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Natural attenuation of indicator bacteria in coastal streams and estuarine environments

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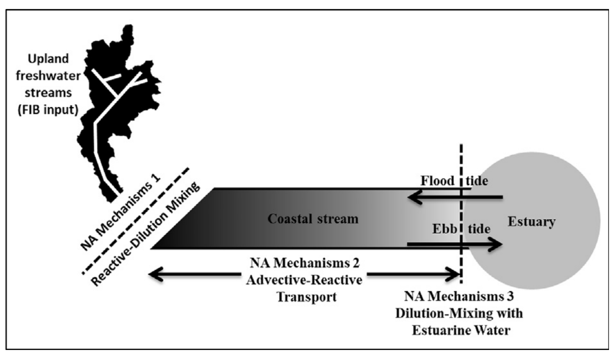
HIGHLIGHTS

- Proposes a flow dependent natural attenuation framework for pathogens in estuaries: freshwater-coastal, coastal stream, and coastal-estuary
- Lines of evidence developed for each regime with associated reductions in indicator bacteria
- Proposed framework provides linkages between natural water system components and total maximum daily loads (TMDLs)
- Results indicate more achievable source reduction targets are likely using natural attenuation

GRAPHICAL ABSTRACT

Natural attenuation regimes for freshwater-coastal-estuarine systems allow linkages between components and their pathogen concentrations and load interactions and lead to more achievable reduction targets.



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ABSTRACT

One of the most significant causes of poor water quality is the presence of pathogens. To reduce the cost of human exposure to microbial contamination, monitoring of Fecal Indicator Bacteria (FIB), as a surrogate for the presence of pathogens in natural waters, has become the norm. A total maximum daily load (TMDL) framework is used to establish limits for microbial concentrations in impaired waterbodies. In order to meet microbial loads determined by the TMDLs, reductions in microbial sources varying from 50% to almost complete elimination are required. Such targets are fairly difficult, if not impossible, to achieve. A natural attenuation (NA) framework is proposed that takes into account the connectivity between freshwater streams and their receiving coastal estuaries. The framework accounts for destructive and non-destructive mechanisms and defines three regimes: NA 1 - reaction-dilution mixing at the freshwater-tidal interface, NA 2 - advection-reactions within the tidally influenced coastal stream, and NA 3 - dilution-discharge at the interface with the estuary. The framework was illustrated using the Houston Metropolitan area freshwater streams, their discharge into the Houston Ship Channel (HSC) and into Galveston Bay. FIB concentrations in Galveston Bay were much lower when compared to FIB concentrations in Houston streams. Lower enterococci concentrations in tributary tidal waters were found compared to their counterparts in fresh waters (NA1 regime). Additionally, 70% reduction in FIB loads within the HSC was demonstrated as well as a decreasing trend in enterococci geometric means, from upstream to downstream, on the order of 0.092 day^{-1} (NA2 regime). Lower enterococci concentrations in Galveston Bay at the confluence with the HSC were also demonstrated (NA3 regime). Statistical testing showed that dilution, tide-associated processes, and salinity are the most

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important NA mechanisms and indicated the significant effect of ambient temperature and rainfall patterns on FIB concentrations and the NA mechanisms.

1. Introduction

In recent years, it has become evident that one of the most prevalent pollutants associated with water quality of streams, estuaries and coastal systems is pathogens (U.S. EPA, 2017; Henry et al., 2016). In the U.S. alone, marine-born microbial contamination costs include more than $300 million for gastrointestinal disease associated with beach recreation (Dwight et al., 2005; Ralston et al., 2011). To reduce beach closures and the cost of human exposure to microbial contamination, monitoring of Fecal Indicator Bacteria (FIB) as a surrogate for the presence of pathogens in natural waters has become the norm (Desai et al., 2010; Goodwin et al., 2012). This is mainly due to their plentitude in the fecal waste of warm-blooded species, their survival in a wide range of water quality regimes, and their strong association with the risk of gastrointestinal illness. Coliform bacteria are one example of FIB. While not interchangeable, for the purposes of this paper, the term FIB is used to broadly represent the potential presence of fecal contamination and pathogenic organisms as pollutants in surface water systems.

Monitoring of FIB in freshwater has conventionally focused on total coliform, and more recently on *E. coli* as measures of pathogenic contamination (Gabutti et al., 2000; Odonkor and Ampofo, 2013). While total coliforms and fecal coliforms refer to a group of species of bacteria, *E. coli* is a single species belonging to the fecal coliform group. Thus, the presence of *E. coli* in freshwater is a more reliable indicator of the existence of fecal origin contamination compared to the general coliform group (Jin et al., 2004). In marine waters, enterococci (similar to *E. coli*, facultative anaerobic, typically commensal, opportunistic pathogens commonly found in the gastrointestinal tracts of many animals and birds) are considered to be a more appropriate FIB (Ostrolenk et al., 1947; Cabelli et al., 1979; Cupakova and Lukasova, 2003; Fogarty et al., 2003; Fisher and Phillips, 2009; Byappanahalli et al., 2012a; Conway and Cohen, 2015). Several studies have established the relationship between enterococci in environmental waters and health outcomes (Wade et al., 2003; Colford et al., 2004).

FIB concentrations are influenced by different environmental factors. Inactivation of bacteria is due to several environmental conditions such as salinity, pH, dissolved oxygen (DO), sunlight and water temperature, transport mechanisms such as overland flow and in-stream routing, and biological factors such as microbial predation and competition (Lipp et al., 2001; Anderson et al., 2005; Mill et al., 2006; Dorner et al., 2006; Mattioli et al., 2017; Palazon et al., 2017). The effect of seasonality on FIB concentrations has been reported differently: some researchers reported higher concentrations in warmer months (Diez-Vives et al., 2014; Hathaway et al., 2010) and others in cooler ones (Lipp et al., 2001; Pote et al., 2009). Researchers have also studied the factors that contribute to greater potential for fecal contamination at the regional scale (Opisa et al., 2012, Rochelle-Newall et al., 2015, Brinkmeyer et al., 2015, Gordon et al., 2013, Liao et al., 2015, Rochelle-Newall et al., 2015, Durham et al., 2016, Kirs et al., 2017, Bai and Lung, 2005, Zhou et al., 2017a, 2017b), and have reported on the extensive variability of FIB spatially and temporally (He et al., 2007; Traister and Anisfeld, 2006; Desai and Rifai, 2013; Diez-Vives et al., 2014; Chenier et al., 2012; Enns et al., 2012; Cui et al., 2013; De Luca-Abbott et al., 2000).

In surface water, Total Maximum Daily Loads (TMDLs) have been adopted as the conceptual framework for managing pollutants in natural water systems (Johnson et al., 2013) according to the Section 303 (d) that was amended to the Clean Water Act (CWA) in 1972. A TMDL is the maximum amount of a pollutant that a body of water can receive while still meeting water quality standards. TMDLs can be developed for bacteria impairment for different uses of the water body such as contact recreation and oyster harvesting (He et al., 2007; Johnson et al., 2013). When water quality standards are exceeded, pollutant sources are inventoried (by the States according to Section 303(d) of the CWA), and the required pollutant reductions are determined. According to the U.S. EPA, "reduction actions are implemented through a wide variety of programs at the state, local and federal level. These programs may be regulatory, non-regulatory or incentive-based e.g., a cost-share program" (U.S. EPA, 2019a). Studies on FIB have elucidated that significant reductions of this pollutant would be needed, particularly in urbanizing and coastal zones, to maintain water quality. Such reductions (typically in excess of 50% and often approaching complete reduction) are difficult, if not impossible, to achieve due to the diverse sources of FIB (surrogate of pathogens) that include diffuse sourcing from wildlife and polluted runoff (Benham et al., 2005).

Natural attenuation refers to observed reductions in contaminant concentrations as a result of a number of fate and transport processes (Wiedemeier et al., 1999). These processes, originally defined for fuels and chlorinated solvents, include dilution, sorption, advection, dispersion, and biotic and abiotic transformations. From a mass balance point of view, the aforementioned processes can be categorized into two major groups: destructive and nondestructive. Nondestructive processes reduce concentrations but do not reduce mass; these include dilution, sorption, advection, and dispersion. Destructive processes, on the other hand, such as biotic and abiotic transformations, cause degradation of contaminants and will change both mass and concentrations (Wiedemeier et al., 1999). However, the same concept (framework) can be applied to FIB. The concept of natural attenuation originated in ground water (Kao et al. 2010, Ouvrard et al., 2013, Freitas et al., 2015, Zhou et al., 2017a, 2017b, Kawabe and Komai, 2019) but a few studies have been completed at the groundwater-surface water interface (Smith et al., 2009; Batlle-Aguilar et al., 2009), and for contaminants in soils and sediments (Wang and Tam, 2012; Agnello et al., 2016; Dong et al., 2019; Yan et al., 2019), as well as for uranium and pharmaceuticals and nutrients in wetlands (Casey and Klaine, 2001; Manaka et al., 2007; Acuna et al., 2015).

FIB standard concentrations are higher for navigation and industrial water supply uses compared to primary contact recreation and exceptional aquatic life and oyster water uses (TCEQ, 2014). Thus, it would be reasonable to take advantage of this higher potential for natural attenuation (NA) capacity present in coastal estuaries to assimilate the typically inordinate high FIB loads from urbanized upper watersheds. In addition, and because of their living nature, die-off and regeneration, and accumulation in sediment beds, FIB can continually pollute the overlying waters. A consideration of the NA mechanisms that influence the fate and transport of FIBs in a natural water system is merited. Importantly, quantification of attenuation rates would aid in understanding concentration and mass reductions that might naturally occur in such systems thereby lessening the need for unattainable and costly reductions via engineering solutions. In addition, such quantification helps regulators set limits that are more realistic.

In tidal systems, tidal movement and the magnitude of the tide can change the properties of the water body, especially salinity (Etemad-Shahidi et al., 2010), as well as cause dilution/mixing that can change FIB concentrations. Consequently, evaluating bacterial water quality in a tidal estuary system is more complicated since it is subject to both fresh and marine water sources and fate mechanisms affecting FIB (Mill et al., 2006; McLaughlin et al., 2007). Unlike groundwater, natural attenuation mechanisms of FIB in an estuarine system involve the combined effect of several attenuation processes depending on the flow regime (Rippy et al., 2013a). For example, natural attenuation of FIB at the discharge point of a freshwater river into an estuary can be mainly attributed to dilution-mixing whereas reactive transport processes dominate within the estuary. Similar to groundwater, however, the spatial and temporal variability of FIB within the estuary as a function of environmental variables and their re-growth and die-off are less well understood (Surbeck et al., 2010).

Some studies have noted reductions in FIB concentrations in estuary systems compared to fresh waters (McLaughlin et al., 2007; Lewis et al., 2013; Johnston et al., 2015) but did not elucidate natural attenuation regimes in different segments of an estuarine system that may be responsible for the observed declines. Other studies investigated the correlation of FIB concentration, die-off and re-growth rates to other water quality constituents such as dissolved organic carbon and nutrients (Surbeck et al., 2010) in riverine systems, FIB mortality mechanisms (Rippy et al., 2013b) and advection and cross-shore horizontal diffusion, mainly caused by waves and currents, in nearshore waters (Rippy et al., 2013a), and the effect of small storm drains on levels of FIBs within an enclosed beach (Rippy et al., 2014). No studies to date have investigated the natural attenuation of FIBs as a framework that accounts for destructive (degradation processes leading to die-off and inactivation) and non-destructive (advection, dispersion, dilution) natural attenuation mechanisms and defines flow dependent FIB NA regimes in an estuarine system.

This paper makes the case for using natural attenuation as a framework for managing microbial loads in natural water systems, particularly coastal and estuarine environments that may have a sufficient assimilative capacity to significantly reduce microbial loads from upland urbanized drainage areas as well as drainage areas contributing runoff directly to the estuary itself. The residence times within tidally influenced conduits that take freshwater discharges from upland areas into the receiving estuary are demonstrated to be important for reactions that reduce FIB concentrations as noted in Kiaghadi and Rifai (2016).

2. Methods and materials

2.1. Conceptual framework of natural attenuation in coastal and estuarine systems

In this study, nondestructive processes for surface water were defined to include dilution/mixing, advection, and dispersion while destructive processes include bacteria death and re-growth due to environmental conditions within the estuary such as temperature and salinity.

Estuarine natural water systems are typically the discharge points for a freshwater riverine system as it transitions through a saline coastal stream or channel towards the ocean. Fig. 1 illustrates a schematic representation of the developed conceptual framework for natural attenuation of FIB in such a system. The FIB loads entering the estuarine system from upland areas, as shown on the left side of Fig. 1, encounter three hypothetical attenuation regimes:

2.1.1. Reactive-dilution mixing regime at freshwater/marine interface

As freshwater enters the more saline coastal stream, a reduction in certain FIBs (total coliform and *E. coli* in particular and Enterococci at higher light intensities) can be expected due to the die-off (destructive) that is associated with increased salinity (Carlucci and Pramer, 1960, Dorsey et al., 2010, Du et al., 2012) and dilution (non-destructive). Additionally, attenuation due to rising and ebbing tides within the tidally influenced parts of the freshwater streams will experience FIB die-off (destructive).

2.1.2. Advective-reactive transport regime within receiving coastal stream

As FIB is transported within the more saline coastal stream to the receiving estuary, FIB concentrations can be attenuated due to the increase in the volume of water (dilution, non-destructive). The total number of FIB (with a unit of MPN, calculated by multiplying FIB concentration to the volume of water), may also be attenuated due to rising tides (Fig. 1) that increase salinities and change hydraulic conditions (thereby further contributing to FIB die-off), and due to other ambient environmental variables such as diurnal temperature and sunlight variations.

2.1.3. Dilution-mixing regime at the interface with estuarine water

As coastal stream flows are discharged into the receiving estuary (right side of Fig. 1), attenuation of FIB due to dilution mixing and increased salinities may occur.

The aforementioned NA processes are not as well understood and cannot be individually quantified with conventionally collected water quality sampling data. Thus, in a similar manner to natural attenuation studies in ground water, the 'lumped' effects of NA processes is inferred by estimating apparent first-order decay rates from sampling data calculated using enterococci as the FIB of interest (see Section 2.5). This is because the entire system is tidal and enterococci are the only measured FIB in almost all the sites. The proposed NA conceptual framework and estimation methods are demonstrated using the Buffalo Bayou estuary in Houston, TX as a model system as described in the next sections (Section 2.4).

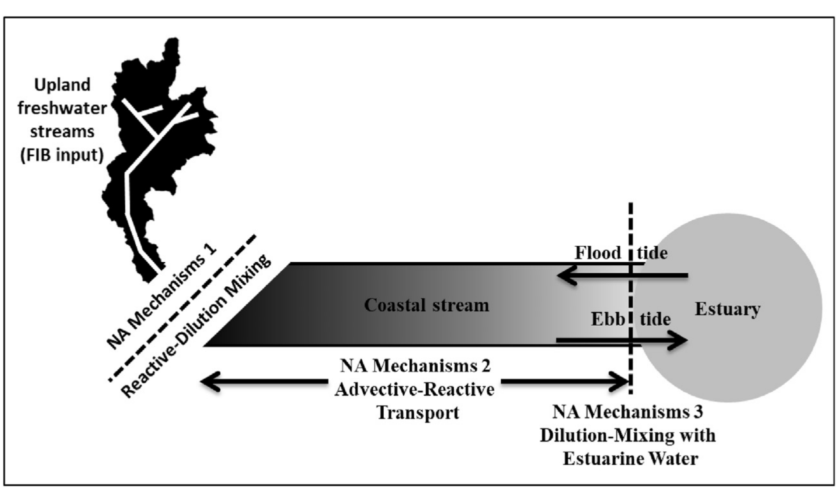

Fig. 1. Conceptual model for natural attenuation in coastal and estuarine systems (darker shading indicates higher FIB concentrations).

2.2. Study area

Fig. 2 shows Buffalo Bayou (latitude: 29.725243, longitude: −95.230272); a freshwater stream that transitions to the saline Houston Ship Channel (HSC, a widened and deepened section of Buffalo Bayou used for navigation) that discharges into the Galveston Bay Estuary. FIB inputs to the system are from the Houston Metropolitan area (Lester and Gonzalez, 2011) that is highly urbanized (developed areas) and exhibits FIB levels that exceed Texas Commission on Environmental Quality (TCEQ) standards (Petersen et al., 2005; Desai et al., 2010; Brinkmeyer et al., 2015). In addition to FIB, the study area has been impaired for depressed dissolved oxygen, nutrients, polychlorinated biphenyls (PCBs) and dioxins. Bacteria loads may come from point and non-point sources such as domestic and industrial wastewater treatment facilities, sanitary sewer overflows, storm water discharges, illicit discharges, wildlife and unmanaged animal contributions, agricultural activities and domesticated animals. Additional FIB inputs enter the HSC from its adjacent drainage areas; however, those tend to be relatively negligible compared to inputs from Houston.

2.3. Data acquisition

Water quality data including Enterococci, *E. coli*, salinity, pH, temperature, and DO were compiled from the TCEQ Surface Water Quality Web Reporting Tool (TCEQ, 2014). According to TCEQ "all data reported by TCEQ are planned through the development of Quality Assurance Project Plans (QAPPs)". Thus, no extra quality assurance/control (QA/QC) was conducted in this study. A total of thirteen (13) Water Quality Monitoring (WQM) stations in the HSC and one located in Upper Galveston Bay (station 15244, Fig. 2) were chosen for the study based on data availability. There were no data for enterococci prior to 2000 (fecal coliform was the FIB prior to 2000) thus, the period of record covers the years 2000–2013 unless otherwise stated in the text. Additional data from 19 WQM stations within the tributaries and their tidally influenced segments were also assembled for FIB load calculations, estimation of the Enterococci/*E. coli* ratio (EPA, 2019b), and correlation analyses of FIB in tributaries to their counterparts in the receiving HSC. It should be noted that WQM stations above the tidal boundary for each tributary (Fig. 2) would have *E. coli* data whereas WQM stations located in the tidally influenced sections have enterococci data. For comparison purposes, freshwater *E. coli* data were converted to saline enterococci equivalents using a conversion ratio that was calculated from co-sampled data for *E. coli* and enterococci in stations located at the tidal boundaries within the study area. At each station with at least six concurrent measurements of *E. coli* and enterococci (eight WQM stations, spatially distributed in the study area), a regression analysis was conducted between the log-transformed values of two FIB concentrations. To have a more realistic ratio (slope) the fitted line at each station was forced to pass through the origin (intercept = 0). For all of the regression models, coefficients' *P*-values were smaller than 0.05 so the average of eight calculated slopes (ratios) was used as the final conversion ratio. Table S1 in Supplementary Information (SI) provides a comprehensive list of WQM stations with their studied parameters.

Flow data for freshwater streams were obtained from the United States Geological Survey (USGS). Since the HSC is tidally influenced, flows for tidal parts of the system were extracted from an existing and validated Environmental Fluid Dynamics Code (EFDC) model developed by Howell (2012). The volume of water at different locations within the

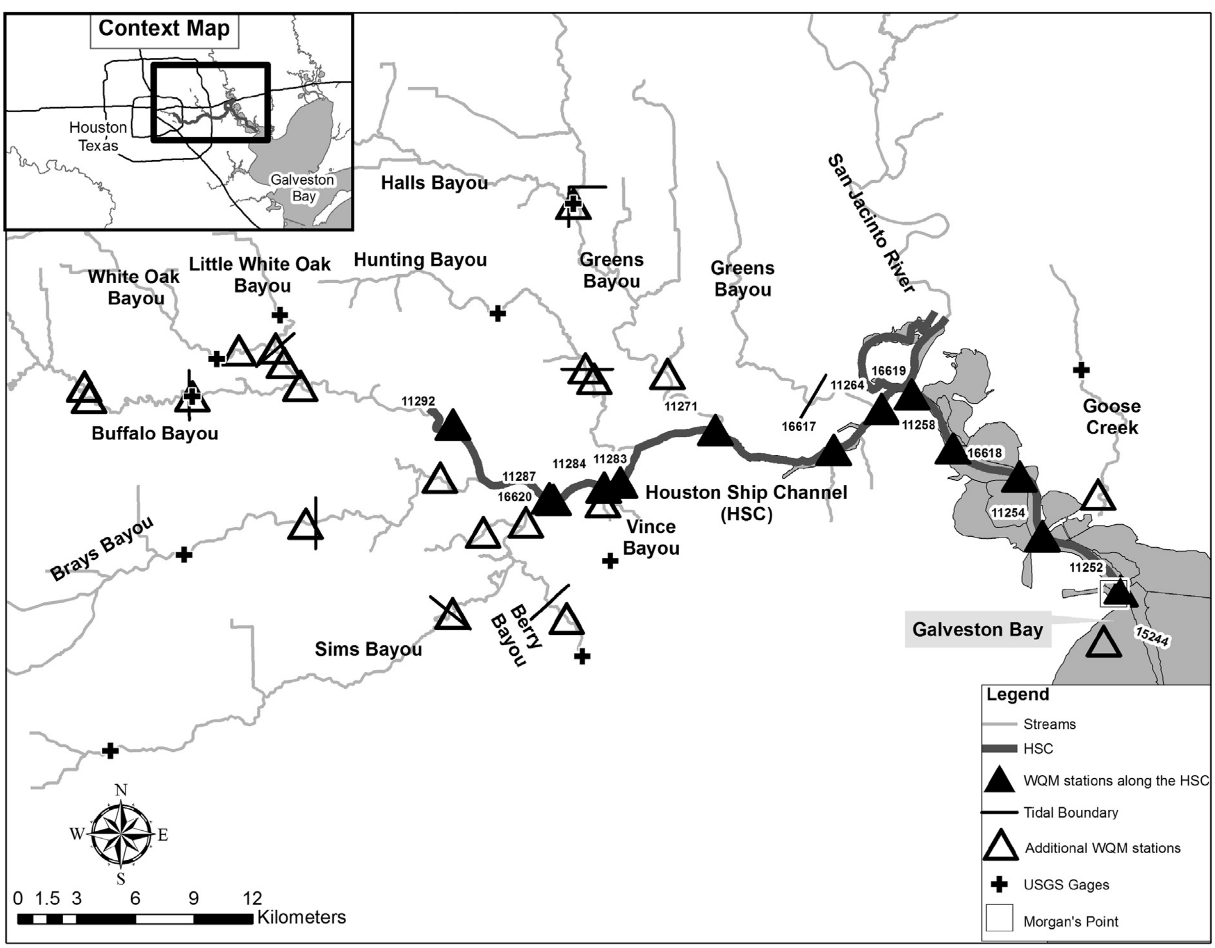

Fig. 2. Schematic of the Houston Ship Channel (HSC), USGS gages and Water Quality Monitoring stations.

HSC was computed by estimating the HSC cross sectional area at select WQM stations. Using ArcMap software (ESRI, 2012), Digital Elevation Model (DEM) data were overlaid on the spatially defined EFDC model grids to allow extraction of the width value from the model grid and its corresponding depth from the DEM data.

Hourly tide data at Morgan's Point (see Fig. 2 for the location) were obtained from the National Oceanic and Atmospheric Administration (NOAA) Center for Operational Oceanographic Products and Services (CO-OPS, 2014) tides and currents database. Monthly averaged atmospheric ambient temperature data for the period of 1981–2010 were obtained from NOAA's National Weather Service (NOAA, 2014).

2.4. Statistical methods

Kolmogorov-Smirnov and Shapiro-Wilk methods (Yap and Sim, 2011) were applied using IBM ® SPSS ® (IBM Corp., 2013) to conduct normality and log-normality tests for the gathered data. The results of the analyses indicated that none of the water quality data sets were normal or lognormal for any station (P-Value ≪ 0.05). Thus, geometric mean and median, Mann-Whitney U test, and Spearman correlation, were used in the study. For enterococci data, one date in particular, Jan 10, 2012 was eliminated from the analysis because it was defined as an outlier in all WQM stations using the Interquartile Range (IQR) method. Approximately, 20% of all enterococci sample results along the HSC were reported below the minimum 10 MPN per 100 ml (MPN/100 ml) detection limit. These values were replaced with half the reported detection limit for the purposes of statistical analyses. The maximum detection limit for enterococci was 24,000 (MPN/100 ml) and only two samples were reported as >24,000 (MPN/100 ml); the two values were replaced with 24,000 (MPN/100 ml).

As noted in Section 2.1, three NA regimes were assumed to exist within coastal streams and natural estuarine environment. Biological mechanisms of removal (predation, competition, viral lysis, etc.) were not considered in this study; however, their lumped effect was considered in decay rate calculations (see Sections 2.1 and 2.5 for more details). For each NA regime, different "lines-of-evidence" were developed to prove their occurrence.

2.4.1. Reactive-dilution mixing regime at freshwater/marine interface

This NA regime (see Fig. 1 for the location) can be evaluated using two "lines-of-evidence": 1) a reduction in FIB concentrations upstream of the tidal divide in freshwater relative to downstream in the more saline water, and/or 2) a reduction in FIB loads when comparing the sum of all the input loads from freshwater streams to the load in the receiving estuary.

The first line of evidence was tested for select tributaries where data were available. Visual analysis and a Mann-Whitney U test were conducted on enterococci concentrations measured at different sides of the tidal divide. For the second line of evidence, the median FIB load at the mouth of the HSC was estimated as the product of the flow in the Channel, extracted from the EFDC model, and the enterococci geometric mean concentration at station 11252 (Fig. 2). To calculate the flow rate, the advective velocity at Morgan's Point (Fig. 2), extracted from the EFDC model was multiplied by the cross sectional area of the HSC at the same location (see Table 1 for the cross section value). Input FIB loads from freshwater streams were calculated using the median flow from USGS gages, and the FIB geometric mean concentration data from WQM stations above the tidal divide. Two streams, Goose and Vince Bayou (Fig. 2), did not have WQM stations near the tidal boundary, for those, the nearest downstream (tidal) WQM station was used (additional WQM stations in Fig. 2).

2.4.2. Advective-reactive transport regime within receiving coastal stream

To evaluate this NA regime, multiple lines of evidence were developed: 1) a reduction in FIB concentrations from upstream to downstream, 2) an evaluation of correlations between FIB concentrations and environmental factors such as pH, temperature, DO concentrations, and salinity that support the prevailing assimilative capacity of the coastal stream, and 3) an evaluation of correlations between FIB concentrations and dilution-mixing and tidal mechanisms. The second and third analyses were designed to distinguish between chemical and physical mechanisms affecting the FIB attenuation in the system.

Table 1
Estimated hydraulic characteristics of the HSC at different WQM stations.

Station ID	Width (m)	Average depth (m)	Cross sectional area (m^2)
11292	426	9.0	3827
16620	222	9.3	2067
11287	282	9.2	2606
11284	206	10.4	2135
11283	215	9.6	2076
11271	315	10.3	3236
16617	365	10.7	3906
11264	414	10.3	4283
16619	414	9.4	4373
11258	528	7.8	4135
16618	894	7.0	6249
11254	511	9.3	4758
11252	496	9.5	4111

The first line of evidence was evaluated by applying visual analysis, regression analysis, and a Spearman's correlation test to determine the correlations, if any, between the concentrations/values of enterococci and the distance from Morgan's Point (mouth of the HSC). In addition, curvilinear regression analysis was also undertaken to find the best line fit of enterococci geometric means along the HSC. To evaluate the second line of evidence, correlation analyses were conducted. Salinity, DO, water temperature, and pH were all studied using Spearman's correlation test to determine the relationship(s), if any, to enterococci concentrations in the HSC. Two types of correlation tests were undertaken: one using all available data and the second using geometric mean concentrations of enterococci and median concentrations/values of ambient water quality variables at each station. In other words, the correlations were conducted once for the entire system as a whole by lumping all data and then using geometric mean and median values of enterococci and environmental variables, respectively, calculated in 13 stations.

For the last line of evidence, the HSC was considered as a series of reactors. The volume of each reactor was estimated using the cross sectional area of the HSC at each station (assuming unity for the length). The estimated depth, width and cross sectional area of the HSC at each WQM station are provided in Table 1. Spearman correlation analysis was done between the cross sectional area of the HSC and the corresponding enterococci geometric mean concentration.

Visual examination of tide patterns showed that the daily averaged hourly water levels (the average water levels at each hour of the day for a specific month), at Morgan's Point showed the exact same monthly pattern for different years. It should be noted that the similarity was found on the tide pattern and not the values of water levels (see Fig. S1 in the SI as an example for January). To confirm this similarity in the pattern, correlation analyses were conducted among daily averaged hourly water levels for each year (2000−2013) for all the 12 months resulting in 1260 analyses. All analyses showed significant (P-value < 0.05) and very strong (R > 0.75) correlations. Thus, the annually averaged hourly water level was used in the study for each month. The starting times for flood and ebb tides of tide at Morgan's Point for different months were calculated and samples were categorized based on the time of sampling. The Mann-Whitney U test was performed to find significant differences between enterococci concentrations in samples collected in flood tides compared to ones collected in ebb tides.

2.4.3. Dilution-mixing regime at the interface with estuarine water

Only one line of evidence was used to evaluate this NA regime: a reduction in FIB concentrations upstream and downstream of the discharge points. This line was tested by comparing FIB concentrations in the most distant downstream WQM station within the HSC (station 11252, see Fig. 2) to the closest WQM station located in Upper Galveston Bay Estuary (station 15244, see Fig. 2).

2.5. Apparent NA decay rates

A conservative dye simulation was performed with the validated EFDC model (Howell, 2012) for the HSC under normal flow to estimate the travel time between the selected points for the calculation. The EFDC model was already validated for flow, velocity and salinity (Howell, 2012) in the study area. Dye was released at station 11292 (at the beginning of the channel, Fig. 2). The time required for the center mass of the dye to pass through subsequent WQM stations was extracted from the simulation results. A curvilinear regression was applied using IBM ® SPSS ® (IBM Corp., 2013) to estimate the overall decay rate for the system using the following equation:

$$C_{Entro-t} = C_{Entro-i}e^{-kt}$$

where,

$C_{Entro-t}$ = enterococci concentration at time t (M/V)
$C_{Entro-i}$ = Initial enterococci concentration (M/V)
k = Apparent first-order decay rate (1/T)
t = time (T)

In addition, Enterococci concentrations at the headwater were compared to the concentrations at the mouth of the channel using a Mann-Whitney U test to confirm apparent reductions between the two locations.

2.6. Seasonality

Seasonality and its potential attenuative effects were evaluated by correlating water temperature and enterococci concentrations and investigating the effect of rainfall on FIB concentrations. It is noted, however, that enterococci can survive in a wide range of temperatures. Thus, and considering that sunlight exposure is the most important "natural disinfector" for surface water bodies (Maiga et al., 2009; Bolton et al., 2010), the enterococci concentrations in samples collected in warm months were compared to their counterparts from cool months. Months with averaged daily temperatures ranging from 24 to 32 °C were considered as warm and those with temperatures ranging from 12 to 18 °C were considered to be cool (Desai et al., 2010). Based on this classification, and using the monthly averaged ambient atmospheric temperature for the period of 1981–2010 (NOAA, 2014), November, December, January, and February were classified as cool (12 to 18 °C), and May, June, July, August, and September were considered as warm (24 to 32 °C). March, April, and October with average temperatures of 17.4, 21.0, and 22.3 °C, respectively, did not fit in either category.

The effect of rainfall on FIB concentrations was studied by comparing "wet" and "dry" concentrations (wet refers to concentrations influenced by a rainfall event occurring within the 5 days preceding the sampling event). The '5 days since rainfall' criterion was deemed representative of the approximately 2 days to >7 day range of travel times reported in Petersen (2006).

3. Results and discussion

Descriptive statistical data for enterococci, days since rainfall, and other water quality variables are provided in Table S2 in the SI. Enterococci geometric means ranged from 14 to 67 MPN/100 ml for the studied stations, median values ranged from 10 to 52 MPN/100 ml. Median DO ranged from 4.2 to 7.4 mg/l; the median pH ranged from approximately 7.3 to 8 while the median salinity ranged from 2.4 to 15.6. The median water temperature ranged from 21.7 to 25.1 °C. The average conversion ratio between enterococci and *E. coli* was found to be 0.39 (ranged from 0.4 to 0.81), which is comparable to the 0.28 value reported by Borel et al. (2015). The average log-scale conversion ratio was 0.78 (ranged from 0.67 to 0.86). As would be expected, it was observed (data not shown) that median salinity increases moving from the headwaters to the mouth of the HSC. An increasing trend in the median DO concentration was also observed as one moves towards Morgan's Point for the studied period 2000–2013, probably due to the higher DO concentrations in upper Galveston Bay; cooler water temperatures and a larger water surface allowing more re-aeration mixing. Although pH did not show significant spatial variability, a slight increase in pH values moving downstream was found that might be due to increased industrial discharges into the channel.

Table S3 in the SI summarize the flow data and Table S4 in the SI shows the geometric mean of *E. coli* and enterococci concentrations, and loads from the freshwater tributaries. Flows ranged from <0.03 m^3/s to >538 m^3/s with Greens Bayou exhibiting the highest mean and median flows followed by Buffalo Bayou (Table S3). As shown in Fig. 3 the highest daily median enterococci load, however, was discharged from Buffalo Bayou followed by Greens Bayou (also see Table S4). The median daily bacteria load discharged to the HSC from all freshwater tributaries was estimated to be 21.6 trillion MPN per day. As shown in Fig. S2 in the SI and confirmed with Mann Whitney *U* test, (P-value < 0.05), the enterococci concentrations for all stations were higher (as would be expected) in wet samples. Thus, when assessing the assimilative capacity of a coastal system, a careful consideration of the timing of FIB measurements relative to rainfall events must be undertaken to account for the confounding effect of elevated concentrations due to storm water.

3.1. Reactive-dilution mixing (NA regime 1)

3.1.1. Attenuation due to tidal influence within freshwater streams

The data in Fig. 4 illustrate a rapid drop in enterococci concentrations between freshwater and the tidally influenced tributary waters for all tributaries. The Mann-Whitney U test showed the statistical significance of this observation in all tributaries except for Greens Bayou (P-value = 0.09), one of the largest tributaries with a relatively large flow input to the HSC. As can be seen in Fig. 2, the distance between tidal and non-tidal WQM stations for Greens Bayou is longer compared to other tributaries. In addition, the non-tidal station located at the confluence of Greens and Halls Bayou can introduce uncertainties in the results due to the rapid mixing of water from Halls and Greens Bayous.

3.1.2. Attenuation due to dilution-mixing

EFDC modeling results from Howell (2012) resulted in an advective velocity of 0.108 m/s at Morgan's Point (Fig. 2). Using the cross sectional area of the HSC at Morgan's Point (4111 m^2, Table 1), a flow rate of 444 m^3/s was calculated. Considering the geometric mean concentration of 17 MPN/100 ml at Morgan's Point (station 11252, see Fig. 1 and Table S2 in the SI), a total FIB load at the mouth of the HSC of 6.5 trillion MPN per day can be estimated. Thus, approximately, 70% reduction in FIB loads emanating from the greater Houston area (total load of 21.6 trillion MPN per day as discussed above) results within the Channel (not accounting for FIB loads entering the HSC from adjacent areas that drain directly into it or from the San Jacinto River).

The calculated 70% reduction in freshwater FIB loads in the HSC means that TMDLs established for streams in Houston could have lower and potentially more achievable reduction targets than the current, near 100% estimated reductions. The rapid drop in enterococci concentration between freshwater and the tidally influenced tributary waters (Fig. 4) and significant reduction in FIB loads support the occurrence of NA regime 1 that is attributable to dilution and other inactivation mechanisms such as the influence of hydraulic conditions, salinity,

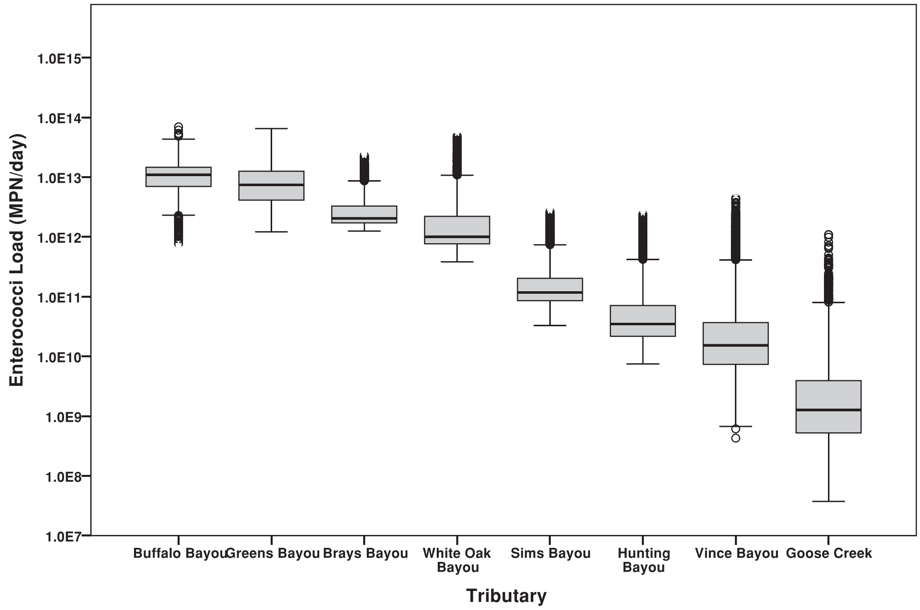

Fig. 3. Boxplots of Enterococci loads discharged to the HSC from the tributaries.

and ambient environmental variables. McLaughlin et al. (2007) reported a similar observation in Newport Bay estuarine system in southern California. The authors reported a significant difference in enterococci concentrations between two close stations, one located in discharging fresh water to the Bay and the other in the coastal stream (McLaughlin et al., 2007). Morrison et al. (2008) also reported a rapid drop from 86 to 23 CFU/100 ml in enterococci concentrations at two sites located on the opposite sides of tidal boundary with relatively close and low salinities.

3.2. Advective-reactive transport within the HSC (NA regime 2)

3.2.1. Attenuation in enterococci concentrations with distance

A general decreasing trend in enterococci geometric means and medians from upstream to downstream is illustrated in Fig. 5: the ranges of observed values (Fig. 5T) as well as the geometric means for each station (Fig. 5B) decline as one moves downstream. The trend is statistically significant using a Spearman's correlation test (*P*-value < 0.05). Curvilinear regression analysis (Fig. S3 in the SI) showed that the best trend for enterococci geometric means along the HSC is an exponential trend (P-value < 0.05, $R^2 = 0.87$).

3.2.2. Attenuation of enterococci due to temperature, salinity, dissolved oxygen and pH

The water temperature along the HSC was relatively constant (Table S2 in the SI). Water temperature ranged from 8 to 34 °C and a statistically significant negative correlation between water temperature and enterococci was found (*p*-value < 0.05). The data in Fig. S4 in the SI illustrate the observed seasonality effect where higher concentrations in the cool period can be readily seen; the difference was statistically confirmed by a Mann-Whitney *U* test (p-value < 0.05 in all WQM stations). As noted before, higher concentration of FIB have been reported

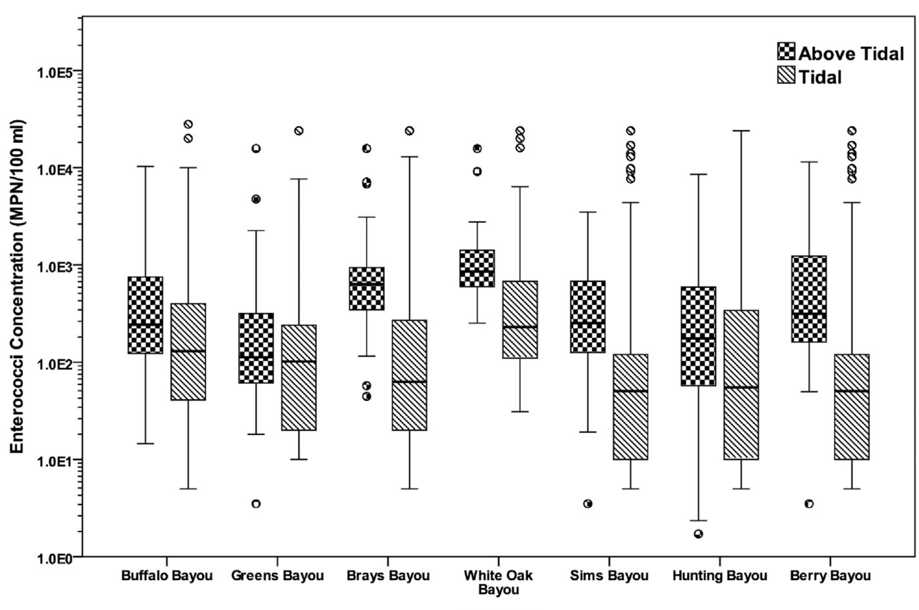

Fig. 4. Boxplots of Enterococci concentrations in freshwater and tidally influenced tributary waters.

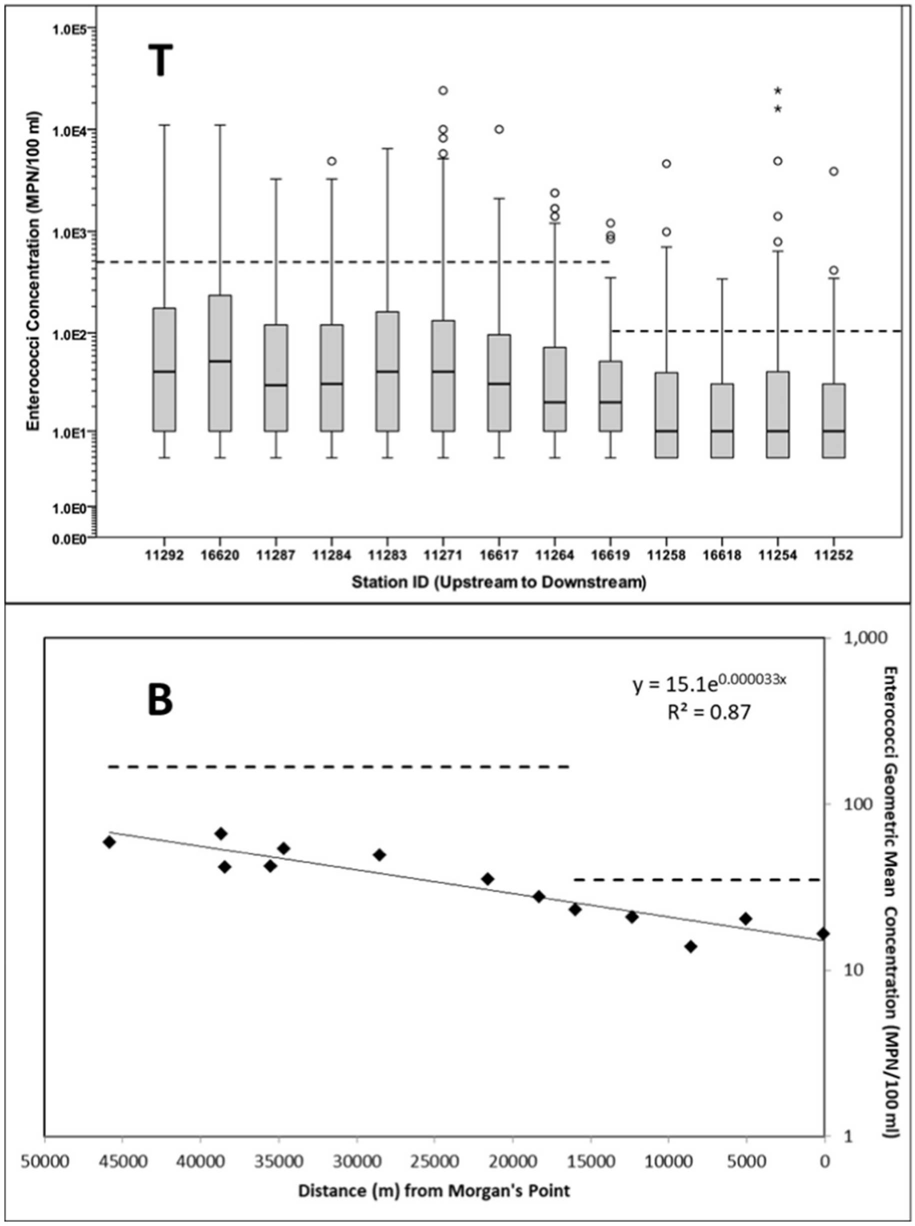

Fig. 5. (T) Boxplots Enterococci concentrations for 2000–2013 (dashed lines represent the single sample standards for Enterococci: 500 MPN/100 ml for navigation/industrial water supply (segment 1006 and 1007), and 104 MPN/100 ml for noncontact recreation/ high aquatic life (segment 1005)); (B) Enterococci geometric mean concentrations as a function of distance from outfall of Channel into Galveston Bay (dashed lines represent the Enterococci geometric mean standards: 168 MPN/100 ml for navigation/industrial water supply (segment 1006 and 1007), and 35 MPN/100 ml for noncontact recreation/ high aquatic life (segment 1005)).

in both warmer months (Hathaway et al., 2010; Diez-Vives et al., 2014) and cooler ones (Lipp et al., 2001; Pote et al., 2009). However, higher concentrations of enterococci in cooler months in the HSC system is logical since the main source of pathogenic contaminations in the HSC is from bacterial loads from greater Houston Metropolitan area watersheds. This finding is also comparable to Desai et al. (2010) that reported higher concentrations of indicator bacteria in cooler months.

Spearman's correlation test (Table S5) showed significant negative correlation between salinity and enterococci concentration (P-value < 0.05, R = −0.36) in contrast with findings in the literature that reported a positive correlation with salinity until a certain concentration is reach after which salinity becomes detrimental. This correlation is much stronger when applying the test to the geometric mean of enterococci and median concentrations of salinity at WQM stations (R = −0.92). The fairly poor correlation (R = −0.36) might be due to the fact that enterococci could survive in waterbodies with salinities up to 6.5% (Byappanahalli et al., 2012b). Stations closer to Morgan's Point (see Fig. 2 for the location) with higher salinity showed stronger negative correlation between salinity and enterococci concentrations. Considering the observed median range in the stations (2.4 to 15.6 ppt as represented in Table S2 in the SI), there might be a threshold in which the salinity has little to no effect on the enterococci concentration. However, Sinton et al. (2002) reported higher sunlight inactivation rates at the presence of higher salinity for all FIBs including enterococci.

A negative correlation between DO and enterococci concentration was also observed (Spearman's correlation test, P-value < 0.05), however the correlation coefficient was very small ($R = 0.07$). This might be caused by the fact that enterococci are facultative anaerobic bacteria. Using geometric mean enterococci concentrations and median concentrations for DO led to stronger correlations ($R = 0.79$). Lastly, Spearman's correlation test showed a very weak but significant correlation between pH values and enterococci concentrations (P-value < 0.05, R Spearman = −0.09). The range of pH values in all WQM stations was from 6.8 to 8.5 and the difference between the lowest and the highest pH median values were <0.6, enterococci are not affected by pH at these ranges. As a result, pH and DO are not likely important factors in enterococci growth or die-off rate in the study area.

3.2.3. Attenuation of Enterococci due to dilution-mixing and tidal effects

A strong negative correlation (P-value < 0.05, $R = 0.77$) was found between the cross sectional area of the HSC and the corresponding enterococci geometric mean concentration indicating the direct effect of dilution on enterococci concentrations.

An examination of the tidal data revealed unique hourly water level patterns with rising and falling hours for different months (Fig. S5 in the SI). Table 2 shows the starting time for flood and ebb tides of tide at Morgan's Point for different months. The Mann-Whitney *U* test showed that enterococci concentrations were significantly higher (*P*-Value < 0.05) in samples collected in ebb tides compared to ones collected in flood tides. This is in accordance with what would be expected, as the salinity is lower when the tide is falling. In addition, hydraulic forces can affect the concentrations of enterococci by changing the turbulent diffusion in both water and sediment. Although sediments can affect natural attenuation processes directly by acting as a sink or source for FIBs and indirectly by changing water quality concentrations (Higashino et al., 2008; Roslev et al., 2008; Murniati et al., 2015; Brinkmeyer et al., 2015), the role of sediments in the natural attenuation framework for microbial contamination has not been examined in great detail in this study.

The observed general decreasing trend in enterococci concentrations from upstream to downstream within the HSC, significant and strong correlation among enterococci concentrations and both chemical and mechanical mechanisms support the occurrence of the NA2 regime.

3.3. Dilution mixing with Galveston Bay water (NA regime 3)

The Mann-Whitney U test for enterococci data from stations 11252 and 15244 (Fig. 2) showed a significant difference between the two stations. Approximately 81% of the samples collected from station 15244 in Galveston Bay had a value below the detection limit while this percentage was only 35% for station 11252 located only 2 miles upstream (Fig. 1). In addition, the enterococci geometric mean concentrations reflected a 60% decline from 17 MPN/100 ml for station 11252 to 7 MPN/100 ml for station 15244 (Table S2).

In addition to the dilution effect, as it has been reported in other studies (Jin et al., 2004; Mill et al., 2006), the lower concentration of enterococci in Galveston Bay could be related to its lower water depth compared to the riverine section of the HSC. Sunlight inactivation could be more effective in shallower waters (Davies-Colley et al., 1994; Sinton et al., 2002).

Table 2
Starting time for flood and ebb tides at Morgan's Point for different months.

Month	Flood tide starting time	Ebb tide starting time	Number of samples in flood tide	Number of samples in ebb tide
Jan	19:00	6:00		11
Feb	18:00	5:00		18
Mar	15:00	2:00		10
Apr	11:00	23:00	10	9
May	9:00	22:00	17	
Jun	8:00	20:00	14	
Jul	7:00	18:00	15	
Aug	6:00	16:00	16	
Sep	5:00	14:00	11	
Oct	22:00	12:00	10	1
Nov	21:00	10:00	14	5
Dec	20:00	8:00	1	11

3.4. Estimated NA decay rates

The result of Mann-Whitney U test showed significant difference between enterococci concentrations at the headwater and the mouth of the channel (P-Value ≪ 0.05).

3.4.1. EFDC results

The travel times estimated with the EFDC model for a conservative dye simulation in the HSC are shown in Fig. 6. The dots on Fig. 6 represent the location of WQM stations along the HSC and the time required for the center mass of the released dye at station 11292 (see Fig. 2) to pass through corresponding WQM stations. The average advective velocity along the HSC was found to be 2540 m/day (0.03 m/s). As shown in Fig. 6, a linear relationship between travel time and the distance from the point of release up to Greens Bayou was observed indicating a constant advective velocity. A significant drop in advective velocity was found right after the confluence of Greens Bayou with the HSC. This could be because of the fact that Greens Bayou joins the HSC in the opposite direction of the dominant flow direction. Thus, water flow coming from Greens Bayou resists against water flow in the HSC and causes lower advective velocities and higher travel times in that part of the channel. Another anomaly was observed at the confluence of the San Jacinto River (SJR, see Fig. 1) with the HSC. Advective flow from the SJR highly affects the flow regime at and near the confluence and causes non-linear behavior in travel time. Although high flow discharging from the SJR into the HSC could lead to higher advective velocities within the channel, the presence of shallow side bays that act like detention reservoirs somewhat 'neutralize' the effect of discharge from the SJR.

3.4.2. Decay rate

Using the output of the EFDC model, an exponential curve was fitted to the travel times and corresponding enterococci geometric means. The power of the best-fitted line was reported as the decay rate. Thus, a decay rate with a value of 0.092 day^{-1} was estimated using travel times between WQM stations and geometric mean concentrations of enterococci at the corresponding locations.

4. Conclusions

In the context of microbial contamination, the developed natural attenuation conceptual framework at the system level rigorously accounts for the complexities associated with this constituent including the significant variability in FIB concentrations at various temporal scales, the paucity in FIB data, and the correlation of FIB concentrations with numerous environmental factors such as pH, temperature, dissolved oxygen and salinity. Using such a conceptual framework

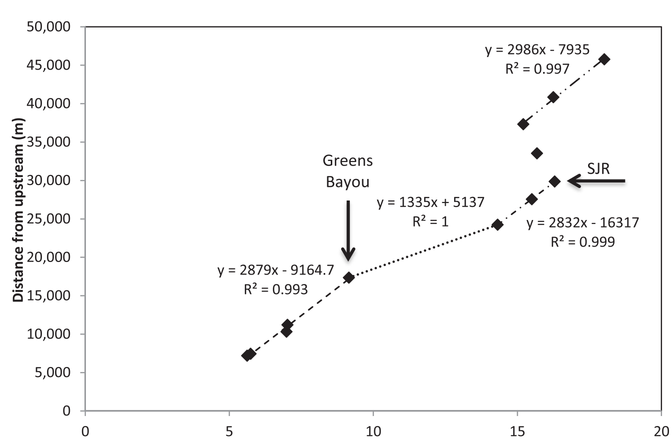

Fig. 6. Travel time for the dye center of mass from EFDC model.

developed in this study, the occurrence of natural attenuation of FIB in coastal streams and natural estuarine environment was proven using several "lines-of-evidence".

Various statistical tests and the numerical model applied in this study showed that both physical (dilution and tidal pattern) and chemical mechanisms (salinity and temperature) are the most important NA mechanisms affecting the FIB concentrations in an estuarine system. While the effect of dissolved oxygen, pH and water temperature were found to be minimal the effect of ambient temperature and rainfall patterns (seasonality) on FIB concentrations and consequently the NA mechanisms were found to be significant.

The developed framework can be applied to achieve a more comprehensive approach in developing TMDLs. While TMDLs are estimated for a given stream, at a specified location, and for specified flow regimes a natural attenuation framework considers the data from the entire system and for all flows within the system including tidal influences. Furthermore, a natural attenuation framework supports establishing the overall assimilative capacity of the system and can guide the development of more rigorous FIB monitoring programs that yield comparable data for the entire system.

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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.2019.04.315.

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