STREET RUNOFF AS A SOURCE OF FECAL INDICATOR BACTERIA TO URBAN EMBAYMENTS

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Stormwater from the urban environment has been identified as an important source of pollution to coastal waterways. While the types of pollutants (e.g., metals, organics) can be quite diverse, there is an increasing appreciation of urban stormwater as a source of fecal microbes relevant to water quality monitoring programs. In this study, street water samples from Queens, New York, were found to consistently contain high levels of enterococci, a group of fecal indicator bacteria, via cultivation-based approaches. These concentrations were orders of magnitude above the EPA recommended Beach Action Value and significantly higher than the concentration observed in receiving waterways, implicating stormwater as a potentially significant source of indicators relevant to waterway monitoring programs. Fecal indicators were also found to persist in a viable form on street surfaces during periods of extended dry weather. Sanger sequencing of bacterial isolates confirmed the identity of these microbes to be *Enterococcus* spp. The presence of *Enterococcus* was also confirmed by high-throughput amplicon sequencing of street water samples; however, the ratio of the fecal indicator to broader groups of fecal taxa in sequencing data suggested an overrepresentation of the indicator in street water. The results of this study confirm that fecal indicators are abundant in urban stormwater but may be partially decoupled from broader fecal taxa creating a possible complexity in the interpretation of monitoring data influenced by stormwater versus sanitary sewer waste.

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INTRODUCTION

Urbanization has transformed many coastal landscapes with significant consequences for the quality of adjacent waterways. The surface of the urban environment is primarily impervious to precipitation resulting in minimal infiltration and shorter retention compared to natural surfaces or low-density suburban development (Alley and Veenhuis 1983). Urban stormwater interacts with contaminants on these impervious surfaces before being delivered to receiving waterways. These contaminants, often occurring as a result of human and industrial activity, can include metals (Bay et al. 2006), sediments (Vaze and Chiew 2004), hydrocarbons (Brown et al. 1985), and microbes (Brownell et al. 2007; National Research Council 2009). By addressing these contaminants and managing stormwater flow, the quality of coastal urban habitats can be improved, recreational opportunities can be expanded, and public health risk can be reduced.

Since the passage of the Clean Water Act in 1972, increased regulation has led to better management and an improved understanding of the mechanisms underlying the degradation of coastal waterways (Brosnan and O'Shea 1996). Urban receiving waters are often contaminated by a combination of sources including partially-treated wastewater discharges (Watkinson et al. 2009), untreated Combined Sewer Overflow (CSO) (Eaton et al. 2013; Young et al. 2013), and direct stormwater discharge (Hatt et al. 2004; Parker et al. 2010; Brownell et al. 2007). Management in most urban areas has focused initially on mitigating sanitary sewage pollution via upgrades to wastewater treatment plants (WWTPs) (Hetling et al. 2003) and reducing or eliminating CSOs (García-Barcina et al. 2006; Soonthornnonda and Christensen 2008). Once sources of sanitary sewage become more effectively managed, the direct stormwater discharge can receive greater management attention. In the New York City area, this has led to the recent expansion of efforts by management agencies to implement various forms of stormwater

discharge control measures, including Municipal Separate Storm Sewer System (MS4) regulations (NYC 2018). Under this new permitting system, increased emphasis is placed on preventing illicit discharges to stormwater pipes, pollution control in stormwater, and green or grey infrastructure initiatives to reduce overall stormwater discharge to receiving waterways.

In addition to chemical pollutants, microbial contaminants have become a significant concern in urban watersheds (Sidhu et al. 2013; Parker et al. 2010). In receiving waters, the patterns of microbial fecal contamination and even more generally sewage contamination, have been studied by quantifying the abundance of certain fecal indicator bacteria (FIB) using EPA approved cultivation-based methodologies. The quantification of FIB, such as enterococci, serves as an important tool in water quality monitoring and the mitigation of sanitary sewage sources (Urban Water Resources Research Council 2014). The studies used to establish FIB as a water quality tool have generally been based on surface water recreational environments (e.g., lakes and coastal beaches) where the primary source of fecal pollution was attributed to human sewage (Cheung et al. 1990; Fattal et al. 1987; McBride et al. 2013; Ferley et al. 1989). In these studies, FIB concentrations were significantly correlated to swimmers' health outcomes. Enterococci have also been found to be abundant in stormwater; while the EPA recommended beach action value for a single sample measurement is 60 cells/100 ml (US EPA 2012), enterococci concentrations in stormwater samples have been shown to exceed this threshold by 3-4 orders of magnitude (Parker et al. 2010; Brownell et al. 2007; Sidhu et al. 2012; Sauer et al. 2011). If FIB are abundant in urban stormwater, and more specifically urban street water, then it becomes critical to understand the source of these FIB to allow efficient mitigation. Although primarily designed for use in waterways, FIB may also be useful for evaluating contamination patterns in sediment (Perkins et al. 2014), groundwater (Boehm et al. 2004), and perhaps even air

(Dueker et al. 2012; Montero et al. 2016). However, the interpretation of FIB beyond water column environments can be complex (O'Mullan et al. 2017) and requires additional study.

In recent years, advances in molecular genetic techniques, such as quantitative PCR and high-throughput DNA sequencing, have led to the development of new methods for microbial source tracking (MST) (Mclellan and Eren 2014). DNA sequencing approaches have allowed the identification of taxonomic groups of microbes shared in most fecal samples, both human and non-human animal, sometimes referred to as the fecal core microbiome (Shanks et al. 2013; Newton et al. 2015). These fecal taxa (FT) provide a microbial community signature from feces that can be analyzed with DNA sequencing approaches from environmental samples and can be used in parallel with more traditional cultivation-based methods for measuring FIB to evaluate the extent of fecal and sewage contamination in the environment (Newton et al. 2013; Mclellan and Eren 2014). DNA sequencing methods, including high throughput environmental amplicon sequencing, and more traditional FIB isolate taxonomic identification using Sanger sequencing, may be useful in verifying the coupling of FIB to a source of fecal contamination or detecting FIB false positives that potentially originate from environmental sources that are decoupled from fecal contamination. If the easily measured, and regulatory linked, cultured FIB (e.g., enterococci) are correlated to the percent sequence representation of DNA sequences from FT in environmental samples, then it supports the management relevance of traditional cultivation-based FIB in representing broader fecal microbial contamination in stormwater. In contrast, if cultivation-based FIB and the percent sequence representation of DNA sequences from FT in environmental samples are not correlated, it would suggest that the FIB in stormwater may be decoupled from the broader fecal microbe contaminants, instead signaling an important complexity in monitoring data that must be understood (O'Mullan et al. 2017).

In light of the emerging interest in stormwater management and sewage mitigation by New York City and many other urban coastal municipalities, confirmation of the value of FIB as a fecal monitoring tool for stormwater is of great significance. The research goals for this study were to determine:

1) the typical concentration range of FIB in urban street water from Eastern Queens, NY, and whether it could act as a significant FIB source to receiving waterways;

2) if viable FIB could be detected from swabs of the urban surface during periods of dry weather;

3) if FIB from urban stormwater consist of distinct species of *Enterococcus*, as determined by 16S rRNA gene sequencing of environmental isolates, as compared to dominant *Enterococcus* species previously found in CSO-impacted surface water; and

4) the extent of stormwater FIB coupling to broader groups of FT.

The overarching hypothesis tested was that FIB would be abundant in urban street water and that the occurrence of FIB and FT would be positively correlated. The findings of this study have management significance for monitoring and mitigation of fecal-polluted waterways in New York City and other similar urban centers.

METHODS

Sample Collection

Ten street locations were selected in Queens, NY, to sample urban street water (stormwater sampled on the urban street) runoff (Fig. 1). In prior studies, "stormwater" is often sampled at the end of a stormwater pipe, as opposed to the curbside "street water" sampled in this study. This distinction may be important in characterizing concentrations and sources of FIB. The curb-side sampling sites were adjacent to storm drains which receive a steady influx of street water runoff during rain events and were generally located on downgrades where water would flow rapidly into drains rather than pool. Sampling took place on eleven occasions from 2014 to 2017, from April to late October. Grab samples were taken during periods of rainfall by sampling flowing runoff at a distance generally within 3 m upstream of a catch basin to which the runoff was being conveyed. During dry weather, defined as the lack of precipitation in the three days before sample collection, triplicate samples of street surfaces were collected from a 10 x 15 cm area of the street surface using a sterile swab (Copan Liquid Amies Elution Swab) and a swabbing effort of approximate 30 seconds. The swab was then suspended in 1 mL transport medium, sealed and stored away from light in a cool container until they were transported back to the lab for further processing.

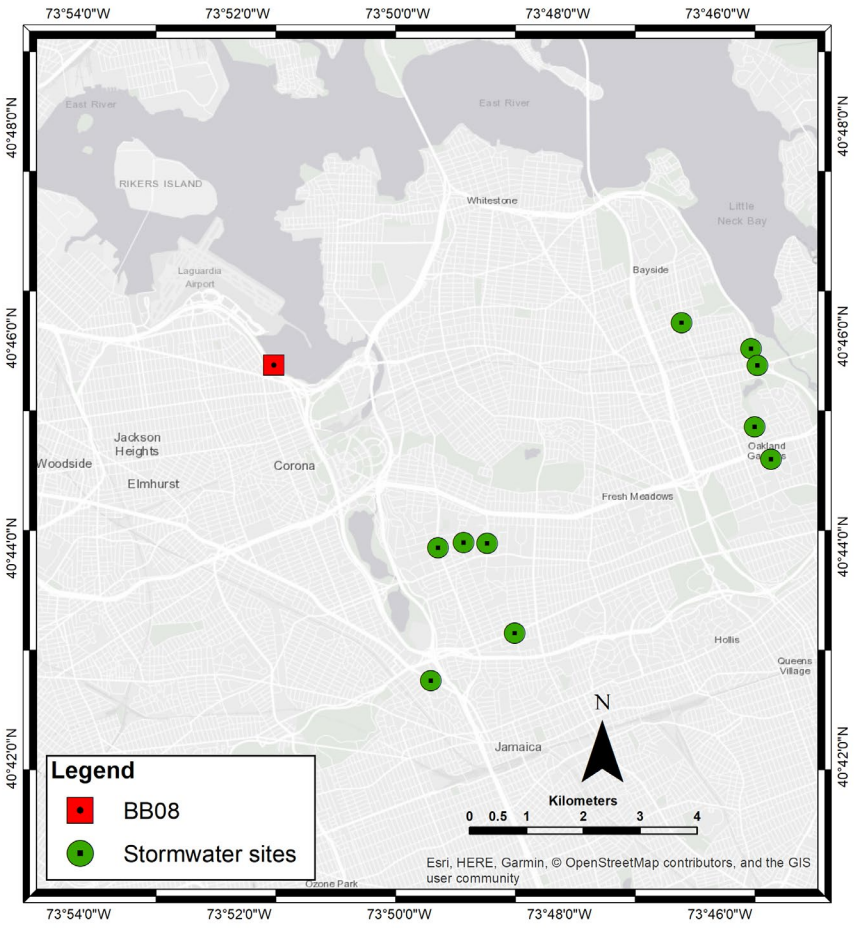

Figure 1: Map of Queens NY, with street water collection sites and CSO outfall (BB-08) labeled.

In order to compare composition in street water and waterways, estuarine surface water samples were obtained from Flushing Bay and Little Neck Bay, which are embayments impacted primarily by CSO and separate storm sewer discharge, respectively, and are the receiving waterways for urban street water samples collected for this project. In addition to surface water samples taken in Flushing Bay and Little Neck Bay, actively flowing CSO discharge from an outfall located on Flushing Bay (outfall BB-08) was sampled during several wet-weather events which occurred through June 2015 and July 2016. All water samples were collected in sterile 1 L plastic bottles and stored on ice and in the dark until further processing.

Enterococcus cultivation and quantification

Culturable enterococci, a group of FIB, were enumerated in the urban street water runoff, receiving waters, and with dry-weather street swab samples, using the Enterolert® Method (www.Idexx.com) within six hours of sample collection as required by EPA guidelines (US EPA 2012). Enterolert® samples from stormwater and receiving water were initially diluted ten-fold per the handling of environmental samples as described in (Young et al. 2013). Because of high enterococci concentrations, urban stormwater samples after 6/19/2017 were often diluted both 10 and 100-fold in order to remain within the Enterolert® method range of detection.

To positively identify *Enterococcus* colonies from street water using molecular genetic approaches, a subset of street water samples, dry-weather swab samples, and receiving surface water samples were incubated on Membrane Enterococcus In`doxyl-β-D-Glucoside (mEI) Agar according to U.S. Environmental Protection Agency (EPA) Method 1600 for culturable enterococci (US EPA 2002). The mEI plates where incubated for 24 hours at 41˚C, after which observable colonies were identified as enterococci by the presence of a blue halo around the colony. Colonies that grew a blue halo were then picked using sterile pipette tips into individual wells of 96-well plates and suspended in 30 µL of nuclease-free sterile water (Hyclone Laboratories, Inc. Logan, Utah, USA).

Bacterial Isolate DNA sequencing

DNA extraction and amplification of bacterial colonies isolated from mEI plates was conducted following the approach of Young et al. (2013). Briefly, cells were lysed by boiling 96-well plates at 95˚ C for 5 minutes. Lysed colony suspensions underwent PCR amplification using primers 8F and 1492R (Teske et al. 2002). Amplification reactions included 35 cycles of

denaturation (45 s at 95˚C), annealing (45 s at 55˚C) and elongation (60 s at 72˚C). PCR products were visualized by electrophoresis to confirm amplification of a single approximately 1,500 base pair product and were then sent for single pass Sanger sequencing, using the same 8F primer, to Eton Bioscience (Union, NJ, USA). After quality control and trimming, the resulting 16S rRNA gene sequences from mEI colonies were queried against the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide) using the Basic Local Alignment Tool (BLAST) (Altschul et al. 1990) to identify the most taxa sharing highest sequence identity within the database.

Illumina Sequencing

Following sample collection, 100-500 ml of stormwater was filtered through 0.2 μm "sterivex" cartridge filters (Millipore, Darmstadt, Germany) which were then immediately stored at –80˚C until further processing. DNA extraction from sterivex filters was performed using the DNeasy PowerWater Isolation Kit (Mo Bio Laboratories Inc) following the manufacturer's instructions. Following extraction, the purified DNA product was amplified and sequenced, by Molecular Research DNA labs (www.mrdnalab.com, MRDNA, Shallowater, TX, USA) using a protocol described by (Dowd et al. 2008). In brief, high-throughput sequencing reactions for 16s rRNA genes was prepared using a 30 cycle PCR step procedure with the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were checked in 1% agarose gel to determine the success of amplification and the relative intensity of bands. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to prepare DNA libraries for Illumina TruSeq DNA sequencing.

Statistical Analyses

Enterococci concentrations that exceeded 24196 cells/100 ml were capped to 24196 cells/100 ml for all downstream statistical analysis. Mann–Whitney nonparametric tests were performed to compare street water enterococci concentrations to those found in Flushing Bay and Little Neck Bay surface waters. Spearman's Rank correlation coefficient was used to compare the ratio of percent FIB representation to fecal core bacteria in stormwater, surface waters, and CSO samples. These statistical tests were performed using PRISM and R (version 3.4.3) statistical software (R Core Team 2015).

RESULTS

FIB Concentration in Street Water and Receiving Waterways

Enterococci were found to be not only present but abundant, in all street water samples taken (Fig. 2). The geometric mean enterococci concentrations were 5786 cells/ 100 ml. Enterococci concentration in street water did not differ significantly among the ten locations ($p = 0.1937$); however, urban street water concentrations were significantly higher than in the two coastal embayments ($p < 0.0001$), Flushing Bay and Little Neck Bay, which are the receiving waterways for the stormwater collected on the streets of eastern Queens. Both waterways have episodically unacceptable water quality based on FIB monitoring (e.g., Young et al. 2013). Little Neck Bay, under both wet and dry conditions, was found to have a significantly lower ($p = 0.0014$) FIB concentration (geometric mean = 3 cells/100 ml) than the heavily CSO-impacted Flushing Bay (geometric mean = 99 cells/100 ml)(Fig. 3).

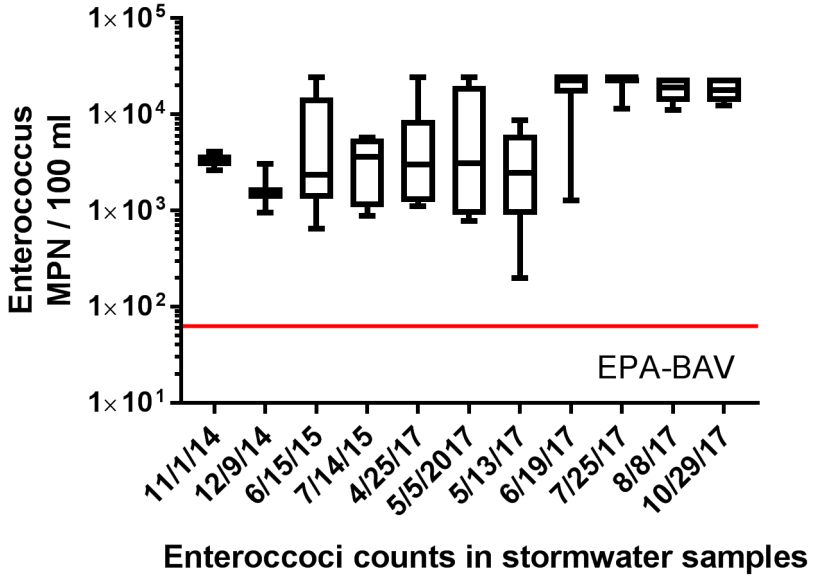

Figure 2: **Range of FIB contamination detected in street water from 10 Queens NY street locations during 11 rain events. Red Line = the EPA Beach Action Value (BAV) for enterococci used for recreational waterway management in the coastal environment.**

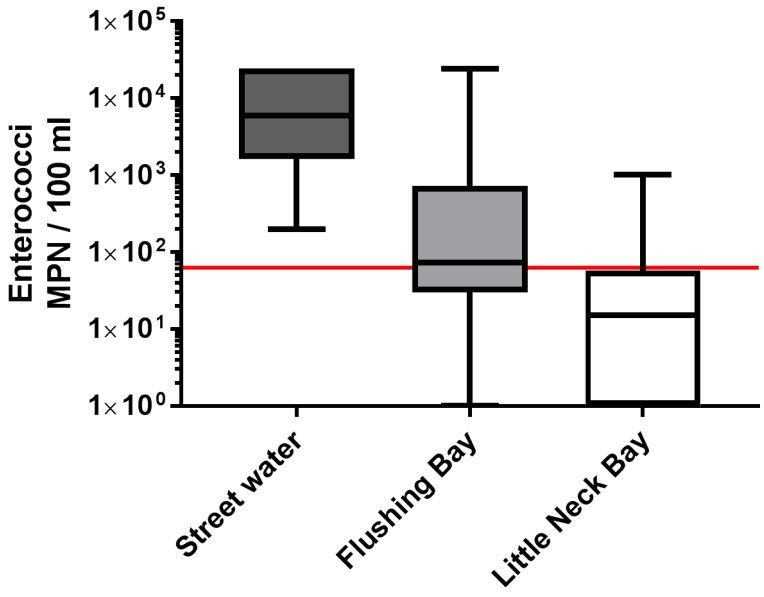

Figure 3: **Comparison of enterococci counts in urban street water, Flushing Bay, and Little Neck Bay. Street water enterococci concentrations were significantly higher than those found in the two embayments (Kruskal-Wallis, $p < 0.0001$). Red line marks the EPA beach action value (63 cells/100 ml).**

FIB Persistence on Urban Streets during Dry Weather

Swab samples were collected on four days during periods of extended dry weather to determine if FIB persist in a viable form on the urban street surface between rain events. Viable enterococci were detected on all four days sampled, with positive detection on 71% of the swab samples processed. The geometric mean enterococci concentration from street surface swabs was 2 cells/100 cm^2.

FIB Isolate Species Identity

Taxonomic identification of isolates from street water, dry-weather surface swabs, and receiving waterways was performed to confirm the taxonomic identity of cultivated strains as *Enterococcu*s species in the dry-weather and street water samples. Twenty-two of the sequenced isolates were from wet weather urban street water, eighteen were obtained during dry weather street swabs, and eleven were isolated from Flushing Bay surface water (Table 1). All 51 sequences, except one, were identified as most similar to the genus *Enterococcus*. The one isolate identified as a false positive was collected from stormwater and shared 99.8% sequence identity to *Aerococcus urinaeequi*. In the 18 dry weather swab sequences, the most common taxa were *E. hirae* (n=5), and *E. lactis* (n=4), while three dry-weather sequences were identified to be *E. faecalis*. For wet-weather street water samples (n = 22), the most frequent species observed was *E. lactis* (n = 10), and *E. hirae* (n = 4). In Flushing Bay surface water, nine out of eleven sequences were matched to *E. faecium* (n = 9) while the remaining two sequences were matched to *E. hirae* (n = 2).

Table 1: Classification of 16S rRNA sequences obtained from isolates of dry weather urban street swabs (n = 18), wet-weather street water runoff (n = 22) and surface water from Flushing Bay, NY (n = 11).

Genus	***Species***	**% of Dry-weather street swab**	**% of Wet-weather street water**	**% of Surface water**
Enterococcus	*canis*	4.5		
	casseliflavus	4.5	5.6	
	faecalis	4.5	16.7	
	faecium	13.6		0.82
	hirae	18.2	27.8	0.18
	lactis	27.3	22.2	
	mundtii	9.1	11.1	
	olivae	9.1	5.6	
	thailandicus	4.5	11.1	
Aerococcus	*urinaeequi*		4.5	

DNA Based Representation of Fecal Contamination

Molecular data from high-throughput amplicon sequencing of street water, receiving water, and CSO discharge showed that *Enterococcus* represented a significantly higher percentage of the total sequences in street water, compared to the *Enterococcus* percent representation in surface water samples (Kruskal-Wallis, $p<0.01$) (Fig 4a). On the other hand, an analysis of the presence and percent representation of FT in sequence data showed that broader fecal bacteria were relatively rare in the street water samples, with significantly lower representation than the FT found in CSO samples (Kruskal-Wallis, $p < 0.01$) (Fig. 4b). The ratio

of enterococci to fecal core percent representations also differed significantly among sample types (Fig. 5). Urban street water had a significantly higher ratio of *Enterococcus* to FT microbes compared to surface water and CSO samples (Kruskal-Wallis, $p < 0.01$). Neither the abundance of culturable enterococci nor the percent representation of *Enterococcus* in the street water samples was significantly correlated to FT (Spearman $r = 0.036$, $p > 0.96$; Fig. 6a). In contrast, Flushing Bay samples had a significant, positive correlation between *Enterococcus* and FT (Spearman $r = 0.61$, $p < 0.05$; Fig. 6b) while CSO examples exhibited the strongest significant association (Spearman $r = 0.87$, $p < 0.01$; Fig. 6c).

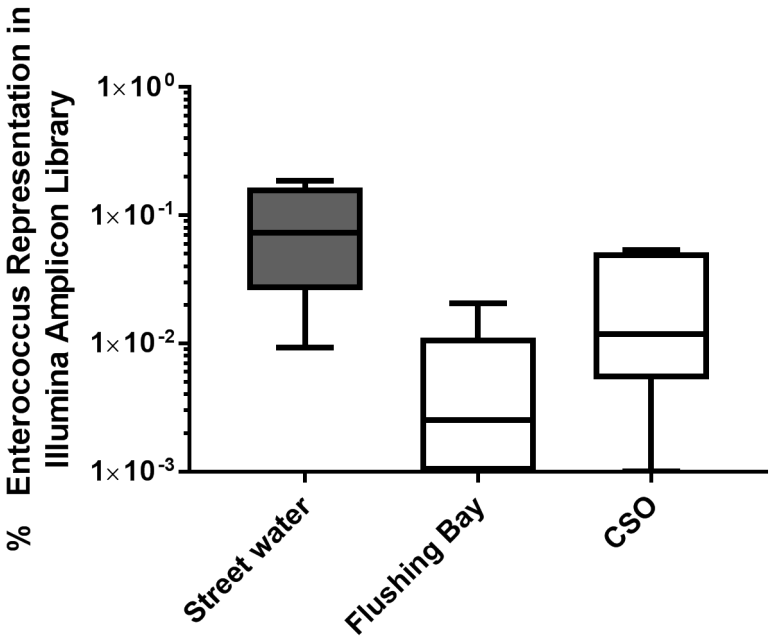

Figure 4a: Percent representation of the genus *Enterococcus* in the high-throughput amplicon sequencing data from three environments: urban street water, surface water from Flushing Bay; and samples from a Combined Sewer Overflow Outfall (CSO). *Enterococcus* was significantly more represented in street water than Flushing Bay or CSO samples (Kruskal-Wallis; $p < 0.01$).

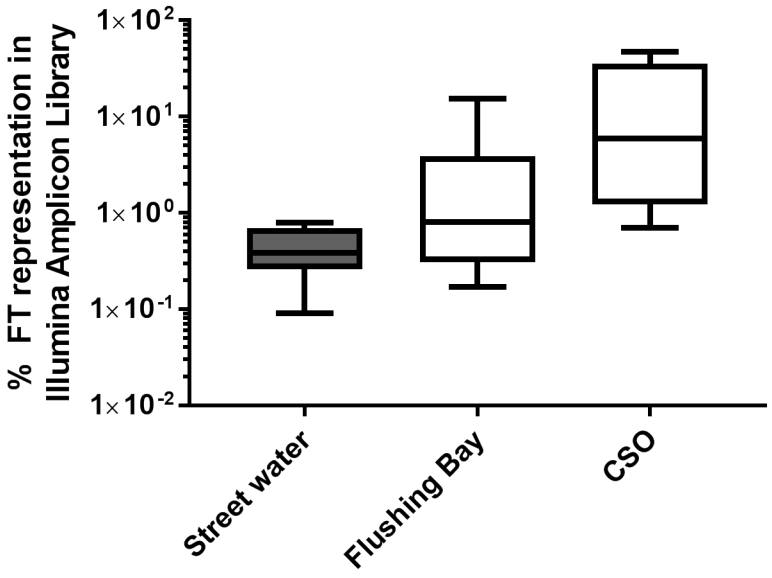

Figure 4b: **Percent representation of Fecal Taxa (FT) in high-throughput amplicon sequencing data from three environments: urban street water, surface water from Flushing Bay; and samples from a Combined Sewer Overflow Outfall (CSO). FT were significantly less abundant in street water than in CSO samples (Kruskal-Wallis; $p < 0.01$).**

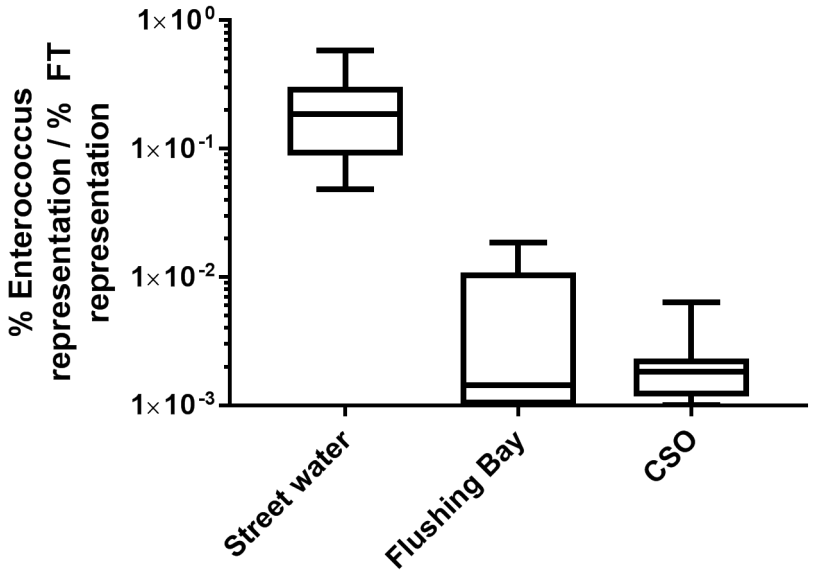

Figure 5: **The ratio of *Enterococcus* to Fecal Taxa (FT) representation in high-throughput amplicon sequencing from three environments: urban street water, surface water from Flushing Bay; and samples from a Combined Sewer Overflow Outfall (CSO). Street water possessed a significantly higher ratio of enterococci to FT than Flushing Bay or CSO samples (Kruskal-Wallis, $p < 0.01$).**

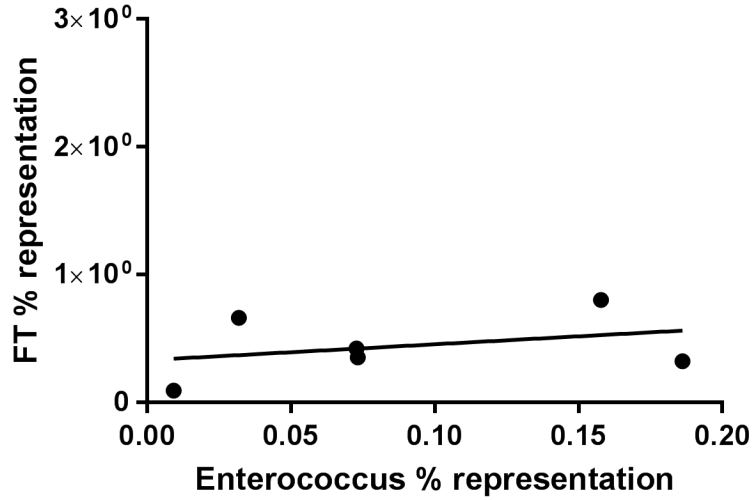

Figure 6a: **Street water *Enterococcus* vs. Fecal Taxa (FT) % representation in high-throughput amplicon sequencing. *Enterococcus* % representation was not correlated to FT % representation (Spearman r = 0.036, p > 0.96).**

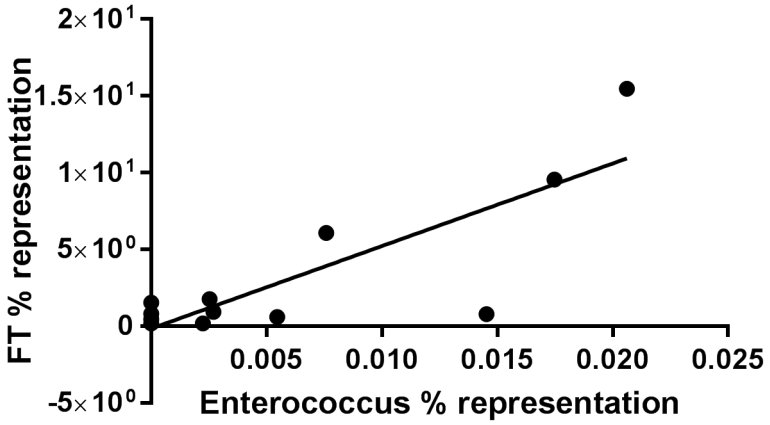

Figure 6b: **Flushing Bay surface water *Enterococcus* vs. Fecal Taxa (FT) % representation in high-throughput amplicon sequencing. *Enterococcus* % representation was significantly correlated to FT % representation (Spearman r = 0.61, p > 0.05).**

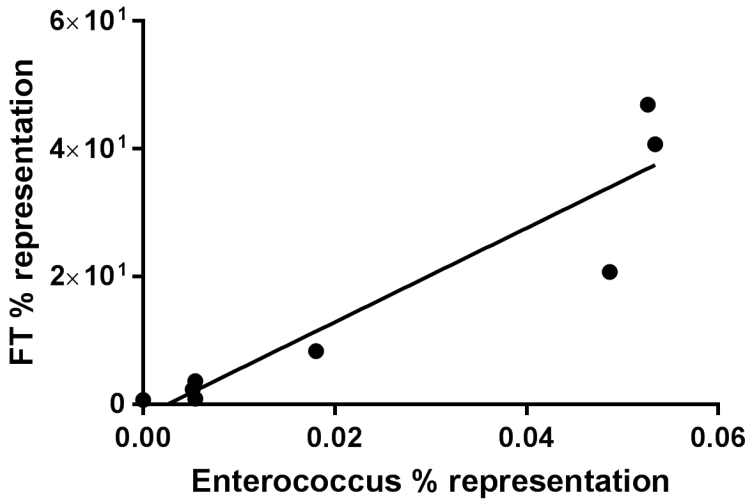

Figure 6c: Combined Sewer Overflow (CSO) *Enterococcus* vs. Fecal Taxa (FT) % representation in high-throughput amplicon sequencing. *Enterococcus* % representation was significantly correlated to FT % representation (Spearman r = 0.87, p > 0.01).

DISCUSSION

Stormwater is a significant source of FIB to waterways

Urban stormwater is known, from prior studies, to contain a high microbial load, including FIB average concentrations reported to be on the order of 10^3-10^5 cells/100 mL (Brownell et al. 2007; Sidhu et al. 2013; Parker et al. 2010), and in some cases has been shown to contain pathogens of concern (Ahmed et al. 2018; Steele et al. 2018). These prior studies have utilized both cultivation and non-cultivation based molecular genetic approaches, commonly including quantitative Polymerase Chain Reaction. In New York City street water, collected curbside before entering a drain, all samples were found to contain FIB and had a geomean concentration of 6.5 x 10^3 cells/100 ml, consistent with prior literature. By comparison, the concentrations in street water were significantly higher than in receiving waters and were orders

of magnitude higher than the geometric mean considered acceptable for recreational contact according to EPA guidelines (35 cells/100 ml ; US EPA 2012). Given the small volume of many urban receiving waterways (e.g., canals and creeks) relative to the annual stormwater volumes these waterways receive (i.e., Flushing Creek) and the high FIB concentrations observed in this study, stormwater could represent a significant source to receiving waterways. This suggests that aggressive management actions should also target stormwater treatment or infiltration and not just sanitary waste removal from waterways.

While the importance of stormwater to the quality of receiving waterways has recently been the focus of increased study and management attention (National Research Council 2009), it is currently unclear if the high-microbial load poses a health concern to those who encounter stormwater while still on the urban street surface. Multiple epidemiological studies have been conducted relating FIB in recreational water to health outcomes (Wiedenmann et al. 2006; Wade et al. 2006); however, there have been no studies directly investigating health outcomes from contact with FIB contaminated water while still on the urban street surface (e.g. encountered while walking or tracked into homes). Public contact with polluted waterways is limited and regulated by management agencies, yet human contact with street water occurs much more frequently in city streets. The microbial content of street water may warrant additional study to understand the health significance, if any, of this exposure as compared to the health risk of contact with receiving waterways. If FIB are tightly coupled to FT in both street water and receiving waterways, it could be assumed that the health risk of exposure may be similar; however, these assumptions are complicated for at least two reasons. First, the route of exposure in receiving waters commonly assumes immersion (e.g., swimming and wading) and does not represent the typical human interaction in city streets. The contact when boating (e.g., kayaking)

in a waterway may be quite similar to the contact while walking in the street. The health outcomes considered in recreational waters have often been restricted to gastrointestinal illnesses and fever (Cabelli et al. 1983; Dufour 1984; Boehm and Soller 2013; Wade et al. 2006; Wiedenmann et al. 2006), while symptoms more relevant for exposure on street surfaces, may include surface infections (e.g., rashes), and respiratory illnesses that have been less commonly evaluated (Fleisher et al. 1996; World Health Organization 2003). Moreover, the source of FIB and broader FT would be expected to differ in street water or stormwater versus sanitary waste. These assumptions contribute to the complexity of interpreting FIB data (O'Mullan et al. 2017) and demonstrate the importance of understanding the ecology of the indicators.

The Presence of Viable FIB on Dry Urban Streets Complicates Management

The usefulness of enterococci as a waterway management tool relies on the overarching assumption that FIB should be strongly correlated with both fecal bacteria and increased incidence of illness; however, the validity of these assumptions are increasingly questioned. Enterococci have been found to be widespread in habitats other than the animal gut, including soil (Byappanahalli et al. 2012) and plant surfaces (Mundt 1963; Müller et al. 2001). These non-animal sources could be important in urban environments and could lead to decoupling from fecal bacteria. In addition, enterococci are known to be abundant in both human and animal waste, which contributes to the difficulty in identifying specific sources in need of management action based on traditional monitoring data. This problem can be especially important in areas where wildlife are abundant, and where terrestrial surfaces can accumulate animal fecal waste (e.g., streets). As a consequence, more emphasis is being placed on utilizing microbial source tracking tools in order to discriminate between human, and animal sources of FIB. Some prior studies that have employed Microbial Source Tracking (MST) methods have found evidence of

human fecal contamination in urban stormwater discharge (Sidhu et al. 2013; Sauer et al. 2011; Parker et al. 2010). These studies typically sampled at the end of stormwater pipes, as opposed to sampling street water, and in stormwater pipes human signals of fecal contamination are often attributed to cross-contamination from sanitary sewers (Sidhu et al. 2013; Sauer et al. 2011).

Human fecal waste is not likely to be a common source of FIB on the street surface. One of the few prior studies to directly sample street water prior to entering a storm drain also used MST and found bird and other wild animal feces to be the predominant source of enterococci but found no evidence of human contamination (Jiang et al. 2007). This has important consequences for stormwater pollution reduction, as management actions targeting domestic versus wild animals will likely differ. Stormwater management programs have attempted to limit pet waste via education, providing waste removal bags, and issuing fines for failure to clean up after pets (NYC 2018). While these actions are appropriate and could limit some forms of animal contamination, wildlife sources can be more difficult to control. Birds, rodents, and other animals are commonly present in New York City (Combs et al. 2018, and their fecal waste may be contributing significantly to bacteria accumulating on city streets. While the microbiome of sanitary sewage reflects human fecal sources (Shanks et al. 2013; Newton et al. 2015) street water may have large contributions from non-human animal and even non-animal sources of FIB. The diverse sources in street water require diverse management approaches, and likely have a health risk that differs from sanitary sewer waste.

Diversity of Street Water Isolates Supports Non-Human Sources

Sequencing of isolates confirmed that the cultivation-based techniques used to enumerate FIB correctly targeted enterococci nearly lacking (less than one percent) false positives. The presence of enterococci in street water and receiving waters was further confirmed by high-

throughput amplicon sequencing of environmental samples. *E. faecium* and *E. faecalis* are the strains most commonly associated with the human gut (Silva et al. 2011) and have been frequently identified as the dominant strains in waterways contaminated with human sewage and in wastewater treatment plants (Ferguson et al. 2013; Manero et al. 2002). In Flushing Bay, which is known to be heavily contaminated by CSO's, including one outfall representing nearly ten percent of all the discharge in New York City (NYC 2016), *E. faecium* represented nearly 82% of the isolate sequences. In contrast, street water isolates were significantly more diverse than surface water isolates and consisted primarily of *E. hirae*, and *E. lactis*. It needs to be acknowledged that it can be difficult to accurately identify *Enterococcus* isolates to species level (Harwood et al. 2004), especially with relatively short 16S rRNA gene sequences; however, the patterns in these species level assignments, do suggest that stormwater isolates may be more diverse than isolates from Flushing Bay. Enterococci are widespread in the environment, and strains such as *E. hirae* and *E. lactis* have been found in animals (Silva et al. 2011) and less commonly in humans (Facklam and Collins 1989; Layton et al. 2010), and environmental sources (Ferguson et al. 2005), so their presence does not necessarily preclude human or suggest a specific animal origin but the isolate sequences are consistent with different or more diverse sources in street water than in CSO contaminated waterways.

Enterococci are Poorly Coupled to Fecal Bacteria in Street Water

The management significance of elevated levels of FIB can be better understood by examining the connection of FIB to FT. FIB and FT are assumed to be positively associated, leading to elevated health risk from increased FIB concentrations. Although this assumption has generally been supported for waterways impacted by sewage pollution (Boehm and Sassoubre 2014; Young et al. 2013), this assumption may fit poorly with other environments where FIB can

be elevated, including street water. In the molecular data, FT were not significantly correlated with *Enterococcus*. A higher ratio of *Enterococcus* to FT was observed compared to receiving waterways. Therefore, FIB in street water appears to be overrepresented compared to broader groups of fecal microbes, relative to the ratios observed in CSO's and receiving waters. The decoupling of FIB and FT suggests different sources for FIB in street water and is consistent with the pattern of increased *Enterococcus* strain diversity discussed above. It is perhaps not surprising that enterococci were found to be so present in stormwater and on the urban dry street surface as a prior metagenomic study of subway surfaces found *Enterococcus casseliflavus* to be the 7th most commonly found bacteria species (Afshinnekoo et al. 2015) and also commonly detected *E. faecium*. This also supports the high abundance of *Enterococcus* species other than just *E. faecium* on the street surface. It remains unclear if some species persist for long on the street surface then others, creating a selective driver of differing diversity, or if this pattern occurs because the sources of FIB to streets differ significantly from sanitary waste.

Relevance to Stormwater Management

In recent years New York City has invested billions of dollars in stormwater management, including Green Infrastructure and other CSO control measures (NYC 2010; NYC 2018). The stated goal of the 2010 GI plan is to capture up to one inch of rain on ten percent of impervious surfaces on CSO watersheds by the year 2030 (NYC 2010). The net result of this ten percent reduction would be the elimination of 1.5 billion gallons a year of CSO flow into New York City waterways. When combined with other CSO control measures this represents a significant decrease in CSO discharge. Even if all CSO discharge into waterways were to be eliminated, many waterways would be unlikely to achieve acceptable levels of FIB in surface waters. For example, the Flushing Creek Long Term Control Plan states that even in the absence

of CSOs the waterway would not meet water quality standards largely as a result of the direct stormwater discharge (NYC 2014).

More recently the city has developed MS4 permitting regulations and has released a comprehensive stormwater report (NYC 2018). Given the role of stormwater in causing CSO discharge and the high concentration of FIB in direct stormwater discharge, the city's goal for stormwater capture would likely need to be substantially expanded to fully meet FIB standards in all New York City waterways. The sources contributing bacteria to urban street water and the public health significance of contact with street water remain important areas for continued study and expanded management action. There remains, relative to sewage-impacted waterways, a lack of information about the microbial content of urban street water and the health risk from contact either directly in the street or in receiving waterways. If the sources of FIB are more diverse in street water than in sewage it is possible that the ratio of FIB to FT and the associated health risk differ as suggested by this study. Current monitoring approaches and regulations do not distinguish FIB originating in stormwater versus sewage. Continued research and perhaps the continued development of monitoring tools could lead to more efficient waterway management.

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