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Patterns of sediment-associated fecal indicator bacteria in an urban estuary: Benthic-pelagic coupling and implications for shoreline water quality

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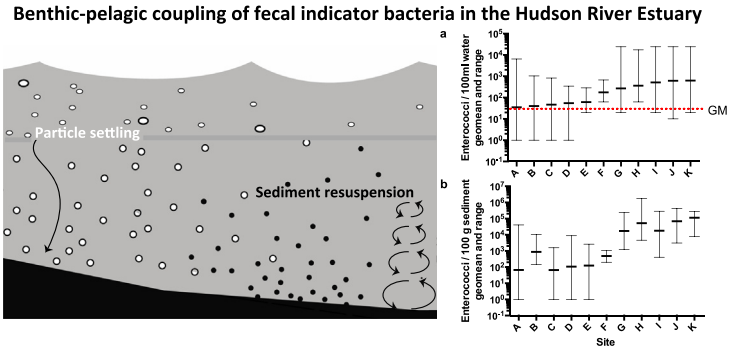
HIGHLIGHTS

- Locations with frequent high FIB counts in water had high sediment FIB counts.
- DNA sequencing confirmed FIB taxonomy and correlation to other fecal taxa.
- Experimental sediment resuspension increased shallow water FIB concentration.
- Sediment FIB may contribute to water quality problems where resuspension occurs.

GRAPHICAL ABSTRACT



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ABSTRACT

Estuarine and coastal waterways are commonly monitored for fecal and sewage contamination to protect recreator health and ecosystem functions. Such monitoring programs commonly rely on cultivation-based counts of fecal indicator bacteria (FIB) in water column samples. Recent studies demonstrate that sediments and beach sands can be heavily colonized by FIB, and that settling and resuspension of colonized particles may significantly influence the distribution of FIB in the water column. However, measurements of sediment FIB are rarely incorporated into monitoring programs, and geographic surveys of sediment FIB are uncommon. In this study, the distribution of FIB and the extent of benthic-pelagic FIB coupling were examined in the urbanized, lower Hudson River Estuary. Using cultivation-based enumeration, two commonly-measured FIB, enterococci and *Escherichia coli*, were widely distributed in both sediment and water, and were positively correlated with each other. The taxonomic identity of FIB isolates from water and sediment was confirmed by DNA sequencing. The geometric mean of FIB concentration in sediment was correlated with both the geometric mean of FIB in water samples from the same locations and with sediment organic carbon. These two positive associations likely reflect water as the FIB source for underlying sediments, and longer FIB persistence in the sediments compared to the water, respectively. The relative representation of other fecal associated bacterial genera in sediment, determined by 16S rRNA gene sequencing, increased with the sequence representation of the two FIB, supporting the value of these FIB for assessing sediment contamination. Experimental resuspension of sediment increased shoreline water column FIB concentrations, which may explain why shoreline water samples had higher average FIB concentrations than samples collected nearby but further from shore. In combination, these results demonstrate

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extensive benthic-pelagic coupling of FIB in an urbanized estuary and highlight the importance of sediment FIB distribution and ecology when interpreting water quality monitoring data.

1. Introduction

Sewage contamination of coastal waterways poses significant concerns associated with public health and management for recreational uses. While sewage can deliver many types of contaminants (e.g., metals, excess nutrients, pharmaceuticals) to a receiving waterbody (Islam and Tanaka, 2004), fecal associated pathogenic bacteria and viruses are the human health risk of greatest concern from short duration exposure to sewage pollution (Cabelli et al., 1982; Prüss, 1998; Shuval, 2003). Although direct measurement of the most relevant human pathogens would have advantages, the diversity, and often low concentrations, of potential pathogens, complicate direct pathogen monitoring in recreational waters (Straub and Chandler, 2003; USEPA, 2012), especially at the spatial and temporal scale required for management. Most monitoring programs therefore base their evaluation of microbial sewage contamination on concentrations of one or more commonly used fecal indicator bacteria (FIB) in water column samples (e.g. Dufour and Schaub, 2007; USEPA, 2012).

FIB, such as *Enterococcus* spp. and *Escherichia coli*, are abundant in human fecal material and were initially selected as indicators under the idealized assumption that they were rarely found in pristine waterways and would have limited environmental persistence, allowing their detection to indicate a recent input of fecal pollution (e.g. Litsky et al., 1953). Evidence clearly shows that in many urban aquatic systems elevated FIB levels are related to discharge or overflow of untreated, or incompletely treated, sewage (Mallin et al., 2007; Young et al., 2013). However, it is also widely recognized that agricultural runoff (Topp et al., 2009), stormwater (Ahn et al., 2005) and wildlife (e.g. Alderisio and DeLuca, 1999; Schoen and Ashbolt, 2010) can be sources of FIB to waterways. It has also become clear that the idealized assumptions for FIB are somewhat flawed due to complexities from their extra enteric ecology (Boehm et al., 2009; O'Mullan et al., 2017), including extended environmental persistence, or even growth, under some conditions (Desmarais et al., 2002; Bordalo et al., 2002; Anderson et al., 2005; Chudoba et al., 2013; Perkins et al., 2016). In particular, soil, sediment, and beach sand can act as naturalized secondary habitat for FIB (Davies et al., 1995; Alm et al., 2003; Lee et al., 2006; Whitman et al., 2006; Garzio-Hadzick et al., 2010; Byappanahalli et al., 2012). If FIB persist in estuarine sediments, then understanding and predicting FIB in estuarine waterways may require including sediments as an indirect FIB source, in addition to direct sewage and other inputs.

The exchange of FIB between planktonic and benthic habitats is becoming recognized as an important, though poorly constrained, aspect of FIB ecology (Whitman et al., 2006; Jamieson et al., 2005a; Halliday et al., 2014; Abia et al., 2016; Hassard et al., 2017). In the context of planktonic to benthic fluxes, a large percentage (up to 95%) of FIB in estuarine water columns are particle associated (Fries et al., 2006; Suter et al., 2011; Mote et al., 2012). To the extent that FIB are deposited in sediment reservoirs, resuspension of sediment or sand colonized by FIB can create a benthic to planktonic flux, as demonstrated in estuaries (Fries et al., 2008; Perkins et al., 2014), streams (Jamieson et al., 2005b), rivers (Abia et al., 2016), and beaches (Phillips et al., 2011; Halliday et al., 2014). Although the development of predictive models for FIB that include benthic-planktonic interactions has been identified as an important research priority for improved prediction of water quality (USEPA, 2012; Jamieson et al., 2005a; Liu et al., 2006; Surbeck, 2009; Kim et al., 2010; Feng et al., 2015), progress remains challenging because of limited information on the distribution of sediment FIB reservoirs in waterways of concern, the factors that influence fluxes to/from the sediments, and the degree to which water column and sediment FIB dynamics are coupled.

Recreational water quality criteria (RWQC) are based on FIB concentrations in water samples, and the criteria are considered violated whether the FIB originate from a sewage outfall, or from a more indirect route, such as resuspension of sediments. However, it is worth assessing whether FIB found in sediments retain their value as indicators of fecal contamination, pathogens and infection risk. Beyond the pathogenic bacteria of greatest concern, sewage contains a diverse microbial community including fecal bacteria other than the commonly used FIB (Shanks et al., 2013; Newton et al., 2015; Abia et al., 2018; Zhang et al., 2018). These fecal bacteria taxa are likely to be present at much higher abundance in sewage than many pathogens, providing useful targets for detection of microbial sewage inputs that are independent of direct FIB quantification (Newton et al., 2013; McLellan and Eren, 2014). Therefore, finding a relationship between FIB and other fecal taxa (FT) in sediment would support the value of FIB as a monitoring tool in sediments, as they are in water.

Few large-scale sediment FIB surveys have been completed in estuaries (e.g., Perkins et al., 2014), and given the wide range of sediment types and environmental conditions found in estuaries, benthic-pelagic coupling of FIB is likely to differ from the sandy beach, oceanic sediment, or stream environments that have received greater research attention. The goals of this study were: (1) to investigate the distribution and possible connection of FIB in the water and sediment of an urbanized estuary; 2) to use DNA sequence data to confirm the presence of FIB in sediment and to assess whether FIB are associated in the sediment with other known FT; and (3) to examine the potential for sediment resuspension to influence FIB levels in shallow water. No prior surveys of sediment FIB levels have been conducted in the Hudson River Estuary, despite chronic sewage contamination (Hetling et al., 2003; NYCDEP Centennial Study, 2009; Riverkeeper, 2015) and the associated impact on water quality (Brosnan and O'Shea, 1996; Young et al., 2013). The observed patterns of sediment FIB are relevant for sewage control, monitoring, and recreational use management of the Hudson River Estuary, as well as other urban estuaries.

2. Methods

2.1. Paired shoreline sediment and water sampling locations

Sediment and water sampling was conducted from the shoreline at seven, low salinity, brackish water sites (Sites A-F, H) and one freshwater tributary (Site G) in the Hudson River Estuary (HRE) to the north of New York City and three higher salinity sites (Sites I—K) in heavily urbanized tidal waterways of eastern Queens, New York (Fig. S1, Table 1). Water and sediment samples were paired by sample collection date to determine if benthic and pelagic FIB concentrations were correlated through time at each of the sampling locations. The lower HRE is heavily impacted by sewage from the New York City metropolitan area, with $>10^{12}$ l per year of sewage treated by waste water treatment plants (WWTPs) and 10^{11} l per year of untreated combined sewer overflows (CSOs) discharged into the tidally influenced waterways (NYCDEP, 2009). Sites were selected to represent a range of shoreline benthic conditions, from coarse-grained sandy beaches to fine grained muds. Sites also varied in proximity to tributaries and known sewage inputs, ranging from official swimming beaches (Site D) considered to have generally acceptable water quality, to highly urbanized embayment (Sites I

Table 1
Station collection locations and basic environmental characterization. Salinity is reported in Practical Salinity Units (PSU) and turbidity is measured in Nephelometric Turbidity Units (NTU).

Site	Name	Latitude	Longitude	Salinity mean PSU	Organic carbon mean %	Turbidity mean NTU
A	Ossining	41.1545	−73.8697	3.4	2.8	21.5
B	Stony Point	41.2248	−73.9643	2.2	0.9	23.7
C	Kingsland Point Park	41.0856	−73.8728	4.5	0.6	25.1
D	Croton	41.1863	−73.8950	3.0	0.6	28.3
E	Sneedens Landing	41.0118	−73.9031	5.0	4.4	46.4
F	Parelli Park	41.0426	−73.9161	4.3	2.9	24.3
G	Sparkill Creek	41.0294	−73.9254	0.3	11.6	0.8
H	Piermont SPDES	41.0421	−73.9045	4.4	27.9	242.9
I	Flushing Bay Sandy	40.7609	−73.8564	24.8	1.3	26.0
J	Flushing Bay Dock	40.7634	−73.8434	25.4	7.0	18.4
K	Meadow Creek	40.7450	−73.8373	14.0	6.1	83.3

and J) sites within a kilometer of CSOs that release >5 billion liters of untreated sewage each year.

At each site, approximately 50 ml of water was collected in sterile polypropylene tubes without disturbing the sediment. Salinity, temperature, and turbidity were measured using handheld Hach sensors. Duplicate 20 ml (approximately 20 g from the top 3 cm) surface sediment samples were then collected just offshore the water line (approximately 20 cm deep) using a modified 60 ml syringe as a small coring device. For sites with unconsolidated coarse grained sediments, a metal putty knife was used to block the end of the syringe upon collection to prevent sediment loss. Sediment samples were then transferred to sterile polypropylene tubes for temporary storage. Water and sediment samples were placed into a dark cooler, on ice, and transported back to the laboratory for immediate processing. For a subset of samples, a third sediment sample was collected for molecular genetic analyses and upon transport back to the laboratory was stored in a −80 °C freezer until DNA was later extracted.

2.2. Enumeration of shoreline FIB and sediment characteristics

In the laboratory, sediment wet weight was recorded for all samples, one duplicate was then processed for sediment dry weight and organic content, and the other for FIB enumeration. For sediment dry weight, samples were dried for at least 48 h at 60 °C, until weight stabilized, at which time dry weight was recorded. Dry weight/wet weight ratios for each set of samples were used to normalize microbial abundances to grams of sediment dry weight. Dried sediment samples were then combusted at 550 °C for approximately 6 h to determine sediment organic carbon by loss on ignition relative to pre-combustion dry weight (Apha, 2005), a subset of the samples was then combusted again to confirm no further loss of mass occurred. FIB were extracted from the second of the duplicate sediment samples within 4–6 h after sample collection by mixing 10 ml of weighed (wet) sediment with 100 ml of a sterile sediment extraction buffer consisting of 0.1% sodium pyrophosphate and 0.1 mM EDTA (Suter et al., 2011). The extraction slurry was shaken for 30 min at 200 rpm based on the highest recovery of FIB from sediment samples in initial experiments (not shown). Liquid samples, either of collected estuary water or sediment extraction buffer, were processed for enterococci and *E. coli* using 10 ml of sample water in 90 ml of sterile deionized water mixed with IDEXX Enterolert or Colilert media and incubated within Quanti-tray 2000 for 24 h at 41 °C and 35 °C, respectively. Water FIB samples were calculated to a final reported value per 100 ml, while sediment samples were calculated to a final reported value per 100 g of sediment dry weight.

2.3. Molecular characterization of shoreline sediment

Because IDEXX protocols for enumeration of enterococci are designed for detection in water, additional tests were conducted to rule out the widespread occurrence of false negative or false positive enterococci detection. After incubation of sediment microbial samples for enumeration of enterococci with IDEXX Enterolert media, 5 positive (fluorescent) wells and 5 negative wells from an incubated quantitray-2000 were pooled, separately, and cells lysed by heating to 95 °C for 5 min. DNA was amplified for the gene encoding 16S rRNA using the universal primers 8F and 1492R (Teske et al., 2002) and protocols described by Young et al. (2013), followed by transformation and cloning using the TOPO-TA cloning kit (Invitrogen) according to manufacturer instructions. The included M13F and R vector primers were used to select fragments of the correct size and sent for single-pass Sanger sequencing by SeqWright Inc. (Houston, TX). Taxonomic identification from each isolate sequence was performed using top hits from Genbank database BLAST searches (www.ncbi.nlm.nih.gov/genbank/).

The taxonomic identity of sediment and water indicator isolates was also investigated from a subset of sampling stations, by isolating colonies identified as enterococci using EPA approved MEI media Membrane Filtration (MF) techniques (USEPA, 2006). Colonies were picked from MEI plates, suspended in 40 μl of sterile water, lysed by heating to 95 °C for 5 min, and then processed for 16S rRNA gene amplification and Sanger sequencing as described by Young et al. (2013), followed by taxonomic identification as described above.

Additional sediment samples were collected from sites A-D and G-J in August of 2015 for high throughput DNA amplicon Illumina sequencing. The DNA sequence library was used to characterize the percent representation, relative to total sequences obtained, of a group of bacterial genera and families commonly found in fecal material (VandeWalle et al., 2012; Shanks et al., 2013; Newton et al., 2015), referred to in this study as fecal taxa (FT). DNA was extracted from sediment using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) according to manufacturer instructions. As described in O'Mullan et al. (2015), DNA was then quantified using a Qubit fluorometer (Invitrogen, Grand Island, NY), amplification potential was confirmed using bacterial primers 8F and 1492R, and DNA extracts were sent to Molecular Research DNA labs (www.mrdnalab.com, MRDNA, Shallowater, TX) for Illumina MiSeq amplicon sequencing (Chiodini et al., 2015). Bioinformatic analyses of high throughput sequence data utilized the Quantitative Insights Into Microbial Ecology ver. 1.9.1 (QIIME) software package (Caporaso et al., 2010) to remove barcodes and perform quality screening, including filtering of sequences based on length, primer mismatches and De Novo chimera detection using USEARCH ver. 6.1 (Edgar, 2010) in Qiime. Operational Taxonomic Unit (OTU) assignment and taxonomic classification was performed relative to SILVA 97% OTU database ver. 132 (Pruesse et al., 2007) and then any non-target sequences from Archaea, mitochondria, chloroplast, and sequences failing to be taxonomically identified were removed. OTUs were considered as FT if they were classified as *Bacteroidaceae, Porphyromonadaceae, Clostridiaceae, Lachnospiraceae, Ruminococceae, Rikenellaceae*, or *Prevotellaceae*, consistent with Newton et al. (2015) where these bacterial families were found to be both abundant in sewage and each representing >3% of total sequence reads originating from human

stool samples. DNA sequence data has been uploaded to the National Center for Biotechnology Information Genbank database using accession numbers: SAMN08998298 - SAMN08998299 and SAMN08822502 - SAMN08822507 for Illumina samples and MH001984 - MH002236 for Sanger sequences.

2.4. Comparison of water FIB concentration from shoreline, nearshore, and mid-channel

Resuspension of FIB from sediments should have greater impact on levels of water column FIB in shallow, relative to deeper, areas because dilution should vary with water depth. Thus, if resuspension plays a role in water column FIB levels, then shallow, shoreline stations should frequently have higher FIB levels than deeper locations that are nearby but further offshore. To test this prediction, water column enterococci geometric mean values from a subset of the shallow shoreline locations of this study were compared to similar data collected during the Riverkeeper Hudson River water quality monitoring program. During the Riverkeeper program (http://www.riverkeeper.org/water-quality/hudson-river/), many locations along the HRE are sampled by boat approximately monthly, and a number of the Riverkeeper sampling locations are geographically close to the sampling locations of this study (Supplemental Fig. S2). The Riverkeeper sampling program uses the same collection and analysis protocols for water column enterococci enumeration as used in this study. Six of the shallow (<20 cm) shoreline stations from this study (Sites A–D, F, H) were selected for comparison to five nearshore locations with 2–3 m depths (Sites N1–N5), and three mid-channel locations with depths > 5 m (M1–M3), sampled by boat for surface water FIB during the Riverkeeper program. Site names, sampling date, and geometric means for the Riverkeeper stations are found in supplemental Table S1. For each shoreline sample selected from this study, the monthly samples from the Riverkeeper program that were closest in time were used for comparison to ensure similar sample number for each site, though the observations were not paired in time for analysis.

2.5. Shoreline sediment resuspension from wading

Common recreational activities such as wading or entering shallow water for swimming could result in sediment resuspension. To examine the potential for shoreline sediment resuspension to influence shallow water FIB levels, ten wading trials were performed at shoreline locations near the Piermont Pier (between sites F and H). For each wading trial, the sampler walked into the water to a depth of approximately 20 cm, disturbing the bottom as evidenced by the observation of increased water turbidity. Water samples were collected before and after the sampler entered the water, and the change in enterococci concentration was calculated (FIB after wading minus FIB before wading) for each trial.

2.6. Statistical analyses

The Prism software package (version 6.0; GraphPad Software Inc) was used to perform non-parametric statistical comparisons, due to the non-normal microbial distributions, including Spearman's r coefficient for correlation analyses and Wilcoxon or Kruskal Wallis tests to test for differences in the median between groups of two, or more than two, respectively. Following initial Kruskal Wallis comparisons, a Dunn's multiple comparison post hoc test was used to compare pairs of groups for significant differences. In all cases, the tests used two-tailed tests and a significance threshold of 0.05. To calculate geometric means for FIB concentrations, zero values were replaced by values 1 significant digit below minimum detection limits.

3. Results

3.1. Distribution of FIB along the shoreline

Both enterococci and *E. coli*, the two most commonly used fecal indicator bacteria, were consistently detected with cultivation-based approaches in water and sediment at all 11 shoreline sampling sites investigated in the lower HRE and Flushing Bay. The geometric mean concentrations of the two indicators, paired by location, were correlated in samples of both water ($r = 0.809$, $p = 0.004$) and sediment ($r = 0.909$, $p < 0.001$) (Fig. S3). Similarly, concentrations of the two indicators in individual samples were positively correlated when paired by location and date (water, $r = 0.504$, $p < 0.001$; sediment, $r = 0.836$, $p < 0.001$). Strong correlations between the two indicators suggest that general patterns of sewage contamination severity can be consistently assessed regardless of the indicator selected. To simplify description of the cultivation results the data presentation will focus on enterococci and *E. coli* data will be reported in supplementary material.

FIB contamination was sufficiently frequent and high at the shoreline sampling locations that the geometric mean value for enterococci in water at each location exceeded the US EPA guidelines for recreational waters of 35 CFU per 100 ml (US-EPA, 2012) (Fig. 1A). Enterococci concentrations in water varied over approximately four orders of magnitude across sites and over three orders of magnitude within most sites on different sampling days. An even greater range of variability was observed in sediment enterococci concentrations across sites, with the highest measured concentrations exceeding 10^6 cells 100 g^{-1} (Fig. 1B). The locations with the four highest enterococci geometric means, for both water and sediment, occurred at three urban, CSO impacted sites in Queens, New York (Sites I—K), and at Piermont SPDES (Site H) to the north of New York City, which is near both a sewer prone to episodic overflow and a waste water treatment plant outfall. In contrast, the four sites with the lowest geometric mean concentrations of water column enterococci were the most northerly sites; none of which are in close proximity to a CSO or other known sewage discharge.

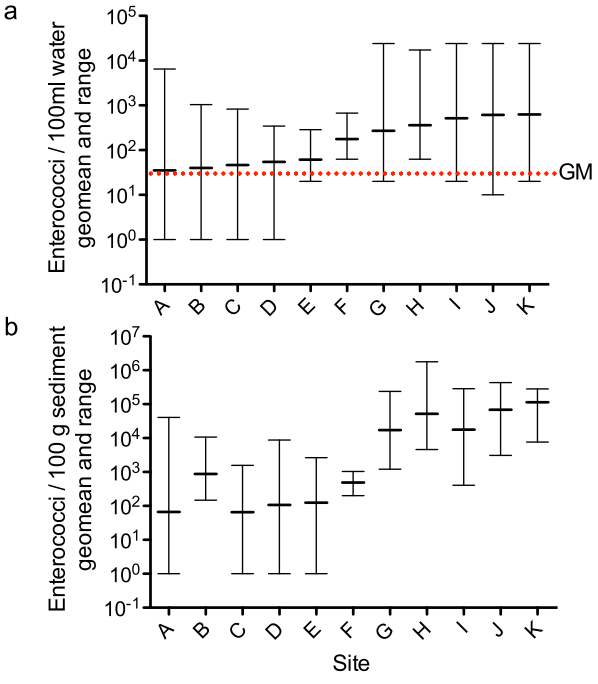

Fig. 1. FIB concentration in water and sediment. Concentration of FIB as represented by the geometric mean (dark horizontal bar) and range by site in water (top panel, a) and sediment (bottom panel, b). Dotted line indicates the US-EPA geometric mean (GM) guideline. Sampling sites are designated by letters (as described in Table 1) on the x-axis.

3.2. Relationship of shoreline sediment and water FIB

When water and sediment samples were paired by collection date, no significant correlations between water and sediment enterococci concentrations were found for any of the eleven sites, individually, and only one location (Site B-Stony Point; $r = 0.778$, $p = 0.03$) showed a significant temporal correlation for *E. coli* in water and sediment (Table 2). However, after combining data from all sites, significant correlations were found between water and sediment samples collected at the same time and location, for both enterococci ($r = 0.482$, $p < 0.01$) and *E. coli* ($r = 0.646$, $p < 0.01$). Much greater predictive value was found by correlating the geometric mean values of enterococci (Fig. 2; $r = 0.882$, $p < 0.01$) and *E. coli* (Fig. S4; $r = 0.836$, $p < 0.01$) in water and sediment, paired by station. Thus, locations with high FIB geometric means in water also had high geometric mean values in sediment.

The eleven sampling locations represented a range of sediment types from coarse-grained sands with <2% organic content, to fine-grained mud with high organic carbon content. Sampling location H, along the Piermont Pier, had by far the highest organic C content (27%; Table 1), which is not surprising given the peat-like marsh sediment conditions found at this site. The FIB geometric mean concentration in sediments was positively correlated with the mean percent organic C at each sampling location for both enterococci (Fig. 3; $r = 0.673$, $p = 0.03$), and *E. coli* (Fig. S5; $r = 0.682$, $p = 0.03$).

3.3. Molecular genetic investigation of fecal associated bacteria

Taxonomic analysis of DNA sequences from the gene encoding 16S rRNA confirmed that the IDEXX Enterolert cultivation-based assay, typically used for water column enumeration of enterococci, was not prone to either false positive or false negative identification of enterococci when applied to sediment samples from Flushing Bay (site J). DNA sequences ($n = 62$) originating from 5 pooled IDEXX quanti-tray wells that fluoresced blue, indicating positive detection of enterococci, were all found to have greatest similarity to species of *Enterococcus* (Genbank accession numbers MH002099-MH002160). The vast majority (97%) of sequences were most similar to *E. faecium*, with the remaining 3% of sequences most similar to *E. durans*. In contrast, none of the DNA sequences ($n = 15$) from 5 pooled, non-fluorescing (i.e., negative), wells were found to be most similar to species in the genus *Enterococcus (Genbank accession numbers MH002084-MH002098)*. The most common taxa (33% of sequences) associated with sequences from non-fluorescing, negative wells, were species from the genus *Sulfurovum*.

Membrane filtration assays, using mEI media, were performed to obtain colonies enumerated as enterococci from eight HRE water and sediment samples so that the taxonomic identity of isolates could be characterized (Table 3). The presence of these colonies on mEI media provides additional confirmation of the presence of viable enterococci in sediment. Overall, >99% of the 176 sediment isolate sequences from FIB cultivation assays were confirmed as enterococci, having greatest similarity to *Enterococcus* species in genbank, with the most common species (39%) identified as *E. faecium*. One sequence (<1% of isolates) was instead found to have greatest similarity to *Klebsiella pneumonia*, indicating a very low level of false positives. Of the 37 water sequences, 100% were most similar to *Enterococcus* species, with 30% identified as *E. faecium*. Examining the data, location by location, revealed that the sites with the highest cultivation based concentrations of water and sediment enterococci (H, I, J) had either *E. faecium* or *E. faecalis* identified as the most common isolate. In comparison, locations with lower geometric mean enterococci concentrations (e.g. B and D), and situated further from either CSO or wastewater treatment discharge, had other *Enterococcus* species, such as *E. gallinarum* or *E. casseliflavus*, identified as the most common sediment isolate sequences.

High throughput Illumina DNA sequencing was used to characterize the representation (% of total sequences obtained from a sample) of both FIB and FT from 8 sediment samples. OTUs classifed as *E. coli* and OTUs belonging to FT were identified in all 8 sediment samples, while OTUs classified as belonging to the genus *Enterococcus* were only detected in the 2 sediment samples collected from Flushing Bay (Sites I and J). These two Flushing Bay sediment samples also had the highest percent sequence representations of *E. coli* and of FT. The percent representation of FIB (*E. coli* + *Enterococcus*) was significantly correlated ($r = 0.87$, $p = 0.008$) with the percent representation of FT in sediment samples across the eight locations (Fig. 4).

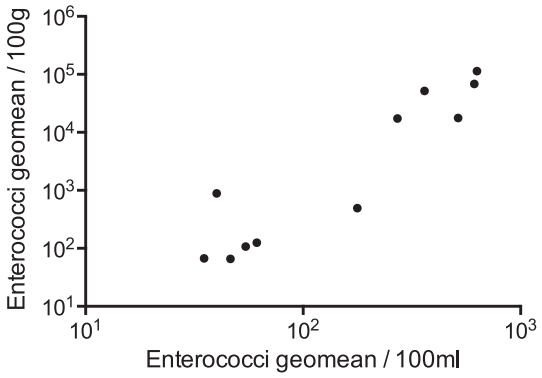

Fig. 2. FIB correlation in water and sediment across sites. Positive association of the enterococci geomeans in water and sediment by sampling site, $r = 0.882$, $p < 0.01$.

Table 2
Correlation statistics for sediment and water FIB within sites, and across ("total") sites.

Site	Name	Enterococci			*E. coli*		
		# of pairs	Spearman r	p value	# of pairs	Spearman r	p value
A	Ossining	11	0.476	0.139	9	0.550	0.133
B	Stony Point	9	0.617	0.086	8	0.778	0.030
C	Kingsland Point Park	10	0.506	0.138	8	0.407	0.315
D	Croton	10	0.231	0.518	8	0.503	0.209
E	Sneedens Landing	6	0.371	0.497	6	0.395	0.450
F	Parelli Park	6	0.257	0.658	6	−0.657	0.175
G	Sparkill Creek	10	−0.188	0.607	7	0.179	0.713
H	Piermont SPDES	11	0.127	0.714	9	0.283	0.463
I	Flushing Bay Sandy	7	0.429	0.354	6	−0.058	0.933
J	Flushing Bay Dock	11	−0.018	0.962	10	−0.227	0.531
K	Meadow Creek	6	0.029	0.999	6	−0.371	0.497
	Total	97	0.482	<0.001	83	0.646	<0.001

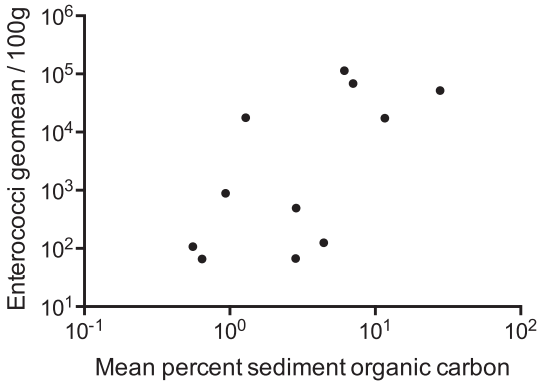

Fig. 3. Correlation of FIB and % organic carbon in sediment. Positive association of the enterococci geomeans with the mean percent organic carbon by sampling site, $r = 0.673$, $p = 0.03$.

Table 3
Taxonomic identification, and percent representation of isolates within a sample location, based on DNA sequencing of isolates obtained from membrane filtration (MF) enterococci assays using Hudson River Estuary sediment and water.

Site	Name	Type	Method	# Seq	# Ent	Enterococcus species	Non-Enterococcus Species
A	Ossining	Sed	MF	1	1	100% *E. faecalis*	None
B	Stony Point	Sed	MF	9	9	56% *E. gallinarum*, 33% *E. casseliflavus*, 11% *E. faecalis*	None
		Water	MF	9	9	44% *E. mundtii*, 33% *E. hirae*, 11% *E. casseliflavus*, 11% *E. faecium*	None
C	Kingsland Point Park	Sed	MF	2	2	50% *E. faecium*, 50% *E. casseliflavus*	None
		Water	MF	1	1	100% *E. faecium*	None
D	Croton	Sed	MF	5	5	40% *E. casseliflavus*, 40% *E. faecalis* 20% *E. thailandicus*	None
		Water	MF	1	1	100% *E. faecium*	None
G	Sparkill Creek	Sed	MF	12	12	33% *E. hirae*, 25% *E. faecalis* 25% E. *mundtii*, 17% *E. faecium*	None
		Water	MF	7	7	86% *E. faecalis*, 14% *E. hirae*	None
H	Piermont SPDES	Sed	MF	27	27	37% *E. faecium*, 33% *E. faecalis*, 26% *E. hirae*, 4% *E. thailandicus*	None
		Water	MF	8	8	37.5% *E. faecalis*, 25% *E. faecium* 25% *E. mundtii*, 12.5% *E. hirae*	None
I	Flushing Bay Sandy	Sed	MF	49	48	43% *E. faecium*, 25% *E. hirae*, 10% *E. lactis*, 8% *E. faecalis* 8% *E. durans*, 2% *E. gallinarum*, 2% *E. thailandicus*,	2% *Klebsiella pneumonia*
J	Flushing Bay Dock	Sed	MF	34	34	59% *E. faecium*, 26% *E. hirae*, 6% *E. faecalis*, 3% *E. gallinarum*, 3% *E. lactis*, 3% *E. mundtii*	None
		Water	MF	11	11	55% *E. faecium*, 27% *E. thailandicus* 18% *E. hirae*	None
	TOTAL	Sed	MF	139	138	39% *E. faecium*, 23% *E. hirae*, 16% *E. faecalis*, 5% *E. gallinarum*, 4% E. *casseliflavus*, 4% *E. lactis* 3% *E. mundtii*, 2% *E. thailandicus*, 3% *E. durans*	<1% *Klebsiella pneumonia*
	TOTAL	Water	MF	37	37	30% E. *faecium*, 24% *E. faecalis*, 19% *E. hirae*, 16% *E. mondtii*, 8% *E. thailandicus*,3% E. *casseliflavus*	None
J	Flushing Bay Dock	Sed	IDEXX negative	15	0	None	33% *Sulfurovum sp.*, 67% other
J	Flushing Bay Dock	Sed	IDEXX positive	62	62	97% *E. faecium*, 3% *E. durans*	None

3.4. Comparison of water column FIB concentration from shallow and deep sites

Enterococci geometric mean concentrations in water were compared across sites from three depth categories, defined as shallow shoreline (<20 cm) (Sites A, B, C, D, F, H), nearshore (2–3 m) and midchannel (>5 m) sites (Fig. S2, Table S1). The median abundance of enterococci differed significantly with depth of the sampling site (Kruskal Wallis, $p < 0.01$). More specifically, shoreline geometric means were significantly higher than mid-channel geometric means (Dunn corrected $p < 0.01$) (Fig. 5).

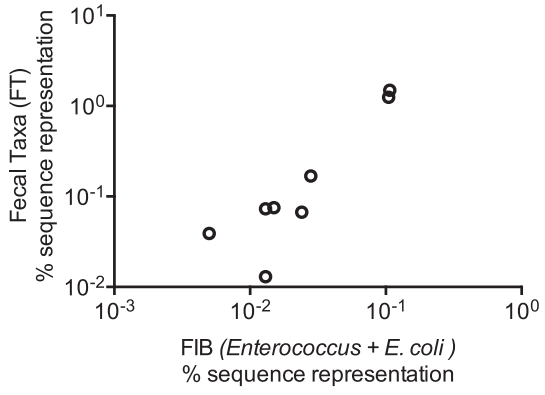

Fig. 4. Correlation of FIB and fecal taxa sequence representation. Significant positive association ($r = 0.85$, $p = 0.006$) of the *E. coli* percent sequence representation with Fecal Taxa (FT) percent sequence representation from Illumina 16S rRNA gene amplicon sequence libraries across eight sediment sampling sites (A, B, C, D, G, H, I, and J).

3.5. Shoreline sediment resuspension from wading

Ten wading trials were performed along the shoreline near the Piermont Pier (near sites F and H), with paired water column samples collected before and after wading into the water, to examine the potential for recreator, or water sampler, activity to resuspend sediment and alter FIB levels in the water column. Nine of the ten trials resulted in increased water column FIB levels after wading (Fig. 6). The mean increase in enterococci concentration was 114 MPN/100 ml, as

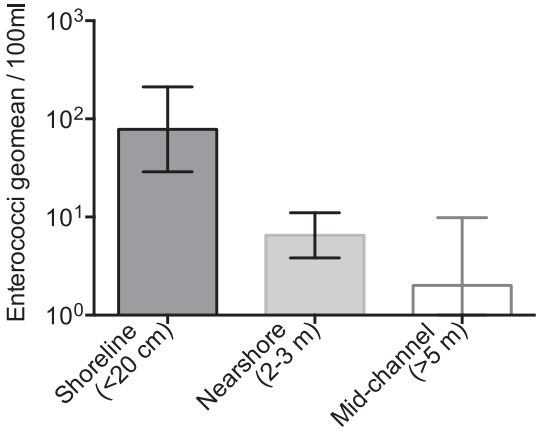

Fig. 5. FIB change by depth. Comparison of enterococci concentrations from six shallow (<20 cm depth) shoreline sampling stations from this study (Sites A, B, C, D, F, H) to data at nearby sampling stations from the Riverkeeper water quality monitoring program in deeper water (2-3 m, $n = 5$; and >5 m, $n = 3$), collected by boat. Only a subset of shallow stations were selected for the comparison due to the lack of adjacent deeper water comparison stations for some locations (sites E, G, I, J, K). Bars represent geomeans ± 95% confidence interval. Geomeans were significantly different by depth, Kruskal Wallis, $p < 0.01$.

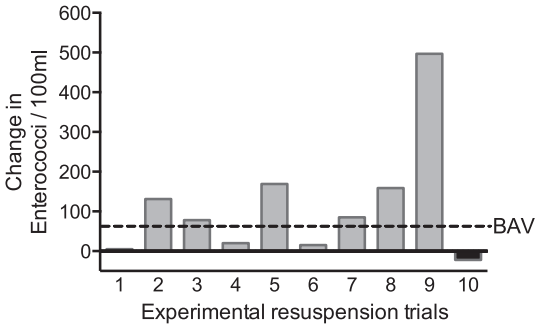

Fig. 6. FIB resuspension experiment. Water samples were collected before and after a sampler entered the water near the Piermont Pier (shoreline in proximity to sites F and H). Each bar represents a distinct resuspension trial and the height of the bar represents the change in enterococci measured (concentration after – concentration before). Grey bars represent increases in FIB after experimental resuspension, the black bar (right) represents a small decrease in FIB after one trial. Dotted line represents the US-EPA Beach Action Value (BAV).

compared to an EPA Beach Action Value (BAV) of 60 MPN/100 ml (US-EPA, 2012).

4. Discussion

4.1. FIB spatial distributions and connection of water and sediment reservoirs

This FIB survey shows the HRE to be highly impacted by sewage, as previously recognized (Hetling et al., 2003; NYCDEP Centennial Study, 2009; Suter et al., 2011; Young et al., 2013; Riverkeeper, 2015). Both enterococci and *E. coli* were widely distributed in water and sediment of all eleven sites (Fig. 1), and with concentrations of the two FIB positively correlated in water and sediment samples (Fig. S3). The maximum sediment FIB concentrations in this study exceeded cultivation based FIB concentrations reported (10^3–10^4/100 g) for most beach sands (Alm et al., 2003; Bonilla et al., 2007; Yamahara et al., 2007; Halliday et al., 2014) and coastal sediments (Perkins et al., 2014; Luna et al., 2018), but were consistent with maximum concentrations (10^5–10^6/100 g) reported in riverbank (Desmarais et al., 2002), wetland (Evanson and Ambrose, 2006), and estuarine sediments (Hassard et al., 2016), as well as heavily bird influenced sands under a pier in Santa Monica, CA (Mika et al., 2017). At the northernmost sites (e.g. Sites A–D), further from New York City, maximum sediment FIB levels in the HRE were generally lower (10^3–10^4/100 g), and more similar to maximum concentrations observed in prior studies of beach sand, except in the one case where a local sewage source was present (e.g. Site H).

The concentration of FIB was generally at least an order of magnitude higher in surface sediments (per 100 g dry weight) than in the water column (per 100 ml) (Fig. 1), consistent with prior estuarine surveys (Roslev et al., 2008, Perkins et al., 2014, Hassard et al., 2016). This pattern is likely related to several processes. A large percentage of FIB are particle associated (Jamieson et al., 2005a; Fries et al., 2006; Suter et al., 2011; Mote et al., 2012), which promotes settling (Ducklow et al., 1982; Garcia-Armisen and Servais, 2009) and accumulation at the sediment interface. In addition, FIB persistence is likely longer in sediments than in water, especially under low temperature, low light, and high organic carbon content (Desmarais et al., 2002; Craig et al., 2004; Haller et al., 2009; Chudoba et al., 2013; Perkins et al., 2016). Estuarine sediment can thus be considered an important reservoir or zone of accumulation, and in extreme cases could even act as a naturalized habitat (Davies et al., 1995; Haller et al., 2009).

In the HRE, water column FIB concentrations were more variable over time than in sediments, and water FIB concentrations at individual sampling sites were poorly correlated with sediment FIB concentrations in samples collected at the same time (Table 2) as observed in other marine systems (e.g. Luna et al., 2018). These observations suggest that sediments provided a more stable FIB reservoir, reinforcing the importance of prolonged FIB persistence, while counts in the water column had greater temporal fluctuations, responding to episodic inputs and faster decay and dispersion. However, geometric mean FIB values across sites showed significant positive correlations between water and sediment (Fig. 2, Fig. S4). This finding is novel, though not necessarily surprising, as FIB inputs are assumed to reach the water first, and sediments secondarily. Sediments will therefore only have high FIB levels if the overlying water column periodically experiences elevated concentrations.

Sediment FIB concentrations increased in proximity to sewage outfalls and CSOs (e.g. Sites H and I-J, respectively). These sites also had the highest percent representation of FIB and FT DNA sequences. Discharge from a Flushing Bay CSO, located near sites I and J, is known to contain high particle concentrations with rapid particle settling rates (median settling rate ca. 10 m h^{-1}, Fugate and Chant, 2006), which implies limited horizontal transport before particles, and associated microbes, would reach the sediments. Particles in CSO or other sewage discharges are also expected to deliver organic carbon to the sediments, consistent with high sediment organic carbon values at sites H, I and J. The higher sediment organic carbon concentrations should also increase FIB persistence.

4.2. Molecular genetic investigation of fecal associated bacteria

Sequence data from positive IDEXX wells and mEI colonies from HRE sediment suggest that cultivation based techniques for FIB enumeration are not prone to high frequency false positives or false negatives when applied to sediments. For comparison, Ferguson et al. (2005) found a slightly higher portion (8% to 15%) of putative enterococci colonies isolated from coastal ocean sediments on mEI media actually belonged to genera other than *Enterococcus*.

The spatial pattern in the distribution of *Enterococcus* species within the HRE, may relate to different sources, or persistence of different species, across sampling sites. *E. faecium* and *E. faecalis* are commonly human associated (Noble, 1978) and *E. faecium*, *E. faecalis* and *E. hirae* are widely distributed in animal feces (Devriese et al., 1987). This may be why these species were most abundant overall (Table 3) and especially dominant in sediment in areas influenced by episodic sewage overflows (Sites H, I, J). *E. faecium* and *E. faecalis* were also the most common *Enterococcus* isolates in other studies of coastal sediments (Vignaroli et al., 2018; Ferguson et al., 2005). The increased frequency of *E. gallinarum, E. mundtii*, and *E. casseliflavus* at our northern sites (B-D) with lower FIB geometric means and no nearby CSOs or sewage outfalls, may indicate differing fecal sources, perhaps with less human influence, or less frequent delivery. For example, both *E. casseliflavus* and *E. mundtii* have been found in association with plant material and soil sources (Leclerc et al., 1996). Alternately, the relative abundance of different *Enterococcus* species could reflect differential environmental persistence, as demonstrated for *E. casseliflavus* versus *E. faecalis* (Mote et al., 2012; Tymensen et al., 2017). Differential environmental persistence of FIB strains (Anderson et al., 2005; Mote et al., 2012) would be most important in areas where FIB delivery occurs less frequently, as expected for the more northerly sites in this study.

Because FIB arrive to the sediments indirectly, via the water, and then experience conditions that should favor their extended persistence, it is appropriate to question whether FIB in sediment continue to be useful indicators for pathogens that originate from fecal sources. The positive correlation between enterococci and *E. coli* in sediments, supports the concept that fecal microbes co-occur and are transported together. Similar evidence was described by Perkins et al. (2014) in the Conway Estuary, where multiple FIB and fecal pathogens were positively correlated with each other in sediment. The significant and positive correlation of FIB and FT sequence representation across HRE sites in high-throughput sequencing data provides additional support for the

use of FIB in representing broader patterns of sewage and fecal associated bacteria. Use of high throughput sequence data to determine the representation of FT has been common in water column studies (e.g. Newton et al., 2013; Zhang et al., 2018), but recent studies (Köchling et al., 2017; Mika et al., 2017; Luna et al., 2018; Abia et al., 2018) have used molecular data to better understand fecal contamination patterns in sediment. Even with high throughput sequencing approaches, OTUs classified as *Enterococcus* were only detected in the samples with the highest sequence representation of *E. coli* and FT, as well as the highest levels of cultivated FIB. Limited detection of common FIB in sequence databases reinforces the use of broader groups of FT that can be more abundant and easier to detect. However, complexities of differential environmental persistence of FIB, FT, and specific pathogens, as well as the offset commonly observed when attempting to enumerate sediment taxa using cultivation versus molecular based techniques, remain as areas in need of continued investigation.

4.3. Resuspension potential

In the HRE and many other environments, turbidity is often positively correlated with FIB concentrations (Fries et al., 2008; Suter et al., 2011; Halliday et al., 2014) and sediment resuspension has been suggested as a factor modulating water column FIB levels (Whitman et al., 2014; Roslev et al., 2008; Halliday et al., 2014). In this study, shoreline locations had significantly elevated FIB concentrations compared to surface water samples collected from nearby locations over deeper water. These observations are consistent with the general trend of greater FIB concentrations at nearshore compared to mid-channel stations in the HRE (Suter et al., 2011; Young et al., 2013). Resuspension of FIB from sediment could contribute to these trends and the plausibility of resuspension as the cause of higher shoreline FIB counts is bolstered by the sediment resuspension trials. Sediment resuspension was similarly proposed to explain elevated concentrations of amoeboid protists in shoreline samples from the HRE (Juhl and Anderson, 2014). Turbulence, related to tides, wind and waves, can cause sediment resuspension (Fugate and Friedrichs, 2003) which can be an important determinant of water quality (Roslev et al., 2008; Halliday et al., 2014; Whitman et al., 2014), especially with respect to organisms, such as FIB, that are commonly particle associated.

4.4. Conclusions and management relevance

Sediments were a reservoir of FIB in the HRE, with higher concentrations in the sediments than in the overlying water and spatial coupling between water and sediment FIB geometric means across locations. Counts of enterococci and *E. coli* were positively correlated to each other and to a broader community of fecal microbes measured in sediment using molecular approaches. Observations indicate an important role for resuspended sediment FIB in estuarine water quality.

Greater temporal stability of sediment FIB, compared to those in the water, suggests that sediment sampling could be a useful monitoring approach where episodic FIB delivery is expected, but frequent water column sampling is unrealistic. However, the lack of regulatory guidance is an important limitation to interpreting sediment FIB monitoring data. The act of a recreator entering shallow water, and causing resuspension, could alter the risk from contact with that water, especially along shorelines with high organic, muddy, sediment. This prediction assumes that FIB counts in sediment are coupled to illness risk, as they are in water, a topic that is still widely debated (Donovan et al., 2008; Sabino et al., 2014; Heaney et al., 2012; Solo-Gabriele et al., 2016; Abia et al., 2016). In addition, deep water boat-based sampling may underestimate the health risks from shoreline recreation, and shoreline sampling programs should explicitly consider whether to collect, or to avoid, water with resuspended sediments. Finally, it is relatively common to eliminate sewage disinfection during the non-recreational season (Laubusch, 1958; Mitch et al., 2010), however, persistence of FIB in sediments suggests that year-round disinfection would have benefits in shallow water environments where resuspension occurs. Management of estuaries would benefit from improved understanding of sediment FIB dynamics and the health risks from contact with contaminated sediment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.405.

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