Application of denaturing gradient gel electrophoresis (DGGE) for assessing fecal pollution sources at a recreational beach

M. A. Esseili, I. I. Kassem, J. Lis and V. Sigler

ABSTRACT

We investigated the effectiveness of *Escherichia coli* community fingerprinting for identifying fecal pollution sources impacting a recreational beach. *E. coli* in water collected from the beach, nearby creek and storm sewer outfall were enumerated using membrane filtration, while *E. coli* communities were characterized following polymerase chain reaction analysis and denaturing gradient gel electrophoresis (DGGE) fingerprinting. Analysis of *E. coli* densities to determine the contributions of the creek and storm sewer during dry weather was inconclusive. However, DGGE fingerprinting indicated that the creek *E. coli* communities had a greater impact on the beach community composition (80–95% similarity), than on storm sewer communities (41–64%). Following rainfall events, *E. coli* communities in the creek were at least 93% similar to those at the beach, while the similarity of the outfall and beach communities varied from 72 to 96%. Furthermore, *E. coli* communities at the beach were more similar to creek communities than to storm sewer communities after the first 2 h and 48 h following the onset of rainfall, and of comparable similarity following 24 h of rainfall, suggesting transient contributions from the storm sewer. DGGE analysis of *E. coli* communities provided evidence that the creek was a consistent source of *E. coli* to the beach, while the storm sewer was a transient source.

Key words | community fingerprinting, DGGE, *E. coli*, fecal pollution, recreational water, storm water

M. A. Esseili
I. I. Kassem
V. Sigler
Ohio Agricultural Research and Development Center,
The Ohio State University, OH,
USA

J. Lis
Lorain County General Health District,
Elyria, OH,
USA

V. Sigler (corresponding author)
Department of Environmental Sciences,
University of Toledo, OH,
USA
E-mail: *von.sigler@utoledo.edu*

INTRODUCTION

Contact with polluted water can result in a number of illnesses ranging from skin rashes and eye infections to more serious gastrointestinal and respiratory diseases (Natural Resources Defense Council (NRDC) 2008). Furthermore, the number of beach advisories in the United States and the incidences of waterborne-disease outbreaks associated with recreational water use have been steadily increasing (Hilborn *et al.* 2013). This trend is exacerbated by expanding coastal urbanization (National Oceanic and Atmospheric Administration (NOAA) 2008), which results in increases in impervious surfaces (Paul & Meyer 2001), stress to recreational waters (Mallin *et al.* 2000) and runoff of pollution following rainfall events (Boehm *et al.* 2002; Ackerman & Weisberg 2003; Sercu *et al.* 2009). Polluted storm runoff accounts for half of all recreational water quality advisories issued at beaches in the USA (NRDC 2008) and can contain several pathogens that enter natural waterways either through storm sewer networks or via drainage ditches and streams (Olivieri 1977; Noble *et al.* 2003; Cardonha *et al.* 2004; Salmore *et al.* 2006). As a result, multiple epidemiological studies have linked human illness with primary contact with waters impacted by contaminated storm runoff (Calderon *et al.* 1991; Haile *et al.* 1999).

It is imperative to identify and mitigate sources of frequently occurring fecal pollution in order to limit the incidences of water-associated illnesses resulting from exposure to contaminated recreational water (Simpson *et al.* 2002). Numerous bacteria source-tracking methods

doi: 10.2166/wh.2014.034

have been proposed for identifying fecal pollution sources. In many cases, these methods focus on identifying the host origin of an indicator organism and require the construction of libraries that contain thousands of isolates and need regular updating (Simpson *et al.* 2002; Meays *et al.* 2004), which is time consuming and resource intensive. An alternative approach to identifying pollution sources is to determine, 'geographically', where fecal pollution originates. Community fingerprinting can facilitate this effort by effectively distinguishing assemblages of bacteria based on differences in whole communities of fecal indicator bacteria (not isolates) sampled from suspected sources and sinks (Farnleitner *et al.* 2000). For example, denaturing gradient gel electrophoresis (DGGE) analysis reproducibly differentiates complex microbial communities by separating polymerase chain reaction (PCR) amplicons that differ in DNA sequence by as little as one nucleotide (Muyzer *et al.* 1993). Previous studies have demonstrated the effectiveness of DGGE analysis to differentiate *Escherichia coli* communities in natural waters (Farnleitner *et al.* 2000; Sigler & Pasutti 2006). Further analysis identified a highly polymorphic *E. coli*-specific gene that is capable of differentiating *E. coli* communities from various hosts (Esseili *et al.* 2008). The optimized method was applied to show that geese and wastewater effluent contributed to fecal pollution of surface waters (Esseili *et al.* 2008). In addition, DGGE analysis also revealed that following off-field movement through drainage tiles, *E. coli* communities originating in land-applied biosolids were responsible for the contamination of the downstream surface (Esseili *et al.* 2012). In these studies, despite changing environmental conditions and temporal changes in bacteria density and community structure that complicate the process of pollution source identification, bacteria transport pathways and pollution sources were effectively characterized. Therefore, it follows that DGGE analysis could be a useful technique to identify pollution sources on a watershed scale.

In this study, DGGE analysis was used to characterize bacteria pollution occurring at Huntington Beach, located on Lake Erie in northern Ohio. During the past 6 years, the beach has been the subject of annual water quality advisories that impact the beach for an average of 15 days (17.5%) during the summer swim season (Nowcast 2012), and is ranked among Ohio's most polluted beaches with regard to water quality standard exceedance days (NRDC 2008). The consistency of advisories suggests that the beach is impacted by a persistent source(s) of fecal pollution. Two sources suspected of contributing pollution to the beach water quality include: (1) Porter Creek, which discharges immediately east of the beach; and (2) the outfall of the city storm sewer network, which discharges west of the beach. Therefore, based on (1) the established utility of community fingerprinting to differentiate *E. coli* communities originating from discrete sources, and (2) the need to identify source(s) of bacteria pollution to coastal areas, the overall objective of this study was to identify the geographic sources of fecal pollution sources to Huntington Beach.

MATERIALS AND METHODS

Study sites

Huntington Beach (41°29′28″ N 81°56′05″ W) is located in Cuyahoga County, Ohio on the southern shore of Lake Erie (Figure 1). The beach is approximately 0.4 km in length and is a destination for an estimated 243,000 visitors per year. Four sampling sites separated by approximately 100 m were chosen along the beach, representing the western end of the beach (Site A), middle sites (Sites B and C) and an easternmost site (Site D) (Figure 1). Porter Creek is a tributary to Lake Erie approximately 14 km in length that discharges immediately east of Huntington Beach. The land use of the immediate watershed around the creek (21.6 km^2) is mostly residential and naturalized, and includes some recreational and light industry areas. Porter Creek exhibits an average base flow of 780 L s^{-1} (Global Water FP101-FP201 Global Flow Probe). The outfall of the Bay Village storm sewer network is located 0.8 km west of Huntington Beach and drains land that is mostly residential in use with some light industry. The storm sewer network collectively drains an area of 18.4 km^2 and exhibits a base flow of 0.4 L s^{-1} during dry weather and 1.5 L s^{-1} following rainfall events.

Water sampling

Water sampling was performed during dry weather (following at least 72 h of no precipitation) and wet weather

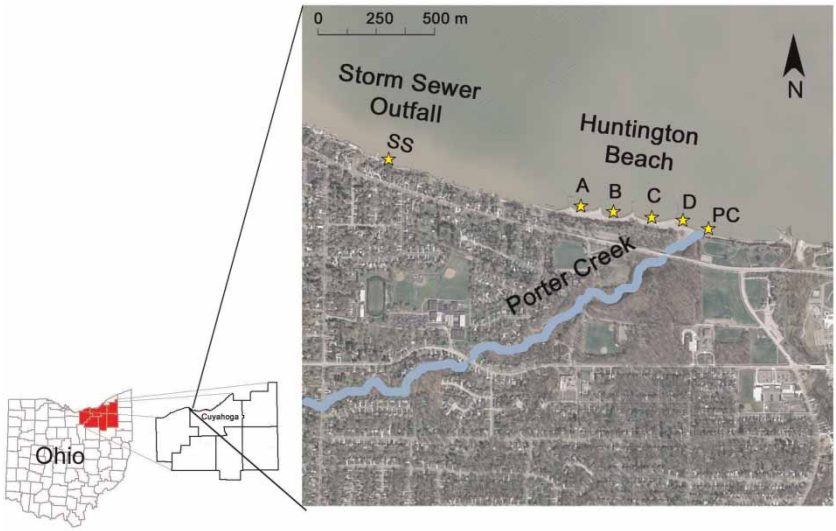

Figure 1 | Satellite photo of sampling locations at Cuyahoga County, OH including Huntington Beach (Sites A, B, C and D), Porter Creek (PC) and storm sewer outfall (SS).

conditions (described below), including four dry events (termed D1, D2, D3 and D4) and three wet weather events (W1, W2 and W3) that occurred over a 2-year period, during the swimming season (May to September). During the swimming season of the first year, D1, D2, W1 and W2 were sampled while D3, D4 and W3 were sampled during the second year. Specifically, samplings during events W1 and W2 were performed following low (16 h after ~4 mm of rain) and high (5 h after 23 mm of rain) volume rainfall events, respectively. To better understand if bacteria densities and communities from Porter Creek and the storm sewer change over the course of a rainfall event, a third wet weather event (W3) included multiple samplings performed 2, 24 and 48 h following the accumulation of 10 mm of rainfall. This threshold depth was chosen, as it represents the average rainfall depth occurring within a 24 h storm event during the swim season (data from www.ohionowcast.info).

Water samples from the beach and Porter Creek were obtained by inverting a sterile Nalgene bottle approximately 30 cm below the water surface in an area that was, on average, 0.7 m deep, while water samples were obtained from the storm sewer outfall by collecting water as it exited the outfall. Samples were collected in triplicate and maintained on ice until analysis was performed, which was always within 6 hours of collection. Hourly rainfall data were obtained from Cleveland-Hopkins International airport through the NOAA website (http://www.erh.noaa.gov/data/obhistory/KCLE.html).

DNA isolation and DGGE fingerprinting of *E. coli* communities

E. coli densities in each sample were determined by membrane filtration using EPA method 1603 (United States Environmental Protection Agency (USEPA) 2002). Briefly, approximately 500 ml of water (or diluted, as necessary) from each site was filtered onto a nitrocellulose membrane (0.45 μm pore size), which was transferred to Modified mTEC agar and incubated at 44.5 °C for 16 h. Colonies diagnostic for *E. coli* were counted and *E. coli* densities were expressed as colony forming units (CFU) 100 mL^{-1}. *E. coli* community DNA isolation, *E. coli*-specific PCR (using primers specific for *E. coli uidA* gene) and DGGE fingerprinting analysis were performed according to the method of Esseili *et al.* (2008). To facilitate inter- and intra-gel fingerprint comparisons, a previously developed DGGE marker (Esseili *et al.* 2008) was loaded such that no more than four fingerprint lanes separated each marker lane. All DGGE fingerprint images were analyzed using GelCompar II software (version 4.5, Applied Maths) for pairwise comparisons of fingerprint similarity. Normalization was performed using the DGGE marker as an external reference. The curve-based Pearson coefficient

function, which evaluates the position and intensity of the bands, in combination with 1% optimization and 1% position tolerance was used to generate a similarity matrix summarizing the relationships between fingerprints (McLellan *et al.* 2003). Cluster analysis was performed on the resulting similarity matrix using the unweighted pair group method with arithmetic means algorithm, resulting in dendrograms that graphically displayed relationships between *E. coli* community fingerprints (McLellan *et al.* 2003). Since DGGE community fingerprinting is a new approach for identifying fecal pollution sources, the minimum similarity value above which a suspected source is considered a polluting source, is not known. For this reason, an *E. coli* community obtained from an unsuspected non-indigenous source (horses) was used to generate a reference fingerprint against which all other fingerprints were compared. Specifically, fecal material was obtained from three horses (housed at the University of Findlay Equestrian Farm, Findlay, OH), suspended (1:10 W/V) in sterile, 1X phosphate buffered saline (1X), filtered through a nitrocellulose membrane and cultured as described above. PCR and DGGE analyses of the *E. coli* DNA were performed, as described above. The similarity of the reference community with all sampled *E. coli* communities never exceeded 75%. Therefore, a suspected source was considered a contributing source if its *E. coli* community fingerprints were >75% similar to those occurring at the beach.

Statistical analyses

GraphPad Prism v. 5 (GraphPad Software, USA) was used for statistical analyses. One-way analysis of variance followed by Tukey's post hoc test was used to determine significant differences in *E. coli* densities among beach sites, Porter Creek and the storm sewer outfall. Differences in means were considered significant when the *P*-value was <0.05. Data were expressed as mean ± standard deviation (SD). The integrity of the dendrogram organization (branching and structure of the resulting clusters) was validated by the calculation of cophenetic correlation coefficients with GelComparII software, which were always greater than 0.85 (maximum is 1.0).

RESULTS

Porter Creek is a persistent source of fecal pollution during dry weather conditions

Since water quality standards are based on fecal indicator bacteria densities, we monitored *E. coli* densities at our study sites. *E. coli* densities at Porter Creek and the storm sewer were variable and significantly different from each other during the four dry weather sampling events. *E. coli* densities in Porter Creek were between 480 ± 26 (event D2) and $1{,}115 \pm 227$ (event D3) CFU 100 mL^{-1} (Figure 2). *E. coli* densities in the storm sewer were also variable, ranging from 71 ± 40 (event D2) to $2{,}040 \pm 144$ (event D3) CFU 100 mL^{-1}. The four beach sites also exhibited variations in *E. coli* densities, from 7 ± 2 (site A) to 27 ± 2 (site D) CFU 100 mL^{-1} during sampling event D2, to a high of 660 ± 268 (site A) to 587 ± 107 (site D) during sampling event D3. *E. coli* densities in Porter Creek and the storm sewer were significantly higher than those observed at the proximal beach sites (Sites D and A, respectively); however, no clear trend in densities was observed that could conclusively identify Porter Creek or the storm sewer as a source of *E. coli* for the beach (Figure 2). *E. coli* community fingerprinting was used to better elucidate the contributions of the creek and sewer outfall to the beach. *E. coli* communities in Porter Creek were relatively dissimilar to those in the storm sewer (42 to 67% similar) (Table 1), indicating that each was partially impacted by differing on-land sources of *E. coli*. With the exception of Site A during event D2, the *E. coli* communities in Porter Creek were 80 to 95% similar to those at the beach, while the similarity of communities in the storm sewer outfall to those at the beach was between 41 and 64% (Table 1). Communities at the four beach sites were more similar to those from Porter Creek than to communities at the storm sewer outfall. Specifically, *E. coli* communities from Porter Creek were 92% (event D2) and 98% (event D4) similar to those from beach site D (Figure 3), and 82% (event D2) to 95% (event D1) similar to communities from proximal sites B, C and often site A (Figure 2). In contrast, *E. coli* communities from the storm sewer outfall were between 41% (event D1) and 65% (event D4) similar to communities from all other sites.

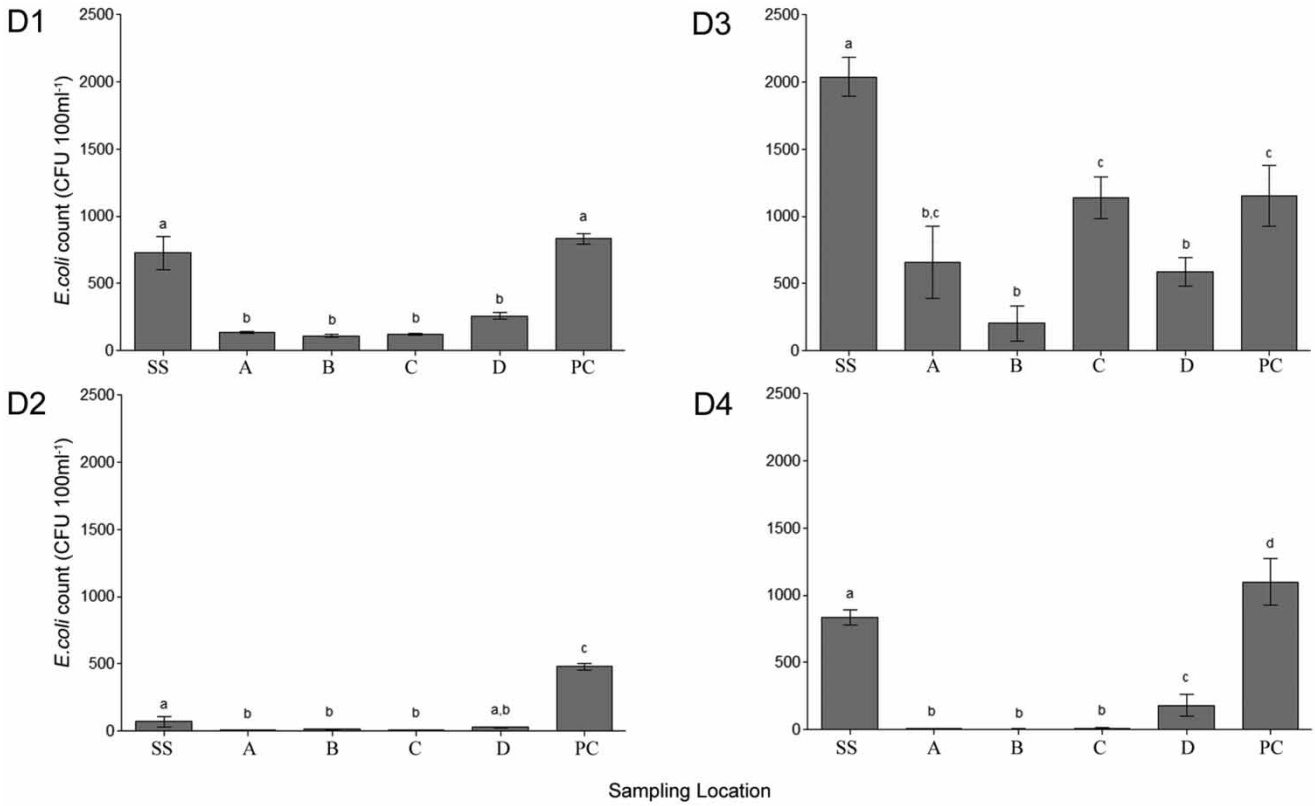

Figure 2 | *E. coli* densities (CFU 100 mL^{-1}) representing samples from the storm sewer outfall (SS), Huntington Beach (Sites A, B, C and D) and Porter Creek (PC) during four dry weather events (D1 to D4). Data are expressed as the mean density ± SD ($n = 3$). Means with different letters differ significantly ($P < 0.05$).

Table 1 | Community similarity values obtained following cluster analysis of fingerprints representing *E. coli* communities sampled from: Huntington Beach (HB), Porter Creek (PC) and storm sewer outfall (SS) during dry (D1, D2, D3 and D4) and wet (W1, W2 and W3) weather sampling events

Similarity (%)	D1	D2[a]	D3	D4	W1	W2[a]	W3 (2 h)	W3 (24 h)	W3 (48 h)
PC to HB	95	38	80	84	93	85	91	83	87
PC to SS	42	58	66	67	70	96	79	94	87
SS to HB	41	34	58	65	72	85	82	84	72

Legend: PC, Porter Creek; HB, Huntington Beach; SS, storm sewer.
[a]Eliminating Beach Site A from the comparisons resulted in a similarity between PC to HB and SS to HB of 82, 52 (D2) and 97 and 96% (W2), respectively.

Porter Creek is a persistent source of fecal pollution during wet weather conditions while storm sewer outfall is a transient source

Water samples were collected following two wet weather events representing low (W1) and high (W2) rainfall volumes. During event W1, *E. coli* densities in the storm sewer outfall and Porter Creek were significantly higher than those from the beach sites (Figure 4); however, the overall *E. coli* levels at all sites were within the range of values observed for dry weather events (125 ± 13 CFU 100 mL^{-1} at Site A to 114 ± 4 CFU 100 mL^{-1} at Site D). During event W2, *E. coli* densities at all beach sites (1,467 ± 251 CFU 100 mL^{-1} at Site A to 3,200 ± 519 CFU 100 mL^{-1} at Site D) exceeded the values observed during dry weather events mentioned above (Figure 4). *E. coli*

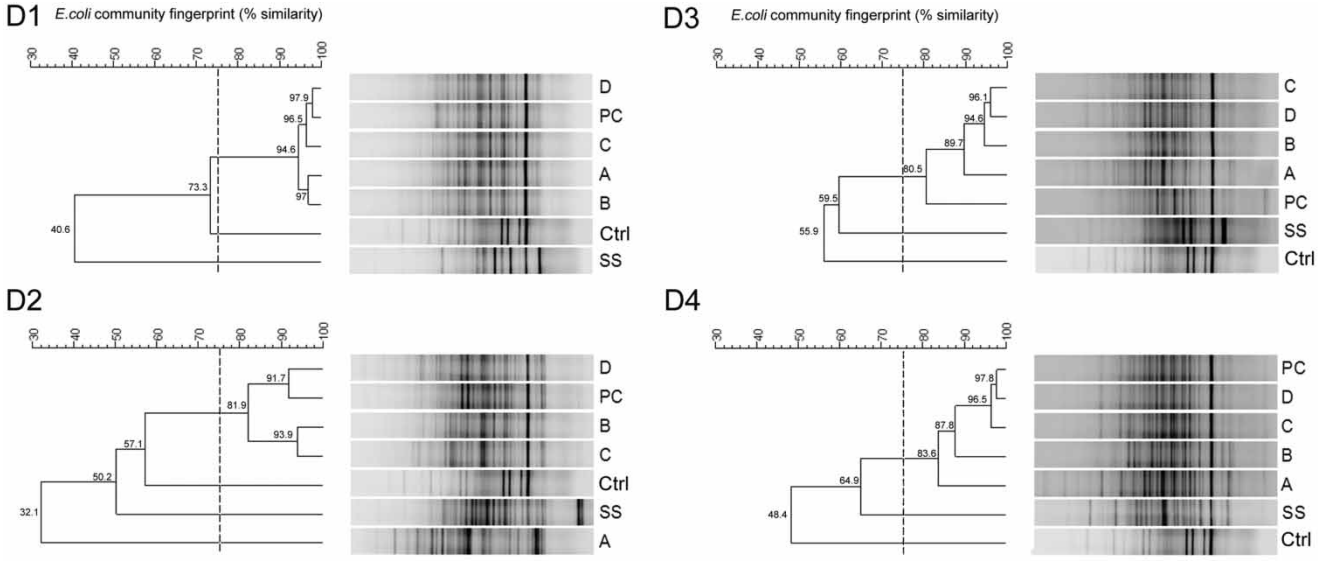

Figure 3 | Cluster analysis of *E. coli* community fingerprints representing samples from the storm sewer (SS), Huntington Beach (Sites A, B, C and D) and Porter Creek (PC) during dry weather events (D1, D2, D3 and D4). Dashed lines on the dendrograms represent the similarity cut-off value (75%) obtained following multiple comparisons with a control *E. coli* community fingerprint (Ctrl), as described in 'Materials and methods'.

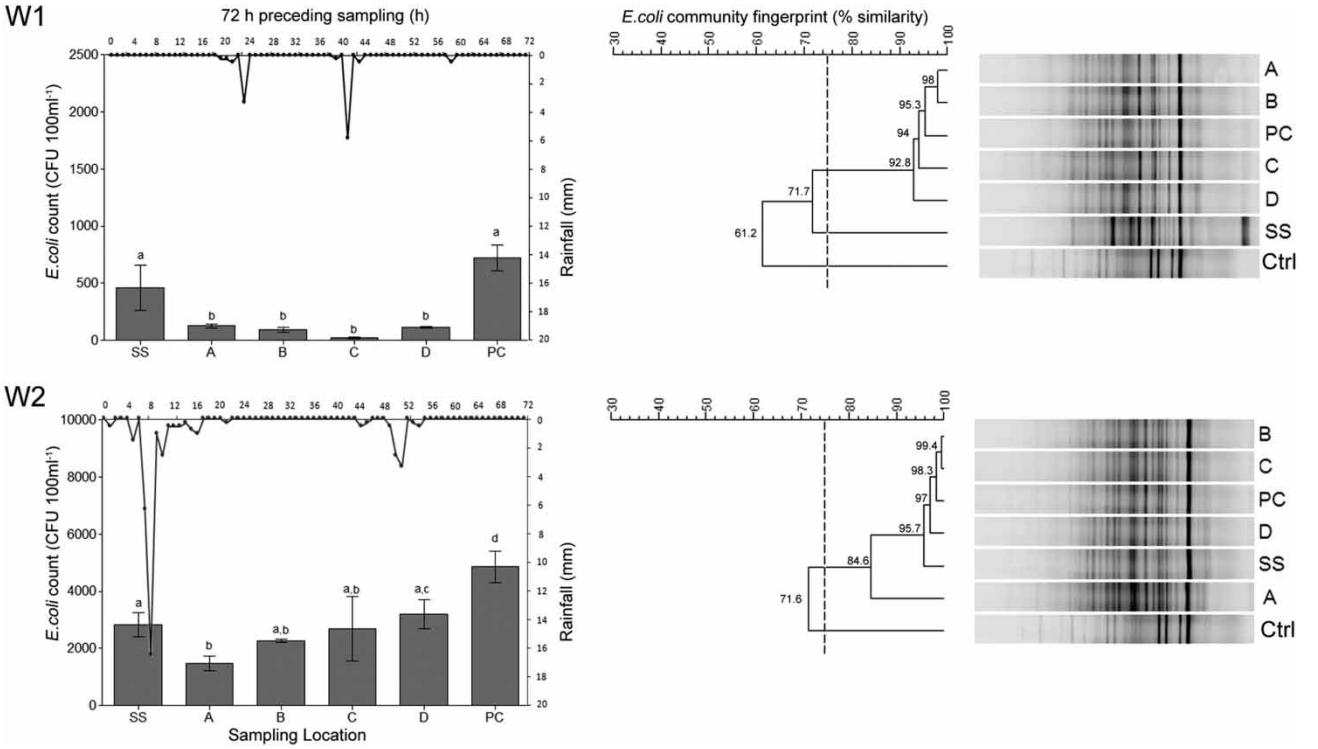

Figure 4 | *E. coli* densities (CFU 100 mL^{-1}) and cluster analysis of *E. coli* communities sampled at the storm sewer outfall, Huntington Beach (Sites A, B, C and D) and Porter Creek during two wet weather events (W1 and W2). The amount of hourly rainfall is shown on the second *y*-axis and the rainfall during the 72 h preceding the samplings is shown on the second *x*-axis. *E. coli* density data are expressed as the mean ± SD ($n = 3$). Means associated with bars labeled with different letters differ significantly ($P < 0.05$). Dashed lines on the dendrograms represent the similarity cut-off value (75%) obtained following multiple comparisons with a control *E. coli* community fingerprint (Ctrl) as described in Materials and methods'.

densities in the storm sewer outfall and Porter Creek were significantly higher than those found at the west and east beach sites (A and D), respectively.

During wet weather, the similarity of *E. coli* communities in Porter Creek to those in the storm sewer outfall was variable and dependent on the rainfall intensity. For example, during the relatively light rainfall event W1, *E. coli* communities from the beach were 93% similar to those in Porter Creek and 72% similar to those in the storm sewer outfall (Figure 4, W1). However, during the heavier event W2, *E. coli* communities from Porter Creek and the storm sewer outfall were 97 and 96% similar, respectively, to those at the beach (with exception of site A), suggesting a dual contribution of *E. coli* from both the creek and the storm sewer to the beach.

To better understand the contributions of *E. coli* in the creek and outfall to the beach pollution, a more detailed, temporal sampling was performed during a third wet weather event. After two hours of rainfall (10 mm), *E. coli* densities in Porter Creek ($63{,}000 \pm 7{,}089$ CFU $100\ mL^{-1}$) were significantly higher than those in the storm sewer ($23{,}500 \pm 2{,}000$ CFU $100\ mL^{-1}$), and both sites exhibited *E. coli* densities significantly higher than those at any beach site (Figure 5(a)). Throughout the next 24 hours, 13 mm of additional rainfall accumulated, and *E. coli* densities in both Porter Creek and the storm sewer outfall gradually decreased to levels similar to those observed during dry weather (Figures 5(b) and 5(c)). During this time, the dynamics of *E. coli* densities at the beach sites indicated the east-to-west movement of an *E. coli* front. Specifically, *E. coli* densities at site D (easternmost site) were at a maximum after 2 h, and then gradually decreased to levels observed in dry weather samples after 48 h. *E. coli* densities at beach sites A, B and C (west of site D) were initially low after 2 h of rainfall, but then peaked following 24 h and decreased to levels similar to those observed during dry weather after 48 h.

During the early stages of the rainfall event, *E. coli* communities at the beach sites exhibited greater similarity to those at Porter Creek than to communities at the storm sewer. However, both values were above the cut-off similarity value, suggesting that both sources played a role in polluting the beach. Specifically, 2 h after the accumulation of 10 mm of rainfall, *E. coli* communities in Porter Creek were 91% similar to those at the beach (99% similar to communities at Site D; Figure 5(a)), whereas communities in the storm sewer outfall were 82% similar to those at the beach (Table 1). After 24 h, the communities from the beach sites A and B, Porter Creek and the storm sewer were 92% similar (Figure 5(b)), and by 48 h, the similarity of all beach sites to Porter Creek and the storm sewer decreased to 87% and 72%, respectively (Figure 5(c) and Table 1). Communities in Porter Creek maintained high similarity with those in the nearest beach site (D, 90%) throughout the 48 h monitoring period.

DISCUSSION

Primary contact with recreational waters polluted with harmful bacteria is a risk to human health (Calderon *et al.* 1991; Haile *et al.* 1999). Reducing this risk can be accomplished through appropriate management that begins by understanding the geographic origin of the pollution and environmental conditions that drive pathogen movement (Dwight *et al.* 2002; Esseili *et al.* 2008). Huntington Beach has experienced frequent water quality advisories since monitoring began in 2006. Advisory days have occurred, on average, for 17% of the swimming season (Nowcast 2012), and in every case, the advisory resulted from *E. coli* densities that exceeded a regulatory threshold considered safe for primary contact with recreational water. The beach is located adjacent to the drainage of Porter Creek and a storm sewer outfall, both of which are suspected conduits for fecal pollution from the surrounding watershed during wet weather. While previous studies showed that the transport of fecal indicator bacteria to beaches increases during rainfall events, significant increases can also occur during dry weather (Stein & Tiefenthaler 2005; Sercu *et al.* 2009). In the current study, dry weather samplings were performed to (i) determine if fecal pollution from Porter Creek and the storm sewer outfall impacted the beach under prevailing dry conditions, and (ii) provide a baseline to which wet weather results could be compared. During dry conditions, we observed increases in *E. coli* densities at the beach concomitant with those at the creek and outfall, an observation that aligns with those of previous studies showing that high densities of fecal indicator bacteria at creeks

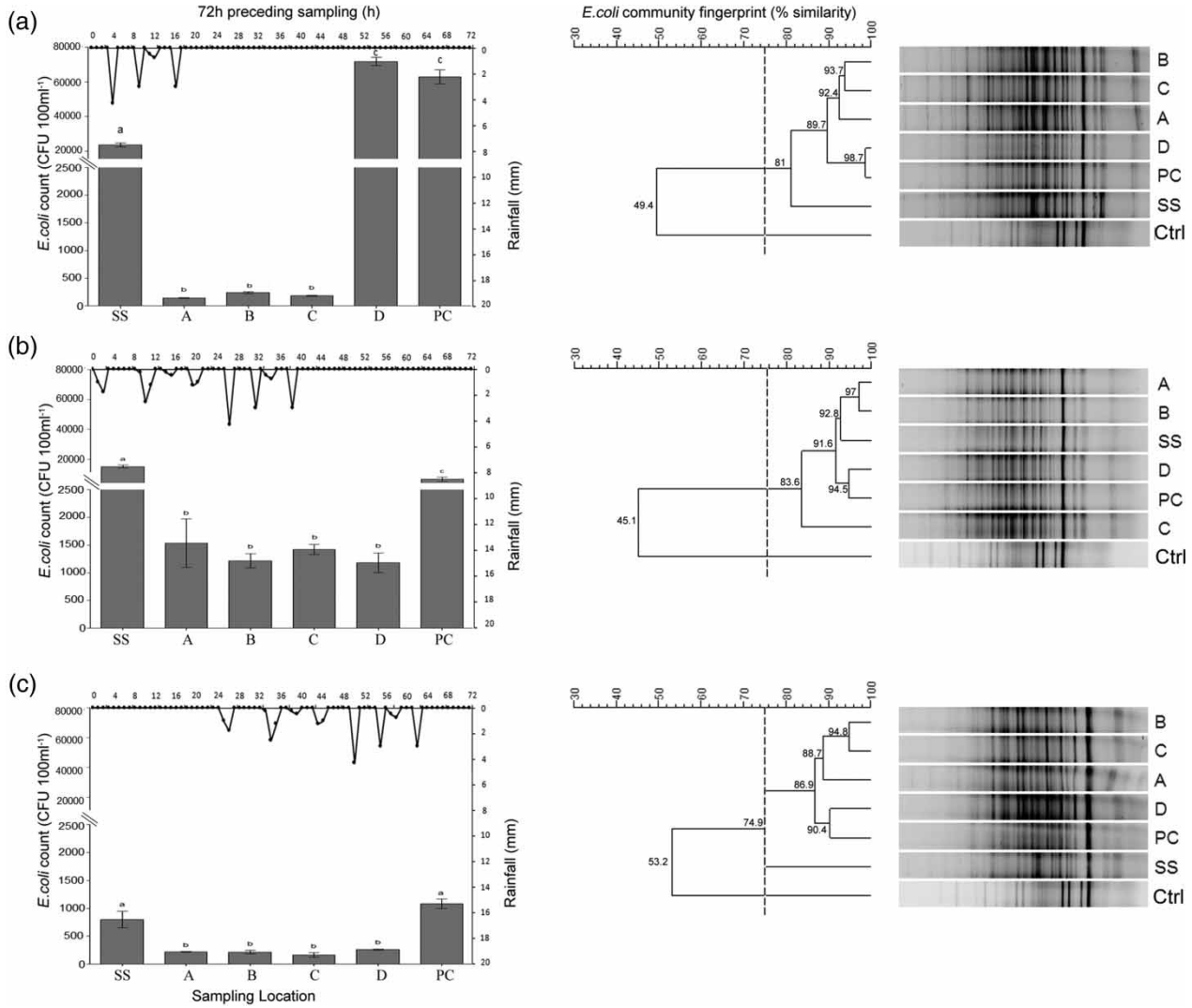

Figure 5 | *E. coli* densities (CFU 100 mL^{-1}) and cluster analysis of *E. coli* community fingerprints at the storm sewer outfall, Huntington Beach (Sites A, B, C and D) and Porter Creek during wet weather sampling events (W3). Samplings were performed at (a) 2, (b) 24 and (c) 48 h following the accumulation of 10 mm rainfall. The amount of hourly rainfall is shown on the second *y*-axis and the rainfall during the 72 h preceding the samplings is shown on the second *x*-axis. *E. coli* density data are expressed as the mean ± SD ($n = 3$). Means associated with bars labeled with different letters differ significantly ($P < 0.05$). Dashed lines on the dendrograms represent the similarity cut-off value (75%) obtained following multiple comparisons with a control *E. coli* community fingerprint (Ctrl) as described in 'Materials and methods'.

and rivers have been shown to influence bacteria densities at adjacent beaches (Whitman *et al.* 1995; Olyphant *et al.* 2003) and subsequently, human health (Ihekweazu *et al.* 2006). However, no clear pattern in *E. coli* densities was observed that identified Porter Creek or the storm sewer outfall as a dominant contributor of *E. coli* to the beach. While elevated *E. coli* densities at the creek and outfall during dry weather suggest their role as *E. coli* sources for the beach, enumeration analyses alone cannot reliably connect *E. coli* pollution from sinks to suspected sources. Consequently, we used genetic fingerprinting analysis of *E. coli* communities to compare communities at the beach to those at the storm sewer outfall and Porter Creek, under the assumption that increased similarity between communities from a suspected source and the beach was evidence of an *E. coli* contribution from the source. *E. coli* communities at Porter Creek consistently exhibited high similarity to those from the closest beach site (Site D, Figure 3) indicating a strong local effect of the creek. It is also noteworthy that *E. coli* communities from Porter Creek also impacted

more distant beach sites, as the creek communities often exhibited similarity with those from beach sites A, B and C. In contrast, *E. coli* communities from the storm sewer were consistently less similar (below the 75% threshold value) to those at the beach during all dry weather events. Taken together, our results indicate that Porter Creek represented a persistent source of fecal pollution to the beach during dry weather events, while contributions from the outfall were sporadic. Our results also explain why previous observations indicated significantly higher *E. coli* densities at Huntington Beach during dry weather and westward lake currents, (Francy 2003), as water movement and wave action will impact *E. coli* transport (Ge & Frick 2009, Ge *et al*. 2012), and at Huntington Beach westward currents will drive the movement of *E. coli* from Porter Creek toward the beach.

During and following rainfall events, surface runoff drives the transport of *E. coli* (Matty *et al*. 1987; Ramos *et al*. 2006), often resulting in increased bacteria densities in offsite waters (Doran & Linn 1979; Selvakumar & Borst 2006; Brooks *et al*. 2009) and at beaches near impacted streams or storm outfalls (Ackerman & Weisberg 2003; Haack *et al*. 2003; Noble *et al*. 2003; Nevers *et al*. 2007; He & He 2008). At Huntington Beach, the occurrence of bacteria pollution is positively correlated with rainfall in the previous 24 h to the extent that the precipitation measure is used in a predictive model to predict water quality at the beach (Francy & Darner 2007). We expanded on those findings by using genetic fingerprinting to identify from where, geographically, the *E. coli* communities originated. *E. coli* communities in Porter Creek were consistently similar to those at the beach during rainfall events, regardless of rainfall intensity. In contrast, it was only during/following the more intense rainfall events (e.g. W2, and W3 at 2 and 24 h) that genetic evidence supported the connection between *E. coli* in the sewer outfall and pollution at the beach. To better characterize the dynamics of bacteria movement and the contributions from differing sources, we assessed the *E. coli* community structures in the creek, outfall and beach at three time-points during a rainfall event. *E. coli* densities in the creek and outfall were high (relative to dry weather densities) at the outset of the rain event, consistent with a 'first flush' phenomenon, and then decreased as the storm progressed. This pattern has been observed previously, as fecal bacteria can accumulate in storm drains and river sediments during dry weather, and then become re-suspended and transported to coastal waters during the early stages of storm events (Brownell *et al*. 2007). For example, Haack *et al*. (2003) observed increased *E. coli* density at a beach, nearby river and storm outfall within 5 h following the beginning of a storm event, while Nevers *et al*. (2007) reported that *E. coli* densities at a beach and two nearby creeks increased for 24 h following a rainfall event. Genetic fingerprinting analysis expanded on our enumeration results by providing evidence that the contributions of the creek and outfall communities changed throughout the rainfall event. *E. coli* communities from both sources exhibited their highest similarity to those at Huntington Beach during the early stages (2–24 h) following the onset of rainfall. The similarity between communities at the storm sewer and beach decreased throughout the remaining 24 h (48 h total), while *E. coli* communities at Porter Creek maintained high similarity to those at the beach. These results confirm those of previous studies that showed *E. coli* densities can change rapidly (Converse *et al*. 2011) and originate from multiple sources (Wicki *et al*. 2011) throughout a single rainfall event. Since the most cost-effective measure to reduce frequently occurring fecal pollution is to identify and limit the pollution at its source (Simpson *et al*. 2002), the implications of these findings to resource managers attempting to identify sources of pollutions in recreational waters are numerous. For example, the timing and frequency of most beach monitoring activities do not currently account for pollution sources that change within the duration of a single rainfall event. This issue was illustrated in the current study during event W3, as our genetic fingerprinting evidence connected *E. coli* communities at the storm sewer outfall with those at the beach during the first 24 h following the onset of rainfall, while afterwards, the apparent contribution of the outfall decreased. This example illustrates (i) how *E. coli* dynamics can impact the identification of a source, and (ii) how multiple samplings might be necessary during and following rainfall events to accurately assign a source to a sink.

Using genetic fingerprinting analyses to assign fecal pollution sources to a contaminated sink is often challenging since (i) fecal indicator bacteria at the sink can exhibit genetic diversity over space and time, (ii) the relative

contribution of fecal pollution sources can change throughout time, as described above, and (iii) no standard fingerprinting method or threshold similarity value has been agreed upon to match a source bacteria community to one at a sink. DGGE analysis of *E. coli* communities using *uidA* can facilitate the identification of pollution sources by differentiating communities occurring in potential sources and comparing them to those present in a polluted sink (Sigler & Pasutti 2006; Esseili *et al.* 2008). By fingerprinting the entire *E. coli* community, DGGE analysis takes advantage of the genetic diversity contained among unique *E. coli* strains that comprise a community to generate a descriptive, discrete fingerprint defining each site. At the same time, community fingerprinting avoids much of the cost and time associated with developing extensive isolate libraries. Despite its advantages, the results of DGGE analysis must be considered in the light of two methodological limitations, including biases associated with PCR amplification of complex mixtures of DNA (Suzuki & Giovannoni 1996; von Wintzingerode *et al.* 1997), and the comigration of bands of different DNA sequence but similar melting behavior (Nübel *et al.* 1996). Although we recognize these limitations, the intended use of DGGE analysis in the this study is exclusive of these two limitations, as we used the analysis as a comparative method by which *E. coli* communities could be compared, while providing a strong complement to culture-dependent, enumeration analyses.

CONCLUSION

The results of this study support storm water management practices aimed at alleviating the effect of urban runoff on recreational waters at Huntington Beach. By combining enumeration and genetic fingerprinting analyses, Porter Creek was identified as a persistent source of *E. coli* to Huntington Beach during dry and wet weather, while the contribution of the storm sewer outfall was transient and occurred only shortly following relatively high intensity rainfall events. Furthermore, our results strongly suggested that Porter Creek and the storm sewer network are each impacted by overland runoff from common sources within the watershed, as the *E. coli* community similarity in the creek and outfall increased during rainfall events. Because the community fingerprinting approach facilitates the identification of the pollution hot spots impacting the watershed, subsequent research efforts should aim to identify potential inland sources of pollution that contribute to the *E. coli* load in the creek and storm sewer network, and ultimately to the beach.

ACKNOWLEDGEMENTS

We would like to thank Steve Hiltner, Kawthar Esseili and Meesha Kaw for their help in water filtration and *E. coli* enumeration. This work was supported by the Cuyahoga County Board of Health, the Ohio Lake Erie Commission's Lake Erie Protection Fund Small Grants Program (grant number LEPF SG 338-08) and the United States Department of Agriculture Cooperative State Research, Education, and Extension Service (grant number 2008-38898-19239).

REFERENCES

Ackerman, D. & Weisberg, S. B. 2003 Relationship between rainfall and beach bacterial concentrations on Santa Monica bay beaches. *J. Water Health* **1**, 85–89.

Boehm, A. B., Grant, S. B., Kim, J. H., Mowbray, S. L., McGee, C. D., Clark, C. D., Foley, D. M. & Wellman, D. E. 2002 Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environ. Sci. Technol.* **36**, 3885–3892.

Brooks, J. P., Adeli, A., Read, J. J. & McLaughlin, M. R. 2009 Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. *J. Environ. Qual.* **38**, 218–229.

Brownell, M. J., Harwood, V. J., Kurz, R. C., McQuaig, S. M., Lukasik, J. & Scott, T. M. 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 Res.* **41**, 3747–3757.

Calderon, R. L., Mood, E. W. & Dufour, A. P. 1991 Health effects of swimmers and nonpoint sources of contaminated water. *Int. J. Environ. Health Res.* **1**, 21–26.

Cardonha, A. M., Vieira, R. H., Rodrigues, D. P., Macrae, A., Peirano, G. & Teophilo, G. N. 2004 Fecal pollution in water from storm sewers and adjacent seashores in Natal, Rio Grande do Norte, Brazil. *Int. Microbiol.* **7**, 213–218.

Converse, R. R., Piehler, M. F. & Noble, R. T. 2011 Contrasts in concentrations and loads of conventional and alternative

indicators of fecal contamination in coastal stormwater. *Water Res.* **45**, 5229–5240.

Doran, J. W. & Linn, D. M. 1979 Bacteriological quality of runoff water from pastureland. *Appl. Environ. Microbiol.* **37**, 985–991.

Dwight, R. H., Semenza, J. C., Baker, D. B. & Olson, B. H. 2002 Association of urban runoff with coastal water quality in Orange County, California. *Water Environ. Res.* **74**, 82–90.

Esseili, M. A., Kassem, I. I. & Sigler, V. 2008 Optimization of DGGE community fingerprinting for characterizing *Escherichia coli* communities associated with fecal pollution. *Water Res.* **42**, 4467–4476.

Esseili, M. A., Kassem, I. I., Sigler, V., Czajkowski, K. & Ames, A. 2012 Genetic evidence for the offsite transport of E. coli associated with land application of Class B biosolids on agricultural fields. *Sci. Total Environ.* **433**, 273–280.

Farnleitner, A. H., Kreuzinger, N., Kavka, G. G., Grillenberger, S., Rath, J. & Mach, R. L. 2000 Simultaneous detection and differentiation of *Escherichia coli* populations from environmental freshwaters by means of sequence variations in a fragment of the beta-D-glucuronidase gene. *Appl. Environ. Microbiol.* **66**, 1340–1346.

Francy, D. S. 2003 Escherichia coli at Ohio Bathing Beaches-Distribution, Sources, Wastewater Indicators, and Predictive Modeling. US Geological Survey Scientific Investigations Report. http://oh.water.usgs.gov/reports/wrir/wrir02-4285.pdf.

Francy, D. S. & Darner, R. A. 2007 Nowcasting beach advisories at Ohio Lake Erie beaches. US Geological Survey Open-File Report 2007-1427. http://pubs.usgs.gov/of/2007/1427.

Ge, Z. & Frick, W. E. 2009 Time-frequency analysis of beach bacteria variations and its implication for recreational water quality modeling. *Environ. Sci. Technol.* **43**, 1128–1133.

Ge, Z., Whitman, R. L., Nevers, M. B. & Phanikumar, M. S. 2012 Wave-induced mass transport affects daily *Escherichia coli* fluctuations in nearshore water. *Environ. Sci. Technol.* **46**, 2204–2211.

Haack, S. K., Fogarty, L. R. & Wright, C. 2003 *Escherichia coli* and enterococci at beaches in the Grand Traverse Bay, Lake Michigan: sources, characteristics, and environmental pathways. *Environ. Sci. Technol.* **37**, 3275–3282.

Haile, R. W., Witte, J. S., Gold, M., Cressey, R., McGee, C., Millikan, R. C., Glasser, A., Harawa, N., Ervin, C., Harmon, P., Harper, J., Dermand, J., Alamillo, J., Barrett, K., Nides, M. & Wang, G. 1999 The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* **10**, 355–363.

He, L. M. & He, Z. L. 2008 Water quality prediction of marine recreational beaches receiving watershed baseflow and stormwater runoff in southern California, USA. *Water Res.* **42**, 2563–2573.

Hilborn, E. D., Wade, T. J., Hicks, L., Garrison, L., Carpenter, J., Adam, E., Mull, B., Yoder, J., Roberts, V. & Gargano, J. W. 2013 Surveillance for waterborne disease outbreaks associated with drinking water and other nonrecreational water – United States, 2009–2010. *MMWR-Morbidity and Mortality Weekly Report* **62**, 714–720.

Ihekweazu, C., Barlow, M., Roberts, S., Christensen, H., Guttridge, B., Lewis, D. & Paynter, S. 2006 Outbreak of *E. coli* O157 infection in the south west of the UK: risks from streams crossing seaside beaches. *Euro. Surveill.* **11**, 128–130.

Mallin, M. A., Williams, K. E., Esham, E. C. & Lowe, R. P. 2000 Effect of human development on bacteriological water quality in coastal watersheds. *Ecol. Appl.* **10**, 1047–1056.

Matty, J. M., Anderson, J. B. & Dunbar, R. B. 1987 Suspended sediment transport sedimentation and resuspension in Lake Houston Texas USA: Implications for water quality. *Environ. Geol.* **10**, 175–186.

McLellan, S. L., Daniels, A. D. & Salmore, A. K. 2003 Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA fingerprinting. *Appl. Environ. Microbiol.* **69**, 2587–2594.

Meays, C. L., Broersma, K., Nordin, R. & Mazumder, A. 2004 Source tracking fecal bacteria in water: a critical review of current methods. *J. Environ. Manage.* **73**, 71–79.

Muyzer, G., de Waal, E. C. & Uitterlinden, A. G. 1993 Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **59**, 695–700.

Nevers, M. B., Whitman, R. L., Frick, W. E. & Ge, Z. 2007 Interaction and influence of two creeks on *Escherichia coli* concentrations of nearby beaches: exploration of predictability and mechanisms. *J. Environ. Qual.* **36**, 1338–1345.

NOAA 2008 National Ocean Service, Population Trends Along the Coastal United States: 1980–2008. http://www.oceanservice.noaa.gov.

Noble, R. T., Weisberg, S. B., Leecaster, M. K., McGee, C. D., Dorsey, J. H., Vainik, P. & Orozco-Borbon, V. 2003 Storm effects on regional beach water quality along the southern California shoreline. *J. Water Health* **1**, 23–31.

Nowcast 2012 Nowcasting Beach Advisories (Ohio Nowcast for Huntington Beach). http://www.ohionowcast.info/nowcast_huntington.asp.

NRDC 2008 Testing the Waters 2008: A Guide to Water Quality at Vacation Beaches. Natural Resources Defense Council. http://www.nrdc.org/water/oceans/ttw/titinx.asp.

Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W. & Backhaus, H. 1996 Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *J. Bacteriol.* **178**, 5636–5643.

Olivieri, V.P. 1977 United States Environmental Protection Agency, Municipal Environmental Research Laboratory. *Microorganisms in Urban Stormwater*. Municipal Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH.

Olyphant, G. A., Thomas, J., Whitman, R. L. & Harper, D. 2003 Characterization and statistical modeling of bacterial (*Escherichia coli*) outflows from watersheds that discharge into southern Lake Michigan. *Environ. Monit. Assess.* **81**, 289–300.

Paul, M. J. & Meyer, J. L. 2001 Streams in the urban landscape. *Annu. Rev. Ecol. Syst.* **32**, 333–365.

Ramos, M. C., Quinton, J. N. & Tyrrel, S. F. 2006 Effects of cattle manure on erosion rates and runoff water pollution by faecal coliforms. *J. Environ. Manage.* **78**, 97–101.

Salmore, A. K., Hollis, E. J. & McLellan, S. L. 2006 Delineation of a chemical and biological signature for stormwater pollution in an urban river. *J. Water Health* **4**, 247–262.

Selvakumar, A. & Borst, M. 2006 Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *J. Water Health* **4**, 109–124.

Sercu, B., Van De Werfhorst, L. C., Murray, J. & Holden, P. A. 2009 Storm drains are sources of human fecal pollution during dry weather in three urban southern California watersheds. *Environ. Sci. Technol.* **43**, 293–298.

Sigler, V. & Pasutti, L. 2006 Evaluation of denaturing gradient gel electrophoresis to differentiate *Escherichia coli* populations in secondary environments. *Environ. Microbiol.* **8**, 1703–1711.

Simpson, J. M., Santo Domingo, J. W. & Reasoner, D. J. 2002 Microbial source tracking: state of the science. *Environ. Sci. Technol.* **36**, 5279–5288.

Stein, E. D. & Tiefenthaler, L. L. 2005 Dry-weather metals and bacteria loading in an arid, urban watershed: Ballona Creek, California. *Water Air Soil Pollut.* **164**, 367–382.

Suzuki, M. T. & Giovannoni, S. J. 1996 Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol.* **62**, 625–630.

USEPA 2002 *Environmental Protection Agency Method 1603: Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). EPA-821-R-02-023. Office of Water, Washington, DC.

von Wintzingerode, F., Göbel, U. B. & Stackebrandt, E. 1997 Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* **21**, 213–229.

Whitman, R. L., Gochee, A. V., Dustman, W. A. & Kennedy, K. J. 1995 Use of coliform bacteria in assessing human sewage contamination. *Nat. Areas J.* **15**, 227–233.

Wicki, M., Karabulut, F., Auckenthaler, A., Felleisen, R., Tanner, M. & Baumgartner, A. 2011 Identification of fecal input sites in spring water by selection and genotyping of multiresistant *Escherichia coli*. *Appl. Environ. Microbiol.* **77**, 8427–8433.

First received 21 January 2014; accepted in revised form 4 May 2014. Available online 7 June 2014