# Trends in Regional Enterococci Levels at Marine Beaches and Correlations with Environmental, Global Oceanic Changes, Community Populations, and Wastewater Infrastructure

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## Abstract

An increase in the number of advisories issued for recreational beaches across south Florida (due to the fecal indicator bacteria, enterococci) has been observed in recent years. To evaluate the possible reasons for this increase, we reviewed weekly monitoring data for 18 beaches in Miami-Dade County, Florida, for the years 2000–2019. Our objective was to evaluate this dataset for trends in enterococci levels and correlations with various factors that might have influenced enterococci levels at these beaches. For statistical analyses, we divided the 20-year period of record into 5-year increments (2000–2004, 2005–2009, 2010–2014, and 2015–2019). The Wilcoxon rank sum test was used to identify statistically significant differences between the geometric mean of different periods. When all 18 beaches were collectively considered, a significant increase ($p = 0.03$) in enterococci was observed during 2015–2019, compared to the prior 15-year period of record. To better understand the potential causes for this increase, correlations were evaluated with environmental parameters (rainfall, air temperature, and water temperature), global oceanic changes (sea level and Sargassum), community populations (county population estimates and beach visitation numbers), and wastewater infrastructure (sewage effluent flow rates to ocean outfalls and deep well injection). In relation to the enterococci geometric mean, the correlation with Sargassum was statistically significant at a 95% confidence interval ($p = 0.035$). Population ($p = 0.078$), air temperature ($p = 0.092$), and sea level ($p = 0.098$) were statistically significant at 90% confidence intervals.

**Key words:** enterococci, beach, fecal indicator bacteria

## 1. Introduction

Beaches are an important part of coastal economies throughout the world, as they provide recreational outlets for tourists and the local community. In the United States, tourism-related earnings, as a percentage of total earnings, are concentrated in counties located within 40 km of the Atlantic, Gulf, and Pacific coasts (Klein et al., 2004). In the state of Florida this is especially true, as Florida is a peninsula with over 2000 km of coastline bordered by both the Gulf of Mexico and the Atlantic Ocean.

Miami-Dade County is a popular tourist destination. In 2019, as much as 46% of tourists and residents visited beaches in the greater Miami area, the county's most popular attraction (GMCVB, 2019). However, in recent years there have been concerns about more frequent beach closures and/ or beach-swim advisories due to bacterial exceedances. Beach closures or beach advisory warnings due to bacterial hazards are generally determined by an exceedance of fecal indicator bacteria (FIB) above a set threshold level. These microbial water quality exposure thresholds are promulgated by individual U.S. states or U.S. territorial regulations and are based upon national recommendations by the U.S. Environmental Protection Agency (EPA) (U.S. EPA, 2012). For marine recreational beaches in the U.S., the FIB used to evaluate this threshold is typically enterococci, although some beach monitoring programs use both enterococci and *Escherichia coli* (each with its own threshold criteria) (U.S. EPA, 2021).

To assist U.S. communities in monitoring their beaches, the Beaches Environmental Assessment and Coastal Health (BEACH) Act was enacted in 2000 to provide funding for coastal communities to conduct water monitoring programs that notify the public about the safety of recreational areas for swimming. For the state of Florida the BEACH Act, coupled with local support, provides funding for regular monitoring of beach water quality through the Florida

Healthy Beaches Program (FHBP). Bacterial water quality regulatory criteria are currently promulgated in the state of Florida by the Florida Administrative Code (FAC), based upon the EPA's water quality recommendations (U.S. EPA, 2012). These criteria require a monthly enterococci geometric mean in recreational waters of ≤ 35 colony forming units (CFU) per 100 mL or ≤ 35 most probable number (MPN) per 100 mL, respectively (FL DEP, 2021, 62-302.530, FAC). The EPA has determined via a series of epidemiological studies that this level of enterococci exposure in marine bathing water (if from a known point-source of human sewage contamination) equates to an estimated illness rate of 36 illnesses per 1000 bathers (U.S. EPA, 2012, pp. 43). In addition to these regulatory water quality criteria, there is also the recommendation for a single-sample Statistical Threshold Value (STV) in conjunction with a "Beach Action Value" (BAV). The STV is based on the results from individual single grab samples during water quality monitoring rather than a monthly geometric mean of multiple repeated samples, as part of the regulatory criteria used for the judgment of "impaired" water bodies. Although most beaches in Florida are usually not closed to beachgoers due to BAV exceedances, the BAV is used as guidance to trigger beachgoer advisory warnings of potential risks from bathing or swimming due to fecal bacteria exposure. These warnings are posted at the beach by managers and reported to the public on the FHBP website.

Enterococci are a group of enteric Gram-positive coccoid bacteria that generally grow at temperatures of between 10°C to 45°C in 6.5% NaCl at pH 9.6. They can survive at temperatures up to 60°C for 30 min and typically inhabit the intestinal tract of humans and animals. This fecal-associated enterococci group was previously classified in the genus *Streptococcus* but is now predominately comprised of the genus genetically reclassified as *Enterococcus*. Enterococci are commonly used for marine water quality monitoring as an indicator of disease-causing, fecal-associated microbes (Byappanahalli et al., 2012). They are, therefore, useful for identifying potential impacts from sewage, especially in areas with dense wastewater infrastructure subject to inadvertent leaks or accidents (Brandão et al., 2020).

Questions have been raised, however, about the interpretation and causes of FIB exceedances at beaches, especially when a sewage source of contamination is not obvious. Enterococci and *E. coli* have been known to persist and regrow in soils and waters in tropical and subtropical environments with no known source of sewage contamination; hence, FIB may not be an appropriate indicator for these regions (Fujioka, 2001; Bordalo et al., 2002; Lamparelli et al., 2015). Other sources of enterococci include stormwater runoff (Korajkic et al., 2011; Kelly et al., 2018; Jennings et al., 2018; Colford Jr. et al., 2007), direct inputs from humans (Elmir et al., 2007; Wong et al., 2009; Korajkic et al., 2011; McQuaig et al., 2012; Turbow et al., 2003; Soller et al., 2010), and animals visiting beaches (PAHO, 2003; Wright et al., 2011). Beach sand has also been found to serve as a source of enterococci (Phillips et al., 2011b, 2014), with its release into shoreline waters a function of wave and tidal conditions (Feng et al., 2013, 2015). The persistence of enterococci in sand has been associated with moisture content (Phillips et al., 2011a; Shah et al., 2011), sand mineralogy (Piggot et al., 2012), and the presence of seaweed (Shibata et al., 2004). Environmental factors have also been associated with FIB levels, in particular, rainfall and ambient temperature (Cordero et al., 2012; Barreras et al., 2019). Other factors associated with FIB levels include global oceanic changes (Dvorak et al., 2018) that result from the effects of climate change (Walters et al., 2010; Barreras et al., 2019). Therefore, although enterococci and *E. coli* are used to identify offshore sources of sewage contamination, other FIB sources exist.

To better assess enterococci values as a whole (regardless of source or cause), the state of Florida threshold for enterococci levels (i.e., the single STV that triggers BAV advisories) was lowered from 104 to 70 CFU/100 mL on January 1, 2016. This change was in response to new EPA guidelines applied throughout the state through grant conditions. Since the threshold was lowered, the number of beach advisories has increased, especially in local jurisdictions such as Miami-Dade County, Florida (Fig. 1). Additionally, the issuance of local beach advisories is based upon the judgment of government officials who must consider other factors impacting the community (e.g., sewage overflow). Consequently, the degree to which the change in threshold or judgment has been responsible for the increase in advisories is unknown, as other factors also influence enterococci levels.

Given the confounding number of management factors, there is a need to evaluate whether the increase in beach advisories reflects a change in water quality. If water quality has changed, the potential causes need evaluation to develop mitigation strategies to reduce the issuance of beach advisories in the future.

The objectives of the current study were to review available datasets and literature to: (a) document the degree to which beach water quality has changed over yearly to decadal time scales, regardless of the set threshold level or judgments used in issuing beach advisories; and (b) evaluate the correlations between measured changes in water quality with potential causes. These potential causes include environmental parameters (rainfall, air temperature, and water temperature), global oceanic changes (sea level and Sargassum), community populations (county population estimates and beach visitation numbers), and wastewater infrastructure (sewage effluent flow rates to ocean outfalls and deep well injection). The focus in the current study was to evaluate long term trends on the time scales of years to decades, as opposed to evaluating short term impacts from seasonal or intermittent storm and weather events. The presumption is that short term variations in these factors would average out over the course of a year such that yearly aggregation of monitoring data would allow for the evaluation of long-term trends. Miami-Dade County was chosen as the study site for this evaluation. Readily available water quality data for beaches were averaged over the course of each calendar year and data were evaluated at the county level, regionally within the county, and for individual beaches to assess the associations between enterococci levels and the identified potential causes.

## 2. Methods

### 2.1 *Data sources*

Data were evaluated using yearly averages to smooth out short term variabilities and seasonal affects that are common to FIB and environmental measurements. Enterococci data were provided by the Florida Department of Health (FDOH, 2020). Samples were collected in waist-deep water near the shore. From August 2000–2002, samples were collected at Miami-Dade County beaches on a monthly basis. After August 2002, samples were collected weekly. In addition to weekly sampling, the dataset also contained “exploratory samples” taken the day following an exceedance to confirm the initial exceedance. However, these resample data points were not included in the study to avoid bias associated with intense sampling efforts during short periods of time. The goal was to consolidate weekly (or monthly prior to 2003) enterococci data independent of intense sampling efforts that may occur between routine monitoring dates. Measurements of enterococci were based upon the EPA's standard membrane filtration method using mEI agar plates (U.S. EPA, 2009).

Data for environmental parameters, i.e., annual mean air temperature and rainfall, came from a National Oceanic and Atmospheric Administration (NOAA) site centrally located in

Miami-Dade County (station KMIA, GPS 25°47′17.16″N, 80°19′0.84″W). Among the NOAA stations within the study area that recorded water temperature, an offshore station located within approximately 22 km of the main coastline was chosen because it had the smallest number of data gaps (station FWYF1, GPS 25°35′26″N, 80°5′48″W). Because these gaps affected the yearly mean, they were filled by computing a linear relationship between the air and water temperature ($R^2 = 0.66$) for the days where both were available. For the days where water temperature values were unavailable, they were estimated from the average air temperature for that day and the computed linear relationship between the air and water temperature. However, a small portion of the air temperature data also had gaps in the daily averages. For these instances, the average of the minimum and maximum air temperature was used for the days where both the mean daily water and air temperatures were unavailable. The overlap in lacking data occurred on August 1 through August 18, 2005, and September 30 to November 14, 2011.

For global oceanic parameters, mean sea level data were obtained from a NOAA site centrally located on the coast (station VAKF1, GPS 25°43′53″N, 80°9′43″W). Sargassum data were available from two sources: one source that was local but available for a portion of the period of record, and one source that covered a broader Atlantic basin area (Wang et al., 2019). Wang et al. (2019) estimated the yearly average mass of Sargassum in the Caribbean Sea and Central Atlantic Ocean using remote sensing imagery for the 2000–2019 period of record. Sargassum quantities were compared between these two datasets using a Spearman correlation between local (Ft. Lauderdale) and regional (Caribbean Sea and Central Atlantic) quantities ($r_s = 0.879$), which was significant ($p = 0.001$). Because regional Sargassum data were found to have a significant correlation with the local data, the correlations between enterococci and

Sargassum were evaluated for both datasets (i.e., Ft. Lauderdale records for 2010–2019 and the Caribbean Sea/Central Atlantic dataset for 2000–2019).

These data were supplemented with a 10-year hauling record of seaweed quantities from a 6.4-km service area for the City of Fort Lauderdale, Florida for the 2010–2019 period of record. The northern boundary of the service area was Oakland Park Boulevard, and the southern boundary was Harbor Drive. The City of Ft. Lauderdale is located within approximately 12 km of the northern border of Miami-Dade County. Its seaweed hauling record was chosen, as it was the only local record with more than 3 years of data. The seaweed was typically removed using rear-mounted blades and measured in tons or cubic yards. A conversion factor of 8 cubic yards to 6 tons wet seaweed, provided by the City of Fort Lauderdale, was used.

For community population measures, we used data from the U.S. Census Bureau (U.S. Census, 2020). Beach visitations were compiled for Miami-Dade County using the U.S. Lifeguard Association (USLA, 2020) data.

Wastewater was treated in Miami-Dade County throughout the period of record using both primary and secondary treatment coupled with chlorine disinfection, with outfalls designed to provide mixing and dilution of the treated effluent to avoid measurable impacts to nearby beaches. Throughout the 20-year enterococci monitoring record, the wastewater plant did undergo improvements in treatment including in 2002 the conversion of traditional air activated sludge process to pure oxygen activated sludge and in 2018 the addition of deep well injection for effluent disposal at the Central District Wastewater Treatment Plant. Wastewater infrastructure data were provided by the Miami-Dade Water and Sewer Department (MD-WASD, 2020). These data included ocean outfall flow rates at the two major wastewater outfalls in Miami-Dade County: the North District (GPS 25°55′13″N, 80°5′10″W) and Central District (GPS 25°44′31″N, 80°5′10″W), plus effluent flows disposed via deep well injection.

### 2.2 *Statistical analysis*

Data from the FHBP were used to compute basic statistics (the yearly geometric mean and percent exceedances) for enterococci. Yearly statistics were used to analyze long-term trends and avoid the variability associated with short term environmental factors such as the influence of storm events. As mentioned above, the FDOH issues health advisories based on the geometric mean or single sample maximums that exceed a set threshold. Since January 2016, the single sample threshold BAV for enterococci has been 70 CFU/100 mL (prior to 2016, the threshold was 104 CFU/100 mL). Enterococci levels were converted to percent exceedances of BAV to evaluate the dataset in terms of potential health advisories. The percent exceedance is defined as the percentage of time a beach exceeded the regularly monitored single-sample threshold value. To focus the evaluation on true changes in water quality, percent exceedances were evaluated for both thresholds (70 and 104 CFU/100 mL) as if they were consistent over the entire 20-year period so that regulatory changes would not influence the exceedance data (Fig. 1). The geometric means and percent exceedances were calculated using MATLAB software (MathWorks version R2015a).

The Shapiro-Wilk test was used to evaluate the normality of the enterococci data. Because the enterococci dataset did not have a normal or log-normal distribution, its relationship with environmental, global oceanic, population, and wastewater infrastructure data was analyzed for correlation using Spearman correlations. The strength of the correlation is given by $r_s$, the Spearman's rank correlation coefficient, with values greater than 0.30 considered strong and *p* values less than 0.1 considered significant at 90% confidence, and less than 0.05 considered significant at 95% confidence.

The last 5 years of the data collection period (2015–2019) showed a visible increase in beach advisories (Fig. 1). To evaluate the significance of this increase, the 20-year period was divided into four 5-year periods:  2000–2004, 2005–2009, 2010–2014, and 2015–2019. The data used for comparison included grouping the first 15 years versus the last 5 years. Additionally, the periods were divided into 5-year increments, and comparisons were made between the means among all possible combinations of the four 5-year periods to identify if statistical differences in the means were observed between the periods. The geometric means for the different periods were compared using the Wilcoxon rank sum test.

### 2.3 *Beach locations and geomorphology*

The locations of the sampling points for every Miami-Dade County beach in our study (see Table S1, supplement text) were input into a KML file that could be viewed as a satellite image using Google Earth (see Fig. 2). Wastewater treatment plant (WWTP) outfall locations and data collection sites in the area of interest were also plotted. To determine whether the increase in enterococci levels was consistent throughout the county, the data for all 18 beaches were averaged together. Additionally, the data were divided geographically to determine the geometric mean and percent exceedance of each region within the county over time (see Fig. 2). The beaches were categorized geographically as bay-facing ($n$ = 3), Atlantic-facing–North ($n$ = 5), Atlantic- facing–Central (n = 5), and Atlantic-facing–South (n = 5). Using these criteria, the increase in enterococci from 2015 to 2019 could be evaluated on a regional (within county), as well as entire county, basis.

## 3 Results

Enterococci levels based upon the geometric mean and percent exceedance using the 70 CFU/100 mL threshold are described first. Statistics using the 104 CFU/100 mL threshold were similar to the 70 CFU/100 mL threshold. Details of the statistical analysis using the 104 CFU/100 mL threshold are available in the supplemental text (Fig. S1). Correlations between the enterococci geometric means and environmental, global oceanic, community population, and wastewater infrastructure factors are described at the end of this section.

### 3.1 *Evaluation of enterococci levels*

#### *3.1.1. 2015–2019 versus 2000–2014*

For all beaches collectively in Miami-Dade County, enterococci levels (by geometric mean and percent exceedance using the 70 CFU/100 mL threshold) increased over time. This was observed for the county-wide average (Fig. 3), and the increase was most distinct during the 2015–2019 period.

When we compared enterococci levels by geographic region, specifically comparing the last 5 years (2015–2019) to the prior 15 years (2000–2014) (Fig. 3, Table 1), results for the geometric mean showed that enterococci abundance was statistically different for these two periods for all regions ($p = 0.043$), for bay-facing and Atlantic-facing– North beaches ($p = 0.080$), and for Atlantic-facing–Central and Atlantic-facing–South beaches ($p = 0.043$). For percent exceedance (Fig. 3, Table 1), the results showed that enterococci values were significantly different for the two time periods for Atlantic-facing–North and Atlantic-facing–South beach regions ($p = 0.080$ and $\text{p} = 0.043$, respectively). For bay-facing and Atlantic-facing–Central beaches, the difference in percent exceedance between the two periods was insignificant.

Of the 18 beaches we studied, 13 had data for the entire period of record (2000–2019). We evaluated each of these 13 beaches by comparing the last 5 years (2015–2019) to the prior 15 years (2000–2014). The geometric means showed that enterococci levels were statistically different for the two periods for 11 of the 13 beaches (see Fig. S2, supplemental text).

### *3.1.2. 5-year period comparison*

For all beaches collectively, the enterococci mean level for 2015–2019 was statistically different from the other 5-year periods ($p < 0.1$) (Table 2, last bolded row). However, when the earlier periods were compared to one another, the means were not significantly different ($p > 0.1$). Similarly, for the percent exceedance for all beaches collectively, the 2015–2019 period was significantly different from the other 5-year periods ($p < 0.1$) (Table 3, last bolded row). There was no statistical difference when the earlier periods were compared to each other.

When the enterococci geometric mean by geographic region (Table 2, last row) were compared, the 2015–2019 period was significantly different from the 2000–2004 and 2010–2014 periods for all regions (bay-facing, Atlantic-facing–North, Atlantic-facing–Central, and Atlantic-facing–South beaches, $p < 0.1$). The 2015–2019 period was significantly different from the 2005–2009 period for three of the four regions (bay-facing, Atlantic-facing–North, and Atlantic-facing–South beaches, $p < 0.1$). The only exception was the Atlantic-facing–Central beaches ($p = 0.138$). When these earlier 5-year periods were compared to one another for all regions, the means were not significantly different ($p > 0.1$).

Similarly, when we compared enterococci percent exceedances by geographic region (Table 3, last row), the 2015–2019 period was significantly different from the other three 5-year periods for all 12 combinations of comparison, with the exception of two regions (i.e., the bay-facing region during 2000–2004 ($p = 0.225$) and 2005–2009 ($p = 0.500$) and the Atlantic-facing–

Central region during 2000–2004 ($p = 0.138$) and 2005–2009 ($p = 0.225$)). For the earlier 5-year periods there was no statistical difference for any geographic region, with the exception of the bay-facing beach region, which showed statistical differences with the 2000–2004 and 2005–2009 periods.

## 3.2 *Correlations*

When evaluating environmental factors (Fig. 4a and b), Spearman correlations were considered strong for the annual mean air temperature ($r_s = 0.394$ geometric mean and 0.387 percent exceedance) and significant ($p < 0.1$) (Table 4). The annual mean water temperature was weakly correlated with the geometric mean ($r_s = 0.165$) and strongly correlated with percent exceedance ($r_s = 0.311$) (Table 4). Neither relationship with the annual mean water temperature was significant ($p > 0.1$). Yearly rainfall was weakly correlated ($r_s = 0.140$ and 0.137), but the correlation was not significant ($p > 0.1$).

When the enterococci geometric mean and percent exceedance were compared to Miami-Dade County annual population estimates (Fig. 4c), Spearman correlations were 0.420 and 0.391, respectively, and both were significant ($p < 0.1$) (Table 4). For Miami-Dade County beach attendance, data were not collected for some years. Although correlations were found to be 0.285 and 0.365, respectively, these correlations were not significant ($p > 0.1$); again, likely due to the smaller number of data points.

When considering the global oceanic factor of sea level rise (Fig. 4d), the Spearman correlations were considered significant when compared to the geometric mean ($r_s = 0.380$, $p < 0.1$) and weak when compared to the percent exceedance ($r_s = 0.272$, $p > 0.1$) (Table 4). For the annual mean area of Sargassum in the Caribbean Sea and Central Atlantic (Fig. 4e), Spearman correlations were considered strong and significant when compared to the enterococci geometric mean ($r_s = 0.474$, $p < 0.1$) and weak when compared to the percent exceedance ($r_s = 0.281$, $p > 0.1$) (Table 4). When comparing the enterococci geometric mean and percent exceedances to the annual volume of Sargassum hauled from Fort Lauderdale, Florida, correlations were considered strong but not significant ($r = 0.394$, $p > 0.1$, and $r = 0.358$, $p > 0.1$ respectively), likely due to the smaller number of data points.

For wastewater infrastructure (Fig. 4f), Spearman correlations were −0.211 and −0.174, respectively, for the enterococci geometric mean and percent exceedance when compared to effluent volumes for the North District WWTP outfall (Table 4). In comparison with the effluent from the Central District WWTP outfall, the Spearman correlations were −0.131 and −0.251, respectively. In comparison with the annual mean effluent from the injection well of the North District WWTP, the Spearman correlations were 0.219 and 0.123, respectively. However, none of these correlations in relation to wastewater effluent were statistically significant ($p > 0.1$).

## 4. Discussion

In observance of the enterococci geometric mean levels and percent exceedances for all beaches in Miami-Dade County, we found an increase over time in both bacteria levels and percent exceedances. This increase was statistically significant for the 2015–2019 period when compared to the prior periods. Because the trend was consistent for all areas, we assumed the cause for this increase in geometric mean level was not an intermittant point-source factor. After evaluating different parameters and their correlations to the enterococci geometric mean for the 20-year period, Sargassum had the highest correlation, followed by county population, air temperature, and sea level (Table 4). Sargassum was significant at the 95% confidence interval while county population, air temperature, and sea level were significant at the 90% confidence interval. Other parameters, including rainfall, water temperature, beach visitation, and wastewater effluent to an injection well, had smaller positive correlations (<0.3) but were not statistically significant when evaluating the geometric mean. Similarly, when compared to percent exceedances at the 70 CFU/100 mL threshold, county population, followed by air temperature, Sargassum (Ft. Lauderdale dataset), beach visitation, and water temperature had the highest correlations. County population and air temperature were significant at 90% confidence limits when considering percent exceedances. Other parameters, including yearly rainfall, sea level, and wastewater effluent to an injection well, had smaller positive correlations (<0.3) but were not statistically significant for percent exceedances. These findings are consistent with past studies that associated tide, rainfall, temperature, and human bathers with enterococci levels (Elmir et al., 2007, 2009; Wright et al., 2011; Enns et al., 2012; McLellan et al., 2015; Dila et al., 2018; Barreras et al., 2019; McLellan and Roguet, 2019; Kelly et al., 2020; Powers et al., 2020). Another study also found that mean sea level, 48 h rainfall, and sea surface temperature were among the most relevant parameters to predict culturable enterococci surface water concentrations at Escambron Beach in San Juan, Puerto Rico (Laureano-Rosario et al., 2019). However, another study found that wastewater disposal through deep well injection might not affect water quality (Griggs et al., 2003).

We additionally found that wastewater effluent to ocean outfalls had a negative correlation with enterococci levels, although not statistically significant. This finding is counterintuitive because higher flow of sewage is associated with higher amounts of enterococci (Watkinson et al., 2007; Kelly et al., 2021). However, it is likely that the continuous improvements to the wastewater infrastructure is one contributing factor towards the inverse relationship between wastewater flows and yearly enterococci levels. It is likely that the main source of enterococci at the beach is not wastewater from the WWTPs.

Other studies conducted in Hawaii and Guam (Fujioka et al., 1988; Roll and Fujioka, 1997; Fujioka et al., 1999) and Puerto Rico (Toranzos and Marcos, 2000) have shown that FIB is consistently present and measured in high concentrations in the subtropical environment, even in the absence of any known source of human or animal waste (Fujioka, 2001; Elmir et al., 2007; Boehm et al., 2009; Fujioka et al., 2015; Lamparelli et al., 2015). This suggests there may be potential sources of enterococci other than wastewater treatment plants.

Our results support the idea that the cause for the increase in enterococci values at the 18 Miami-Dade County beaches during the period of study was not from the treated wastewater outfalls. Our study also found a significant correlation with enterococci concentrations and the human population, as observed in other studies (Brooks et al., 2016; Malnik et al., 2019), although this has been generally attributed to an associated increase in wastewater effluent. The correlation with population increases may be due to other sources of sewage such as septic tanks and due to increased human presence at the beach which may bring with it additional animal sources (e.g., dogs and wild animals attracted to food from humans) and sources associated with solid waste and bathroom facilities (or lack thereof) at the beach.

Nonetheless, FIB used to regulate marine and fresh water have been found to persist in the environment in the absence of fecal pollution, particularly in tropical climates. This may be due to the fact that increased bacterial growth and replication occur in warmer temperatures (Byappanahalli et al., 2012; Mote et al., 2012). However, there is no general consensus on the degree to which air and water temperatures influence enterococci populations. Some studies have found that enterococci thrive in warmer temperatures (Zhang et al., 2012; Laureano-Rosario et al., 2017), while a different study found a negative association between enterococci concentrations and air temperature (Brooks et al., 2016). Another study found that water temperature was not an indicator for the total number of enterococci in wastewater; instead, enterococci were dependent primarily on wastewater flow (Lépesová et al., 2019).

We also found there was a significant correlation between enterococci concentrations and mean sea level. This finding is similar to that of Walters et al. (2010) who found that FIB and pathogen concentrations were influenced by their flux from the land, which is exacerbated during rainfall. Another study found that when sea level periodicity was considered, a clear pattern of higher daily maximum sea level was observed 24 h prior to days categorized as unsafe for swimming based on FIB thresholds at both beaches; although not always statistically significant (Laureano-Rosario et al., 2021). This is contradictory to a past study that found an inverse correlation between enterococci concentrations and mean sea level anomalies (Laureano-Rosario et al., 2017). It has been suggested this negative correlation is due to bacterial dilution that occurs during higher mean sea level anomalies, as well as associated backwash mixing and enhanced drainage from coastal sources that may promote increased concentrations during lower mean sea level anomalies (Grant et al., 2001; Yamahara et al., 2009; Maraccini et al., 2012). The positive correlation determined in our study is likely due to increased contact between seawater and shoreline sediments (Phillips et al., 2011b; Wright et al., 2011). These sediments are known to contain elevated levels of enterococci and, during higher sea levels, bacteria from these sediments can be washed into coastal waters through wave and tidal action (Feng et al., 2013, 2015; Roca et al., 2019).

Another parameter we found to have a significant correlation with enterococci concentrations was the presence of Sargassum. There are limited findings on how Sargassum relates to enterococci, but there is research on how the presence of other aquatic plants such as seagrass and algae can decrease and increase enterococci concentrations, respectively (Dodds and Gudder, 1992; Whitman et al., 2003; Ishii et al., 2006; Lamb et al., 2017). In recent years, there has been an evident increase in the amount of Sargassum but insufficient data to conclude what factors play a role. However, there are many hypotheses as to what conditions have led to these changes, including warming temperatures, climate change, and nutrient enrichment (Franks et al., 2011, 2014; Johnson et al., 2012; Lapointe et al., 2015; Djakouré et al., 2017; Wang et al., 2019).

Excess Sargassum blooms have not only become a concern for Florida, but have also created issues for many Caribbean countries, as well as for Mexico, Africa, South Korea, and China (Franks et al., 2011; Gower et al., 2013; Partlow and Martinez, 2015; Hawang et al., 2016; Su et al., 2018). When large algal masses wash ashore and accumulate on beaches they emit a foul odor, which is a nuisance to both tourists and local communities (Partlow and Martinez, 2015; Ansary et al., 2019). Additionally, floating mats of decomposing Sargassum can cause coral mortality in nearshore waters and adverse environmental conditions for a variety of marine organisms (van Tussenbroek et al., 2017). In response, many countries have begun using satellite programs to locate large mats of seaweed before they wash ashore. Additionally, Mexico has been exploring ways to collect Sargassum at sea (Partlow and Martinez, 2015).

Seaweed removal practices have also been implemented to mitigate its effects. Seaweed grooming has been practiced at some Miami-Dade County beaches since the 1980s. However, changes in seaweed removal practices occurred in 2015 because previous methods were no longer permitted by the Florida Fish and Wildlife Conservation Commission. Currently, rear-mounted blades are typically used to remove seaweed. For the future, offshore removal is recommended, as well as composting and fertilizer initiatives, to put excess Sargassum to productive use (Sembera et al., 2018).

Other potential variables generally associated with enterococci (whose correlations were not evaluated in our study) are stormwater events (Patz et al., 2008; Hernandez et al., 2014; Roca et al., 2019), nutrients (Kelly et al., 2020), solar irradiance (Laureano-Rosario et al., 2017), and coastal sediments (Mueller-Spitz et al., 2010; Russell et al., 2013; Whitman et al., 2014;). Recent studies have also found that beach sediment can sustain populations of enterococci and are potential non-sewage sources of these bacteria in recreational waters (Whitman and Nevers, 2003; Shibata et al., 2004; Abdelzaher et al., 2010; Badgley et al., 2010; Wright et al., 2011). We recommend that future studies include beach sediment measurements as part of the monitoring effort to allow for correlation analysis between bacteria levels in sediment in relation to enterococci in beach water.

The strength of the study comes from 20 years of continuous enterococci monitoring data. However, it is difficult to find data for other factors for this length of time. We used the environmental data that was available. The correlations were significant only for $p$ values of 0.1, however, given the uniqueness of this data set and the fact that the datasets were not designed for the purpose of this study, we believe that a $p$ value of 0.1 is significant. Future studies would benefit from environmental monitoring designed specifically to assess temperature closer to the coast, direct measurements of Sargassum beached on the shore, and more consistent measurements of beach visitation rates. Evaluating sea level for a longer period may also increase the significance of the sea level relationship.

## 5. Conclusions

Enterococci levels increased during the 2015–2019 period at the beaches evaluated in our study, and the factors that influenced the increase were primarily associated with an abundance of Sargassum, county population, air temperature, and sea level. This is the first study to correlate regional fecal indicator bacteria against increases in Sargassum strandings. The increase in Sargassum strandings may be influenced, as mentioned earlier, by warming temperatures, climate change, and nutrient enrichment suggesting that increases in the volume of Sargassum strandings may continue. Increases in Sargassum strandings may exacerbate bacterial exceedances and beach advisories which emphasize the need to further evaluate global factors that may influence coastal water quality.

This study was limited in scope by the lack of consistent long-term temperature data specific to the study area. Additionally, the study was limited by the number of data points ($n = 20$ for each parameter), which were insufficient for multiple regression analysis. For the future, the combined effects of these parameters on enterococci concentrations should be considered. Further research is needed to determine which parameters, both included in and excluded from this study, correspond to enterococci variability. Finally, predictive models that incorporate considerations of all respective parameters should allow for more timely beach advisories that reduce public health risks.

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**Table 1.** Wilcoxon rank sum test that compares the geometric mean and percent exceedance of enterococci levels from the last 5 years (2015-2019) to the prior 15 years (2000-2014) for all Miami-Dade County beaches and within the different regions of Miami-Dade County. Significant values are marked with an asterisk.

| | ***p* Value** | |
|---|---|---|
| **Region** | **Geometric Mean** | **Percent Exceedances** |
| All Miami-Dade County | 0.043* | 0.043* |
| Bay-facing | 0.080* | 0.225 |
| Atlantic-facing–North | 0.080* | 0.080* |
| Atlantic-facing–Central | 0.043* | 0.225 |
| Atlantic-facing–South | 0.043* | 0.043* |

**Table 2.** Wilcoxon rank sum test that compares the geometric mean of enterococci levels over the different 5-year periods for all of Miami-Dade County with a breakdown by region. Bolded values correspond to the comparison of all beaches collectively in Miami-Dade County. Comparisons by region correspond to (1) Bay-facing, (2) Atlantic-facing–North, (3) Atlantic-facing–Central, and (4) Atlantic-facing–South. Significant differences between the categories are marked with an asterisk.

| Time Period | 2000-2004 | 2005-2009 | 2010-2014 | 2015-2019 |
|---|---|---|---|---|
| 2000-2004 | - | | | |
| 2005-2009 | **0.500**<br>(1) 0.893<br>(2) 0.138<br>(3) 0.686<br>(4) 0.893 | - | | |
| 2010-2014 | **0.500**<br>(1) 0.225<br>(2) 0.255<br>(3) 0.893<br>(4) 0.686 | **0.893**<br>(1) 0.345<br>(2) 0.893<br>(3) 0.686<br>(4) 0.138 | - | |
| 2015-2019 | **0.043***<br>(1) 0.043*<br>(2) 0.080*<br>(3) 0.043*<br>(4) 0.043* | **0.080***<br>(1) 0.080*<br>(2) 0.043*<br>(3) 0.138<br>(4) 0.043* | **0.043***<br>(1) 0.080*<br>(2) 0.043*<br>(3) 0.080*<br>(4) 0.043* | - |

**Table 3.** *p*-values compared to percent exceedances over the different 5-year periods for all of Miami-Dade County. Regional values are evaluated as (1) Bay-facing, (2) Atlantic-facing–North, (3) Atlantic-facing–Central, and (4) Atlantic-facing–South. Significant differences between the categories are marked with an asterisk.

| **Time Period** | **2000-2004** | **2005-2009** | **2010-2014** | **2015-2019** |
|---|---|---|---|---|
| 2000-2004 | - | | | |
| 2005-2009 | **0.500**<br>(1) 0.893<br>(2) 0.345<br>(3) 0.893<br>(4) 0.500 | - | | |
| 2010-2014 | **0.500**<br>(1) 0.080*<br>(2) 0.225<br>(3) 0.500<br>(4) 0.686 | **0.893**<br>(1) 0.043*<br>(2) 0.345<br>(3) 0.686<br>(4) 0.225 | - | |
| 2015-2019 | **0.080***<br>(1) 0.225<br>(2) 0.080*<br>(3) 0.138<br>(4) 0.043* | **0.080**<br>(1) 0.500<br>(2) 0.043*<br>(3) 0.225<br>(4) 0.080* | **0.043***<br>(1) 0.080*<br>(2) 0.043*<br>(3) 0.080*<br>(4) 0.043* | - |

**Table 4.** Correlations between environmental, global oceanic, community population, and wastewater infrastructure factors with enterococci levels for all Miami-Dade County beaches.

| Parameter | Geometric Mean | | Percent Exceedance | |
|---|---|---|---|---|
| | Spearman Correlation | Significance | Spearman Correlation | Significance |
| Air Temperature | 0.394 | 0.085* | 0.387 | 0.092* |
| Water Temperature | 0.165 | 0.486 | 0.311 | 0.182 |
| Rainfall | 0.140 | 0.556 | 0.137 | 0.565 |
| Sea Level | 0.380 | 0.098* | 0.272 | 0.246 |
| *Sargassum* (Caribbean Sea) | 0.474 | 0.035 | 0.281 | 0.230 |
| *Sargassum* (Fort Lauderdale) | 0.394 | 0.260 | 0.358 | 0.310 |
| County Population | 0.420 | 0.066* | 0.391 | 0.088* |
| Beach Attendance | 0.285 | 0.284 | 0.365 | 0.163 |
| North District-Injection Well | 0.219 | 0.354 | 0.123 | 0.604 |
| North District-Outfall | -0.211 | 0.373 | -0.174 | 0.462 |
| Central District- Outfall | -0.131 | 0.582 | -0.251 | 0.286 |

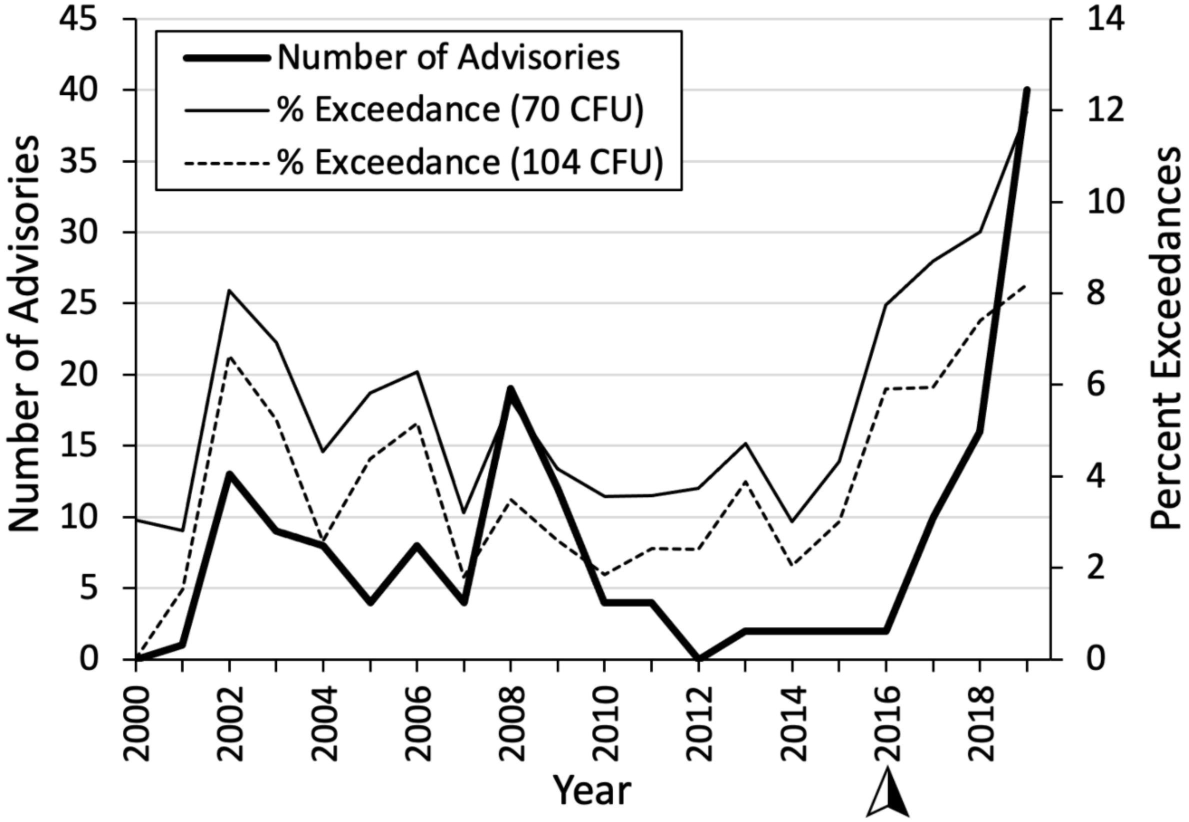

**Figure 1**. Number of beach advisories compared to the percent exceedances of enterococci at the 70 and 104 CFU/100 mL threshold in Miami-Dade County for 2000-2019. Percent exceedance is defined as the number of days that weekly enterococci measures exceeded the set threshold divided by the number of weekly measures for a given year. The arrow indicates the year when the threshold was changed from 104 CFU to 70 CFU.

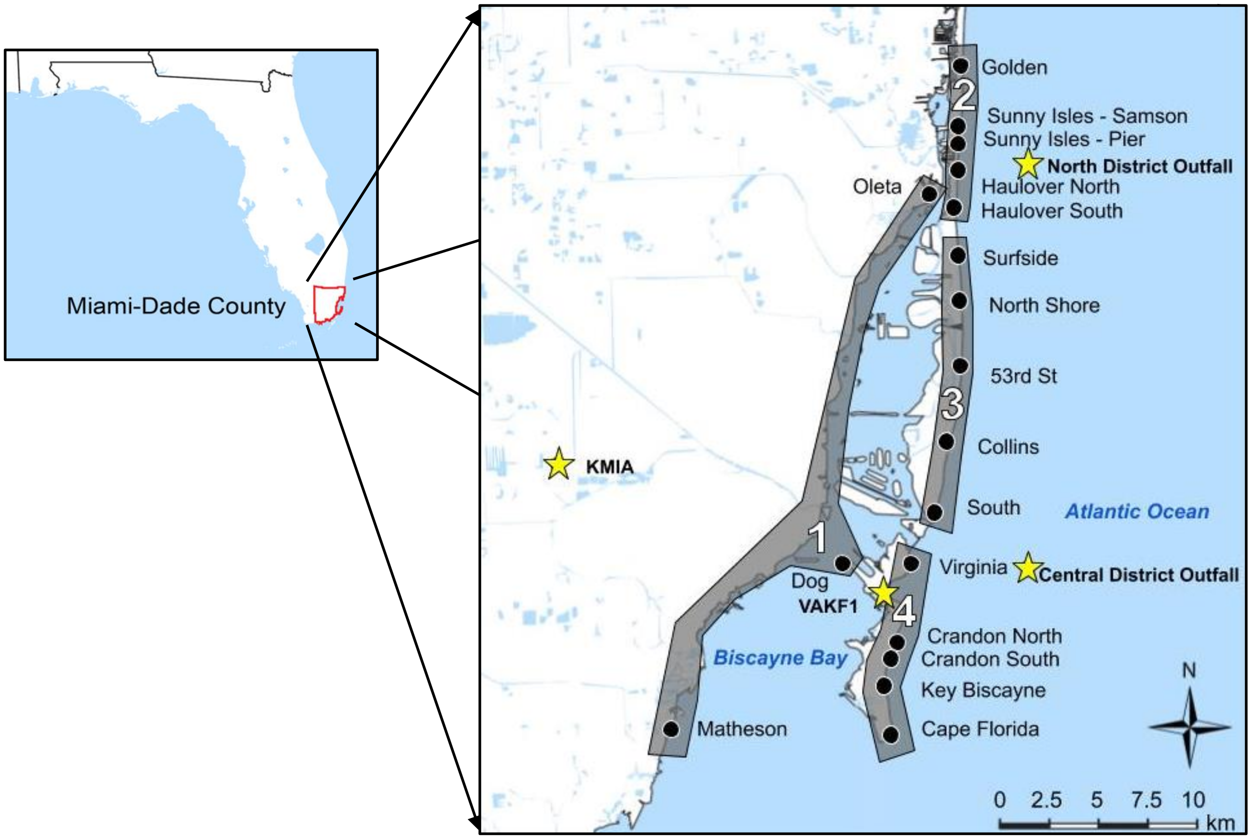

**Figure 2.** Beach locations in Miami-Dade County, Florida, USA (black circles), including: a) geographic distribution of 18 Miami-Dade beaches; b) regional division of 18 beaches: (1) Bay-facing, (2) Atlantic-facing–North, (3) Atlantic-facing–Central, and (4) Atlantic-facing–South. The location of ocean outfalls and environmental monitoring stations (yellow stars) is also shown. Environmental monitoring station FWYF1 is not shown due to the large distance (>20 km southeast) from the main coastline.

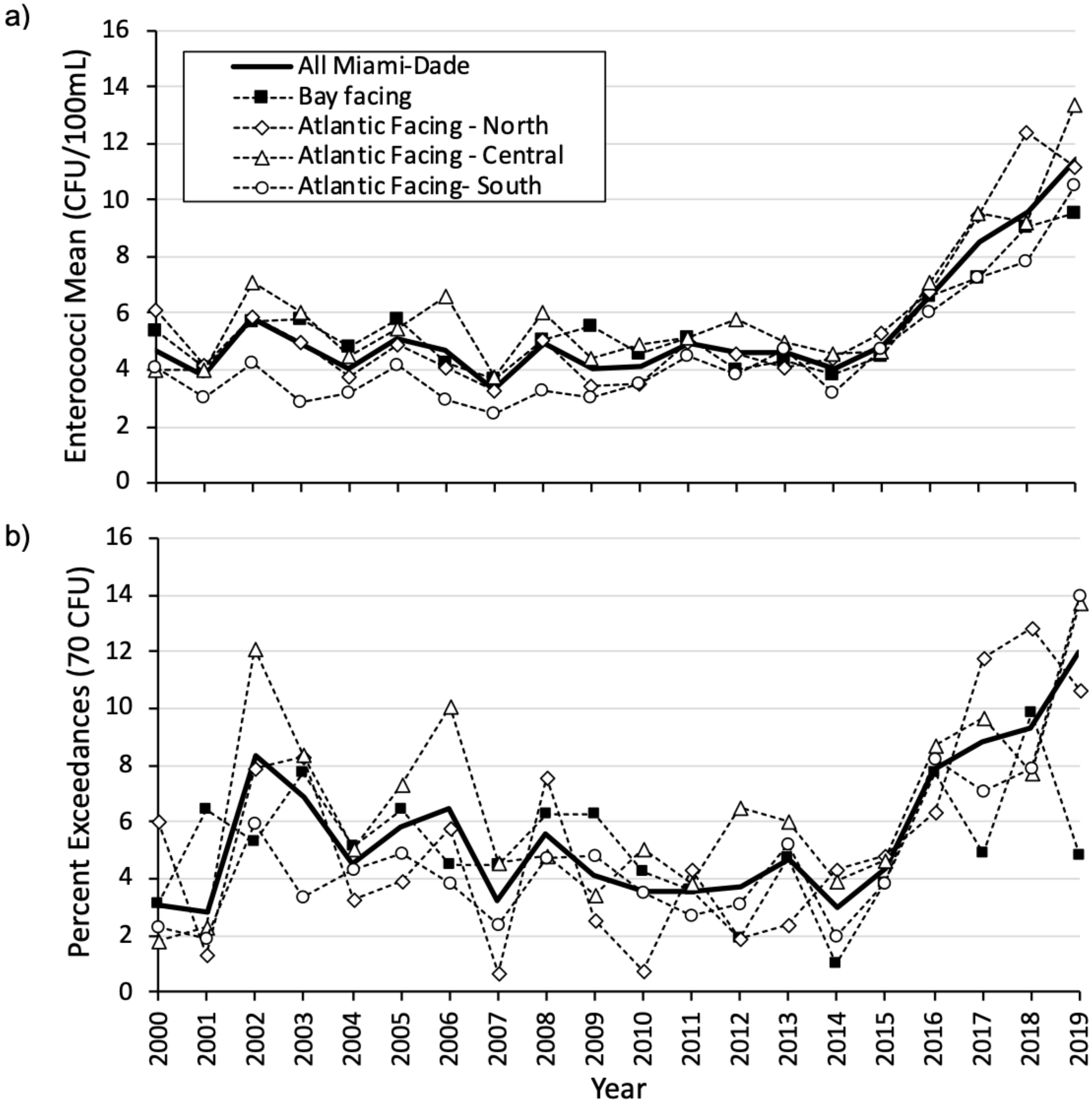

**Figure 3**. a) Annual geometric mean of enterococci abundance for all beaches in Miami-Dade County collectively and the four regions during 2000-2019. b) Percent exceedances of enterococci at the 70 CFU/100 mL threshold for all beaches in Miami-Dade County collectively and the four regions during 2000-2019.

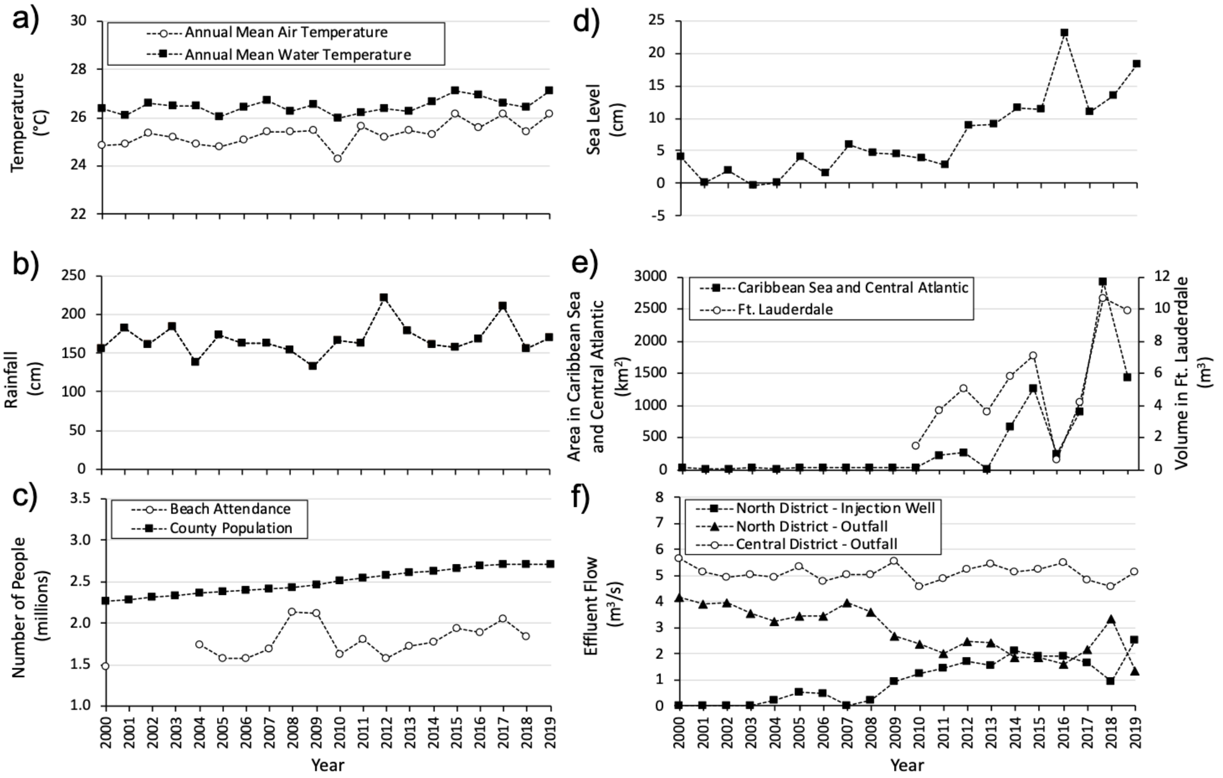

**Figure 4.** a) Annual mean air temperature and water temperature, b) Rainfall in Miami-Dade County, c) Miami-Dade County population estimates and annual beach attendance for 2000-2019, d) Annual mean sea level, e) Annual average area of Sargassum in the Caribbean Sea and Central Atlantic and annual volume of Sargassum in Fort Lauderdale, FL, and f) Annual mean effluent at the North and Central District WWTPs.

**Table S1.** Geographic coordinates of sampling locations for 18 Miami-Dade County beaches.

| **Beach Name** | **Geographic Location** | |
|---|---|---|
| | **Latitude** | **Longitude** |
| Oleta State Park | 25°54'27.86"N | 80° 7'57.83"W |
| Dog Beach | 25°44'44.83"N | 80°10'39.68"W |
| Matheson Hammock Park | 25°40'16.94"N | 80°15'40.66"W |
| Golden Beach | 25°57'57.55"N | 80° 7'5.52"W |
| Sunny Isles - Samson Park | 25°56'14.76"N | 80° 7'10.92"W |
| Sunny Isles - Pier Park | 25°55'47.51"N | 80° 7'13.80"W |
| Haulover Beach – North | 25°55'7.34"N | 80° 7'16.13"W |
| Haulover Beach | 25°54'10.08"N | 80° 7'18.42"W |
| Surfside Beach | 25°52'54.28"N | 80° 7'12.27"W |
| North Ocean Terrace | 25°51'30.89"N | 80° 7'7.52"W |
| 53rd Street | 25°49'51.55"N | 80° 7'9.32"W |
| Collins Park | 25°47'45.01"N | 80° 7'31.57"W |
| South Beach | 25°46'5.20"N | 80° 7'50.05"W |
| Virginia Beach | 25°44'38.63"N | 80° 8'37.12"W |
| Crandon Park – North | 25°42'30.95"N | 80° 9'6.33"W |
| Crandon Park – South | 25°42'11.04"N | 80° 9'13.11"W |
| Key Biscayne Beach | 25°41'21.56"N | 80° 9'23.51"W |
| Cape Florida Park | 25°40'7.52"N | 80° 9'15.53"W |

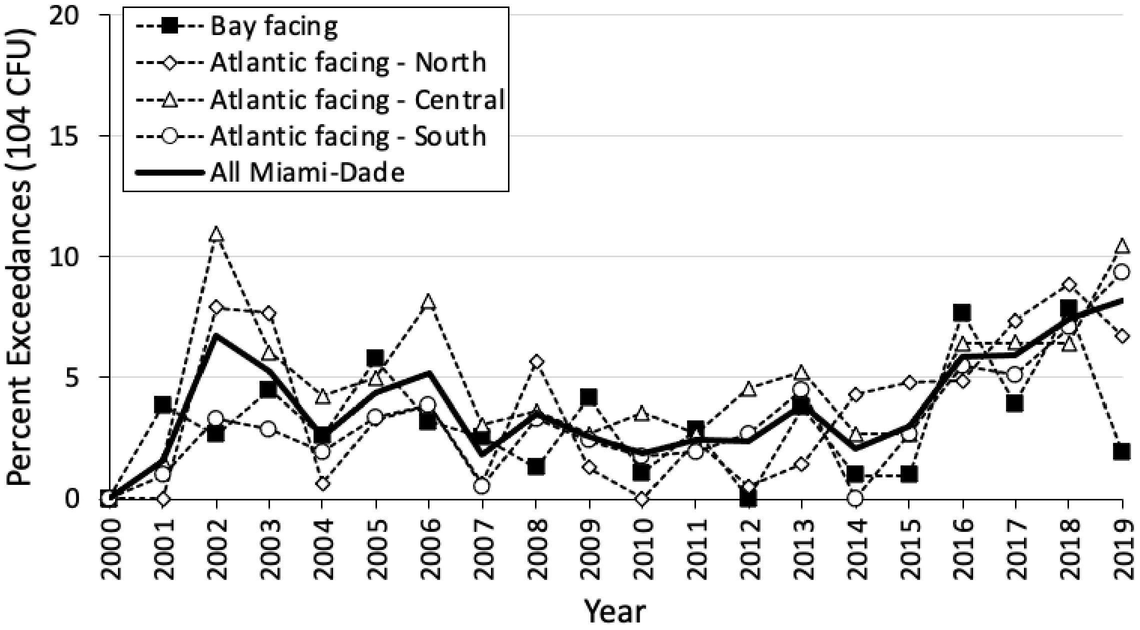

**Figure S1**. Percent exceedances of enterococci at the 104 CFU/100 mL threshold for the four regions in Miami-Dade County, 2000-2019.

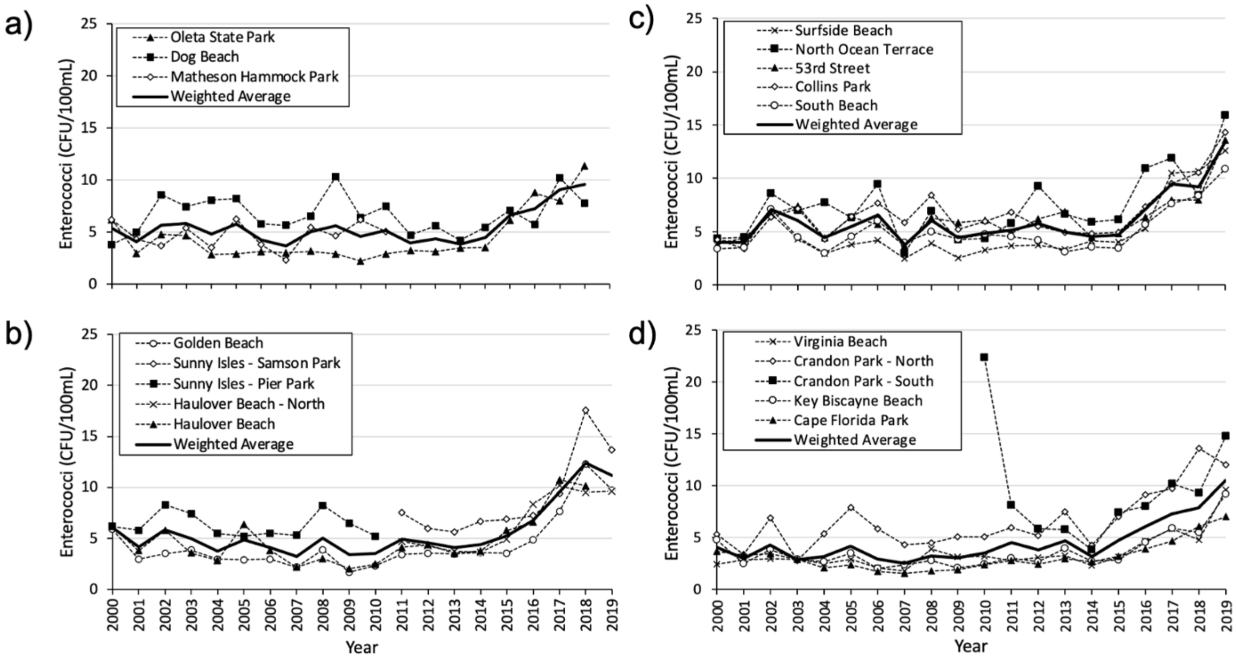

**Figure S2.** Enterococci geometric means for individual beaches in Miami-Dade County categorized by geographic location: a) Bay-facing , b) Atlantic-facing–North, c) Atlantic-facing–Central, and d) Atlantic-facing–South. Crandon Park beach was split into two monitoring sites (Crandon Park–North and Crandon Park–South) in 2010 during an episode of elevated bacteria levels towards the end of the calendar year, thus skewing the yearly monitoring data towards elevated values in 2010.