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Advice on Identifying Sources of Faecal Contamination in Houhora Harbour

RESEARCH BASED SOLUTIONS

Advice On Identifying Sources Of Faecal Contamination In Houhora Harbour

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EXECUTIVE SUMMARY

Houhora Harbour is situated in Northland and has been used to commercially grow Pacific oysters (*Crassostrea gigas*) for the New Zealand and export food markets. Over the last few years, the officers working for Northland District Health Board and New Zealand Food Safety Authority (NZFSA) have expressed concern about elevated faecal indicator bacteria (FIB) levels measured in both shellfish and seawater samples. The FIB levels often exceed the regulatory limits for shellfish, thus putting the commercial status of the oyster farms at risk. The Authorities have requested that the source(s) of the microbial pollution be identified in order to appropriately manage the problem(s) leading to contamination.

This report, funded through the Foundation for Research, Science & Technology (FRST) Envirolink advice scheme, provides background information on shellfish sanitation and current monitoring practices and a review of historical microbial water quality data taken from the Houhora Harbour oyster farms and surrounding waters. Based on the review, we identify potential pollution sources that may be leading to elevated counts of microbial indicator organisms in Houhora Harbour. We then discuss the limitations of FIB data and provide recommendations for future FIB monitoring and the application of Microbial Source Tracking (MST) markers for identifying the sources leading to contamination of shellfish resources in Houhora Harbour.

During the past few years, oyster harvesting areas in Houhora Harbour have complied with NZFSA guidelines (NZFSA 2006) for conditionally approved growing areas. However, on several occasions there have been *Escherichia coli* (*E.coli*) counts in the shellfish tissue samples above the criteria threshold (≥230 *E. coli* per 100 g tissue). Swans are frequently observed in the Harbour and subsequently they have been implicated as a likely source of the elevated bacteria counts. However, data suggest that their numbers have not increased appreciably over the past few years, and in comparison to other animals in the catchment, their potential contributions to faecal contamination are likely to be relatively low. For instance, we estimate that ruminant animals such as cows and sheep produce ~90 % of the faecal matter in the catchment. Large amounts of bacteria from these sources are likely flushed into the harbour during rain events; data collected in Tasman Bay (Cornelisen & Kirs, unpublished data) suggest that ruminant bacteria can be transported the distance of the oyster harvest areas via tides and wind-driven currents. With the presence of waterfront developments and septic systems, human sources of contamination are possible.

To better understand the sources and processes leading to high levels of faecal bacteria in oyster harvesting areas, we recommend continuation of FIB monitoring with integration of MST markers when high FIB counts are observed in order to assist in identifying the source(s) of contamination. There are a number of validated markers currently available that can identify presence/absence of human and ruminant faecal contamination and methods for quantifying the amount of marker present within samples also exist (*e.g.* real-time PCR, which provides an indication of the relative contribution of different sources in a contaminated sample). Markers for birds, including waterfowl are available; however, they are not as effective as the markers for other sources of contamination. It would be advantageous to first assess the presence/absence of dominant sources in the catchment (*e.g.* ruminant) and sources that pose the greatest health risk (*e.g.* humans).

Through process of elimination, the importance of swans can in turn be assessed and later confirmed using MST techniques. We also recommend a detailed shoreline survey of FIB and MST markers in water samples in order to assess all potential contaminant sources and pathways into the harbour.

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1. INTRODUCTION

Bivalve molluscan shellfish (BMS), such as mussels, oysters, clams and scallops have long been enjoyed as a food source throughout the world. However, shellfish are efficient filter feeders and as a result can accumulate pathogenic micro-organisms, viruses, protozoa, marine biotoxins, heavy metals or other toxic substances within their tissues. Shellfish are often consumed whole and/or raw; hence there is increased risk for food safety problems. Raw shellfish receive the second highest hazard rating for all foods (International Commission on Microbiological Specifications for Food). New Zealand Food Safety Authority (NZFSA) requires that all shellfish harvested commercially for food products must comply with the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006 (NZFSA 2006).

Houhora Harbour is situated in Northland and has been used to commercially grow Pacific oysters (*Crassostrea gigas*) for the New Zealand and export food markets. Over the last few years, officers working for Northland District Health Board and NZFSA have expressed concern about elevated faecal indicator bacteria (FIB[1]) levels measured in both shellfish and seawater samples. The FIB levels often exceed the regulatory limits for shellfish, thus putting the commercial status of the oyster farms at risk. The authorities have requested that the source(s) of the microbial pollution be identified in order to appropriately manage the problem(s) leading to contamination.

The objectives of this report are to:

i) Provide background information on shellfish sanitation and current monitoring practices;

ii) Provide a brief review of historical microbial water quality data taken from the Houhora Harbour oyster farms and surrounding waters to assess the extent of the problem and compliance with NZFSA food legislation;

iii) Based on the review, identify potential pollution sources that may be leading to elevated counts of microbial indicator organisms in Houhora Harbour;

iv) Provide advice on possible ways to confirm pollution sources leading to contamination of shellfish resources in Houhora Harbour.

This report represents the first of several steps necessary to address water quality problems that are common in many of New Zealand's coastal and estuarine waters. Hence, the advice provided here is directly transferable to other areas that are experiencing faecal pollution problems where the source(s) of contamination remains unknown. The primary goal of the recommended approach is to identify the source of the problem, which is a critical step in prioritising mitigation measures and managing the problem.

[1] Standard FIB for monitoring shellfish include *Escherichia coli* and faecal coliforms, which are associated with the gastrointestinal tracts of warm blooded animals.

2. BACKGROUND ON SHELLFISH SAFETY AND MONITORING

2.1. Potential microbial food safety problems

Societies around the world have historically recognised that consumption of shellfish, including oysters, can cause illness and, traditionally, social wisdom was used to protect consumers. Examples of traditional wisdom include the United States of America (USA) rule that one should only harvest oysters in months with no "r" in them or the Māori use of rāhui after drowning near to shellfish food stocks (McCoubrey 2007).

With advances in microbial science it became possible to recognise and understand more about pathogens that cause illness after eating contaminated shellfish. In the early 19th century many countries, including New Zealand, experienced typhoid and/or cholera cases due to shellfish being contaminated by human sewage. These diseases are no longer prevalent in developed countries due to better sanitation systems, but consumption of contaminated shellfish is still implicated in outbreaks associated with *Salmonella, Shigella, Campylobacter, Yersinia, Listeria, Clostridium, Staphylococcus* and *Escherichia coli* (Hackney & Pierson 1994). These microbial pathogens are associated with human, warm-blooded land and sea mammals and bird populations, where they are harboured in the gastrointestinal tracts and then excreted in very large numbers in faecal material.

People usually recognise the high public health risks associated with discharging untreated sewage in the environment, but are unaware of the potential zoonotic disease[2] risk when food or water supplies are contaminated by faeces from farm animals, wild animals and birds (Hackney & Dicharry 1998).

Therefore, if marine waters are contaminated with discharges from human waste systems (septic tanks, municipal outfalls or marine vessel discharges), wild and domestic animals or bird colonies, there is the high possibility of harvesting shellfish which will cause illness.

2.2. Shellfish sanitation programmes

Food safety authorities around the world have long recognised the potential illness risk associated with shellfish and most countries have food safety laws that address these risks. Due to historical typhoid and cholera outbreaks associated with oyster beds, both the USA and United Kingdom started shellfish public health programmes in the late 1800s. By 2009 these programmes have evolved into the USA National Shellfish Sanitation Programme and the European Commission Directives.

[2] Zoonotic diseases are those that can be transmitted from animals to humans

New Zealand has also established a world-recognised shellfish sanitation programme, known as the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006 (NZFSA 2006). This legislation is administered by NZFSA and covers all the requirements for growing, harvesting and transporting shellfish to the market. The New Zealand programme is based on the principles of the USA and European Union programmes, while taking account of our own unique, environmental features and industry practices. All of these programmes use some basic principles, namely:

- A public health sanitary survey of the shellfish catchment area.
- Monitoring of contaminants (*e.g.* biotoxins, FIB) in water and shellfish samples.
- Control of harvest times according to FIB monitoring results.
- Labelling of shellfish so that people know the origin of the product.

2.2.1. Sanitary survey

A sanitary survey involves an examination of the watershed draining into a shellfish harvesting area to identify and, where possible, eliminate or minimise potential sources of contamination. The information generated is used to identify waters that are likely safe (and unsafe) for the harvest of shellfish for direct human consumption. The sanitary survey originated in the late 19th century as a means to protect drinking water supplies (Fair & Geyer 1963). Because of its success the concept was applied to shellfish growing areas.

2.2.2. Monitoring of water and shellfish contamination

The second principle is the bacteriological and toxicological examination of water and flesh samples. The purpose of this is to confirm the tentative conclusions of the shoreline survey regarding the risk of contamination. The qualitative assumption is that the more faecal material in the water, the greater the risk of contracting disease. Rather than testing the water and shellfish for a large number of individual microbial pathogens, faecal indicator bacteria (FIB) are used to assess the presence of faecal contamination in the marine environment and in turn are used as a surrogate for the potential presence of pathogens (National Research Council 2004). Faecal indicator bacteria were originally established on their relationship to an infective dose of typhoid organisms, but today these levels are still used to reflect the risk of microbial illness. We discuss the limitations of using FIB as a monitoring tool in Section 3.6.

2.2.3. Controlled harvest times

The purpose of regulated harvest times is to ensure that shellfish are only taken when they are considered safe to eat. It is important that harvesting takes into account potential contamination hazards[3], such as those associated with emergency sewage overflows. Areas can be closed when environmental events cause pollution; referred to as conditional

[3] Hazard is defined as the length of time that pathogenic doses of microbes are likely within the shellfish

management (National Shellfish Sanitation Programme 2005). However, before an area can be designated as suitable for conditional management it must meet some fundamental criteria.

The pollution sources need to be identified, well understood and predictable in their performance. All parties need to know when and where the harvest area is contaminated.

As in other countries there are strict requirements on where and when shellfish can be harvested. When the areas are open for harvest the water and shellfish must meet the following minimum FIB limits/conditions:

- The faecal coliform median most probable number (MPN) of the water samples must not exceed 14 per 100 ml and not more than 10% of the samples must exceed an MPN of 43 per 100 ml.
- The *E. coli* median MPN of the shellfish tissue samples must not exceed 230 *E coli* per 100 g tissue and not more than 10% of the samples must exceed an MPN of 700 per 100 g tissue.
- All commercial shellfish harvesting areas must have monthly water and shellfish samples taken when the area is open for harvest, and the medians are calculated on a minimum of 15 sampling events, but usually on three years of environmental data.

3. BACKGROUND ON HOUHORA HARBOUR

3.1. General physical geography of the land

Houhora Harbour and its catchment are located on the eastern side of the northern peninsula, 50 km north of Kaitaia. The Harbour is part of the Aupouri Peninsula, which is 75 km long and 10 km wide. Over much of the area the land climbs gently to approximately 80-100 m above sea level as low rolling dune country with various terraces, interdunal flats, lakes and swamps with Mt Camel the dominant topographical feature rising to 236 m (Figure 1). The rest of the catchment consists of sand country with marine and alluvial terraces, abandoned shorelines and intertidal flats, lakebeds and swamps. Principal rock types in the catchment are gravel, sand, ash and mud of quaternary origins. The majority of the catchment has good drainage. Swamps and lakes are formed inland, many of these swamp areas have had drains put through them with runoff being channelled to the harbour. Major streams discharging into the Houhora Harbour include the Waihopo and the Motutangi streams (Figure 1.

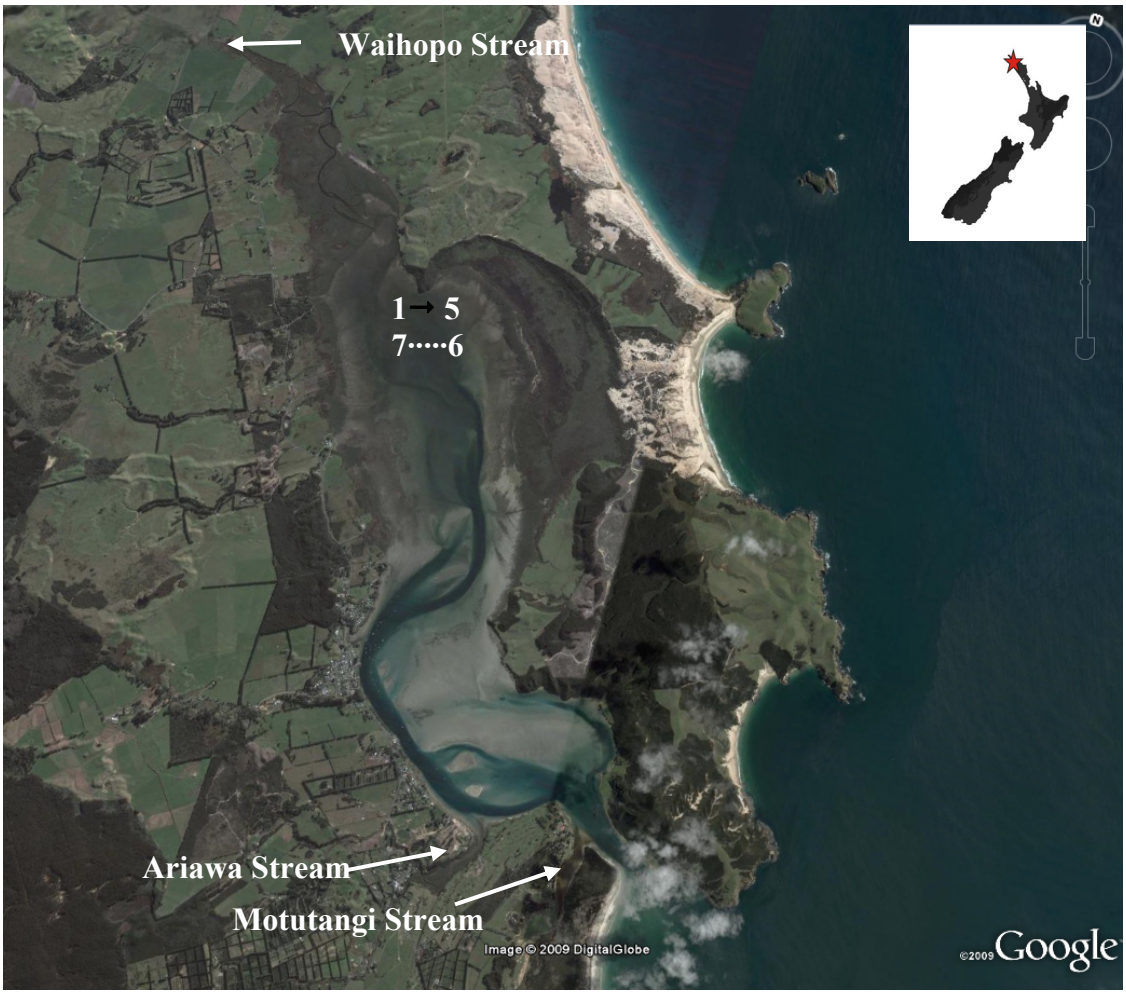


Figure 1. The Houhora Harbour. 1, 5, 6, 7 indicate official sampling sites around oyster harvest areas.

3.2. Physical description of the water body

Houhora Harbour is a shallow, sheltered, elongated harbour, with a deep well-defined channel and an extensive area of intertidal sand flats at the mouth of the harbour running landwards. The channel runs the length of the harbour; at low tide it is about 5 m deep in the southern half and about 2-4 m deep in the northern half. The channel runs close to the western shores.

The harbour is 8.5 km long oriented northwest to southeast and covers an area of 1430 hectares, at high tide it is 2 km wide at its widest point. The harbour is in a zone that has tides in the range of 2.4–3.6 m. It drains at low tide to the principal channel (visible in Figure 1) and it is estimated that up to 60% of water is exchanged in each tidal cycle (Silver & Schmitt 2003). The water in the inlet is generally well mixed and has a short residence time, aiding the flush of contaminants.

Seawater temperatures are typical of Northland and range from 12-23 degrees Celsius and the rainfall in the catchment is around 1500 mm per year. Marsh areas are extensive in the upper

reaches of the harbour and provide a habitat for wildlife. Additionally, the upper harbour has extensive seagrass beds (*Zostera capricorni*) that attract black swans that feed in and around the beds.

3.3. Commercial oyster farming in the harbour

The northern part of the harbour has been used to farm Pacific oysters since 1991 and there is currently 40 hectares being farmed using rack culture methods. Oysters are harvested at any time of the year, although most are harvested between May and November. The Northland District Health Board provides NZFSA with the public health services to implement the shellfish quality assurance programme for the Houhora Harbour.

There are four official sampling sites (see Figure 1) on the farms from which water and shellfish samples are tested for microbial compliance. Currently the Houhora oyster farms are classified as "Conditionally Approved", with rainfall a criterion that opens and closes the harbour. This is because land runoff during rain events affects the microbial quality of the seawater and shellfish; violations of FIB criteria generally coincide with rain events.

3.4. Microbiological water quality at Houhora oyster farms

A classification of "Conditionally Approved" requires that seawater and oyster samples are collected at the four sampling sites (Figure 1) on a monthly basis (unless the farmers have taken a voluntary summer season closure). Northland District Health Board has expressed concern at the occasional unpredicted elevated FIB counts over the past few years. Table 1 shows data for a recent three year period (2006 – 2008).

Table 1. Routine microbial water and shellfish data for Houhora Harbour growing area (2006-2008). Source: Northland District Health Board. Sites pertain to the numbered locations in Figure 1. NA- data not available

Date	Open Status	Faecal coliforms Waters Site 1	Faecal coliforms Waters Site 5	Faecal coliforms Waters Site 6	Faecal coliforms Waters Site 7	*E. coli* Flesh Site 1	*E. coli* Flesh Site 5	*E. coli* Flesh Site 6	*E. coli* Flesh Site 7
17/01/2006	open	1	1	1	1	17	17	17	17
20/04/2006	open	1	1	1	1	140	45	17	68
3/05/2006	open	2	6.1	1	6.8	130	78	17	17
15/06/2006	open	6.8	1	1	1	40	45	20	45
27/07/2006	open	1	1	1	1	17	17	17	17
14/08/2006	open	1	2	2	1	130	17	17	20
26/09/2006	open	1.8	1	1	1	17	45	17	45
22/11/2006	open	1	1	1	1	130	130	45	45
6/11/2006	open	1	2	1	1	45	20	17	45
5/12/2006	open	1	1	2	1	17	20	45	130
14/06/2007	closed	7.8	6.8	1	23	230	130	45	170
27/06/2007	open	2	1	1	2	20	45	45	17
14/08/2007	open	1	2	2	7.8	130	230	110	130
5/09/2007	open	1	1	1	1	110	78	140	20
17/09/2007	open	2	4.5	1	1	3500	17	68	20
2/10/2007	open	7.8	1	1	1.8	45	45	45	330
30/10/2007	open	1	1	1	1	NA	330	45	93
16/01/2008	open	1	2	1	1	17	230	17	130
21/05/2008	closed	11	1	1	4.5	45	490	78	230
28/05/2008	retest	4.5	4.5	2	1	17	17	68	17
5/08/2008	open	1	1	1	1	78	NA	330	330
9/09/2008	NA	2	1	2	4.5	230	490	490	220
23/09/2008	open	1	4.5	1	1	NA	45	17	78
7/10/2008	open	1	1	1	1	20	310	20	19
29/10/2008	open	2	4	1	2	70	NA	70	40
17/11/2008	open	4.5	1	1	1	110	70	70	200
2/12/2008	open	13	4.5	2	1	310	500	70	500
10/12/2008	open	1	1	1	1	2400	2400	500	2400
18/12/2008	closed	1	1	1	1	110	90	40	NA

During the three year period, all four sites complied with NZFSA guidelines (NZFSA 2006) for conditionally approved growing areas (Table 2).

Table 2. Estimated microbial water quality parameters. NZFSA standards for approved growing areas affected by point and non-point sources are indicated in red.

Site	Faecal coliforms Water column			*Escherichia coli* Flesh samples		
	Number of samples	Median	% >43	Number of samples	Median	% >700
Limit:		14	10%		230	10%
1	29	1	0	27	78	7.4
5	29	1	0	27	70	3.7
6	29	1	0	29	45	0
7	29	1	0	28	56	3.6

While faecal coliform concentrations remain low in the seawater, there are seemingly random elevated *E. coli* counts in the shellfish samples (on some occasions >>230 *E. coli* per 100 g tissue; Table 1). The water samples have comparatively low faecal coliform counts; hence it is likely that *E. coli* is concentrated by shellfish from plumes containing relatively low levels of faecal contamination. The concentration of faecal coliforms (likely dominated by *E. coli*) in these plumes remain mostly low, close to the lower detection limit of fermentation tube tests and therefore the water column appears to contain little or no faecal contamination when sampled by this technique. It is therefore likely that an infrequent sampling scheme and current methodology fail to adequately identify faecal contamination events. This faecal contamination can originate from any source and the fate of the contamination will be heavily influenced by currents, tides and weather patterns.

Furthermore, although collected water samples always comply with the guidelines, the threshold (>230 *E. coli* in 100 g) for shellfish samples was exceeded in three samples at Site 1 (n=27) and Site 6 (n=27), in four samples at Site 7 (n=28) and in six samples collected at Site 5 (n=29) over the period of three years. Most importantly, while no samples exceed the threshold in 2006 (n=10), three samples exceeded the threshold in 2007 (n=7) and 13 samples exceeded the threshold in 2008 (n=12), indicating a deteriorating water quality trend in the Houhora Harbour (Figure 2).

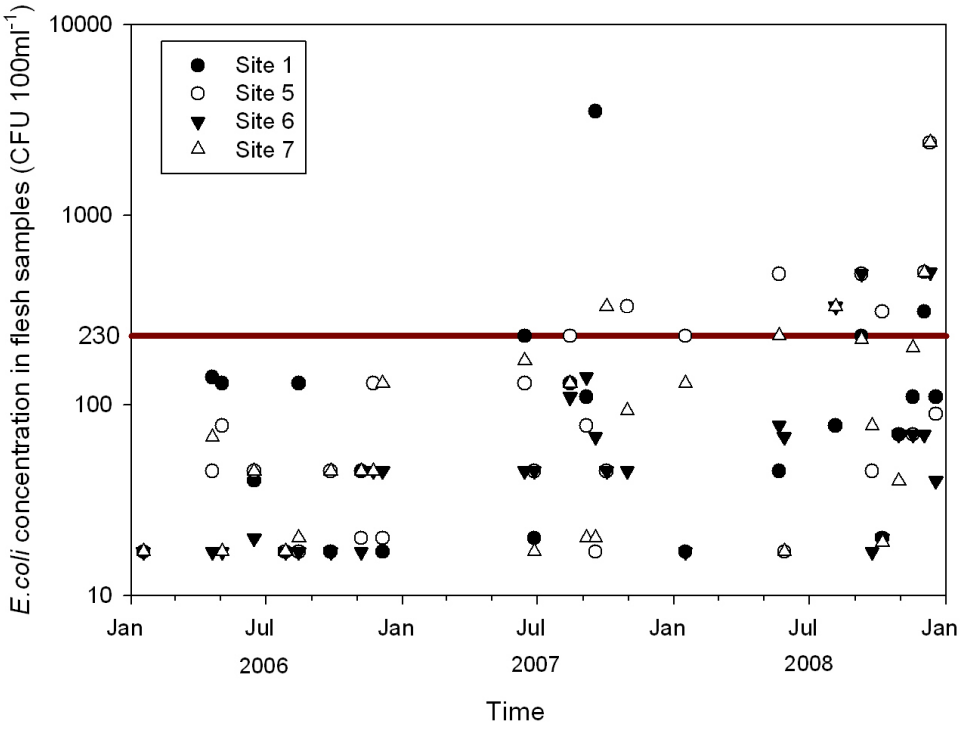
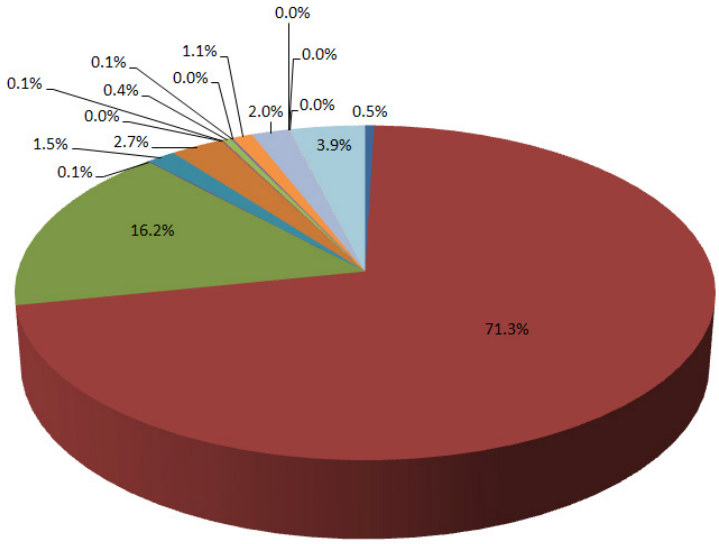


Figure 2. *Escherichia coli* concentrations in the shellfish samples collected 2006-2008. Red line indicates standard for the median estimate.

3.5. Pollution sources in the harbour

Northland District Health Board last undertook a full comprehensive sanitary survey in 2003 (Silver & Schmitt 2003). Based on the stock and wildlife numbers reported in the survey, daily faecal inputs for the primary groups of animals at the Houhora Harbour area can be estimated (Figure 3).



	Estimated numbers	Daily faecal output T/day
Humans	1,000	0.15
Dairy	962	22.70
Sheep	4 570	5.16
Poultry	120	0.02
Horse	24	0.48
Deer	583	0.87
Goat	8	0.01
Donkey	2	0.02
Dogs	443	0.13
Domestic cats	183	0.04
Feral cats	40	0.01
Rats	37 200	0.33
Possum	27,000	0.62
Oyster catchers	80	0.00
Seagull	100	0.01
Turkey	50	0.01
Black swans	3000	1.25

Figure 2. Stock and wildlife numbers and estimated daily faecal inputs in the Houhora Harbour catchment.

In terms of biomass, ruminant livestock (cows, sheep, deer) are the most abundant mammals in the area. Based on the daily faecal material production estimates, ruminants are the dominant source of faecal material produced in the catchment, releasing a combined 28.8 tonnes (90.3% of the total) of faecal matter per day (Figure 3). The main contributor in this group are dairy cows, producing 22.7 T/day (71.3%) of faecal matter in the area (Figure 3). Approximately 1.34×10^{14} CFU of *E. coli* and 2.95×10^{11} CFU of enterococci are released per day by cows in the area. The faecal matter is dispersed over vast areas, but released bacteria remain viable for long periods (or even naturalise) and can enter into the system directly during rain events or leach into groundwater. In addition, cows defaecate ~50 times more per metre when crossing streams (Davies-Colley *et al.* 2004).

Ruminant sources of faecal contamination are important to consider with regard to the Houhora Harbour and contamination of its shellfish resources. Bacteria can be transported long distances; for instance, microbial source tracking (MST) markers have been used to detect faecal bacteria from ruminant animals within the gut tissue of mussels in Tasman Bay located ~6 km away from the Motueka River mouth (Kirs, unpubl. data). These findings suggest it is entirely plausible that significant amounts of faecal bacteria from ruminant animals in the catchment are entering Houhora Harbour via the streams indicated in Figure 3, and depending on tide/wind conditions, are then carried to close proximity of the oyster harvest areas.

There are approximately 1000 people living in the greater Houhora catchment (NZFSA 2006); most rely on onsite disposal systems (septic systems) for sewage disposal. The houses are spread out over the rural settlements; the only dense residential area is located in Pukenui and the coastal margins between Houhora Heads and Subritzky Road. An estimated 0.15 T of human faecal matter (containing approximately 1.5×10^{14} CFU of *E. coli* and 1.5×10^{11} CFU of enterococci) is released daily in the area (Figure 3). While this material should be treated on site, leaking septic systems could have a significant impact, particularly in areas where there are a high number of waterfront properties (*e.g.* along the southwest coast of the bay). Furthermore, some of the human faecal contamination might be released by swimmers, as an average swimmer sheds 6×10^5 CFU of enterococci and 6×10^6 CFU of enterococci and *Staphylococcus aureus* in the first 15 minute exposure period, respectively (Elmir *et al.* 2007), and another fraction can be released from boats. It is critical to identify and quantify possible human faecal contamination component as it is likely to have higher health risk compared to an animal source.

Houhora and Parengarenga Harbours are a home for the second largest population of Black Swans in Northland (roughly 1600-2500 birds, Figure 4). Because of their visible abundance, at times in close proximity to oyster harvesting areas, they have been identified as a likely source of faecal contamination in oysters. Health officers consider that the Black Swan populations have increased in recent years and that they are now grazing near to the oyster farms. This has also been verified by the local residents, who believe that the swans are taking refuge in Houhora due to culling operations in nearby Parengarenga Harbour.

Contrary to this anecdotal information, aerial monitoring data collected in January each year (provided by Dr Rudi Hoetjes; Northland Fish and Game Council) do not indicate an increasing trend in Black Swan numbers in the area since 2002, although there was a peak in numbers in 2006 (Figure 4). Based on these data, there is not a clear correlation between elevated *E. coli* concentrations identified frequently in 2007 (later in the season) and 2008 (Figure 2), and Black Swan population estimates in the area (Figure 4). It is estimated that approximately 2.6×10^4 CFU of *E. coli* and 4.7×10^2 CFU of enterococci are released per gram of faeces by Black Swans (Gilpin *et al.* 2007). Therefore substantially less *E.coli* and enterococci are released by Black Swans in the area when compared to dairy farms or humans (Figure 3). While the contribution from swans is smaller than other sources, their close proximity to harvest areas could elevate their relative contribution to the contamination problem.

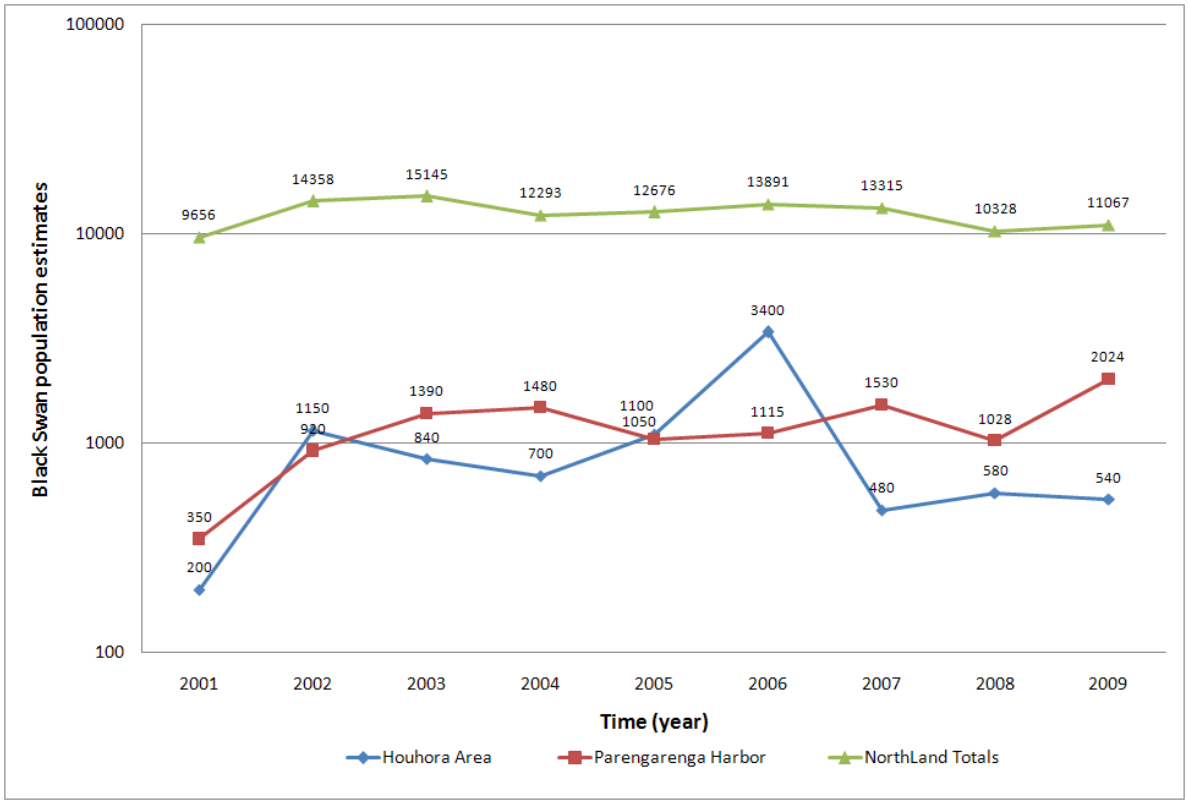


Figure 3. Black Swan population estimates. Data provided by Dr Rudi Hoetjes; Northland Fish and Game Council.

3.6. Limitations of faecal indicator data

The above information based on FIB data provides background and the nature and extent of faecal contamination in Houhora Harbour. While the data provides an overall picture, the data is limited in its ability to identify the sources of the problem and in turn the steps that are necessary to mitigate the problem. As stated in Section 2, FIB are used to identify the presence of faecal contamination and therefore potential food safety risks. An important biological attribute of an indicator organism is a strong quantitative relationship between the indicator concentration and the degree of risk to the public. According to Bonde (1977), faecal indicators should:

- Have similar survival characteristics (or greater) to pathogens.
- Be transported in a similar manner as pathogens.
- Be present in greater numbers than pathogens.
- Be specific to faecal source or identifiable as to the source of origin.

Unfortunately, the FIB currently used for monitoring faecal contamination in coastal waters and shellfish do not satisfy the above criteria as FIB can persist, and in some cases multiply in the environment outside the host organism (Hartz *et al.* 2008; Ishii & Sadowsky 2008; Yamahara *et al.* 2009). Humans, livestock and wildlife are all possible sources of faecal contamination in Houhora Harbour; however, environmental pools of *E. coli* and enterococci may also exist and high concentrations of these indicator organisms can be released during storm events. Standard bacteriological tests are not able to distinguish these pools and therefore can falsely suggest recent faecal contamination.

This can make it difficult when trying to understand the connection between land-use activities or the abundance patterns in Black Swans and actual versus perceived contamination problems. Of greater public health concern is the fact that faecal bacteria cannot survive in the marine environment as long as some viruses, which can persist for a matter of weeks to months (Younger *et al.* 2002). Furthermore, FIB provide no information on the source(s) of contamination; which is critical for assessing the level of actual health risk and enabling effective management and remediation strategies. Scientists are now developing (and applying) more suitable indicators based on microbial source tracking (MST) markers, which are better at confirming a contamination problem and identifying the source(s) of the contamination. In the next section, we provide recommendations on future FIB monitoring and the integration of MST technology, which will assist in understanding the contamination problems in shellfish harvesting/growing waters in Houhora Harbour.

4. RECOMMENDATIONS FOR FUTURE MONITORING AND APPLICATION OF MST TECHNOLOGY

To better understand the mechanisms leading to high levels of faecal bacteria in oyster harvesting areas, we recommend continuation of FIB monitoring with integration of MST markers to assist in identifying the source(s) of the contamination. A number of approaches exist for microbial source tracking (*e.g.* faecal sterols, optical brighteners, antibiotic resistance profiling *etc*.); however, no method has proven more promising for future research directions than library-independent methods based on polymerase chain reaction (PCR) (Stapleton *et al.* 2007). There are a number of validated markers currently available that can identify presence/absence of human and ruminant faecal contamination and methods for quantifying the amount of marker present within samples also exist (*e.g.* real-time PCR, which provides an indication of the relative contribution of different sources in a contaminated sample). Markers for birds, including waterfowl, exist. However, one of them is not carried by a large portion of the swan/geese population (Devane *et al.* 2007) and others have not been validated in New Zealand. It would be advantageous to first assess the presence/absence of dominant sources in the catchment (*e.g.* ruminant) and sources that pose the greatest health risk (*e.g.* humans). Through a process of elimination the importance of swans can in turn be assessed.

Steps could include the following:

1. **Continue routine monitoring of faecal indicator bacteria (and MST markers if counts are high) in water and shellfish samples and review in light of ancillary information (*e.g.* weather, abundance of birds, land use changes)**

Purpose: Assess growing area and shellfish compliance with the NZFSA standards.

Objective: Identify FIB concentrations and trends. Identify high count samples for MST markers.

Approach: Continue random sampling as specified by NZFSA (NZFSA 2006) for conditional approved growing areas. Identify faecal coliform and *E. coli* concentrations as required by NZFSA (NZFSA 2006). Sufficient water per sample (1 Litre) should be collected so that in the event high FIB counts are observed, the remaining water can be used for MST analysis. In addition, freeze extra shellfish samples for possible MST marker analyses depending on FIB results. When *E. coli* concentrations in shellfish exceed 230 MPN 100 g^{-1} or faecal coliform concentrations in water samples exceed 14 MPN 100 ml^{-1}, conduct MST marker tests using qPCR and PCR technologies (Figure. 5).

2. **Conduct intensified non-random monitoring of faecal indicator bacteria (and MST markers if counts are high) in water and shellfish samples**

Purpose: Identify shellfish samples that contain high concentrations of FIB for MST.

Objective: Assess the source and impact of bacterial contamination flushed into the harbour by rain events.

Approach: Conduct non-random sampling of water and shellfish (before and after each rain event) for FIB for one year and/or perhaps coinciding with changes in abundance of swans (*e.g.* during hunting season they are largely absent). Identify faecal coliform and *E. coli* concentrations as required by NZFSA (NZFSA 2006). Filter the water sample, record the volume filtered and store frozen at -80°C for possible MST analyses. Freeze extra shellfish sub-samples for possible MST marker analyses. When *E. coli* concentrations in shellfish samples exceed 230 MPN 100 g^{-1} or faecal coliform concentrations in the water samples exceed 14 MPN 100 ml^{1}, conduct MST marker tests using qPCR and PCR technologies (Figure 5).

3. **Conduct a coastal survey and run MST marker tests on water entering the harbour**

Purpose: Identify possible vectors (point of entry) of faecal contamination and confirm sources of contamination entering the harbour.

Objective: Locate possible point sources of faecal contamination.

Approach: Conduct a detailed field survey of shoreline. Collect water samples as required from streams, cut-off drains and running pipes entering the harbour. Groundwater could be tested in locations having obvious sign of failing septic systems. Beach sediments should be tested next to the shellfish growing areas. Identify additional monitoring sites if needed.

Conduct FIB and MST marker tests on samples collected as specified above. Conduct second round of testing if needed.

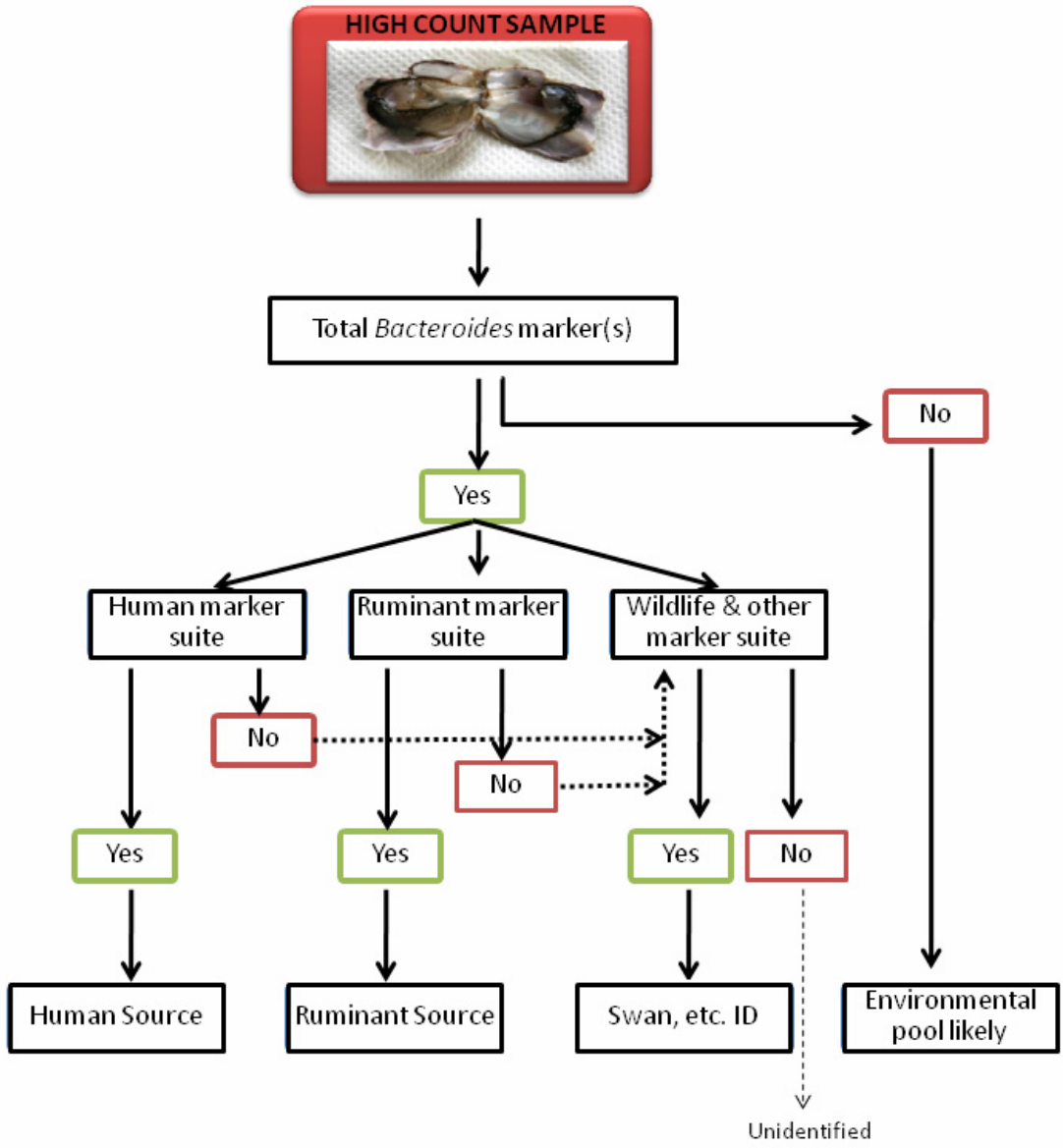


Figure 4. The above flow chart shows the steps that could be taken using existing MST markers. The first marker detects the presence of bacteroides species, which are carried by a range of mammals and birds. If this is confirmed in samples, then a suite of more specific markers can be used to assess importance of different source.

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