols	Interpretation
Faecal 1	Coprostanol:cholestanol	>0.5 suggests faecal contamination, <0.3 in situ bacteria (sediments)
Faecal 2	24-ethylcoprostanol/24-ethylcholestanol	>0.5 suggests faecal contamination, <0.3 in situ bacteria (sediments)
Human 1	coprostanol/(coprostanol+cholestanol)	>0.7 suggests human, <0.7 herbivore
Human 2	%coprostanol/total sterols	>5-6% human, <5-6% herbivore
Human 3	Coprostanol:24-ethylcoprostanol	Human faecal pollution typically has a ratio greater than one
Herbivore 1	24-ethylcoprostanol/total sterols	>5–6% herbivore
Herbivore 2	Coprostanol:24-ethylcoprostanol	Animal faecal pollution typically has a ratio less than one
Herbivore 3	24-ethylcholesterol/24-ethylcoprostanol	<1.0 suggests herbivore >4.0 -12 suggests plant decay

Sediment samples were kept at 4℃ and sent overnight to ESR for faecal sterol analysis.

3. RESULTS

3.1. Rainfall

Results of rainfall measurements prior to sampling are shown in Figures 4-6. There were two rainfall events in the Whangaroa Harbour (46mm and 22.5mm), two events in Kerikeri Inlet (37mm and 26mm) and one rainfall event on 28 April 2010 (40mm in Kerikeri Inlet and Whangaroa Harbour on the 27 April). In Houhora Harbour, 34 mm of rain was measured prior to sampling. Note that the sampling in the Whangaroa Harbour and Kerikeri Inlet was 24-48 hours after rain. Unfortunately, in the Houhora Harbour there was approximately a week between the large event on the 3 March and another small event on 6-7 March and sampling which was eventually undertaken on 11 March 2010. This was due to a lack of resources during the summer period.

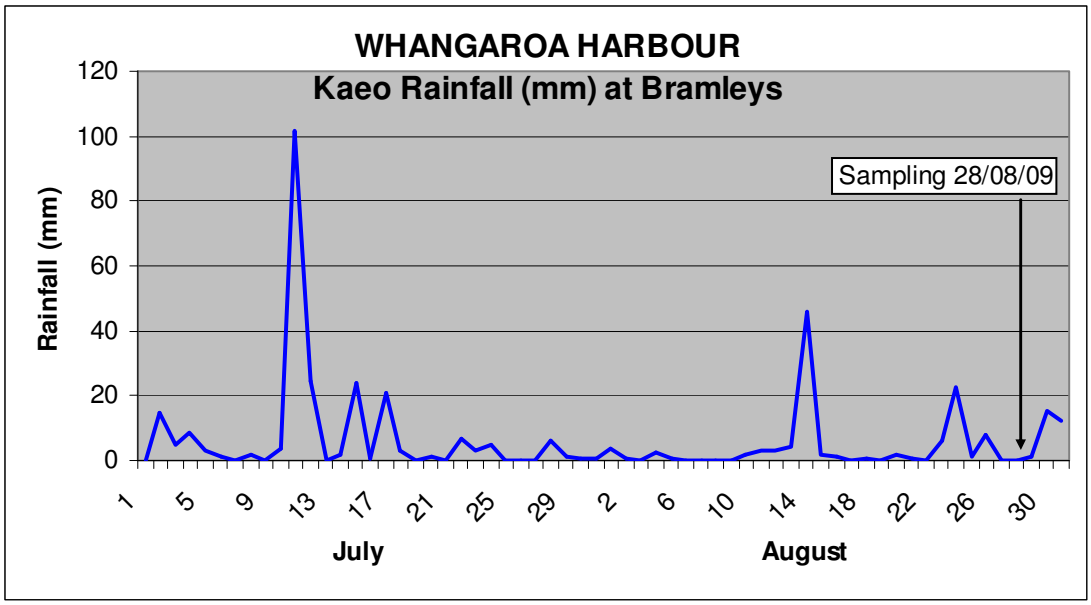

Figure 4. Rainfall (mm) during July-August 2009 in Whangaroa Harbour, at Bramleys monitoring site (south west headwaters of Kaeo River).

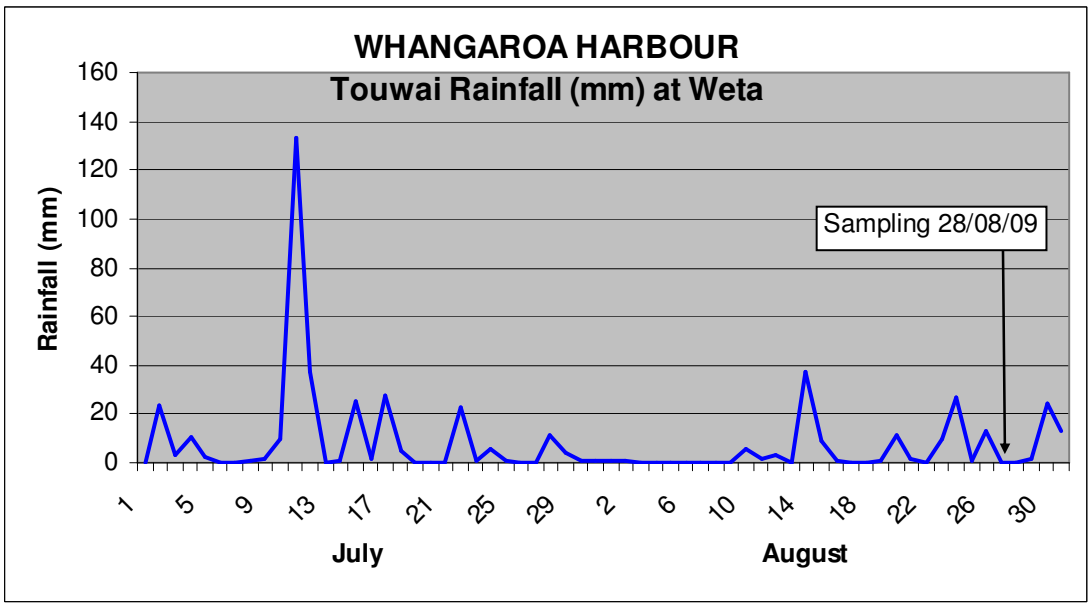

Figure 5. Rainfall (mm) during July-August 2009 in Whangaroa Harbour, at Touwai monitoring site.

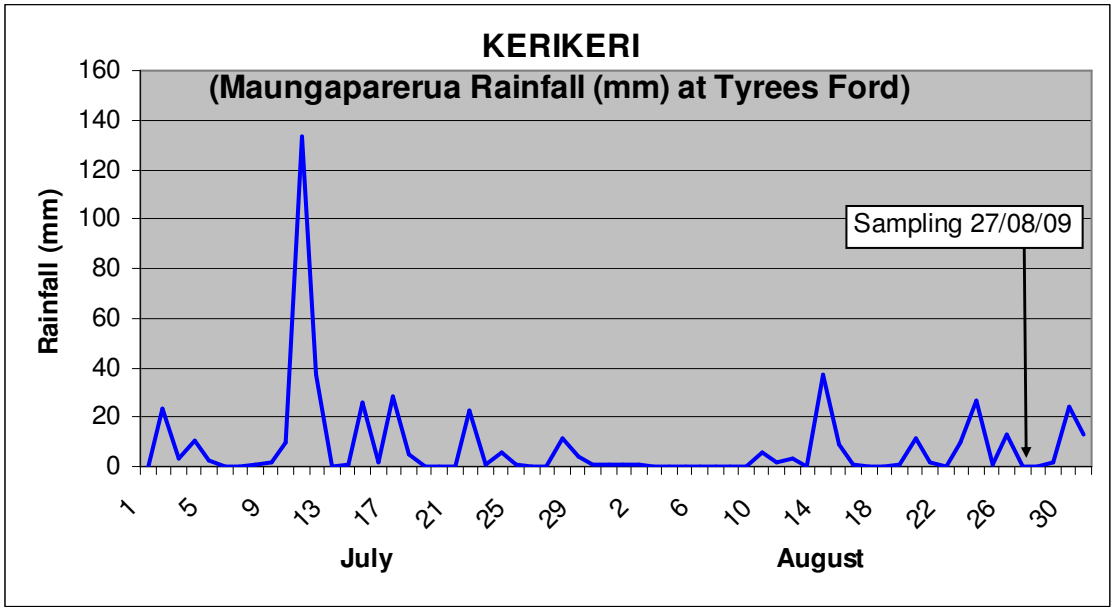

Figure 6. Rainfall (mm) during July-August 2009 in Kerikeri Inlet, at Maungaparerua monitoring site (in the upper catchment of Kerikeri).

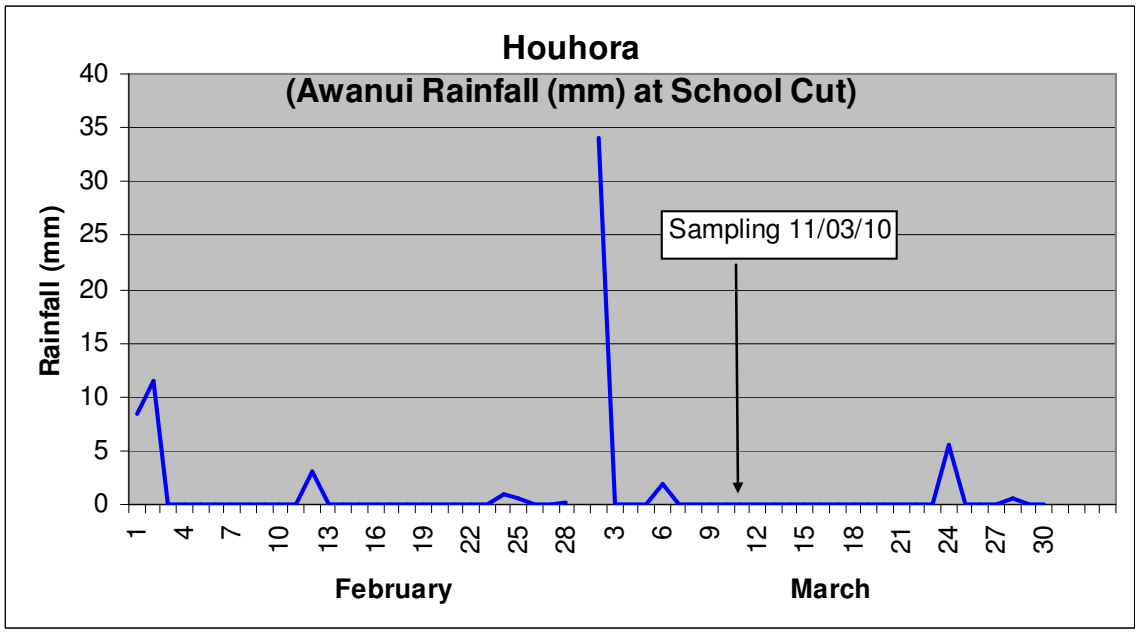

Figure 7. Rainfall (mm) during February-March 2010 in Awanui (this is the most reliable and nearest rain gauge to Houhora).

3.2. Water, Sediment and Shellfish

Results of the faecal sterol analysis of selected water samples are shown in Tables 2 and 3 and sediment samples in Table 4. Levels of *E. Coli* from oyster samples are shown in Tables 5 and 6.

Water samples with *E. coli* concentrations >100 CFU/100mL were selected for further testing of *E. coli* sources. All the water samples taken in Houhora Harbour were low or not detected for *E. coli* and so no analysis of sterols was undertaken.

Table 2. Faecal Sterol Analysis of Water Samples in August 2009.

Site ID	Location	*E. coli*(CFU/ 100mL)	Source of *E. coli*
1	Whangaroa Harbour, marina*	41	N/A (too low)
2	Whangaroa Harbour, Totara North.	132	Ruminant.
3	Whangaroa Harbour, oyster farm.	146	Ruminant.
4	Whangaroa Harbour, culvert*	20	N/A (too low)
5	Whangaroa Harbour, Kaeo River.	520	Ruminant.
K1	Kerikeri, Upper Hauparua Inlet*	10	N/A (too low)
K2	Kerikeri, Lower Hauparua Inlet*	<10	N/A (too low)
K3	Kerikeri, behind lease 17818*	52	N/A (too low)
K4	Kerikeri, in front lease 17818*	<10	N/A (too low)
K5	Kerikeri, behind lease 131*	31	N/A (too low)
K6	Kerikeri, No. 9 Buoy.	185	Ruminant.
K7	Kerikeri, Rangitane River	148	Ruminant.

Table 2 (Contin.) Site ID	**Location**	***E. coli*(CFU/ 100mL)**	**Source of *E. coli***
K8	Kerikeri, Pagoda Lodge.	419	Ruminant.
K9	Kerikeri, Stone Store.	410	Ruminant.
K10	Kerikeri, Waipapa Landing.	243	Ruminant.
H1	Houhora Harbour, NW of oyster lease*	<10	N/A (too low)
H2	Houhora Harbour, SW of oyster lease*	<10	N/A (too low)
H3	Houhora Harbour, East of centre oyster lease*	10	N/A (too low)
H4	Houhora Harbour, SE corner of oyster lease*	10	N/A (too low)
H5	Houhora Harbour, mid-channel to oyster farm*	<10	N/A (too low)
H6	Houhora Harbour, channel of Waimamaku Stream*	10	N/A (too low)
H7	Houhora Harbour, Point south of ramp*	<10	N/A (too low)
H8	Houhora Harbour, channel off Ariawa Stream*	41	N/A (too low)
H9	Houhora Harbour, channel off Motutangi Stream*	52	N/A (too low)

*** Water samples were less than the minimum detection limit (>100 CFU/100mL *E. coli*) for faecal sterol analysis.**

Table 3. Faecal sterol analysis of water samples taken on 28 April 2010 (first storm after drought). Sampling was only undertaken at these sites.

Site ID	Location	*E. coli*(CFU/ 100mL)	Source of *E. coli*
2	Whangaroa (Kaeo River at the shellfish site # 2)	2187	Ruminant.
3	Whangaroa (at the shellfish site # 3)	1296	Ruminant.
K6	Kerikeri, No. 9 Buoy.	389	Wildfowl.
K7	Kerikeri, Rangitane River	620	Wildfowl.
K8	Kerikeri, Pagoda Lodge.	6131	Wildfowl(dominant result) and ruminant (weak result).
K9	Kerikeri, Stone Store.	2247	Wildfowl(dominant result)*.
K10	Kerikeri, Waipapa Landing.	1872	Ruminantand wildfowl.

* Note: the wildfowl marker is detectable in 75% of duck faecal samples and 10% of Canada geese and swan faeces and not from seagull faeces.

Table 4. Faecal sterol analysis of sediment samples in August 2009 and March 2010.

Sample ID	Location	Date sampled	Source of E. *coli*
1	Whangaroa Harbour.	28/8/09	No indication human sterols. Suggestive of herbivore/wildfowl sterols
S1 and S2	Kerikeri Inlet.	27/8/09	No indication human sterols. Suggestive of herbivore/wildfowl sterols
1	Houhora Harbour.	11/03/10	No indication human sterols. Suggestive of wildfowl sterols

Table 5. *E. coli* results of shellfish sampled in August 2009 (in Whangaroa Harbour and Kerikeri Inlet) and in March 2010 (in Houhora Harbour).

Sample ID	Location	*E. coli* (CFU/ 100g)
1	Whangaroa Harbour.	3,100
2	Whangaroa (Kaeo River).	200
3	Whangaroa (near Pupuke River and Totara North).	2,100
K183	Kerikeri Inlet (farm lease #183).	8,300
K17	Kerikeri Inlet (farm lease #17).	2,200
K18	Kerikeri Inlet (farm lease #18).	3,600
1	Houhora Harbour.	200

Table 6. *E. coli* results of shellfish sampled on 28th April 2010.

Sample ID	Location	*E. coli* (CFU/ 100g)
1	Whangaroa Harbour, gamefishing club.	212
2	Whangaroa (Kaeo River mouth).	208
3	Whangaroa (Pupuke River mouth).	1,099
K6	Kerikeri Inlet, No. 9 Buoy.	4,453
K8	Kerikeri Inlet, Pagoda Lodge.	21,463
K10	Kerikeri Inlet, Waipapa Landing.	7,018

4. DISCUSSION

Results of this study suggest that, at the time of sampling, coastal waters in the Whangaroa harbour and Kerikeri Inlet have a common source of *E. coli* and that is from ruminant sources. Sediments also have a common source of *E. coli* and these were identified as being either wildfowl or a combination of bovine/wildfowl.

Many of the oysters sampled in Whangaroa and Kerikeri were highly contaminated with levels of *E. coli* which was not unexpected as they were sampled after rain. At these times, oyster farms are closed for harvest and do not re-open until NDHB strict criteria are followed.

Overall, results showed no clear source of human faecal contaminants (by using sterols analysis) at the time of sampling. An investigation into the operations of the waste water treatment plant (WWTP) around the time of sampling was undertaken and monitoring data showed the plants were operating within consent conditions. Both of these results suggest no human sources at the time of sampling. Faecal coliform levels were measured in waters at the discharge outlet of the WWTP and these levels were considered to be low (Orevich, C. pers. comm., 2010).

Further sampling of waters and oysters in Kerikeri Inlet and Whangaroa Harbour would further validate these results; this could show that the results so far are a one-off or there is a repeat of the already identified sources during other times. NRC currently monitors water quality at sites in Kerikeri Inlet on a bi-monthly basis.

5. CONCLUSION

Shellfish were highly contaminated with *E. coli* after rain. At the time of sampling, results of the sterol analysis suggested sources of *E. coli* were more likely to be from ruminant and/or wildfowl sources than any other source. No human sources were identified at any of the sites sampled.

It would be of benefit to undertake further sampling to determine if the *E. coli* sources are wildfowl and/or ruminant at other sampling occasions. In particular, sampling when the WWTP is discharging would enable NRC to undertake a risk assessment of both diffuse (ruminant and wildfowl) and point sources (WWTP) in to the receiving waters and their potential impact on shellfish health.

6. AKNOWLEDGEMENTS

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7. REFERENCE

Devane, M., Saunders, D., Gilpin, B. (2006) Faecal Sterols and Fluorescent Whiteners as Indicators of the Source of Faecal Contamination. Chemistry in New Zealand. 70:74-77.http://nzic.org.nz/CiNZ/articles/Devane_70_3.pdf