Final report for the Lake Erie Protection Fund Small Grants Program

Tracking Fecal Pollution at Recreational Beaches (SG 338-08)

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ABSTRACT

The main objective of this study is to investigate the role of (i) a storm sewer outfall, and (ii) Porter Creek as two geographic fecal pollution sources in the impaired water quality at Huntington Beach (Bay Village, OH). Following two rainfall events and two spells of dry weather between August and November of 2008, water samples (n = 90) were collected from among two adjacent storm sewer outfalls and from three locations in Porter Creek, four locations along Huntington Beach and a control site along Cahoon Creek. Samples were processed for *E. coli* enumeration and *E. coli* community fingerprinting. During dry and wet weather conditions *E. coli* densities were highest in Porter Creek (1200 to 63000 CFU 100 ml^{-1}) as compared to storm sewer outfalls (1000 to 23000 CFU 100 ml^{-1}) or beach (600 to 1500 CFU 100 ml^{-1} respectively). Interestingly, *E. coli* densities in the east end of the beach were consistently higher than those in the west end (ranging from 650 to 71000 CFU 100 ml^{-1}). Cluster analysis using *E. coli* community fingerprinting revealed an association between Porter Creek and beach during both dry and wet events while the storm sewer outfall appeared to impact the beach primarily during wet weather events. Overall, this research provided evidence based on traditional and molecular microbiology that a major contributor to microbial water quality issues at Huntington Beach is Porter Creek, while pollution from the storm sewer network appears to be a transient factor. Based on this research, future efforts should focus on the identification of inland runoff hotspots that ultimately impact Huntington Beach by contributing contamination to Porter Creek and the storm sewer network.

1. Introduction

The problem in brief: Huntington Beach (Bay Village, OH) is extensively monitored for *Escherichia coli* densities during the swim season in an effort to limit the exposure of swimmers to harmful bacteria. As a result of these efforts, Huntington Beach was posted with swimming advisories for 24 out of the 92 days (26%) in the 2006 swim season, which placed the beach third among all Tier I beaches in Ohio in percentage of exceedance days. In 2007, the beach was posted for 18 out of the 100 days (18%). The origins of the *E. coli* are unknown. These statistics not only suggest that a continual loading of fecal waste is occurring near the beach, they also are indicative of a greater problem occurring nationally; the degradation of recreational water quality resulting from fecal pollution. Persistent fecal pollution of recreational waters is an established public health hazard, as pollution introduced through animal or human waste can contain pathogenic bacteria, viruses and protozoa. In particular, bacterial contamination is responsible for over 14% of the impaired waterways recognized by the USEPA (USEPA, 2004a) and is the most common reason for an "impaired" classification of Ohio's waterways. The USEPA estimates that over 5,000 impairments nationwide are attributed to microbial pathogens (approximately 24% of all impairments) reported under the national 303(d) total maximum daily load (TMDL) program. New technologies for microbial source tracking are continually emerging. However, these methodologies are often limited to identifying the host organism(s) from which the microbial pollution (i.e. *E. coli*) originated. Identifying the geographic origins of microbial pollution is also important, as understanding from where in the watershed the pollution originates can have a significant impact remediation strategies. The continual input of fecal pollution to Huntington Beach demonstrates the need for alternative methods to identify pollution sources and facilitate the development of pollution mitigation strategies. In 2004, we established collaboration with the CCBH in an effort to identify geographic sources of bacterial pollution in the Rocky River Watershed that impact water quality at Huntington Beach. Preliminary data revealed that two potential sources of fecal contamination (using *E. coli* as an indicator organism) were a stormwater outfall and Porter Creek, both of which enter Lake Erie near Huntington Beach (data not shown). Therefore, the main objective of this study is to investigate the role of (i) the storm sewer network, the outfall of which enters Lake Erie approximately 800 meters west of the beach, and (ii) Porter Creek, which drains the local watershed and empties into Lake Erie immediately east of the beach, as two geographic fecal pollution runoff that might contribute to impaired water quality at Huntington Beach.

In 2006, the number of closing and advisory days at ocean, bay, and Great Lakes beaches topped 25,000, which is more than ever recorded in past 17 years (NRDC, 2007). The overwhelming majority of these days resulted from monitoring that revealed high levels of fecal bacteria. Approximately 40% of all beach impairments resulted from pollution associated with storm sewer overflow and overland runoff, while over 50% of the closing and advisory days were attributed to unknown bacteria sources (USEPA, 2003; NRDC, 2006). Runoff likely drives the occurrence of bacteria pollution at Huntington Beach, as the densities of fecal indicator bacteria have been shown to increase following runoff-producing rainfall events (data not shown). It is clear that efforts to decrease the number of beach closings per season will enhance not only coastal economies, but also public awareness of environmental improvements in our nation's recreational waterways.

The most cost-effective measure to reduce frequently occurring fecal pollution is to identify and mitigate the pollution at its source (Simpson et al., 2002). However, it is difficult, if not impossible, to rapidly identify and track the variety of pathogens associated with the pollution. Therefore, indicator bacteria such as *Escherichia coli* and *Enterococcus spp.* are typically used to detect the presence of fecal pollution (Santo Domingo and Sadowsky, 2007, Dufour, 1977; USEPA, 1986). For example, in Ohio, beach advisories are posted when *E. coli* densities exceed 235 colonies per 100 ml. By monitoring *E. coli* abundance at Huntington Beach, CCBH officials routinely identify impaired waters and subsequently make decisions based on potential public health risks.

We aimed to couple storm- and natural water drainage with beach pollution by collecting water from Huntington Beach as well as the storm sewer outfall and Porter Creek, locations that before/during/following rainfall events were known to generate runoff that enters the Lake Erie near Huntington Beach. Densities of *E. coli* were determined for all sampling sites during wet and dry weather conditions. In addition, genetic fingerprinting was used to characterize differences in *E. coli* communities originating from distinct geographic locations. Computer analysis of the fingerprints was then used to associate *E. coli* communities originating from the storm sewer outfall and Porter Creek (likely pollution sources) and the beach (impacted sink).

2. Methods

Sampling and site description: Several sampling sites are already established based on previous work performed in collaboration with CCBH, including four sites across Huntington Beach, three sites along Porter Creek (East of the beach), two storm sewer outfalls (west of the beach), and a control site along Cahoon Creek (Fig.1), which is out of the influence of Porter Creek and storm sewer outfall.

Sampling Site	Sample Code
Huntington Beach West	A
Huntington Beach Central	B
Huntington Beach East	C
Huntington Beach east at Porter Creek	D
Porter Creek at mouth	PC1
Porter Creek at Huntington sled Hill	PC2
Porter Creek at Lake Erie Nature and Science center	PC3
29800 Lake Rd. West Outfall	SS1
29800 Lake Rd. East Outfall	SS2
Cahoon creek at lake road	Ctrl

Fig 1. Map of the study area and codes assigned to the sampling sites investigated in this study.

Water samples were collected between August and November 2008 during dry (following at least one week of no precipitation) and rainfall events (whenever rainfall totals were expected to be greater than 1.5 cm of rainfall in a 24 hour period). During rainfall events, water samples were collected following 1-5 h, 15-24 h and 48 h of the beginning of rainfall.

Two liters of water from each site were collected by inverting sterile Nalgene bottles approximately 30 cm below the water surface. Collection of water from storm sewer outfall was performed by capturing water as it exited the storm sewer outfall. Samples were maintained on ice or at 4^{o} C until analysis was performed (within 24 hours of collection).

***E. coli* enumeration:** The *E. coli* density in each water sample was determined following membrane filtration. Specifically, water was serially diluted and filtered (100 ml aliquots) onto a 0.45 µm pore-size polycarbonate filter followed by incubation of the filter on modified mTEC for 24 h at 44.5º C. *E. coli* were enumerated by counting colonies displaying *E. coli*-characteristic morphology (purple coloration) on filters exhibiting between 10 and 100 colonies.

DNA isolation and polymerase chain reaction (PCR): By filtering several hundred ml of water, the above filtration protocol was used to produce membranes containing a highly concentrated lawn of *E. coli* representative of the *E. coli* population at each site. DNA isolation was performed by the method of bead-beating and chloroform/phenol purification to generate community DNA. DNA was quantified with spectrophotometry and stored at -20^{o} C until PCR analysis. Two separate PCR analyses were performed to target *uidA* and *mdh*, two genes that are mostly specific to *E. coli*. Agarose gel electrophoresis was performed to compare the size of the resulting PCR product with a DNA size ladder to confirm that the proper PCR product was generated.

Generating *E. coli* community fingerprints with denaturing gradient gel electrophoresis (DGGE): DGGE analysis of the PCR products from each water sample was performed according to the method of Esseili *et al.* (2008). Fingerprint images were documented using a Kodak Gel Logic 200 image analysis system. All fingerprint images were imported to GelCompar II software (version 4.5, Applied Maths) for analysis of fingerprint similarity using the Dice band-based coincidence index. Cluster analysis was performed using the unweighted pair group method with arithmetic means (UPGMA) algorithm, resulting in dendrograms that graphically compare relationships among the fingerprints.

3. Results

A- Dry Weather samplings:

E. coli densities were variable depending on location, but were higher in Porter Creek samples than in storm sewer outfall or beach water samples (Figure 2). The densities of *E. coli* in these three general areas ranged on average from 50-650 (beach), 1200-1300 (Porter creek), 450 -1000 (Strom sewers) and 400-600 (control site) CFU 100 ml^{-1} (Fig. 2).

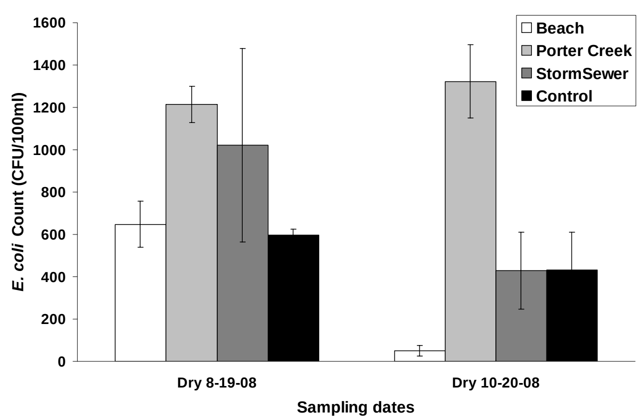

Fig 2: *E. coli* densities in water collected during (A) Aug 19 and (B) Oct 20, 2008 dry weather conditions.

DGGE analysis of *uidA* revealed that each site harbored a complex population of *E. coli*, as numerous DGGE bands of varying migration character defined each fingerprint. During the first dry weather sampling (Fig. 3A) cluster analysis revealed that the *E. coli* communities at the beach sites were relatively different from those at Porter creek and storm sewer sites. However during the second sampling event (Fig. 3B), cluster analysis revealed a clear association between *E. coli* communities in Porter Creek and those from Huntington Beach. Specifically, all Porter Creek samples clustered with those from Huntington Beach (C and D) at 100% similarity. These results suggest the transient nature of Porter Creek's contribution of *E. coli* to Huntington Beach during dry weather conditions. In contrast to the communities in Porter Creek, the lower similarity of *E. coli* communities from the storm sewer outfalls to those from the beach indicate a less positive relationship between the beach and storm sewer communities during dry weather conditions.

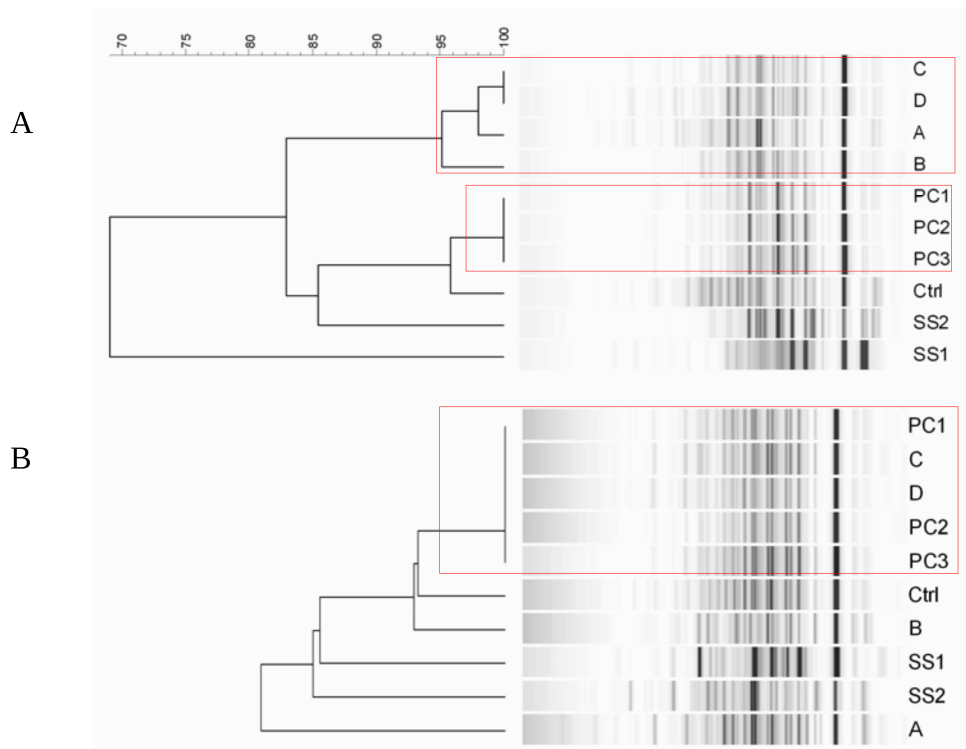

Fig 3: DGGE analysis of *E. coli* communities (*uidA*) collected during dry weather conditions on (A) Aug 19 and (B) Oct 20, 2008.

B- <u>Wet Weather samplings:</u>

During wet weather samplings (sampled from Sep 30 to Oct 2, 2008), *E. coli* densities were variable depending on location, but were generally higher in Porter Creek than storm sewer outfalls or beach water samples (Fig. 4). The lone exception to this trend was in samples collected from the east end of the beach (Site D), which frequently exhibited *E. coli* densities in exceedence of those in Porter Creek. Since Site D is located directly adjacent to Porter Creek, it is possible that the site is under a greater influence of the creek than the remainder of the westerly beach sites, and therefore exhibited higher *E. coli* densities.

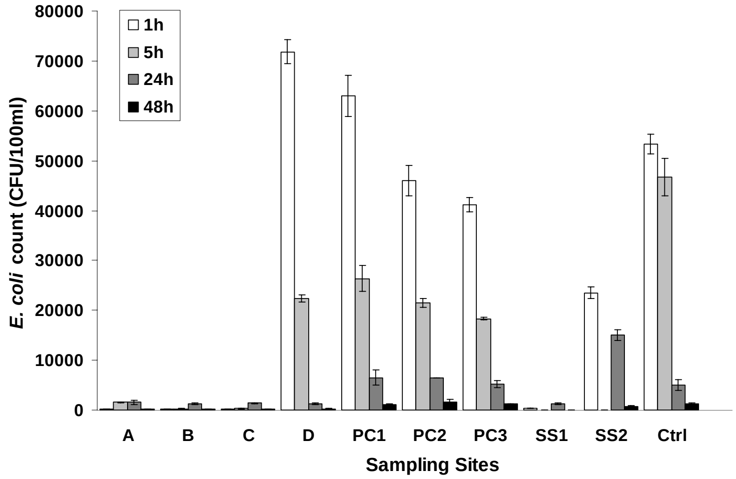

Fig 4: *E. coli* densities in water collected 1, 5, 24 and 48 h after the beginning of a rainfall event. Sampling site codes consistent with those in Fig. 1.

Efforts were made to determine the temporal dynamics of *E. coli* densities during each rainfall event. The highest *E. coli* densities in Porter Creek and storm sewer samples (SS2) were attained 1 h following the beginning of the rainfall event (Fig. 4). While the highest densities of *E. coli* at the west end of the beach were achieved at 5 and 24 h after the rain event began (Fig. 5). Specifically, during the first hour of rainfall, *E. coli* densities in these three general areas ranged from (all CFU 100 ml^{-1}) 150 to 71,000 (beach, with Site D being the highest), 41,000 to 63,000 (Porter Creek), 350 to 23,000 (storm sewers) and 53,000 (control site) (Fig. 4). Furthermore, *E. coli* densities at the east end of the beach (Site D) increased before the more westerly sites (Sites A, B and C), further indicating Porter Creek's influence on beach *E. coli* densities. Five hours after the beginning of rainfall, the *E. coli* densities at Porter Creek decreased (ranging from 18,000 to 26,000 CFU 100 ml^{-1}) while the densities at the west end of the beach (Site A and B) increased after 24 h (ranging from 1200 to 1500 CFU 100 ml^{-1}) (Figs. 4 and 5). It is interesting to note that *E. coli* densities at the west end of the beach (Site A) increased before the more easterly sites B and C. This could have resulted from a contribution from the storm sewer (SS2), which exhibited high *E. coli* densities after one hour of rainfall.

DGGE analysis revealed that the overall similarity of *E. coli* communities from both Porter Creek and the Storm Sewer to those at the beach increased from approximately 70% and 80% (dry weather similarities; Fig. 3) to 92% following the first hour of rain (Fig 6A). Specifically, *E. coli* communities from Porter Creek were 100% similar to those at the beach east end (Site D), while those from the storm sewer outfall were ~92 % similar to those at the beach (Fig. 6A). The high similarity among communities from Porter Creek and Huntington Beach

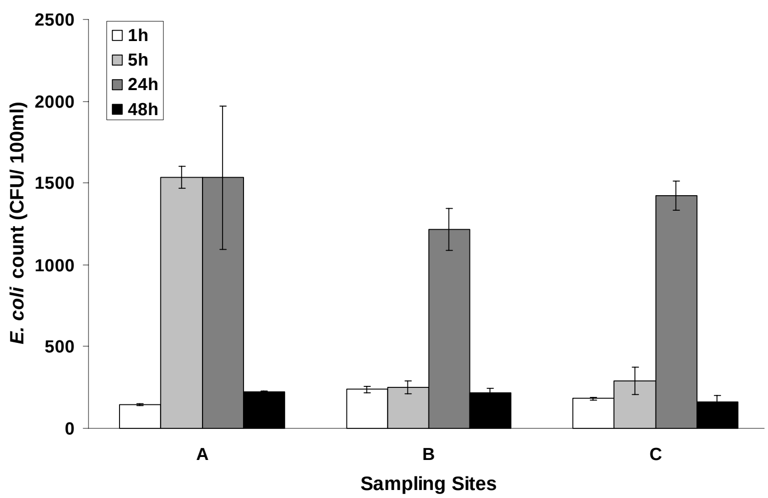

Fig 5: *E. coli* densities in beach water collected at 1, 5, 24 and 48 h after the beginning of a rainfall event. Sampling site codes consistent with those in Fig 1.

persisted for 5, 24 and 48 h after the beginning of rainfall, further illustrating the contribution to Huntington Beach of *E. coli* from Porter Creek. Despite the consistent association between communities from Porter Creek and the beach, the similarity between beach and storm sewer communities decreased following 24 and 48 h of rainfall (Fig. 6C and D). This trend indicated that while a positive relationship between storm sewer and beach *E. coli* communities was detected, the relationship between *E. coli* communities from Porter Creek and Huntington Beach appeared to be longer in duration, suggesting that Porter Creek was a more significant contributor during wet weather events.

Repeated sampling performed during another wet weather event revealed a similar trend in *E. coli* densities and community relationships as described above (data not shown).

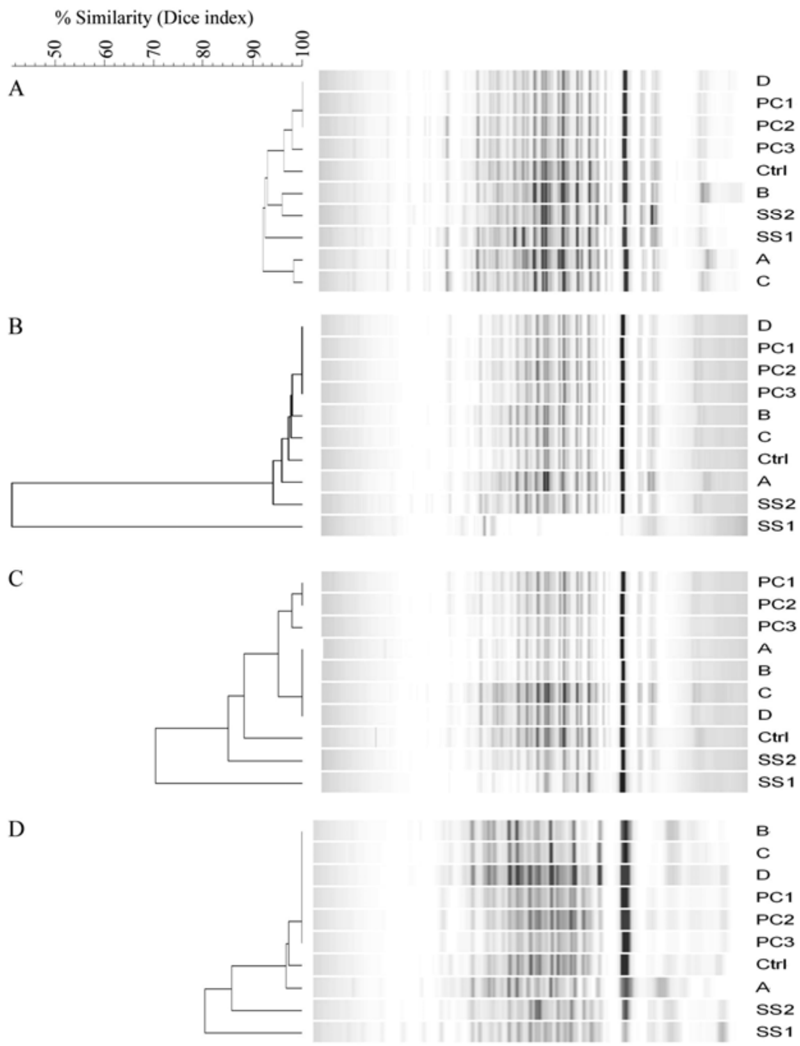

Fig. 6: DGGE analysis of *E. coli* communities (*uidA*) collected at (A) 1, (B) 5, (C) 24 and (D) 48 h after the beginning of a rainfall event. Sampling site codes are consistent with those in Fig. 1. Repeated sampling performed during another wet weather event revealed a similar trend in *E. coli* densities and community relationships

4. Conclusions

Under both dry and wet weather conditions, Porter Creek appeared to influence the *E. coli* at Huntington Beach, while the influence of the storm sewer outfalls was limited to wet weather conditions. While these data suggest that each source is a likely contributor to pollution at the beach, neither Porter Creek nor the stormwater outfall is an exclusive contributor of fecal pollution and both are dependent on prevailing precipitation conditions. These results indicate that stormwater runoff influences the contribution of bacteria by the outfall and Porter Creek, which are likely impacted as storm runoff becomes contaminated with fecal material as it washes over roads, roofs, lawns, construction sites, and other impervious surfaces. These represent the largest identified pollution sources that result in beach closures and advisories (NRDC, 2006). While our results have identified a role of Porter Creek and the storm sewer outfall in the bacterial contamination of Huntington Beach, further efforts are required to identify the hotspots of contaminated overland runoff that enter the creek and storm sewer and ultimately impact Huntington Beach.

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