

NOT ALL FAECAL POLLUTION IS EQUAL; IDENTIFYING THE SOURCE IS KEY TO EFFECTIVE MANAGEMENT

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ABSTRACT

Many of Auckland's beaches and waterways are subject to intermittent microbiological contamination, which can pose a risk to human health through recreational contact. Regular monitoring of faecal indicator bacteria (FIB) is undertaken at beaches over the summer months, consistent with national guidelines, and can result in beach closures.

The presence of microbiological contamination within the marine environment is often presumed to be the result of human related sources, such as wet weather-induced combined sewer and designed wastewater overflows into streams or directly from coastal outfalls. In addition, aging infrastructure, private septic systems or cross connections may contribute microbiological contaminants to the stormwater network and associated open waterways. However, non-human related sources of contamination from domestic, wild and farmed animals and birds also enter the aquatic environment directly or via overland flow.

Effective management of microbiological contamination requires knowledge of the source animal so that appropriate interventions can be applied. FIB results are unable to determine the source of microbiological contamination and are therefore limited in terms of informing management interventions. Recent advancements in molecular techniques have permitted the use of genetic markers to distinguish between sources of faecal pollution.

This paper will present a meta-analysis of several investigations initiated by Auckland Council to investigate the sources of FIB contamination across the region. A tiered approach using FIB and genetic based analysis ('microbial source tracking') was undertaken in a range of marine and freshwater environments in an attempt to determine the animal source of contamination (i.e. human, canine, avian or ruminant).

Identifying the source of faecal contamination within a catchment is challenging and is influenced by a variety of factors in different receiving environments. The use of faecal source tracking can provide information on the sources of microbiological contamination, allowing the implementation of efficient and effective management responses to meet water quality outcomes.

KEYWORDS

Microbial source tracking; *E. coli*; marine; water quality; faecal pollution.

PRESENTER PROFILE

Justine (BSc, PGDipSci, MLS (EnvLaw)) is a Senior Freshwater Scientist at T+T with ten years' experience in the management of freshwater and stormwater. Justine has been involved in multiple water quality and faecal contamination investigative studies within the Auckland Region.

1 INTRODUCTION

Recreational waters in urban areas are subject to a wide array of potential contaminants including microbiological contamination, which can pose a risk to human health (Walker, et al, 2015). This contamination is frequently associated with contaminated stormwater runoff, designed wastewater overflows, aging infrastructure, broken pipes, illegal cross connections or septic tanks, but the extent to which non-human inputs contribute to contamination is often overlooked.

Over the summer bathing season Auckland Council undertakes weekly monitoring of recreational waters which is reported on the Safeswim website. This provides a snapshot of water quality across the region. Consistent with the Ministry for the Environment (MfE) and Ministry of Health (MoH) microbiological water quality guidelines (MfE, 2003), the faecal indicator bacteria (FIB) *Escherichia coli* and enterococci are tested for in freshwater and marine environments, respectively. Results are categorised into modes; green (safe), amber (alert) or red (action required). Where sample results indicate a risk to human health, sites are retested, and warning signs erected. Where there are regular exceedances of trigger levels, over a five year period, long-term health warning signage is erected.

Faecal indicator bacteria are commonly used to identify human health risk in recreational waters as a surrogate for other pathogens known to occur in sewage (i.e. viruses, bacteria, protozoa). The indicators commonly used are near-ubiquitous in warm-blooded animals and so their presence could be due to any number of animal sources (Gilpin et al 2002, Devane et al., 2007, Harwood et al, 2014). As such, FIB alone does not provide sufficient information regarding potential health risk or the source of the contamination, to enable effective management (Okabe et al. 2007, Walker et al, 2015).

Without reliable source information, management interventions driven by FIB results have been made on a best-practice basis, but are not necessarily cost effective or outcomes focused (Santo Domingo et al 2007).

Advancement in analysis techniques can enable a more comprehensive understanding of the source of faecal contamination. Microbial source tracking (MST) tools have been bolstered by developments in molecular techniques (e.g polymerase chain reaction (PCR)) that can identify DNA-markers associated with host-specific faecal microorganisms (Harwood et al, 2014).

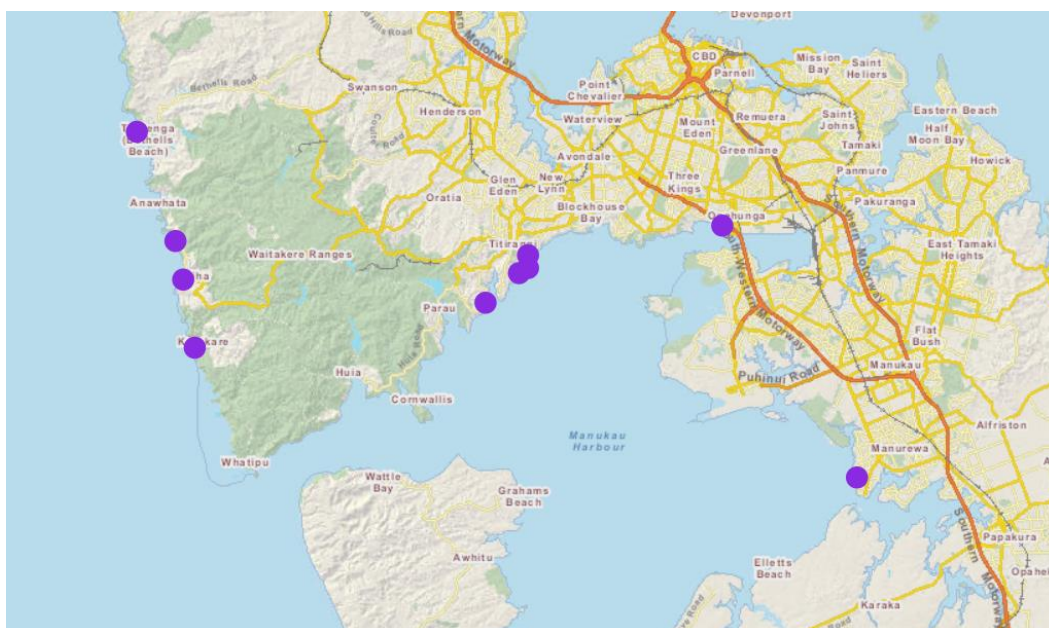
Prior to analysis, host-specific markers need to be selected based on the testing environment and the potential sources of contamination in that area. For example, looking for ruminant markers in a highly urbanised catchment is unlikely to yield useful results. By targeting specific markers, a more comprehensive picture of the contamination profile of an area can be identified, leading to more targeted remedial works and management interventions to improve water quality.

2 SCOPE

A review of bathing beach monitoring data for beaches and lagoons within the Auckland SafeSwim bathing programme revealed patterns of poor water quality in some areas. Bathing sites with poor long-term microbiological water quality are part of a targeted investigations programme by Auckland Council's Healthy Waters Department. A selection of these investigations are summarised below and discussed in this paper.

- The Onehunga Lagoon has been subject to intermittent water quality monitoring which has periodically recorded values in excess of the recreational contact guidelines. Prior to the extensive redevelopment of the Onehunga Foreshore, which opened in November 2015, additional assessment was required to ascertain the causes of the Lagoons exceedances (Walker et al, 2015).
- Weymouth Beach, located in the Manukau Harbour, has had a long-term health-warning sign in place since 2002, due to high levels of FIB. The Papakura Channel (adjacent to Weymouth Beach) has historically good microbiological water quality and so it was considered useful to investigate the sources of contamination affecting the beach (Whatley et al, 2016).
- Six beaches along the northern Manukau Harbour coastline were part of a pilot study undertaken in 2013, where FIB analysis was supplemented with faecal sterol analysis. The faecal sterol analysis did not provide sufficiently robust information to identify sources of contamination. Of the six beaches sampled, Laingholm Beach, Titirangi Beach, Wood Bay and French (Otitori) Bay have since been investigated using PCR analysis (Quinn and Neale, 2016; Quinn and Neale, in press).
- The marine waters of the west coast beaches at Karekare, Piha, North Piha and Te Henga (Bethells) have excellent water quality, however the associated lagoons have historic microbiological water quality issues. The lagoons are often unsuitable for recreational activities and signage is regularly erected to advise against swimming. Extensive investigations have occurred in these catchments to identify the sources of faecal contamination and provide direction for a range of Council and community-led water quality improvement initiatives (Noble and Neale, 2016).

Figure 1: Distribution of study sites across the Auckland region (Basemap source: Auckland Council GeoMaps).



This paper presents a high level summary of the results of these investigations and provides some insights into the use of a tiered approach to assessing microbiological contamination and informing management directives.

3 METHODOLOGY

This paper presents a meta-analysis of several investigations which each applied slightly different methodologies and approaches to assessment. The methodologies can be reviewed in full in the respective technical reports, however the following provides a brief overview.

Sample sites were spatially distributed within each project area and included freshwater streams, lagoons, stormwater outlets and within the marine environment itself. Where possible, Safeswim sites were utilised to enable comparison with the results obtained in the regular monitoring programme. In general, site selection was based on a desktop assessment and related to network infrastructure and known extent of freshwater systems. A total of 62 sampling sites were sampled across these investigations and are discussed in this paper.

All samples were chilled following collection and transported to Aqualab Limited (Auckland), an IANZ accredited Laboratory where FIB analysis was undertaken within 24 hours in accordance with the 2003 MfE and MoH national guidelines for recreational waters. The samples were analysed using the Colilert test (APHA, 2012) which provides a Most Probable Number (MPN) of *E. coli* (freshwaters) or enterococci (saline waters) per 100mL (detection limit 10/100mL).

MST samples were filtered through 0.45µm membrane filters until blocked, a GITC buffer agent was added and the filter papers frozen and stored within 24 hours of collection. This method is consistent with Gilpin et al (2013) and enables samples to be stored until FIB results have been obtained and decisions can be made regarding which samples to send for additional testing.

Following completion of each respective sampling season, subsets of the frozen filters were selected and couriered to the Institute of Environmental Science and Research (ESR) laboratory for MST analysis using the Polymerase Chain Reaction (PCR) method. In general, those filtered samples with corresponding microbiological concentrations >550 *E. coli* MPN/100mL for freshwater or >280 enterococci MPN/100mL for seawater were selected for MST analysis. This is consistent with previous studies, where the use of molecular techniques is more likely to yield useful results when faecal indicator bacteria are high (Cornelisen et al., 2012). The final decision as to which samples should undergo MST was determined based on a number of factors, including total number of samples available, spatial distribution of 'red' FIB results across sites, and budget constraints.

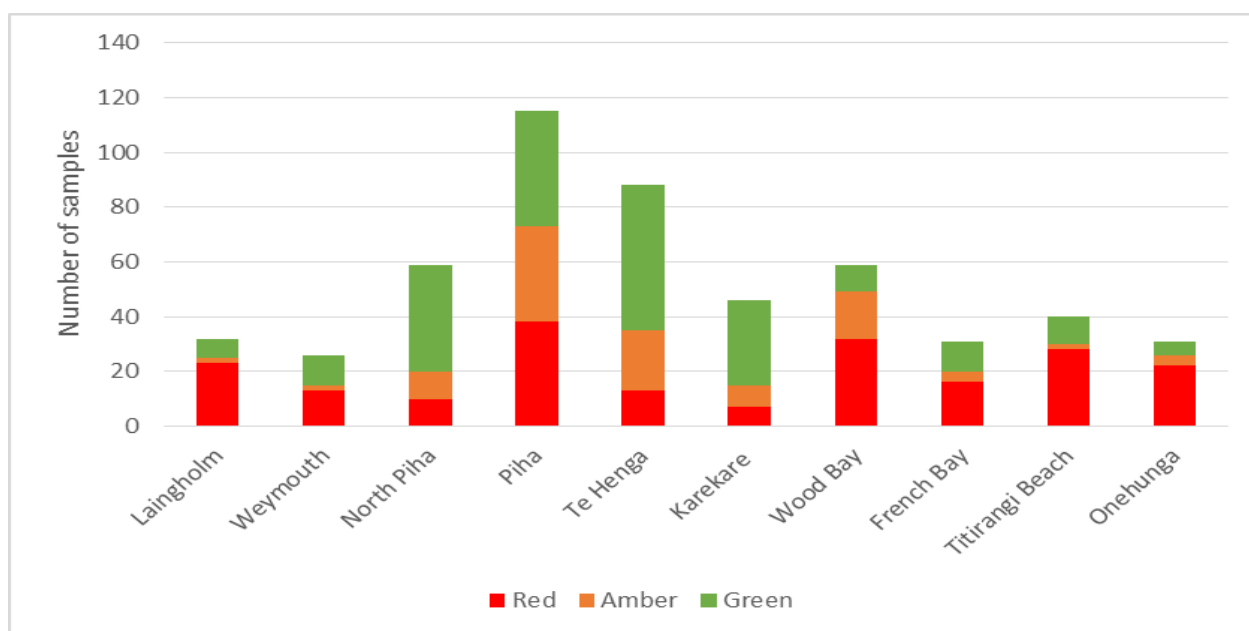
The MST method amplifies the DNA from host-specific bacteria in the filtered water samples and detects the presence of markers for the animal species of interest. The markers chosen for analysis in these investigations were specific to the target catchments. The general faecal marker (GenBac), and specific markers for dog (DogBac), avian (GFD) and human (BiADO and BacH) sources were tested for in all catchments. The avian marker (GFD) detects duck, swan, seagull, geese and chicken faecal sources. In selected project areas additional markers (human (HumM3), horse (Schill) and ruminant (BacR)) were included in the MST suite.

MST results are reported on a semi-quantitative or qualitative scale and multiple markers can be present in a single sample. The GenBac marker is reported on a scale of extremely strong positive > very strong positive > strong positive > positive > weak positive > very weak positive.

4 RESULTS AND DISCUSSION

Of the 526 samples collected across the ten project areas, 202 samples (38%) exceeded 'red' FIB trigger levels (Figure 2) the majority of which were from freshwater or stormwater sites (Figure 3).

Figure 2: FIB results as a number of samples in each recreational contact guideline category across all project areas and sample types.



4.1 LAND-BASED CONTAMINATION

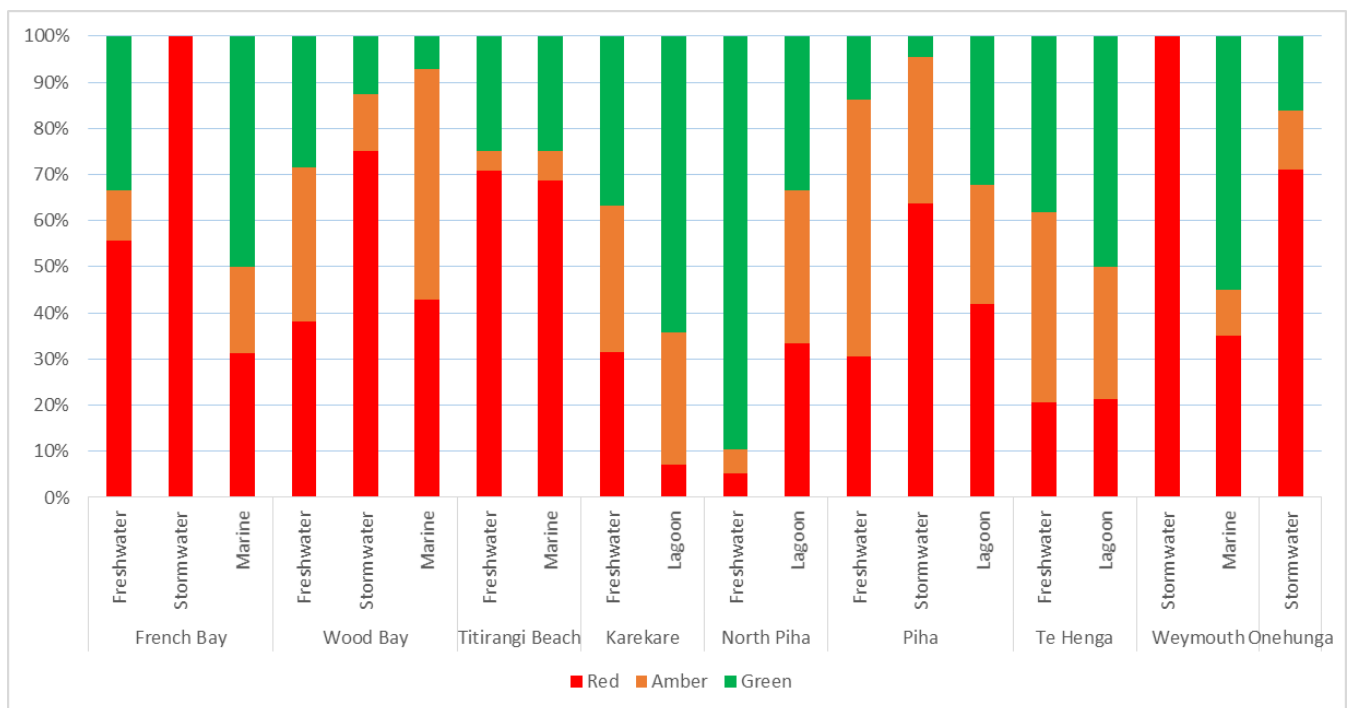
Across all project areas, the source of contamination was determined to be from local land-based geographical sources.

Exceedances were recorded more frequently in freshwater or stormwater inputs, rather than the marine receiving environment, indicating chronic contamination of land based inputs (Figure 3). On several occasions, a dry weather, land based input with FIB concentrations well in excess of the 'red' FIB trigger level was recorded, while the marine environment exhibited no evidence of FIB contamination.

Where marine samples exceeded trigger levels, this was usually in response to wet weather conditions or where the marine site was located in close proximity to a stormwater outfall with high levels of FIB. Some studies have found that disturbance of marine sediments, through wind or tidal action, can lead to elevated FIB concentrations (ref). While this may be a contributing factor, it was not considered to be the primary driver of contamination in these catchments.

Management interventions should be targeted to improving the understanding of network connections and catchment factors that may be contributing to the contamination.

Figure 3: Distribution of FIB results within each sampling environment across each project area. Depicted as proportion of samples within each recreational contact guideline category and in each sampling environment type. Note that total sample numbers for each project area and environment type differ.

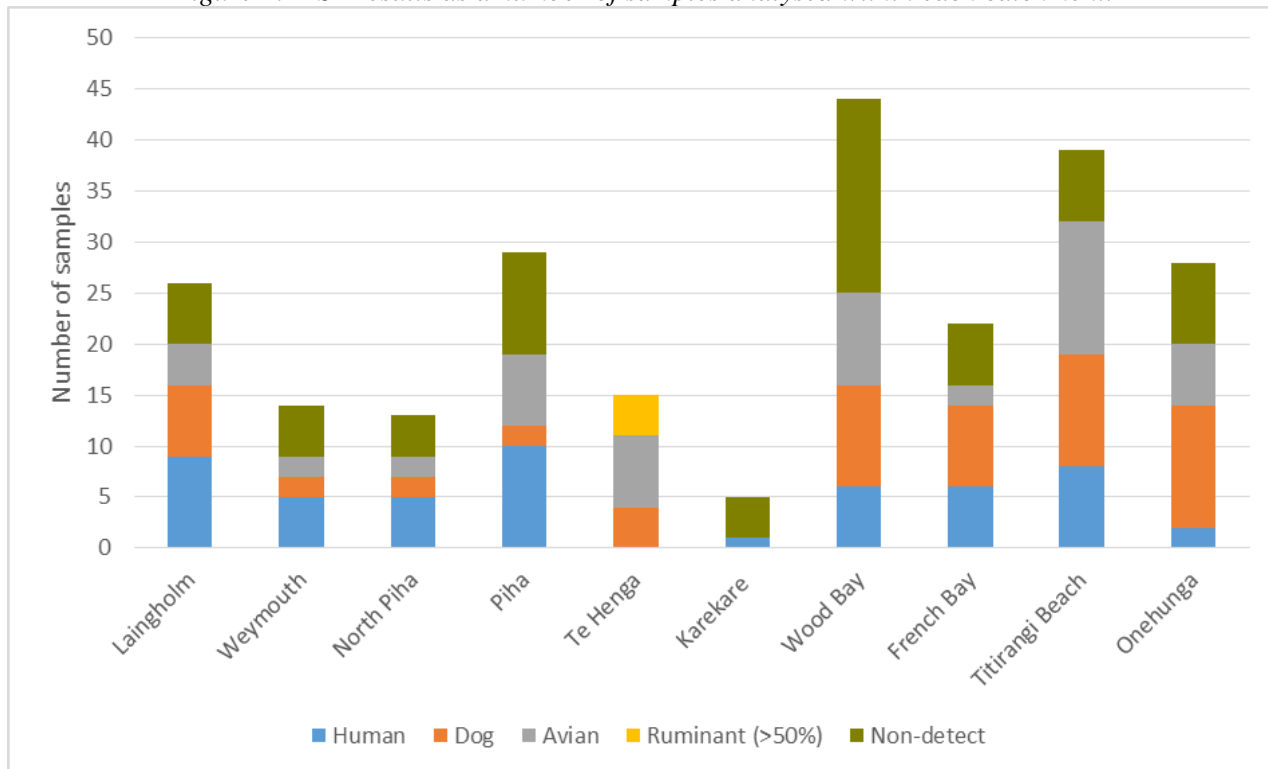


4.2 SOURCES OF CONTAMINATION

Conventional wisdom would indicate that water quality issues in urban areas would be from failing infrastructure, designed wastewater overflows or other human contamination sources. The information obtained in this assessment provides evidence that a significant proportion of the microbial contamination in the bathing beaches of Auckland is not necessarily a result of human contamination (Figure 4).

While contamination by human sewage is considered to demonstrate a higher risk to human health than that of some other animals (Walker et al 2015, Harwood et al, 2014) and therefore may be a priority, contamination by birds and dogs contributes to the overall contamination profile and should be considered when implementing management interventions.

Figure 4: MST results as a number of samples analysed within each catchment.



Increasing awareness and educating dog owners can have a measureable impact on water quality in areas with canine sources of faecal contamination. In a Californian study, dogs were identified as being a contributing factor to elevated faecal indicator bacteria levels (Erwin et al, 2014). Local residents were educated about proper pet waste disposal and the potential impacts on the environment, and subsequently canine faecal contamination within the environment reduced. Working with other departments to provide signage and bins in parks and undertaking education programmes could lead to improved water quality.

Avian sources are more challenging to manage, given the non-point source nature of the source and they are typically wild animals outside the immediate influence of people. However some interventions to manage wild life could be introduced to reduce the levels of contamination, such as signage to discourage people feeding ducks and gulls.

4.3 HOST-SPECIFIC MARKERS

Despite the application of MST, there are some limitations to its application. Host specific markers were detected in every project area, however in 38% of the samples tested (n=69), a host-specific marker was not identified, despite the presence of GenBac and elevated FIB levels.

Table 1 below provides summary statistics of enterococci and *E.coli* results for those 181 samples where MST analysis was undertaken. For each indicator, the mean, median, Water New Zealand’s 2017 Stormwater Conference

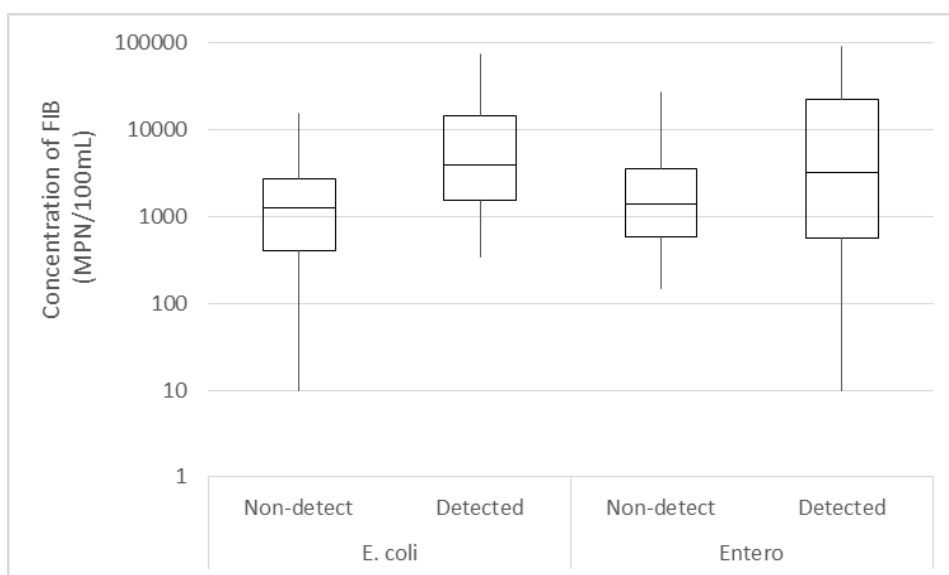
minimum and maximum FIB concentrations are shown against whether a host-specific marker was detected or not. Figure 5 depicts this data in a box plot. Referring to the median is the most meaningful attribute given the presence of outliers in the data.

A key finding from this investigation is that no markers were detected in samples with less than 340 *E. coli* MPN/100mL (Table 1). This is consistent with previous studies which indicate that the use of molecular techniques is more likely to yield useful results when faecal indicator bacteria are high (Cornelisen et al., 2012). However, having high FIB does not mean a marker will be detected. Values of 13,000 *E. coli* and 24,000 enterococci (MPN/100mL), did not result in the positive identification of host specific markers (Table 1).

Table 1: Summary *E. coli* and enterococci results for those samples where MST analysis was undertaken.

Host specific marker detected?	E. coli (MPN/100mL)		Enterococci (MPN/100mL)	
	No	Yes	No	Yes
Summary statistics for FIB indicators				
Mean	2,518	10,294	1,868	7,768
Median	870	2400	830	2650
Minimum	10	340	150	10
Maximum	13,000	240,000	24,000	69,000

Figure 5: Box plot showing relationship between detection of markers and concentration of each faecal indicator bacteria. Note data is presented on a log-scale and an outlier of 240,000 MPN/100mL is excluded from the 'E. coli detected' plot.



Where no marker is detected, this could be due to there being no marker present, or a marker was not detected by this method (or the markers analysed for). The absence of a marker, even when FIB are high could be the result of decaying organic material, or a Water New Zealand's 2017 Stormwater Conference

population of FIB that persist and replicate in favorable sediment environments (see for example Harwood et al, 2014). Environments where this may apply would be lagoons, stream sediments or intertidal ponding areas at low energy beaches. MST techniques may also struggle to identify markers in aged or diluted faecal sources (Field and Samadpour, 2007). Persistence of DNA markers in the environment is influenced by complex biological and physicochemical processes, including marker decay due to temperature, particulate size, salinity and sunlight (Green et al, 2011). Understanding the influence of these variables is one of the biggest challenges for marker detection and subsequent risk assessment (Green et al, 2011).

Notwithstanding the value that MST can bring to a project, the methodology does have limitations. It is also important that a strategic sampling methodology, that is designed according to the characteristics and issues of the test catchment is utilised.

4.4 SAMPLING APPROACH

The way that a sampling methodology is applied is essential to characterizing potential sources of contamination in a project area. The underlying approach to the Auckland investigations was to apply a tiered sampling methodology requiring additional decision-making throughout the course of the investigation to gain meaningful results.

One challenge to the implementation of water quality investigations such as these is the intermittent nature of contamination events. Peak wastewater times may be a driver to identify human contamination, however the study undertaken in Onehunga revealed human contamination leaking between damaged pipes, with discharges presenting at the outfall outside of expected peak times.

While some contamination is wet-weather driven allowing weather events to be targeted, a significant portion of the contamination within the freshwater inputs was present in dry weather.

Large sample sizes across multiple days and weather conditions may be required to capture this variation in contamination depending on the size and nature of the catchment. Catchments are highly variable and subject to their own processes and as such, knowing the catchment and the potential (or expected) sources of contamination should be considered at the early stages of investigation design.

To isolate geographic sources of potential contamination, catchment scale investigations should be undertaken, whereby every input to the network (including surface waters and marine environment) can be identified. Using this as a starting point enables targeted network investigations or stream walks which introduce project efficiencies.

Understanding the landuses and physical inputs (i.e. pipes) contributing to the receiving environment allows for a comprehensive, catchment-wide investigation to be undertaken from the outset. That is, it requires more thought than simply collecting samples from easy to access locations.

Further, by initially identifying those samples which had elevated indicator bacteria it was possible to then isolate which samples and/or sites would yield the best results following the microbial source tracking. As MST samples can be stored for at least six months without impacting sample integrity (Gilpin et al, 2013 and ESR, 2016), this approach enables flexibility in sampling design, allows samples to be batched and reduces courier and analysis costs (Cornelisen et al, 2012).

5 CONCLUSIONS

This paper provides a summary of the key findings and lessons learned from several investigations initiated by Auckland Council to provide a more comprehensive understanding of water quality issues in recreational waters.

Analysis of faecal indicator bacteria provides an indication of risk to human health, however is limited in its ability to provide detail about the animal source of the contamination. Incorporating microbial source tracking analysis into a tiered sampling approach, can provide significantly more useful information to identify contamination sources.

Chronic contamination of freshwater and stormwater inputs should be prioritised for management. Identification of the sources is necessary to ensure that the management interventions are fit for purpose and will result in improved microbial water quality. This is particularly relevant when a significant proportion of the contamination is not a result of human contamination but still contributes to total FIB concentrations in recreational waters.

Management interventions should be specific to each project area and aim to incorporate network utility providers, local residents, farmers and recreational users of beaches and reserves to provide for a holistic approach to managing multiple contaminant sources.

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