From Drainfield to Drinking Water:
Tracing Enterococci in the Coral Gables Waterway

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Introduction

While marine debris may be highlighted as a leading cause of reduced coastal ecosystem health, poorly managed septic systems are a quiet killer, particularly in areas with porous sediments and rising sea levels like Miami-Dade County, Florida. Septic systems, onsite water treatment and disposal systems, are used extensively in the US to treat over 4 billion gallons of wastewater daily, meaning 25% of households country-wide (U.S. EPA, Office of Water, 2001,2005). Since before 1972 Florida septic systems did not require permits the regulatory measures were not taken and records were rarely and inaccurately kept (U.S. EPA 2003).

Embedded in Miami-Dade's county code section 24-43.1 is the mandate that any property owner within a "feasible distance" of a local sewage main must abandon their septic system within 90 days. However, as a result of the estimated cost of $10,000 - $15,000 to connect to county sewage, the vast majority of Miami-Dade's homes remain with septic tanks despite having lateral connections (Miami-Dade County WASD, 2020). Over 95% of properties within the counties are exempt from Miami-Dade County's mandate (Miami Waterkeeper, 2020) . 8423

This dependence poses clear implications for the environment; in 2020 it was concluded that 9,000 septic systems were currently vulnerable to failing (Miami-Dade County WASD, 2020). When considering periodic vulnerability, an estimated 58,349 septic systems in Miami-Dade may be close to failing (Miami-Dade County, 2018).

Septic systems were designed with the assumption that groundwater levels would remain at a stable level. They also rely upon at least two feet of the dry soil between the system and water tables. South Florida's geology presents unique challenges due to its porous limestone bedrock and thin topsoil. Concomitantly, the average sea level in Biscayne Bay has increased by four inches since 1994 (National Oceanic and Atmospheric Administration, 1994-2017). Furthermore, Miami-Dade County's dependence on failing septic tank infrastructure has clear consequences for South Florida's ecology.

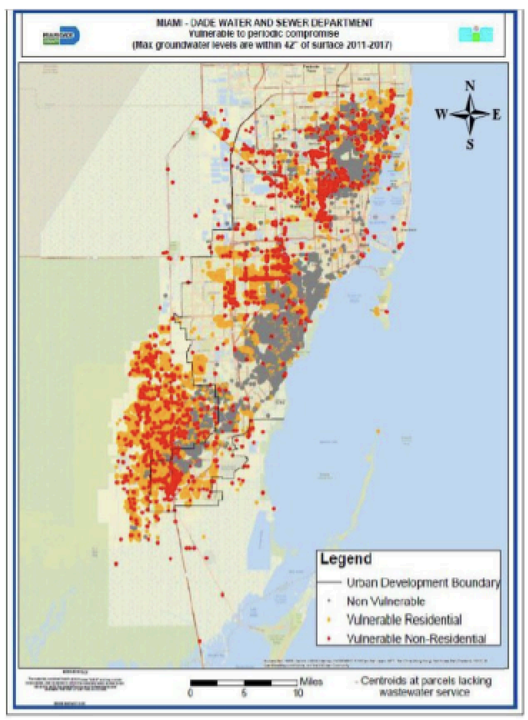

Figure 1 (Miami-Dade Country Septic Systems, Miami-Dade Country RER,WASD,DOH 2018)

In August 2020, Biscayne Bay suffered a massive fish kill indicating an ecological turning point for the ecosystem. Although its causes are disputed, high nutrient pollution and ensuing high oxygen levels were key factors (Miami Waterkeeper, 2020). It has been concluded that septic system failures contribute to these excess nutrients and thereby alter the ecology of Biscayne Bay (Chin, 2020). Even uncompromised systems discharge excess levels of nitrogen into groundwater by themselves through their effluent (Badruzzaman et al., 2012). Studies of Biscayne Bay's chlorophyll concentrations indicate that nutrient pollution has changed its environment from one that is oriented around seagrass to one that is oriented around algae (Millette et al., 2019). Moreover, septic systems may also pose health concerns for Miami-Dade's inhabitants.

Septic systems when employed properly pose minimal health risks for local communities. Ignoring unique cases of waterborne pathogens seeping into potable water supplies by septic discharge, the design of septic tank leach fields away from unsaturated soil prevents diseases (Yates, 2011). However, as stated before, a substantial amount of septics are compromised due to rising sea levels and the unique qualities of South Florida's geology. This paves the way for septic tank effluent to spread bacteria and viruses into local groundwater (Hain and O'Brien 1979). There is a clear need to understand and model the effects of septic systems in South Florida.

In Miami-Dade County's 2020 "Plan of Action Report" concerning the impacts of sea level rise on septic systems, there is an outlined need to identify vulnerable parcels. It is estimated to cost roughly $3.8 - $4.3 billion to expand local sewage systems, roughly 35% of the county's budget (Miami-Dade County WASD, 2016). Therefore it is clear that for a sustainable future, it is necessary to target certain areas.

The objective of this study was to employ bivariate and univariate analysis to investigate the correlation between concentrations of *Enterococci* and the density of septic tanks as well as the distribution of FIB in the Coral Gables Waterway. Specifically, this study aimed to test the hypothesis that *Enterococci* levels are highest in areas around canals with a higher density of septic systems. The null hypothesis will be that *Enterococci* levels are the same regardless of septic density. An understanding of septic systems is critical for a sustainable future in South Florida.

Materials and Methods

A total of sixteen sites's data were used for this study's data. To receive a broader perspective of the correlation between septic tank density and *Enterococci* levels, sites currently tested by Miami Waterkeeper and additional sites were incorporated in data collection. Sites were selected to be evenly distributed across the Coral Gables Waterway. Organizationally, they can be split into three different regions.

Table 1. Organized list of sampled locations

South	Central	North
Entrance to Waterway (CG1)	Lennar Center (CG6)	Coral Gables Senior High School (CG8A)
Cocoplum Yacht Club (CG2)	Ponce De Leon Waterway Access (CG7)	Jeronimo Drive and Granada Boulevard (CG8B)
Gables Waterway Towers (JSK5)	Lake Osceola (JSK1)	Ruth Bryan Owen Waterway Park (CG10)
Ingraham Park (CG3)	West Lab Elementary School (CG9)	Coral Gables Waterway Salinity Control Structure (JSK4)
Granada Boulevard Bridge (CG4)	Blue Road Open Space (JSK2)	South of Coral Villas Park (JSK3)
Hardee Road Bridge (CG5)		AD Doug Barnes Park (CGAD)

Samples were collected on a weekly basis and transported to the University of Miami's Rosenstiel Campus for testing. The needed materials for sampling consisted of latex gloves, a cooler filled with ice, 125 mL sampling bottles, a sampling pole, alcohol, and datasheets. After arriving at a site, initial observations would be made to data sheets recording weather, current, and visibility as well as pictures of water conditions. Then sampling bottles would be fitted to sampling poles with zip ties. It was critical to ensure that the inside of bottles would not be touched to ensure reliable results. Towards the end of sampling days, a field blank of deionized water would also be taken to be tested later as a field blank.

To preserve the initial *Enterococci* levels, samples were transported within six hours to Rosenstiel lab for analysis. The processing procedure consisted of injecting 10 mL of each sample and field blanks into 90 mL of deionized water. Then, a packet of Enteroalert, a defined substrate technology nutrient indicator, would be dissolved in the sampling and deionized water mixture by inverting the bottle several times making sure not to aerate the bottle. The sample solution would then be transferred to a Quanti-Tray 2000 for incubation by sealing them with an IDEXX Quanti-Tray Sealer PLUS. There would be continued measures to reduce the aeration of the sample solution. Trays would then be incubated for 24 hours at 41.0°C. The same procedure was repeated across all testing days and samples.

Technicians would then return to the now-incubated samples to remove them from incubation for analysis. UV light would be shined at samples and the number of fluorescent wells would be counted and marked on data sheets. The number of fluorescent wells would then be inputted in IDEXX MPN charts to determine a CFU/100mL number consistent with each sample.

While *Enterococci* levels were recorded through sampling, there were luckily public records of septic tanks already available for research. The Florida Department of Health's Septic System Database contains over 80,000 data points for Miami-Dade County. For the purposes of this study, a half-kilometer radius was defined around each testing site. The number of septic tanks in each radius was then found using GIS software.

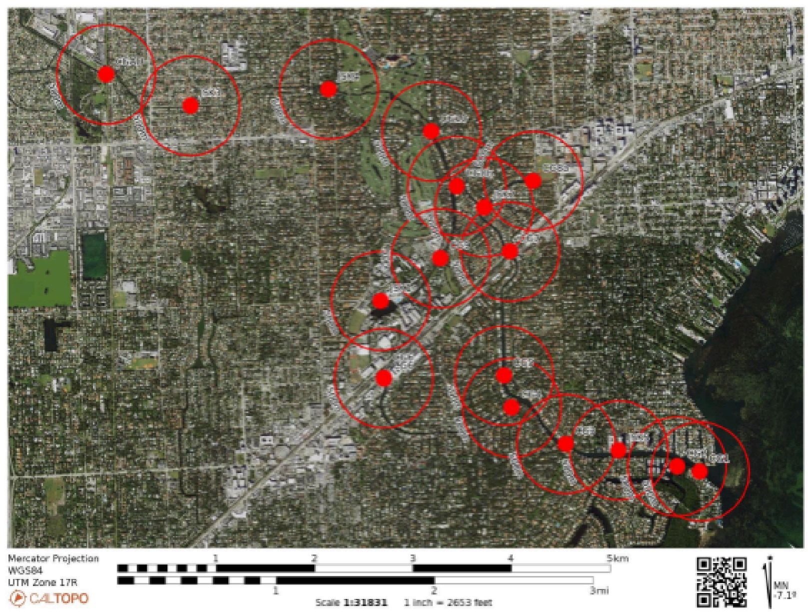

Figure 2. Map of Testing Sites with Septic Tank Radi

In order to prove a statistical relationship between septic tanks within .5 kilometers from testing sites and *Enterococci* levels, a linear regression analysis was used. To better understand each variable, univariate analysis accompanied the linear regression. Outliers were removed from the data set using a Modified Z-Score test. An independence of observations was proven through a Durbin-Watson statistic. Moreover, homoscedasticity was also proven through a Breusch-Pagan Test and analysis of residuals. An ANOVA test was also used between the two variables to prove/disprove my null hypothesis.

Results

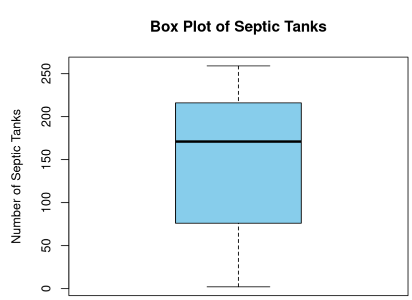

Figure 3

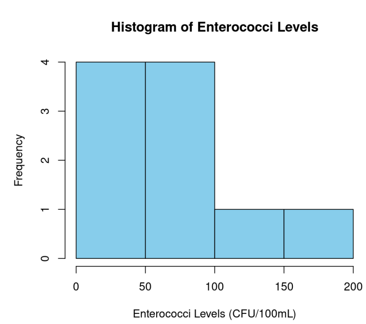

Figure 4

The Median of Septic Tank data was calculated to be M = 180. In contrast, the standard deviation of enterococci levels was SD = 47.87.

Table 2. One-way AnovaTest run in RStudio between MPN and Number of Septic Tanks. The P-value is greater than .05 failing to reject the null hypothesis.

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Septic Tanks	1	2292	2292	1.138	.317
Residuals	8	16108	2014		

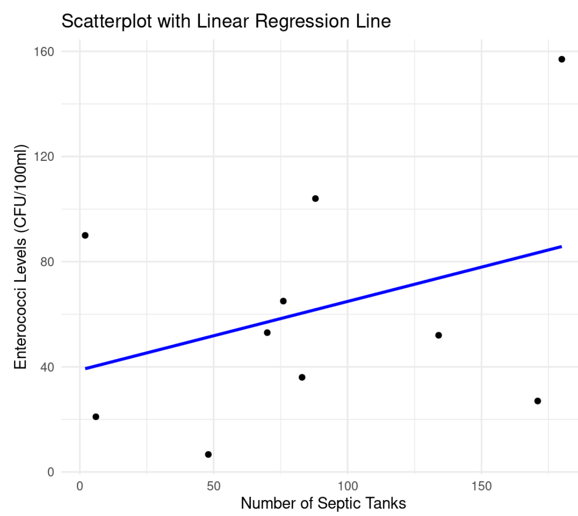

Figure 5 (Outliers Removed)

Linear regression analysis indicated a value of R^2 =.1246, $F(1, 8) = 1.138$, $p = .3171$, The regression coefficient for the number of septic tanks was also $(B = .2611, SE = .2477, t = 1.067, p = .317)$, indicating that enterococci levels increased by 0.2611 units for each additional septic tank. The intercept was 38.7674 ($SE = 25.3392$, $t = 1.530$, $p = .165$), demonstrating that without septic tanks the Coral Gables Waterway would have an approximate CFU/100mL of 38.

Discussion and Conclusion

Many of our results were inconclusive. The variance within our data resulted in an increased p-value for our one-way ANOVA test that resulted in a failure to reject our null hypothesis. On the other hand, as seen in figure three, linear regression analysis failed to demonstrate a clear relationship within our data. Much of this is the result of experimental bias within our experiment.

It is clear that if this study were to be conducted again several recommendations would need to be pursued. To reduce experimental bias, the number of sites tested as well as the number of repetitions would need to be increased. With further data collection, more accuracy could have been found in the statistical analysis of this study's data. Moreover, it can also be concluded that a better dataset for septic tanks should also be found. Duplicates and inconsistencies were common within the public datasets employed. Aside from data collection, a variety of post hoc tests could also have been used to find inconsistencies in the studies results. For instance, Tukey's HSD test could have been conducted to test individual p-values for each site. All in all, several measures should be pursued to reduce experimental bias if one were to repeat this experiment again.

Despite the inconclusive nature of this study's statistical analysis. Valuable connections can be made from its uncertainty. Evidently, to gain a better understanding of the ecological crisis confronting South Florida, both the public and non-profit sectors must place a greater emphasis on taking care of databases such as the Florida DoH's septic tank database. Prior to 1972, Florida septic systems were installed without needing permits, which makes it difficult to estimate their numbers and precise locations (U.S. EPA, 2003). The improper hygiene of these databases created roadblocks for this study and will continue to do so in the future for similar ones. In comparison, *Enterococci* values were also altered by experimental bias created by a lack of resources. This can be explained further by the fact that higher levels of *Enterococci* are present in socio-economically disenfranchised communities which by their very nature lack the infrastructure for frequent water quality testing (Anyabwile 2023). In summary, this study has demonstrated the lack of appropriate infrastructure needed to understand the causes of Biscayne Bay's decline.

Take Home

This project was very frustrating for us as it challenged my original conception of what it meant to do research. Even though we both knew coming into our project that our hypothesis may be proven wrong, when we failed to reject our null it was much harder to swallow. It demonstrated to me that a true scientist abandons ego to find meaning in humility. For science to work, we must be ready to challenge our initial perceptions no matter what they originally were.

Moreover, the research that we conducted also created a clear picture of the lack of information there is surrounding septic tanks and our environment in general. It was appalling that the county itself did not know how many septic tanks existed in our community. It pushed us to be evermore active in our community about our environment.

In summary, this project has been an amazing experience that has bettered us not only as citizen scientists but as members of our community. We would like to thank Toni and Aliza for all the awesome support they have provided us with along the way as well as all the fantastic people at the International Seakeeper Society and Miami Waterkeeper. Thank you!

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