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A short work-flow to effectively source faecal pollution in recreational waters – A case study

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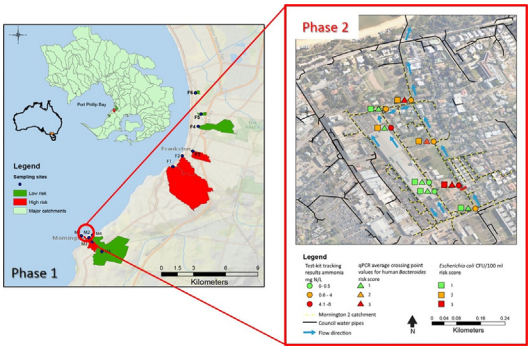
HIGHLIGHTS

- A multi-tiered approach for real-time sourcing of human faecal pollution is proposed.
- Chemical and microbial indicators were used as pollution identification tools.
- A risk assessment framework allowed the detection of high-risk catchments.
- A cracked sewer pipe and a sewer spill were located from 11 catchments in 4 months.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial pollution of recreational waters poses a significant public health risk which, unless mitigated, will continue to increase with population growth. Water managers must implement strategies to accurately discriminate and source human from animal faecal contamination in complex urbanised environments. Our case-study used a new combination of chemical (i.e. ammonia) and microbial (i.e. *Escherichia coli*, *Bacteroides* spp.) faecal monitoring tools in a targeted multi-tiered approach to quickly identify pollution hot-spots and track high-risk subterranean stormwater drains in real-time. We successfully located three point sources of human faecal pollution (both episodic and constant pollution streams) within 11 catchments in a total monitoring time of four months. Alternative approaches for obtaining such fine-scale accuracy are typically labour intensive and require expensive equipment.

1. Introduction

Human faecal pollution is a major source of human pathogens with urban waterbodies highly prone to contamination. Consequently, microbial pollution is a priority target for recreational water managers to reduce the health risk posed to people undertaking water-based

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activities such as swimming, wading and canoeing in these areas (Wade et al., 2013). For instance, enteric illnesses such as gastroenteritis and infections of the central nervous system are common upon exposure to faecal pathogens (Leder et al., 2002; WHO, 2003). Public safety guidelines have been produced by the Australian Government to assist state and territory agencies develop appropriate legislation and standards for local environments (NHMRC, 2008).

Faecal contamination in urban waterways can originate from a variety of sources. However, in urbanised catchments, especially those with relatively high impervious areas, faecal contamination is most likely to discharge through stormwater conveyance networks (Ahn et al., 2005). Poorly functioning sewerage systems and insufficient hydrologic capacity to contain sewage are significant sources of microbial pollution. Overflows can occur during storms when excess stormwaters infiltrates sewers or during peak daytime periods when large amounts of waste is discharged to the sewer. Sewage spills can also occur from structural failures in the sewerage system that can lead to dry weather discharges (Noble et al., 2006; Rajal et al., 2007; Sidhu et al., 2013).

Monitoring of faecal contamination is normally conducted using general faecal indicator bacteria (FIB) (*Escherichia coli* and *Enterococcus* spp.). Although they do not identify the source of faecal pollution, which is a major limitation given possible human and animal faecal sources, they can indicate potential issues that need to be managed (Tran et al., 2015). Another major challenge in urban environments is that pollutants are primarily discharged into subterranean stormwater drains before entering receiving environments, increasing the difficulty of tracking pollution events as they are occurring and locating pollution sources. These pollution events may also be intermittent (e.g. spills occurring each morning from sewer overflows) or persistent (e.g. leakages from cracked sewers) further challenging water managers. Consequently, additional highly specific bacterial and chemical markers have been developed to overcome these pollution-sourcing limitations.

Bacteroides spp. assays are rapidly developing as new FIB (Ahmed et al., 2016). Unlike standard FIB (i.e. *Enterococcus* and *E. coli*), *Bacteroides* spp. are highly host-specific and can consequently discriminate human and animal faecal sources. In the current study we only quantified human-specific *Bacteroides* markers. Development, sensitivity and specificity of these markers is described in Kildare et al. (2007). *Bacteroides* spp. are several magnitudes more abundant in faeces than faecal coliforms increasing the likelihood of detecting microbial contamination in receiving waters (Savichtcheva and Okabe, 2006). *Bacteroides* spp. assay's bacteria are amplified to quantifiable levels using culture-independent methods reducing bias from differences in bacterial viability of collected water samples (Shanks et al., 2014). Recent studies have correlated *Bacteroides* spp. concentrations with *E. coli* (Waso et al., 2018; Ahmed et al., 2008).

Chemical indicators are increasingly being trialled in faecal monitoring studies to improve monitoring sensitivity (Hagedorn and Weisberg, 2011). Levels of caffeine and certain pharmaceuticals have been proposed as chemical indicators of human faecal contamination, however environmental levels are not conservative (Fang et al., 2017), good sampling strategies for non-polar compounds such as these can be difficult in some circumstances and analyses require a minimum of 24 h (Kresinova et al., 2016). Ammonia is another chemical indicator which has been shown to be strongly correlated with human FIB and is a by-product from microbial degradation of proteins, nucleic acid and urea (Cabral and Marques, 2006; Rose et al., 2015).This paper presents an approach to detect and locate sources of human sewage through stormwater system without physically entering these drains. Our multi-tiered approach uses a combination of chemical and microbial indicators to track and verify sources of human faecal pollution. Testing ammonia levels in combination with highly-specific human bacterial indicators (*Bacteroides* spp.; BacHum-UCD) and general faecal indicator bacteria (*Escherichia coli*) can distinguish human pollution events from those originating from industrial or animal sources. It also provides an assessment against Australian safety guidelines. The specific application of novel ammonia passive samplers in conjunction with instantaneous ammonia field test kits at different stages of a monitoring project enables the identification of pollution 'hot-spots' from multiple coastal catchments, while tracing pollution events (including intermittent sewer overflows) as they occur along stormwater networks to identify sewage sources. We report a case study undertaken in Frankston and Mornington Peninsula, Victoria, Australia. Our study area has separate stormwater and sewage system.

2. Materials and methods

2.1. Study region

Eleven catchments were selected across Frankston (F) and Mornington Peninsula (M), Victoria, Australia (Fig. 1) and were monitored between February until June 2014. The climate in this region is Mediterranean with high summer temperatures exceeding 30 °C and winter temperatures as low as 6 °C. The mean rainfall is 739.8 mm and the annual number of rain days is 148.7 (BOM, 2017). Land use across this region is predominately residential and light industrial with some semi-rural sections within Frankston shire. A detailed table of land use proportions is provided in the supplementary data (Table S1).

2.2. Monitoring program

The monitoring approach consisted of two sampling phases. In the first phase, ammonia passive samplers were deployed at the bottom of 11 catchments. The passive samplers were deployed weekly for four weeks in triplicate. Stormwater spot samples were collected when the passive samplers were deployed and again when they were retrieved, and the levels of *Escherichia coli* and human *Bacteroides* markers quantified. Standard water quality indices (pH, temperature and conductivity) were also measured. Total ammonia concentrations were averaged for each deployment period. Baseline levels of ammonia in uncontaminated waters were determined to define "trigger" ammonia levels for phase two of the study (0.5 mg L^{-1}). This was the measured ammonia concentrations where no positive faecal hits were observed. All water samples were stored at 4 °C until analysed within 24 h of collection. Time-weighted average (TWA) concentration of ammonia, *E. coli* and human *Bacteroides* were then ranked against a predetermined set of criteria. Each indicator was then given a value ranging from 1 to 3 (Table 1). If the total catchment ranked a combined score from all three indices of "5" or higher, the catchment was then determined as having a high risk of microbial pollution and selected for phase two of the study.

During phase two, sites within the high-risk catchments were investigated and subjected to an intense and targeted sampling strategy. Ammonia levels present at the time of sampling were determined using an API ammonia test kit. Test kit trigger values were based on colourmetric analysis. If the ammonia concentration was above the "trigger" value (0.5 mg L^{-1}) determined in phase one of the study from baseline levels, the ammonia signal was then traced upstream along the stormwater infrastructure until no elevated ammonia was recorded. The presence of human *Bacteroides* markers and *E. coli* concentrations were measured at the last location of elevated ammonia to confirm whether the source of contamination was faecal and of human not animal origin. If ammonia levels were present but below the "trigger value", the site was retested in 45 min to allow the pollution event to peak. If high risk sites did not breach trigger values, they were resampled within a week. Branches within the stormwater network were eliminated if ammonia concentrations were <0.25 mg L^{-1}, narrowing the catchment to specific areas where the source of contamination appeared to be entering the stormwater network.

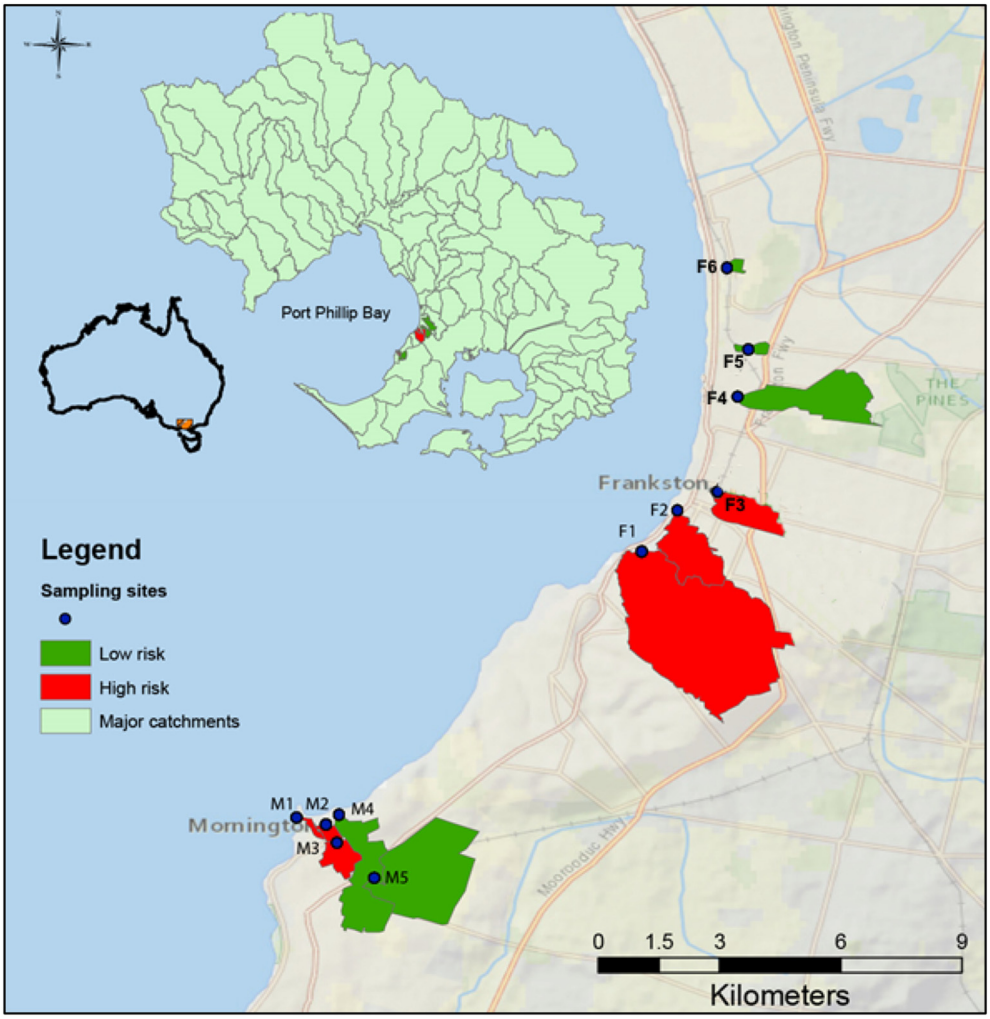

Fig. 1. Phase 1 sampling and microbial pollution risk for the 11 selected catchments. Mornington sites are listed as M# and Frankston sites as F#.

2.3. Microbial analysis

Escherichia coli analyses were performed by Australian Laboratory Services (ALS, Australia) using a standard colilert system and reported as Colony Forming Units (CFU)/100 mL. Human *Bacteroides* markers were extracted by filtering 400 mL of stormwater sample through a 0.45 μm nitrocellulose membrane filter (Millipore) and the membrane stored frozen (−20 °C). Membranes were shredded and DNA isolated using the Isolate I faecal Kit (Bioline) following the manufacturers' instructions. Final elution's (100 μL) of isolated DNA were stored at −20 °C in buffer for subsequent real-time quantitative Polymerase Chain Reaction (PCR). PCR reagents and cycling conditions are described in the supplementary data.

Sensitivity and specificity of the BacHum-UCD marker in our study region was tested against target and non-target faecal samples (human = 5, horses = 3, cows = 3, goats = 3, dog = 3). The diagnostic sensitivity was calculated as the ratio between correctly identified target faeces and the total number of target faeces. The diagnostic specificity was calculated as the proportion of correctly identified non-target results over the total number of non-target faeces. Details are provided in the supplementary data.

PCR was performed using a light cycler (Roche 480) with TaqMan probes (BacHum-UCD) designed to detect partial 16S rRNA genes from human-derived *Bacteroides* spp (Kildare et al., 2007). Four replicates were analysed for each stormwater water sample and their average crossing point value (defined as the number of hot/cold thermo-cycles that the sample fluorescence exceeds background levels) and standard deviation were reported. A standard curve consisting of four replicates of four serial dilutions of known-concentration reference standards encompassing the expected concentration range of the samples and the expected limit of quantification was generated within each independent run. These known concentrations were then plotted against the crossing point determined by the qPCR. A linear regression was then fit to the data using the second derivative method. qPCR determined concentrations were then compared against this standard curve and used to generate the limit of quantification, limit of detection and quality control indices (error, efficiency). The average crossing point value of four replicates of distilled water were also analysed in each run as a negative control.

A run was considered 'successful' if the error and efficiency values were within manufacturers recommendations and if the level of target bacteria in the negative controls did not exceed the limit of quantification, which would indicate sample contamination (see below). The limit of quantification was calculated as the crossing point value in the standard curve that the linear relationship between log concentration and crossing point no longer exists. Environmental samples were considered positive if detected crossing point values were less than the limit of quantification.

Bayesian theorem was then applied to assay sensitivity and specificity to determine the likelihood of *Bacteroides* results representing a true positive. Details are provided in the supplementary data.

2.4. Ammonia passive sampling procedure and ammonia analysis

The ammonia passive sampling device used in phase 1 was designed by Almeida et al. (2016) and it allows the measurement of the TWA concentration of total ammonia (i.e. sum of both molecular ammonia and ammonium cation) for a 7-day sampling period. This sampler has been calibrated under laboratory conditions (up to 2.1 mg N L^{-1}) using synthetic freshwater at 20 °C with an electrical conductivity and pH set at approximately 1500 μS cm^{-1} (i.e. very hard water) and 7.8, respectively.

The ammonia passive samplers were assembled <24 h prior to deployment and attached to a floating structure (Supplementary data) to ensure that the PIM was always in contact with both sampled medium and receiving solution.

The receiving solutions of each sampler were collected after 7 days of deployment and were analysed for ammonia using a gas diffusion-flow injection analysis system (Almeida et al., 2015, 2016).

Ammonia spot samples collected during the second monitoring phase were analysed in real-time using a API Ammonia test kit. Five millilitres of stormwater was mixed with the reagents provided by the manufacturer and allowed to rest to 5 min. The resulting colour change of the solution was then compared against the colour chart provided (concentration range up to 8 mg N L^{-1}).

3. Results

3.1. Phase 1

Out of the 11 catchments monitored during phase 1, six sites were identified as key pollution priority. Sites M1–3 (Table 2a) and F1–3 (Table 2b) were all assessed as having a pollution risk ≥5 and therefore were selected for subsequent investigation in phase 2. F5 also had a pollution risk ≥5 but recorded high salinity so was omitted from further analyses. Water quality measurements levels are provided in the supplementary data (Table S2). The 'trigger' ammonia value for phase two of the study was determined as 0.5 mg L^{-1} of NH_3/NH_4^+ based background levels.

Catchments within Mornington were generally less affected by microbial pollution compared with Frankston. Sites M1 and M2 recorded

Table 1
Criteria used to assess microbial pollution risk. The highest values found during the first monitoring phase were ranked against the criteria below and assigned the associated score. If the total score was ≥5, the site was considered at high pollution risk and included in further analyses.

Parameters assessed	Range	Score
TWA concentration of ammonia using passive sampling	<0.90 mg N L^{-1} [a]	1
	1.0–2.1 mg N L^{-1}	2
	>2.2 mg N L^{-1} [b]	3
Escherichia coli	<1000 CFU/100 mL[c]	1
	1001–1 million CFU/100 mL	2
	>1 million CFU/100 mL[d]	3
Average Cp values for human *Bacteroides* marker using qPCR	>27.0[e]	1
	23.1–27.0	2
	<23.0[f]	3

TWA, time-weighted average; Cp, crossing point, qPCR, quantitative polymerase chain reaction; CFU, colony-forming unit.

[a] The value 0.90 mg N L^{-1} corresponds to the ammonia trigger value reported by the ANZECC guidelines (ANZECC, 2000).

[b] The value 2.2 mg N L^{-1} corresponds to the limit above which the passive sampler does not provide a linear response (Almeida et al., 2016).

[c] The value 1000 CFU/100 mL is double dry weather *Escherichia coli* trigger (>500 cFU/100 mL) http://yarraandbay.vic.gov.au/., providing a conservative trigger value for stormwater environments.

[d] The value 1 Million CFU/100 mL is a conservative estimate that if exceeded, *Escherichia coli* concentrations would pose extreme risk to receiving waters.

[e] The value 27 is a conservative estimate slightly above the limit of quantification for this approach (see Supplementary data).

[f] The crossing point value 23 corresponds to the 15 percentile of all stormwater tested.

Table 2a
Results obtained from the first monitoring phase in Mornington Peninsula using the combined approach. The highest values were identified for each parameter (highlighted in bold) and scored according to the criteria presented in Table 1. A catchment was further investigated if the total score (i.e. the sum of the individual scores) was ≥5, unless high salinity values were detected.

Site name	Sampling	TWA concentration of ammonia - passive sampling (mg N L^{-1})			Ammonia score	*Escherichia coli* (×1000 CFU/100 mL)				*E. coli* score	Average crossing point values for human *Bacteroides* marker - qPCR				qPCR score	Total score (out of 9)	Follow up catchment? (yes/no)
		Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4		Week 1	Week 2	Week 3	Week 4			
M1	S	–	–	–	–	3	5.3	4.3	8.6	2.4	2	NT	35.0	41.2	NT	1	6	Yes
	E	0.40	0.22	1.5	**4.9**		69	**240**	0.29	2.0		**33.8**	34.0	NT	34.5			
M2	S	–	–	–	–	2	20	0.18	**240**	7.7	2	25.1	27.4	23.2	24.7	3	7	Yes
	E	1.0	0.50	**1.3**	0.81		49	24	14	6.8		**19.7**	23.7	30.3	24.0			
M3	S	–	–	–	–	1	0.3	0.63	0.53	1.2	2	28.8	31.2	33.8	31.8	2	5	Yes
	E	0.26	0.26	0.37	**0.52**		4.7	**26**	12	0.041		**25.5**	28.5	33.9	30.8			
M4	S	–	–	–	–	1	0.41	3.2	**6.9**	1.3	2	37.7	33.4	**32.0**	34.2	1	4	No
	E	0.16	0.19	**0.26**	0.24		0.63	2.5	1.1	0.99		33.2	35.4	38.0	33.0			
M5	S	–	–	–	–	1	5.4	0.31	5.8	0.26	2	34.9	37.0	34.1	39.8	1	4	No
	E	0.49	0.19	0.68	**0.74**		1.7	1.1	0.63	**9.1**		37.4	45.0	37.9	**33.9**			

TWA, time-weighted average; S, sampling at the start of the week; E, sampling at the end of the week.

Table 2b
Results obtained from the first monitoring phase in Frankston using the combined approach. The highest values were identified for each parameter (highlighted in bold) and scored according to the criteria presented in Table 1. A catchment was further investigated if the total score (i.e. the sum of the individual scores) was ≥5, unless high salinity values were detected.

Site name	Sampling	TWA concentration of ammonia - passive sampling (mg N L^{-1})				Ammonia score	*Escherichia coli* (×1000 CFU/100 mL)				*E. coli* score	Average crossing point values for human *Bacteroides* marker - qPCR				qPCR score	Total score (out of 9)	Follow up catchment? (yes/no)
		Week 1	Week 2	Week 3	Week 4		Week 1	Week 2	Week 3	Week 4		Week 1	Week 2	Week 3	Week 4			
F1	S	–	–	–	–	1	0.099	**23**	14	0.15	2	34.0	31.9	**25.2**	32.8	2	5	Yes
	E	0.14	<LOD	NA	NA		0.72	0.57	0.13	0.63		27.0	31.1	33.5	28.1			
F2	S	–	–	–	–	3	3.5	**2400**	0.41	87	3	35.8	**19.9**	33.7	35.5	3	9	Yes
	E	0.51	**4.9**	NA	0.31		1.2	2.0	0.2	0.17		29.7	30.6	41.6	37.5			
F3	S	–	–	–	–	1	25	520	0.72	0.41	3	36.0	29.7	36.3	NT	2	6	Yes
	E	**0.77**	0.27	0.26	0.22		**2100**	0.074	1.2	0.21		**23.2**	35.9	NT	NT			
F4[a]	S	–	–	–	–	1	**23**	0.052	0.31	0.075	2	32.9	**29.1**	35.1	37.3	1	4	No
	E	**0.47**	0.15	0.12	0.15		1.5	0.085	0.17	0.063		35.4	36.4	NT	29.7			
F5[a]	S	–	–	–	–	2	21	0.15	**260**	0.099	2	30.7	**29.2**	39.2	38.2	1	5	No
	E	<LOD	0.30	0.45	**1.0**		0.31	0.099	1.1	0.26		35.9	37.6	37.5	35.8			
F6[a]	S	–	–	–	–	1	**6.6**	0.030	0.099	4.3	2	36.6	36.6	38.9	NT	1	4	No
	E	0.16	0.23	**0.37**	0.22		0.41	0.091	1.1	0.23		NT	42.3	NT	**29.6**			

S, sampling at the start of the week; E, sampling at the end of the week; NA, not available (passive samplers were lost); LOD, limit of detection of the GD-FIA system; NT, no sample was collected.
[a] High salinity.

the highest ammonia TWA concentrations and levels of *E. coli* in comparison with other Mornington catchments. However, human specific genetic markers were only identified at site M2. M3 and F1 did not record ammonia TWA concentrations above 1 mg N L^{-1} but the levels of *E. coli* and *Bacteroides* markers in the spot samples identified modest levels of faecal pollution of human origin. Sites F2 and 3 recorded the highest levels of *E. coli* within the study combined with high levels of human-specific faecal markers, even though ammonia TWA levels were only elevated at site F2. Despite modest levels of *E. coli* at Sites M4, 5 and F5, 6 human-specific markers of faecal contamination were absent and low ammonia recorded and were thus not investigated further. High salinity was recorded at sites F4–6 as they are located in estuarine zones.

The lowest ammonia TWA concentration recorded was 0.12 mg N L^{-1} at site F4 with two sites in Frankston recording levels below the limit of detection of the analytical method (i.e. FRA 1 and 5). The highest ammonia concentration was 4.9 mg N L^{-1} which was recorded at sites M1 and F2. It should be noted that the passive sampler only provides a linear response up to 2.1 mg N L^{-1}, meaning that above this value the ammonia TWA concentration recorded is underestimated. Of the sites sampled in the current study, 13% had concentrations of ammonia above 1 mg N L^{-1}. M1 (weeks 3 and 4) had ammonia concentrations ≥1 mg N L^{-1} but corresponding weekly *E. coli* and human specific genetic markers from spot sampling were low. Sites M1 and F5 had elevated levels of *E. coli* (weeks 2 and 3, respectively), but corresponding low levels of human specific faecal indicators and ammonia TWA levels.

30% of the spot samples tested recorded levels of human specific faecal indicators above the limit of quantification (Cp < 31). Sites M2, 3 and F1, 4, 5, 6 all recorded quantifiable levels of genetic human faecal indicators in spot samples, but corresponding levels of *E. coli* were <1000 CFU/100 mL categorised as an *E. coli* score of "1" in the current study.

3.2. Phase 2

Targeted blitz sampling identified damaged sewage infrastructure and a sewer spill in three (M2, F1 and F3) of the six catchments flagged in the current study as high pollution risk. The M2 contamination point source was a broken bypass within a sewer pit (Fig. 2a). This broken bypass was leaking raw sewerage into the Cook Street stormwater main branch. South East Water, the local water and sewage company, repaired the broken pit and the damaged stormwater drain. The F1 pollution hotspot originated from Seaview Road, South Frankston where sewerage was flowing into the Sweetwater catchment from a cracked sewer (Fig. 2b). This pollution source was also confirmed by South East Water who found broken sewerage infrastructure (Fig. 2c). Subsequently, South East Water deployed a field team to repair the broken pipe, reducing the discharge of raw sewage into the stormwater drain.

Monitoring of the remaining two high risk pollution sites (M1 and M3) failed to identify any specific sources of microbial contamination originating from broken or aging sewer infrastructure. Infrequent, weak ammonia levels above the trigger value were detected during phase 2 but these could not be sourced, with municipal authorities suspecting dumping of food waste may be the reason for this pollution.

4. Discussion

Our multi-tiered work-flow combining passive sampling with rapid chemical and molecular analyses provides a tool-kit to quickly locate sources of continual (cracked sewage infrastructure) and episodic (sewer-spill) faecal pollution within large catchments.

Our specific application of passive ammonia samplers to continually sample stormwater and multiple lines of evidence to reduce the likelihood of false/missed detections in tier 1, significantly reduced per site sampling effort. Consequently, we were able to monitor a large number of base catchments and identify pollution hotspots within 1 month. Such an approach is highly favourable for local governments or agencies with no historic base-line *E. coli* or other available faecal indicator data.

Our application of commercial real-time ammonia test kits and highly specific human genetic markers in tier 2 located multiple point sources of human faecal pollution within four months. Pollution events were easily identified using the commercial test kits which could then subsequently be traced from sink to source along catchments reducing the on-ground investment. Real-time results enabled easy detection of episodic pollution events i.e. sewer spills. Other approaches require either frequent sampling strategies to identify pollution sources and assume that the pollution event will re-occur (Panasiuk et al., 2015). Consequently, only one or two high-risk catchments are monitored in three months, or if larger number of catchments is monitored, the timeframe can be up to 4 years (Sauer et al., 2011). These studies often only detect constant stream pollution and rarely episodic events such as a sewer spill. Staley et al. (2016) investigated faecal pollution in the Humber River and one tributary sampling upstream and downstream river sites, as well as drainage inflows weekly for four months. Although human faecal contamination persisted in all of the Humber River sites and its tributary, no specific location could be identified as the source of the pollution event, as it occurred outside the sampling

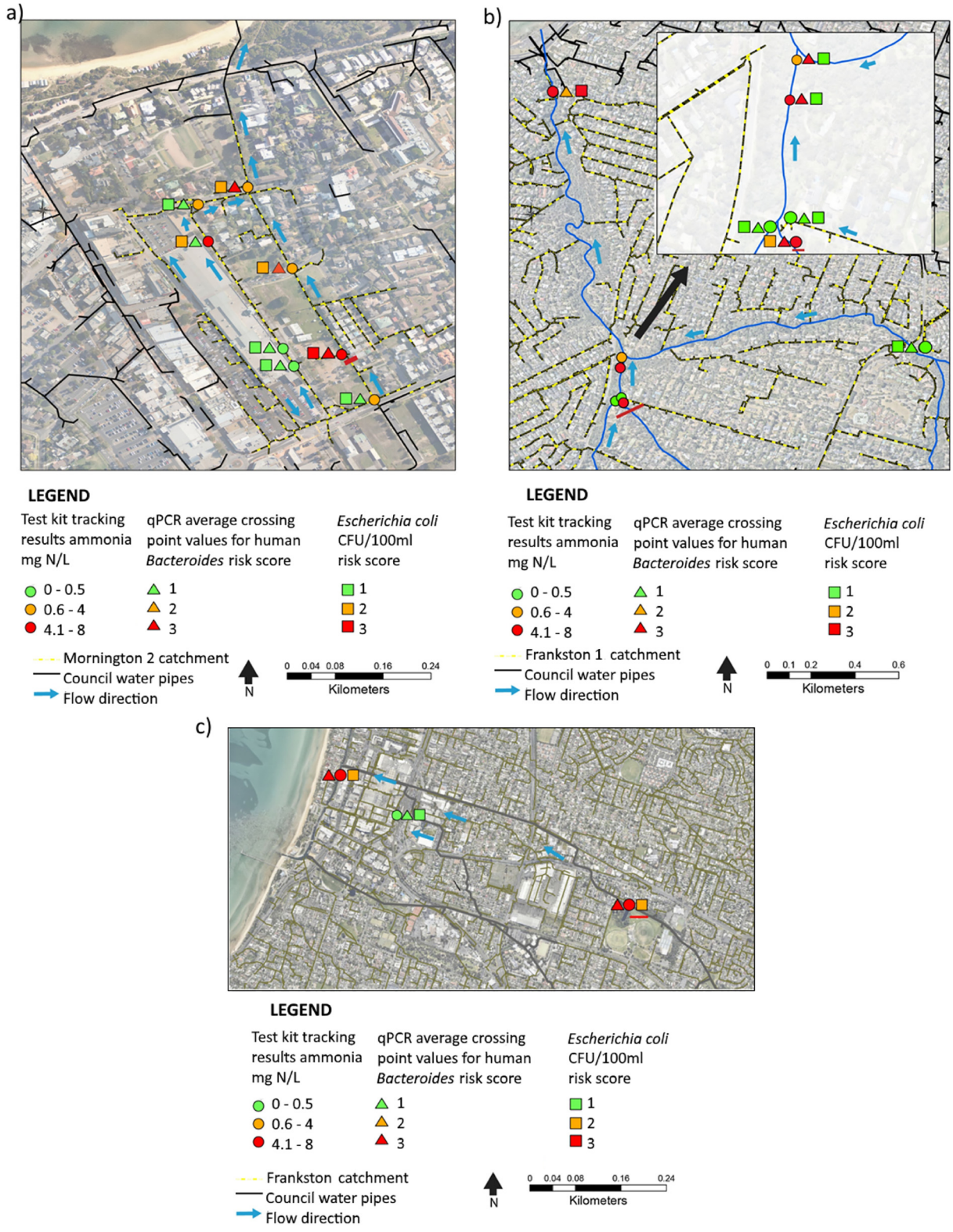

Fig. 2. Phase two blitz survey of catchments. a) M - (Mornington) 2 identifying faecal hot spots. Environmental Protection Agency confirmed cracked by-pass infrastructure in sewer pit (approximate location indicated by red line); b) F (Frankston) 1 sourcing an independently verified sewer spill (approximate location indicated by red line) along the catchment; c) Follow-up blitz investigation in Frankston. South East Water confirms broken sewerage infrastructure upstream of tracking location (approximate location indicated by red line) along the catchment. Faecal indicators are described in the legend.

area. Walker et al., 2015 also implemented a multi-tiered faecal monitoring program however faecal hotspots were identified from *E. coli* concentrations of 17 inflows lagoon, only twice increasing the likelihood of missing an episodic pollution event. This study with the aid of additional sampling and expensive pipe cameras was able to identify cracked sewage infrastructure within four months however, the drainage and potential faecal sources in this study were constrained as the monitoring site was an enclosed recreational lagoon.

The use of chemical markers in faecal sourcing is emerging (Buerge et al., 2003, 2006). Other chemical indicators of faecal (human) pollution are caffeine or pharmaceuticals and personal care products (PPCP's) (Kresinova et al., 2016). Despite high sensitivity, evidence suggests that these compounds are poor faecal indicators because these compounds can degrade or be adsorbed, and it is hard to discriminate non-faecal sources of these markers (Lim et al., 2017). Furthermore, these chemical markers are only moderately correlated to standard faecal indicator bacteria (Linden et al., 2015; Daneshvar et al., 2012). Ammonia has been highly correlated to total coliform, faecal streptococci and enterococci in the Febros River, Spain (Cabral and Marques, 2006). Unfortunately, we were unable to directly correlate ammonia with either *E. coli* or *Bacteroides* spp. because different sampling approaches were (i.e. passive vs. spot sampling). Our successful identification of multiple point sources of human faecal pollution does however support ammonia as a chemical faecal indicator, but like any faecal monitoring tools is most effective when applied in conjunction with other indices.

The addition of microbial markers subsequently determined if the high levels of ammonia were likely the result of faecal pollution entering the stormwater as opposed to industrial sources of ammonia, while also discriminating between human and animal origins. The application of both culture-dependent and independent methods in our case study strengthened evidence for faecal pollution building on standard indices which are applied to determine safe levels of faecal contamination, while also providing additional human source discrimination. Strong correlations between *Bacteroides* spp. and faeces are well documented (Srinivasan et al., 2011) with these assays increasingly being applied to discriminate both point and non-point sources of faecal contamination. It is widely accepted by scientists and water managers that *E. coli* alone is no longer a sufficient faecal indicator bacterium (Tran et al., 2015). Other studies have also complemented *E. coli* assays with different molecular faecal indicators, such as pathogens or bacteriophages, however these lack robust host specificities when compared with *Bacteroides* spp. assays (Savichtcheva and Okabe, 2006). Another alternative approach applies genetic barcoding technologies to define microbial "fingerprints" of faecal pollution sources based on bacterial taxonomic identifications; host-specific fingerprints are then identified in recreational waters (McCarthy et al., 2017). Unfortunately, high genetic variability among and within bacteria and viruses reduce accurate species delineation (Ong et al., 2013). Furthermore, episodic contamination events can be hard to sample due to lengthy processing and analyses times.

5. Conclusion

Our work-flow utilised the advantages of each selected faecal indicator - i.e. time-integrative or passive sampling of ammonia as a chemical marker and high specificity of *Bacteroides* spp., in combination with standard faecal indicator bacteria to rapidly locate constant stream (cracked sewer pipe) and episodic (sewer spill) pollution from a large monitoring area in four months. Unlike other strategies, our approach does not require historic knowledge of faecal hotspots and through the novel implementation of both chemical and microbial faecal indicators in a multi-tiered approach, reduces redundant sampling by only targeting pollution events as they occur and consequently reduces on-ground investment in pollution abatement.

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Appendix A. Supplementary data

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