

New Zealand Journal of Marine and Freshwater Research

ISSN: 0028-8330 (Print) 1175-8805 (Online) Journal homepage: http://www.tandfonline.com/loi/tnzm20

## A tiered approach for the identification of faecal pollution sources on an Auckland urban beach

JW Walker, R van Duivenboden & MW Neale

To cite this article: JW Walker, R van Duivenboden & MW Neale (2015) A tiered approach for the identification of faecal pollution sources on an Auckland urban beach, New Zealand Journal of Marine and Freshwater Research, 49:3, 333-345, DOI: 10.1080/00288330.2015.1014376

To link to this article: http://dx.doi.org/10.1080/00288330.2015.1014376

1	ſ	•	(	1

Published online: 05 May 2015.



🖉 Submit your article to this journal 🕑

Article views: 164



View related articles 🗹



View Crossmark data 🗹

Full Terms & Conditions of access and use can be found at http://www.tandfonline.com/action/journalInformation?journalCode=tnzm20



## **RESEARCH ARTICLE**

# A tiered approach for the identification of faecal pollution sources on an Auckland urban beach

JW Walker<sup>a</sup>, R van Duivenboden<sup>b</sup> and MW Neale<sup>a,c</sup>\*

<sup>a</sup>Research, Investigations and Monitoring Unit, Auckland Council, Auckland, New Zealand; <sup>b</sup>RMPro, Auckland, New Zealand; <sup>c</sup>School of Biological Sciences, University of Auckland, Auckland, New Zealand

(Received 5 November 2014; accepted 27 January 2015)

Microbiological contamination arising from faecal pollution is a widespread effect of human activity on aquatic systems, with management of the issue complicated because contamination sources are often unknown. Recent advances in molecular techniques have permitted the use of host-specific genetic markers to apportion sources of faecal pollution. In this study, we used a tiered approach employing faecal indicator bacteria and Bacteroidales markers to identify faecal sources in an urban setting. Canine sources were the most common source of faecal contamination, which led to a programme of education and targeted management. A single, but substantial, source of human faecal contamination was identified that was subject to corrective action and its effectiveness validated by supplementary monitoring. This study supports the use of a tiered approach for the identification of faecal contamination sources in New Zealand, including the use of faecal indicator bacteria and more complex source-tracking analysis using genetic markers.

**Keywords:** canine faeces; *E. coli*; enterococci; faecal pollution; freshwater; marine; microbial source tracking; New Zealand; remediation; water quality

## Introduction

Microbiological contamination arising from faecal pollution is one of the most pervasive effects on aquatic systems arising from anthropogenic activities. Elevated concentrations of faecal indicator bacteria are commonplace, having been observed extensively in freshwater and marine environments. The scale of the issue is such that it has been described as one of the primary issues for water quality at a global scale (Rabinovici et al. 2004; USEPA 2005; Santo Domingo et al. 2007; Ahmed et al. 2008; Wang et al. 2010; Roslev & Bukh 2011).

The presence of faecal pollution in aquatic systems poses a health risk to humans, with the most common infection pathways including recreational activity in contaminated water, consumption of contaminated shellfish and ingestion of polluted drinking and irrigation water. The presence of faecal matter in aquatic systems clearly has obvious public health impacts, which have concomitant economic impacts associated with the treatment of infections. Shuval (2003) estimated the annual global cost of the disease caused by recreational contact with contaminated water to be US\$12 billion. Furthermore, economic impacts arise from the restrictions placed on beach use and the food industry in the presence of elevated levels of faecal contamination (Rabinovici et al. 2004; Santo Domingo et al. 2007; Walters & Field 2009).

Given the primary concern is one of public health, ideally we would directly monitor the presence and abundance of pathogenic organisms; however, such an approach raises a number of

© 2015 The Royal Society of New Zealand

<sup>\*</sup>Corresponding author. Current address: Golder and Associates (NZ) Ltd, Auckland, New Zealand. Email: mneale@golder.co.nz

challenges: pathogens are rare; variable in distribution; can be highly infectious at low doses; and monitoring all possible pathogens would require a very large number of technically complicated and expensive assays (Field & Samadpour 2007). Consequently, since the mid-20th century, bacteria (typically enterococci or Escherichia coli) have commonly been used as an indicator of faecal contamination in aquatic systems because they are present in faecal matter at high concentrations, their presence and abundance is associated with health risk, and relatively inexpensive and simple assays exist for their detection and enumeration (Boehm et al. 2003; Dickerson et al. 2007; Ishii & Sadowsky 2008; Converse et al. 2009).

More recently, the use of faecal indicator bacteria (FIB) has been questioned because several assumptions that underpinned their use have been proven false. For example, until relatively recently, FIB were thought not to survive outside host animals (Ishii & Sadowsky 2008; Halliday & Gast 2011). However, FIB can survive, grow and establish selfsustaining populations in the environment (Boehm et al. 2003; Noble et al. 2006; Field & Samadpour 2007), including in soils (Sinton et al. 2007), aquatic sediments (Jin et al. 2004; Muirhead et al. 2004), piped stormwater networks (Brownell et al. 2007), living and decaying vegetation (Anderson et al. 1997; Byappanahalli et al. 2003; Whitman et al. 2003; Verhougstraete et al. 2010) and beach sand (Ishii et al. 2007; Halliday & Gast 2011).

Perhaps the greatest limitation of FIB in relation to managing faecal pollution is their ubiquity, being found in the intestines of most warm-blooded animals (Gilpin et al. 2002; Scott et al. 2002; Boehm et al. 2003; Noble et al. 2006; Devane et al. 2007). Hence, even if the presence of FIB at a particular location in space and time is related to faecal contamination, the numerous potential sources of the contamination limit the value of the FIB information in relation to management of faecal pollution requires reliable information about the source of the contamination (Sinton et al. 1998; Scott et al. 2002; Dickerson et al. 2007; Ervin et al. 2013). The benefits of knowing the source of faecal contamination allows a financially efficient, targeted management response (Gilpin et al. 2002). In the absence of reliable source information, millions of dollars may be invested in remedial actions that achieve little water quality or public health benefits (Santo Domingo et al. 2007).

In addition to the specific limitations of FIB, ability to identify sources is challenged by the spatial and temporally variable nature of potential sources, the highly dynamic distribution pathways of contamination, and the complex biological and physicochemical processes that affect the persistence of the contamination in the environment (Boehm et al. 2003). Furthermore, the obvious point sources of faecal contamination have largely been identified and mitigated; hence, the most probable source of most contemporary faecal contamination is a complex mix of non-point sources (USEPA 2005; Santo Domingo et al. 2007; Converse et al. 2009), which collectively provides a challenging management issue (Conn et al. 2011).

A more recent addition to our toolbox for managing faecal pollution is the Bacteroidales order of bacteria. It has long been recognised that Bacteroidales are the dominant microflora in warmblooded animals (Allsop & Stickler, 1985; Fiksdal et al. 1985), and as obligate anaerobes they cannot survive in the wider environment for long periods (Noble et al. 2006; Kirs et al. 2011; Wood et al. 2013). For example, human and ruminant Bacteroidales were not detected after 6 days of faecal contamination in freshwater microcosms, whereas FIB persisted and remained above standards for 14 days (Walters & Field 2009). As a result, the presence of human-specific Bacteroidales is considered a strong indicator of recent human faecal contamination, whilst also being considered an effective indicator of human enterovirus pathogens as they have similar persistence times (Walters et al. 2009).

Historically, the limited use of Bacteroidales for managing faecal pollution has been linked to the challenges of detection and enumeration (Bernhard & Field 2000a). However, recent advances in molecular techniques, specifically polymerase chain reaction (PCR), have largely removed this barrier and the use of Bacteroidales in identifying and managing faecal pollution has increased exponentially since the breakthrough work of Bernhard & Field (2000a). Initially, ribosomal DNA markers were developed that were able to reliably distinguish between human and bovine sources of faecal contamination (Bernhard & Field 2000a,b), and additional host-specific markers have subsequently been developed for chickens, dogs, geese, horses and pigs (reviewed in Roslev & Bukh 2011).

Notwithstanding the development of the range of Bacteroidales markers, it is still recognised that no single method has the ability to identify and track faecal pollution effectively (Noble et al. 2006). Hence, many studies recommend the use of a tiered approach to identify the sources of faecal contamination, starting with general, broad-scale and relatively inexpensive surveys for FIB, with progression to more specific, spatially targeted and relatively expensive investigations of host-specific markers based on the results of the initial broadscale surveys (Boehm et al. 2003; Field & Samadpour 2007; Stoeckel & Harwood 2007; Converse et al. 2009). Furthermore, the use of molecular techniques is more likely to yield useful results when FIB presence is high (Cornelisen et al. 2012).

In this study, we used a tiered approach in an urban beach setting in Auckland, in what is the first example in New Zealand of numerous sources of FIB being reliably identified in a complex urban setting. The results informed appropriate management interventions and those interventions have been validated by subsequent testing. Our study builds on the work of others (Kirs et al. 2011; Cornelisen et al. 2012) to further validate the use of Bacteroidales markers in a New Zealand context, providing an important tool for managing the widespread faecal pollution issues that exist in New Zealand (Deely et al. 1997; Donnison & Ross 1999).

#### Methods

#### Study site

Our study was carried out in the Auckland suburb of Onehunga (Fig. 1). The Onehunga foreshore is a highly modified environment, with the construction of State Highway 20 in the 1970s leading to the creation of an artificial lagoon. Tidal exchange between the lagoon and the Manukau Harbour is artificially controlled by two sluice gates. The gates are typically left open to allow the tidal cycle of the lagoon to match that of the harbour; however, the gates are closed and the water is maintained at a high level at weekends and for sporting events.

Due to the recreational use of the area, intermittent microbiological water quality monitoring has been undertaken in and around the lagoon, which has shown elevated concentrations of enterococci (Auckland Council, unpublished data) which periodically breach the relevant recreational guidelines (Ministry for the Environment 2003). These results have recently taken on greater importance due to the high profile redevelopment of the Onehunga foreshore, which, among other things, will create a number of sandy beaches and public facilities that will likely increase the extent of recreational activity in the area. In order to potentially mitigate the issue, the authors were commissioned by Auckland Council's parks department to identify the causes of the intermittent exceedances of the recreational guidelines.

We began with a review of the relevant microbiological testing data that existed in the area. While the locally targeted monitoring around Onehunga Lagoon indicated intermittent exceedances of recreational guidelines, wider monitoring in the Manukau Harbour indicated low concentrations of enterococci. The two closest beaches to Onehunga sampled as part of the Auckland Council's regional Safe Swim programme met the recreational guidelines at the time of the study (2010/2011 austral summer). Similarly, state of the environment monitoring at three nearby sites (Mangere Bridge, Puketutu Point and Shag Point) in the Manukau Harbour indicated that the microbiological water quality of the general harbour did not provide a substantive threat in relation to recreational activities (2008, 2009 and 2010 enterococci medians for each site were all five [MPN/ 100 mL] or below; Walker & Vaughan 2013)).

Based on the existing monitoring data, we hypothesised that the drivers of the microbiological water quality of the lagoon and the surrounding nearshore marine environment were likely to be localised, non-point source inflows. Such an

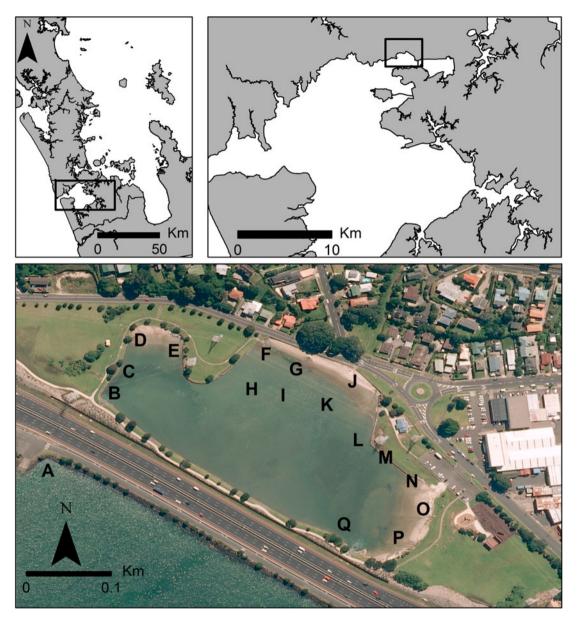


Figure 1 Map showing the Onehunga foreshore, together with the location of the 17 sampled inflows. The inflows are labelled A to Q to correspond with the results in Table 1.

hypothesis is further supported by the findings of studies in similar urban environments, where sources of FIB were typically urban runoff outlets close to shore (Boehm et al. 2003; Noble et al. 2003) and beach water samples taken closer to these outlets failed standards more often than those distant (Noble et al. 2003). Based on this hypothesis we carried out a sanitary survey of the area and identified 17 inflows to the lagoon and surrounding area for further investigation (Fig. 1).

## Sampling regime

We employed a sampling regime based on the framework recommended by Boehm et al (2003). The existing monitoring data fulfilled the first tier of a large-scale FIB survey to identify the broad area of investigation. The second tier involved the identification of the geographical source (i.e. which inflow) of FIB in the 17 inflows identified in the sanitary survey. The third tier focused on identifying the biological source (i.e. which animal) of FIB, where we employed a PCR-based analysis for Bacteroidales markers for those inflows where the FIB results were above the relevant recreational guidelines (Ministry for the Environment 2003). The PCR analysis was undertaken on samples if the FIB result was above either of the marine (enterococci) or freshwater (E. coli) alert level guidelines (140 enterococci/100 mL and 260 E. coli/100 mL).

The 17 inflows were assessed, and water samples collected if they were flowing, on two occasions following dry and wet antecedent weather conditions. We targeted different weather conditions because failure to comply with guidelines after storm events can be much greater (60% failure) compared with dry weather conditions (6% failure) (Noble et al. 2003). While the size and discharge of each of the inflows varied, we made no presumptions about the potential of each discharge to influence water quality. This was because the dry weather conditions at the time of the sanitary survey could have resulted in an underestimation of the potential for effects from rain-event-driven discharges and contamination. Dry weather sampling was undertaken on 30 May 2011, where in the preceding 24 h there had been 0.5 mm of precipitation. The wet weather sampling was undertaken on 10 June 2011, following 10 mm of rain in the preceding 24 h.

Following the results of the tiered investigation, further investigation and supplementary samples were collected on a weekly basis from inflow A between 19 December 2011 and 2 April 2012. These samples were tested for human contamination and were followed by standard piped network investigation techniques to locate the source, including gross litter traps and camera inspections. This supplementary sampling was repeated on five occasions following the repair to a leaking sewer pipe to identify if the human contamination identified in inflow A had been resolved.

To confidently characterise the microbiology of each inflow, all samples were collected at low tide to minimise the influence of potential marine contamination. If the inflow was flowing, two samples were collected in sterile containers. The first sample (100 mL) was used to enumerate the concentration of FIB (*E. coli* and enterococci) and the second sample (1 L) was used for molecular analysis if the FIB results exceeded the recreational guidelines (Ministry for the Environment 2003).

#### Laboratory methods

All bacteriological analyses were conducted by Aqualab Limited (Auckland, New Zealand), an IANZ accredited laboratory. Enterococci assays were carried out in accordance with ASTM D6503-99, and *E. coli* with APHA 9223. The tests returned most probable number (MPN) results with a maximum test result of 24,200 (samples above this were reported as > 24,200).

Molecular analysis for the Bacteroidales markers was undertaken by the Institute of Environmental Science and Research Ltd (ESR), Christchurch Science Centre, New Zealand. DNA was extracted from water samples using a Supor 200, 0.2 µm Polyethersulfone (PES) filter (Pall Corp., Washington Port, NY) and 1 mL of guanidine isothiocyanate (GITC) buffer (5 M GITC, 0.1M EDTA, 10% sarcosyl) was added (Dick & Field 2004). Filters were immersed in the GITC buffer and vortexed, then frozen at -20 °C. DNA was extracted using the Qiagen DNeasy Kit (QIAGEN, Valencia, CA). Briefly, 700 µl AL buffer (supplied by manufacturer) was added to the filter and the mixture was vortexed and incubated for 5 min at room temperature. The supernatant was added to a spin column from the DNeasy kit, and the column centrifuged for 1 min at 15,700 g. During each extraction, a blank of sterile Gibco UltraPure water (Invitrogen, Paisley, UK) was extracted to monitor for potential DNA contamination.

Assays for the general faecal indicator (Gen-Bac), human indicative markers, (BiAdo, HumM3 and BacH), canine marker (DogBac) and wildfowl marker (GFD) were undertaken. Most of these assays were specifically designed and tested in a New Zealand-wide study to introduce these methods for use by Regional Councils (Cornelisen et al. 2012), the exception being the DogBac assay, which is based on the work of Dick et al. (2005). Semi-quantitative results are reported on a scale thus: very strong > strong > positive > weak > very weak > no signal, based on the calculated mean copies per assay.

PCR amplifications were performed in a total volume of 25 µl using 2 µl of DNA template. PCR conditions for the SYBR Green assays were as follows: 2 × LightCycler 480 SYBR Green I Master mix (Roche Diagnostics Ltd, Penzburg, Germany), 0.25 µM of each primer and 0.2 mg/mL of bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO). Each assay run included a nontemplate control (NTC), an extraction blank and a standard curve. The standard curve was generated from 10-fold serial dilutions of the appropriate target cloned into E. coli DH5a (Invitrogen, Carlsbad, CA) using the pGEM-T Easy cloning kit (Promega, Fitchburg, WI, USA). A NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), determined the DNA concentration and allowed for calculation of the copy number of target DNA extracts from plasmid constructs.

## Results

A total of 31 samples were collected from the 17 inflows as part of the tiered sampling regime; 15 on the dry weather sampling event and 16 on the wet weather sampling event (Table 1). A further 29 supplementary samples were collected from inflow A to further investigate the source of contamination (Fig. 2; Table 2).

## Inflow testing

All of the inflows recorded concentrations of FIB above recreational guidelines at least once during

the study (Table 1). With one exception, all of the samples with high FIB concentrations (i.e. > 1000) tested positive for the general faecal marker (GenBac). The one exception was the dry weather sample from inflow M.

The source analysis indicated the presence of canine, avian and human faecal markers for both sampling events. The canine marker was the most commonly detected signal, being detected at three of the inflows during dry weather and nine inflows following rainfall. The avian marker was the next most commonly detected signal, being detected in three inflows on both weather events. The human marker was identified from only one inflow (inflow A), but was detected from this inflow on both dry and wet weather sampling events and was associated with high concentrations of FIB.

Our analysis was unable to attribute an animal source for six of the samples that tested positive for the general faecal marker, including some samples which had very high concentrations of FIB (e.g. inflows B [ $E. \ coli = 17,300$ ] and K [ $E. \ coli = 1070$ ] following wet weather).

## Dry weather sampling

Fifteen of the 17 inflows were sampled during the dry weather sample event (inflows N and C were not flowing at time of sampling), of which four had low concentrations of indicator bacteria and were not subject to further analysis (Table 1). Of the 11 samples analysed for source markers, seven samples tested positive for the general faecal marker, with a strong signal detected in only three samples (inflows A, E and J). The canine marker tested positive in all three of these samples, with the avian and human markers also detected in inflow A.

## Wet weather sampling

Sixteen of the 17 inflows were sampled during the wet weather sample event (inflow M had no flow), of which one (inflow H) had low concentrations of indicator bacteria and was not subject to further analysis (Table 1). All of the 15 samples analysed for source markers tested positive for the general

		Dry weather sampling			Wet weather sampling			
Inflow	E. coli	Enterococci	Faecal signal	Source	E. coli	Enterococci	Faecal signal	Source
A	>24,200	1700	Very strong	Human (weak), avian (weak), and canine (weak)	17,200	12,000	Very strong	Human (strong) and avian (weak)
В	490	150	Very weak	Indeterminate	17,300	14,100	Weak	Indeterminate
С	No flow		-		19,900	>24,200	Positive	Canine (weak)
D	190	20	Not tested	Not tested	12,000	>24,200	Strong	Canine (positive)
Е	750	1200	Strong	Canine (very weak)	>24,200	>24,200	Very strong	Canine (positive)
F	65	120	Not tested	Not tested	860	5500	Strong	Avian (very weak)
G	120	250	No signal	No signal	150	680	Very weak	Indeterminate
Н	10	820	No signal	No signal	30	50	Not tested	Not tested
Ι	210	170	Very weak	Indeterminate	600	630	Weak	Indeterminate
J	3300	5800	Very strong	Canine (weak)	1920	7300	Very strong	Canine (positive)
Κ	110	50	Not tested	Not tested	1070	310	Weak	Indeterminate
L	260	240	No signal	No signal	2600	3700	Positive	Canine (weak)
М	7700	2500	No signal	No signal	No flow			
Ν	No flow		C C	0	>24,200	>24,200	Strong	Canine (very weak)
0	1200	780	Very weak	Avian (very weak)	>24,200	>24,200	Very strong	Canine (positive) and avian (very weak)
Р	10,500	3600	Weak	Avian (weak)	>24,200	>24,200	Very strong	Canine (strong)
Q	75	<10	Not tested	Not tested	>24,200	19,900	Strong	Canine (weak)

**Table 1** Faecal indicator bacteria and source tracking results from the 17 inflows. The dry weather sampling (30 May 2011) was undertaken after 0.5 mm

 rain in the preceding 24 h. The wet weather sampling (10 June 2011) was undertaken after 10 mm rain in the preceding 24 h.

FIB units are MPN/100 mL. Faecal signal results are expressed on a semi-quantitative scale as described in 'Methods'.

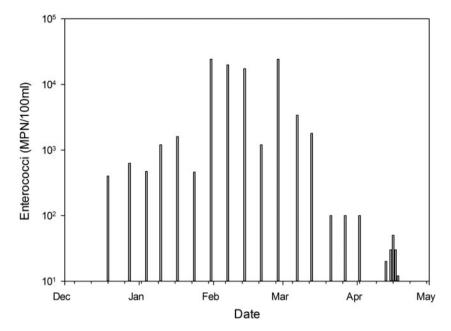


Figure 2 Additional enterococci sampling results from inflow A.

faecal marker, with a strong signal detected in nine samples. The canine marker tested positive in seven of these samples, with the avian and human markers detected in three and one samples, respectively.

## Inflow A supplementary sampling

High concentrations of FIB were consistently identified during the supplementary sampling from inflow A (Fig. 2), with 13 of the 16 samples collected before the identification and repair of the leaking sewer pipe exceeding recreational guide-lines. Human faecal signals were detected in 11 of the 13 samples that failed the recreational guide-lines (Table 2). No other faecal signal was detected from this inflow during the supplementary testing and no source of faecal contamination was detected for the other two samples.

The persistently high FIB results and the humanassociated signal of the faecal contamination for inflow A led to the initiation of traditional piped network investigations in an effort to identify the source of the faecal pollution. Initial investigations using gross litter traps were unsuccessful, failing to find any sign of the solid materials typically associated with sewage. Only when a camera was deployed into the network did the source of the pollution become apparent. A fractured wastewater pipe was discharging via subsurface flow into the stormwater network, and as the discharge was being filtered by the soil there was no sewage litter in the stormwater pipe. The broken pipe was identified on 4 April 2012 and repaired on 10 April 2012. Supplementary samples collected after this date returned FIB concentrations well within recreational guidelines (Fig. 2; Table 2).

## Discussion

We have clearly demonstrated the value of a tiered approach to microbial source tracking in the identification and management of faecal contamination in recreational waters in a New Zealand setting. In a challenging and complex urban environment, with multiple sources of faecal contamination, we used broad-scale FIB assessments and targeted FIB testing of multiple inflows followed by selective Bacteroidales marker assays to identify a substantial source of human-derived faecal contamination that

Date	Enterococci	Faecal signal	Source
19/12/2011	400	No signal	
28/12/2011	630	Strong	Human
04/01/2012	470	Strong	Human
10/01/2012	1200	Weak	Human
17/01/2012	1600	Strong	Human
24/01/2012	460	No signal	
31/01/2012	>24,200	Very strong	Human
07/02/2012	19,900	Positive	Human
14/02/2012	17,300	Very strong	Human
21/02/2012	1200	Very weak	Human
28/02/2012	>24,200	Very strong	Human
07/03/2012	3400	Strong	Human
13/03/2012	1800	Very strong	Human
21/03/2012	100	Not tested	
27/03/2012	100	Not tested	
02/04/2012	100	Not tested	
04/04/2012	Fractured sewer	r pipe identified	
10/04/2012	Fractured sewer	r pipe repaired	
13/04/2012	20	Not tested	
15/04/2012	30	Not tested	
16/04/2012	50	Not tested	
17/04/2012	30	Not tested	
18/04/2012	<10	Not tested	

 
 Table 2 Enterococci and source tracking results from the supplementary sampling of inflow A.

FIB units are MPN/100 mL.

traditional piped network investigation methods (based on the presence of sewage litter) failed to identify. The successful application of the tiered approach to identifying and managing urban sources of FIB is important as the sources of FIB in urban settings can vary substantially in space and time (Whitlock et al. 2002) because impervious surfaces concentrate runoff laden with FIB from numerous sources (Converse et al. 2009; Whitman et al. 2014).

Urbanisation is a strong predictor of FIB abundance as FIB are ubiquitous in urban environments (Noble et al. 2003), but it is not clear to what extent different sources may impact water quality in urban areas (Boehm et al. 2003) and high concentrations of FIB are not necessarily indicative of human contamination (Noble et al. 2006; Brownell et al. 2007). Our findings largely agree with this latter statement, in that we found high FIB in all inflows on at least one of the sample occasions, but only one of the inflows tested positive for the human Bacteroidales marker.

#### Health risk

The ability to reliably identify the presence of human-derived faecal contamination is important for two reasons. First, because of the species barrier concept, there is an underlying assumption that human faecal contamination has a higher health risk than other sources (Scott et al. 2002; Field & Samadpour 2007; Ishii & Sadowsky 2008; Roslev & Bukh 2011). Hence, the presence of humanderived faecal matter may provide a human health risk greater than that of other common animal sources (Sinton et al. 1998).

However, this assumption has not been adequately tested and the distribution of human pathogens among non-human hosts and the wider environment, and the subsequent health risk, are not fully understood (Field & Samadpour 2007; Soller et al. 2010). Non-human hosts are known to be sources and reservoirs of enteric pathogens, including salmonella, pathogenic *E. coli*, campylobacter and cryptosporidium (Scott et al. 2002; Devane et al. 2007; Sinton et al. 2007).

There is clearly further work required to fully understand the relative health risk of faecal contamination from different sources, but it is clear that the presence of FIB may not always be indicative of pathogen presence (Boehm et al. 2003; Colford et al. 2007; Field & Samadpour 2007; Sinton et al. 2007; Walters et al. 2009; Payment & Locas 2011).

#### Management

Whilst the value of source identification for assessing heath risk is subject to debate, the value of reliable source information is critical to the success of management interventions. For example, the management of a bovine faecal source would be radically different from that employed to address a human source. In the absence of reliable source information, management interventions are likely to be limited in efficacy and lead to investment in ineffective remedial actions (Gilpin et al. 2002; Santo Domingo et al. 2007). In our investigation, we repeatedly recorded elevated concentrations of FIB in the inflows to the study area that were well in excess of recreational guidelines. Given the urban location of the study site, and the absence of reliable source information, the responsible environmental management and wastewater service provider would have likely embarked on a series of catchment and pipe network investigations in an ultimately futile attempt to identify potential sources of sewage contamination. In contrast, with the notable exception of inflow A, our study provided evidence that the predominant source of the FIB in and around the Onehunga lagoon was either not faecal, or from non-human animal sources.

Whilst wildlife can be the dominant source of FIB during storm events in urban environments (Whitlock et al. 2002), the identification of canine as the most frequent source of faecal contamination in our study was somewhat unexpected. However, such a finding is not without precedent. Ervin et al. (2014) identified canine contamination as a significant source in a recreational area, reporting that a single canine defecation could have measurable effects on water quality. Similarly, canine faeces were reported as being the largest animal source at a Florida beach, in part because of the high enterococci concentrations in canine faeces and relatively large size of canine faecal events (one canine faecal event being equivalent to 6940 avian faecal events; Wright et al. 2009). Our finding in relation to canine sources led to the commencement of a management plan for dogs and their owners in and around the Onehunga lagoon, including educational signage and the installation of more dog litter bins. Unfortunately, we do not have any sampling results to assess the effectiveness of the management plan, but a similar approach has achieved measurable improvements on a California beach (Ervin et al. 2014).

## Conclusion

Whilst FIB are valuable in large-scale monitoring programmes and investigations as a general indicator of faecal contamination (Scott et al. 2002), the approach of studying FIB alone is of limited effectiveness in informing management decisions. The ecology of FIB is complex; they are widely distributed in the environment and their presence is not always indicative of faecal contamination. Furthermore, even when FIB are faecal in origin, they provide no information as to the source of the contamination. However, our findings support the use of a tiered approach for the identification of faecal contamination, including the use of FIB primarily as a screening tool prior to more complex and expensive source-tracking analysis. Indeed, the Bacteroidales markers are more effective on samples with higher FIB concentrations (Cornelisen et al. 2012); however, the costs of the molecular analyses currently prohibit their inclusion in routine microbiological water quality assessments.

Our study adds to the growing body of literature where Bacteroidales markers have been successfully used to identify human sources of faecal contamination in urban environments (Noble et al. 2006; Ahmed et al. 2008; Kirs et al. 2011), but is one of few where the source of human contamination has been identified, remedial action taken and its effectiveness subsequently validated (e.g. Dickerson et al. 2007; Korajkic et al. 2011) and the first published from New Zealand. Field & Samadpour (2007) described a great need for studies of this type, whereby water quality improvements eventuate from the application of source tracking rather than methodological refinements.

#### Acknowledgements

Auckland Council funded this study. We thank our Auckland Council colleagues David Barker, Greg Hannah and Phillip Johansen for their support during the investigations. We would also like to thank Aqua-Lab for analysing bacteria samples and ESR for advising on and undertaking the molecular analysis, in particular Brent Gilpin and Paula Scholes.

Associate Editor: Dr Judy Sutherland.

#### References

Ahmed W, Powell D, Goonetilleke A, Gardner T 2008. Detection and source identification of faecal pollution in non-sewered catchment by means of hostspecific molecular markers. Water Science and Technology 58: 579–586.

- Allsop K, Stickler DJ 1985. An assessment of *Bacter-oides fragilis* group organisms as indicators of human faecal pollution. Journal of Applied Microbiology 58: 95–99.
- Anderson SA, Turner SJ, Lewis GD 1997. Enterococci in the New Zealand environment: implications for water quality monitoring. Water Science and Technology 35: 325–331.
- Bernhard AE, Field KG 2000a. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16s rRNA. Applied and Environmental Microbiology 66: 4571–4574.
- Bernhard AE, Field KG 2000b. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology 66: 1587–1594.
- Boehm AB, Fuhrman JA, Mrše RD, Grant SB 2003. Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. Environmental Science and Technology 37: 673–680.
- Brownell MJ, Harwood VJ, Kurz RC, McQuaig SM, Lukasik J, Scott TM 2007. Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations. Water Research 41: 3747–3757.
- Byappanahalli MN, Shively DA, Nevers MB, Sadowsky MJ, Whitman RJ 2003. Growth and survival of *Escherichia coli* and enterococci populations in the macro-alga *Cladophora* (Chlorophyta). FEMS Microbiology Ecology 46: 203–211.
- Colford JM, Wade TJ, Schiff KC, Wright CC, Griffith JF, Sandhu SK et al. 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology 18: 27–35.
- Conn KE, Habteselassie MY, Blackwood AD, Noble RT 2011. Microbial water quality before and after the repair of a failing onsite wastewater treatment system adjacent to coastal waters. Journal of Applied Microbiology 112: 214–224.
- Converse RR, Blackwood AD, Kirs M, Griffith JF, Noble RT 2009. Rapid QPCR-based assay for fecal *Bacteroides* spp. as a tool for assessing fecal contamination in recreational waters. Water Research 43: 4828–4837.
- Cornelisen CD, Kirs M, Gilpin B, Scholes P 2012. Microbial source tracking (MST) tools for water quality monitoring. Cawthron Report No. 2047. Nelson, Cawthron Institute. 68 p.

- Deely J, Hodges S, McIntosh J, Bassett D 1997. Enterococcal numbers in waters of marine, lake and river swimming sites of the Bay of Plenty, New Zealand. New Zealand Journal of Marine and Freshwater Research 31: 89–101.
- Devane ML, Robson B, Nourozi F, Scholes P, Gilpin B 2007. A PCR marker for detection in surface waters of faecal pollution derived from ducks. Water Research 41: 3553–3560.
- Dick LK, Field KG 2004. Rapid estimation of numbers of fecal *Bacteroidetes* by use of a quantitative PCR assay for 16S rRNA genes. Applied and Environmental Microbiology 70: 5695–5697.
- Dick LK, Simonich MT, Field KG 2005. Microplate subtractive hybridization to enrich for *Bacteroidales* genetic markers for fecal source identification. Applied and Environmental Microbiology 71: 3179–3183.
- Dickerson JW, Hagedorn C, Hassall A 2007. Detection and remediation of human-origin pollution at two public beaches in Virginia using multiple source tracking methods. Water Research 41: 3758–3770.
- Donnison AM, Ross CM 1999. Animal and human faecal pollution in New Zealand rivers. New Zealand Journal of Marine and Freshwater Research 33: 119–128.
- Ervin JS, Russell TL, Layton BA, Yamahara KM, Wang D, Sassoubre LM et al. 2013. Characterization of fecal concentrations in human and other animal sources by physical, culture-based, and quantitative real-time PCR methods. Water Research 47: 6873–6882.
- Ervin JS, Van de Werfhost LC, Murray JLS, Holden PA 2014. Microbial source tracking in a coastal California watershed reveals canines as controllable sources of fecal contamination. Environmental Science and Technology 48: 9043–9052.
- Field KG, Samadpour M 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Research 41: 3517–3538.
- Fiksdal L, Maki JS, LaCroix SJ, Staley JT 1985 Survival and detection of *Bacteroides* spp., prospective indicator bacteria. Applied and Environmental Microbiology 49: 148–150.
- Gilpin BJ, Gregor JE, Savill MG 2002. Identification of the source of faecal pollution in contaminated rivers. Water Science and Technology 46: 9–15.
- Halliday E, Gast RJ 2011. Bacteria in beach sands: an emerging challenge in protecting coastal water quality and bather health. Environmental Science and Technology 45: 370–379.
- Ishii S, Hansen DL, Hicks RE, Sadowsky MJ 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. Environmental Science and Technology 41: 2203–2209.
- Ishii S, Sadowsky MJ 2008. *Escherichia coli* in the environment: implications for water quality and

human health. Microbes and Environments 23: 101–108.

- Jin G, Englande AJ, Bradford H, Jeng H 2004. Comparison of *E. coli*, enterococci, and fecal coliform as indicators for brackish water quality assessment. Water Environment Research 76: 245–255.
- Kirs M, Harwood VJ, Fidler AE, Gillespie PA, Fyfe WR, Blackwood AD et al. 2011. Source tracking faecal contamination in an urbanised and a rural waterway in the Nelson-Tasman region, New Zealand. New Zealand Journal of Marine and Freshwater Research 45: 43–58.
- Korajkic A, Brownell MJ, Harwood VJ 2011. Investigation of human sewage pollution and pathogen analysis at Florida gulf coast beaches. Journal of Applied Microbiology 110: 174–183.
- Ministry for the Environment 2003. Microbiological water quality guidelines for marine and freshwater recreational areas. Wellington, Ministry for the Environment.
- Muirhead RW, Davies-Colley RJ, Donnison AM, Nagels JW 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research 38: 1215–1224.
- Noble RT, Griffith JF, Blackwood AD, Fuhrman JA, Gregory JB, Hernandez X et al. 2006. Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica Bay, California. Applied and Environmental Microbiology 7(2): 1604–1612.
- Noble RT, Weisberg SB, Leecaster MK, McGee CD, Dorsey JH, Vainik P et al. 2003. Storm effects on regional beach water quality along the southern California shoreline. Journal of Water and Health 1: 23–31.
- Payment P, Locas A 2011. Pathogens in water: value and limits of correlation with microbial indicators. Groundwater 49: 4–11.
- Rabinovici SJM, Bernknopf RL, Wein AM, Coursey DL, Whitman RL 2004. Economic and health risk trade-offs of swim closures at a Lake Michigan beach. Environmental Science and Technology 38: 2737–2745.
- Roslev P, Bukh AS 2011. State of the art molecular markers for fecal pollution source tracking in water. Applied Microbiology and Biotechnology 89: 1341–1355.
- Santo Domingo JW, Bambie DG, Edge TA, Wuertz S 2007. Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. Water Research 41: 3539–3552.
- Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J 2002. Microbial source tracking: current methodology and future directions. Applied and Environmental Microbiology 68: 5796–5803.

- Shuval H 2003. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. Journal of Water and Health 1: 53–63.
- Sinton LW, Braithwaite RR, Hall CH, Mackenzie ML 2007. Survival of indicator and pathogenic bacteria in bovine feces on pasture. Applied and Environmental Microbiology 73: 7917–7925.
- Sinton LW, Finlay RK, Hannah DJ 1998. Distinguishing human from animal faecal contamination in water: a review. New Zealand Journal of Marine and Freshwater Research 32: 323–348.
- Soller JA, Schoen ME, Bartrand T, Ravenscroft JE, Ashbolt NJ 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44: 4674–4691.
- Stoeckel DM, Harwood VJ 2007. Performance, design and analysis in microbial source tracking studies. Applied and Environmental Microbiology 73: 2405–2415.
- USEPA 2005. Microbial source tracking guideline document. Cincinnati, OH, USEPA. 150 p.
- Verhougstraete MP, Byappanahalli MN, Rose JB, Whitman RL 2010. *Cladophora* in the Great Lakes: impacts on beach water quality and human health. Water Science and Technology 62: 68–76.
- Walker J, Vaughan M 2013. Marine water quality annual report: 2011. Auckland Council Technical Report TR2013/031. Auckland, Auckland Council. 43 p.
- Walters SP, Field KG 2009. Survival and persistence of human and ruminant-specific faecal *Bacteroidales* in freshwater microcosms. Environmental Microbiology 11: 1410–1421.
- Walters SP, Yamahara KM, Boehm AB 2009. Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: implications for their use in assessing risk in recreational waters. Water Research 43: 4929–4939.
- Wang D, Silkie SS, Nelson KL, Wuertz S 2010. Estimating true human and animal host source contribution in quantitative microbial source tracking using the Monte Carlo method. Water Research 44: 4760–4775.
- Whitlock JE, Jones DT, Harwood VJ 2002. Identification of the source of fecal coliforms in an urban watershed using antibiotic resistance analysis. Water Research 36: 4273–4282.
- Whitman RL, Harwood VJ, Edge TA, Nevers MB, Byappanahalli M, Vijayavel K et al. 2014. Microbes in beach sands: integrating environment, ecology and public health. Reviews in Environmental Science and Bio/Technology 13: 329–368.
- Whitman RL, Shively DA, Pawlik H, Nevers MB, Byappanahalli MN 2003. Occurrence of *Escherichia*

*coli* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. Applied and Environmental Microbiology 69: 4714–4719.

Wood SA, Smith KF, Banks JC, Tremblay LA, Rhodes L, Mountfort D et al. 2013. Molecular genetic tools for environmental monitoring of New Zealand's aquatic habitats, past, present and the future. New Zealand Journal of Marine and Freshwater Research 47: 90–119.

Wright ME, Solo-Gabriele HM, Elmir S, Fleming LE 2009. Microbial load from animal feces at a recreational beach. Marine Pollution Bulletin 58: 1649–1656.