Enterococci in Wrack, Sediments, and Surface Water

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Introduction

Enterococci are gram-positive bacteria used as a quantitative indicator of water quality degradation- driven by sewage pollution- and used to designate potential human health hazards (see WHO, 2003). However, enterococci can accumulate in sediments (Alm et al. 2003), and have been reported in high concentrations in both sands (Yamahara et al 2007, Lee et al. 2006) and wrack (detritus and decaying vegetation) in the intertidal zone along the California coastline (Imamura et al. 2011).

Here, we evaluated enterococci concentrations across substrate types (water, sediment under wrack, and wrack) and sites within the Loxahatchee River Estuary, a subtropical estuary on the East coast of Florida.

Photo 1. Wrack line near lifeguard stand at Dubois Park.

Studies & Objectives

Study 1 (RDP): Determine if enterococci concentrations differ by: (a) proximity to wrack in surface water samples and (b) substrate type.

Study 2 (JCU, RDP, DUB & JBP): Identify differences in enterococci concentrations (a) between sites, (b) between substrates, (c) site by substrate variability, and (d) evaluate patterns between enterococci concentrations and organic matter (wrack and sediments).

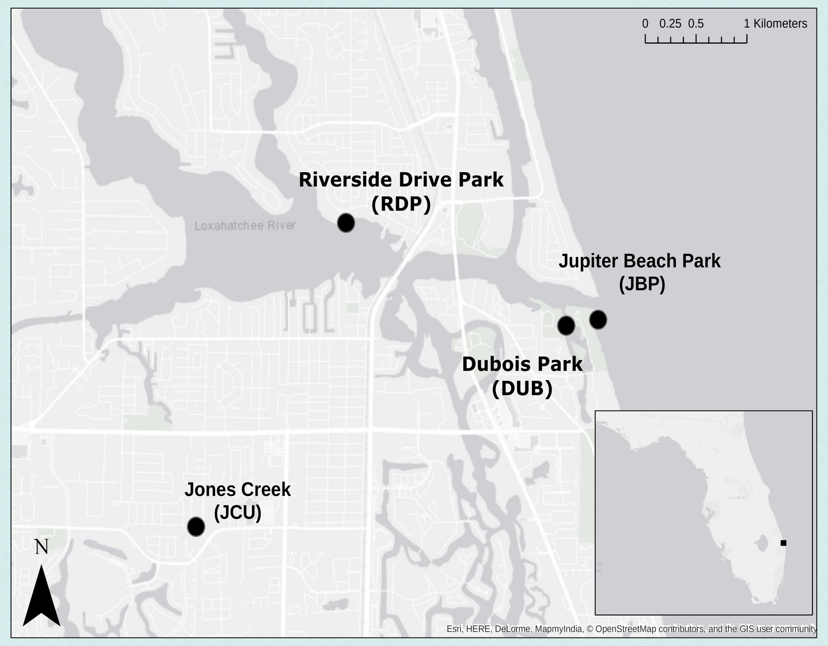

Fig 1. Map of monitoring sites within the Loxahatchee River Estuary. Sites examined in Study 1 (RDP) and Study 2 (JCU, RDP, DUB, JBP) labeled.

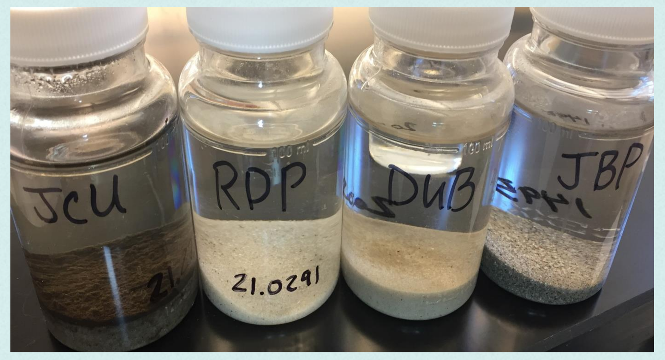

Photo 2 (Top, Left). Sediment samples from study 2 collected at JCU, RDP, DUB and JPB (Fig 1). All samples contained sand, but more organic debris was observed in sediment samples collected from JCU

Photo 3 (Top, Right). Marine derived wrack, including significant amounts of seagrass, deposited by the tide on beach sand at JBP in study 2.

Photo 4 (Bottom Right). Sediments and mangrove detrital wrack at JBP in study 2.

Table 1. Site conditions and enterococci concentrations observed during Study 2 (Jan 2018). Temperature was 20±1 ˚C at all sites.

Site	Control Sediment (OM%)	Wrack Sediment (OM%)	Wrack Type (OM%)	Salinity	Dissolved Oxygen (mg/L)	pH	Enterococci Concentration Geometric Mean (MPN 100mL/100g DW)			
							Water	Control Sediment	Wrack Sediment	Wrack
Jupiter Beach Park (JBP)	Sand (0.7%)	0.6–2.7%	Sargassum, Seagrass (43–58%)	36	7.8	8.0	51	1,710	23,789	16,2768
Dubois Park (DUB)	Sand (0.3%)	0.3–0.8%	Sargassum, Seagrass, Mangrove (53–65%)	31	7.4	7.9	53	506	4,549	34,377
Riverside Drive Park (RDP)	Sand (0.1%)	1.8–3.8%	Sargassum, Seagrass, Mangrove (47–61%)	31	7.9	8.0	43	381	39,177	22,433
Jones Creek Upper (JCU)	Mud & Sand (1.3%)	3.1–17.1%	Mangrove, Other Detritus (27–48%)	15	6.9	7.7	219	11,075	12,322	34,969

Methods

- In November 2017 (study 1) and January 2018 (study 2) we collected samples from different 'substrates': (1) surface water, (2) sediments under wrack and (3) wrack. One control sediment sample without wrack on top was also collected at each site.
- Field readings collected using YSI multiparameter meter, were recorded at each site to characterize general conditions (Table 1). 3 (study 1) or 4 (study 2) replicate samples were collected ≈ 1m apart for each substrate. Wrack was collected from the high tide line using latex gloves. Sediments were collected using a 70% Isopropyl Alcohol sterilized hand spade (≤ 2cm, see Vogel et al. 2017). Surface water samples were collected using a sample pole (< 0.3 m depth).
- Samples were transported to the Laboratory on ice and processed within 4h. Dry weights (DW) and organic matter (OM%) were determined gravimetrically for sediments and wrack (modified ASTM D6503 – 14). After the initial weight determinations, a subsample was collected, vigorously shaken in 100mL of deionized water for 1 min, particulates allowed to settle for 1 min, and an aliquot collected (adapted from Lee et al. 2006) (Photo 5). These aliquots were then processed as surface water samples (following Alm et al. 2003).
- IDEXX Enterolert method (ASTM D6503 – 14) was used to quantify Enterococci concentrations. This method uses a nutrient indicator which fluoresces when metabolized by enterococci (Photo 6) and values are expressed as Most Probable Number (MPN) / 100mL. A modification of this method was used on wrack and sediments, these values are expressed as MPN/ 100 g DW (following Alm et al. 2003, Lee et al. 2006).
- Normality of data was tested using a Shapiro-Wilk test, and non-parametric tests were used for data violating normality. An analysis of Variance (ANOVA) was used to determine weather Enterococci significantly differed with distance from wrack. Kruskal-Wallis rank-sum tests were used to distinguish differences in enterococci concentrations between (a) sites, (b) substrates and (c) sites by substrate types. A Dunn's test with Bonferroni adjustments was then used to make pair-wise comparisons (Dinno 2017).
- A simple regression was used to consider relationships between enterococci concentrations and organic matter (in wrack and sediments). All statistics were conducted in R.(v 3.3).

Photo 5. Susan Noel processing wrack samples.

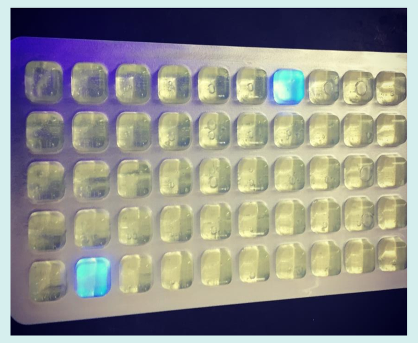

Photo 6. Enterolert fluorescence.

Study 1 Results: Distance from Wrack

Frequent wave exposure and a buildup of wrack is typical of RDP. We examined enterococci concentrations in surface water with increasing distances from wrack (at 0, 1 and 3 meters) and by substrate type. Surface water samples (shown as 0m distance from wrack, Fig. 2a) represent samples collected from water just above wrack during high tide.

- We noted a sharp decline in enterococci concentrations with increasing distance from wrack (ANOVA, , p ≤ 0.01; Fig 2a).
- Results also demonstrated significant differences in enterococci concentrations between substrate types (Kruskal-Wallis, p ≤ 0.01; Fig 2b).

Photo 7. LRD intern Caitlyn Hayes collecting a water sample.

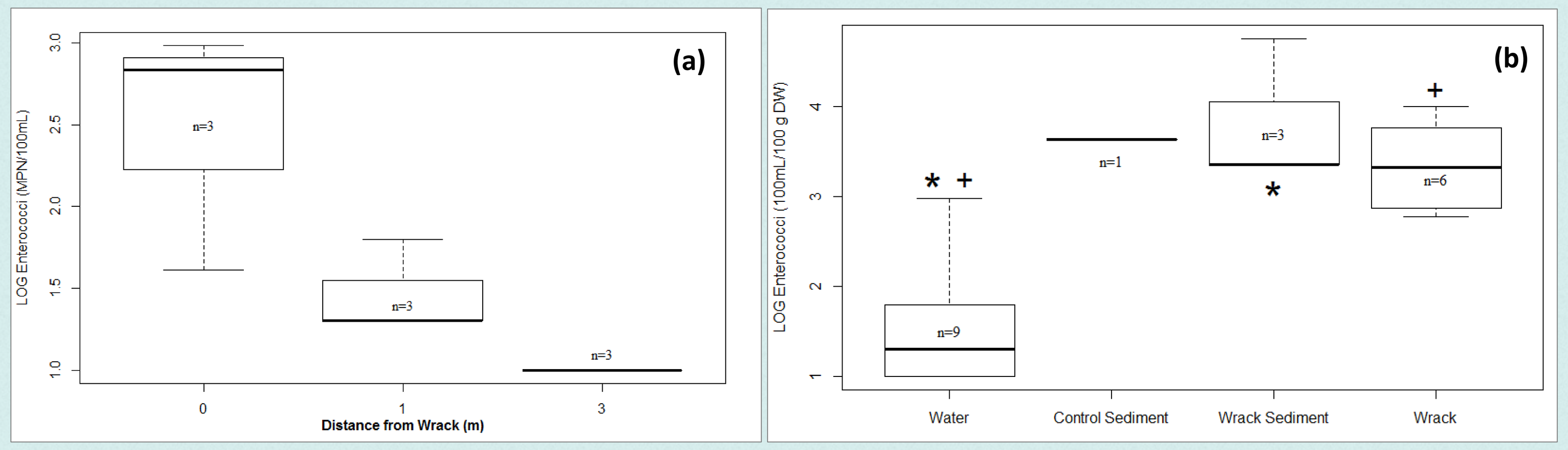

Fig 2. LOG enterococci concentrations (y-axis) **(a)** surface water samples with increasing distance from wrack (x-axis) and **(b)** substrates (x-axis). Box plot showing maximum, upper-quartile, median, lower-quartile, and minimum values (top to bottom), and n=samples. * and + show significant differences (p≤0.05) identified using Dunn's pair-wise comparisons.

Photo 8. Close-up of wrack and sandy sediments at RDP.

Study 2 Results: Site by Substrate Variability

- When averaged across substrates (water, control sediment, wrack sediment, and wrack), enterococci concentrations did not significantly differ between sites (Kruskal-Wallis, p > 0.05; Fig. 4a).
- With all sites combined, enterococci concentrations were significantly higher in wrack and sediments under wrack, than in control sediments and water samples (Kruskal-Wallis, p ≤ 0.001; Fig. 4b).
- When substrates were examined independently, we measured significant differences in enterococci between sites, in both surface water and sediments (Kruskal-Wallis, p ≤ 0.05; Fig. 4c).
- Despite differences between substrates (Studies 1 & 2), we combined sediments and wrack to consider a general trend between enterococci concentrations and organic matter. Although there appears to be a distinct difference between wrack and sediments (colors, Fig. 5), we noted a general positive correlation between organic matter and enterococci concentrations across substrate types (Pearson's R =0.48; Fig. 5).

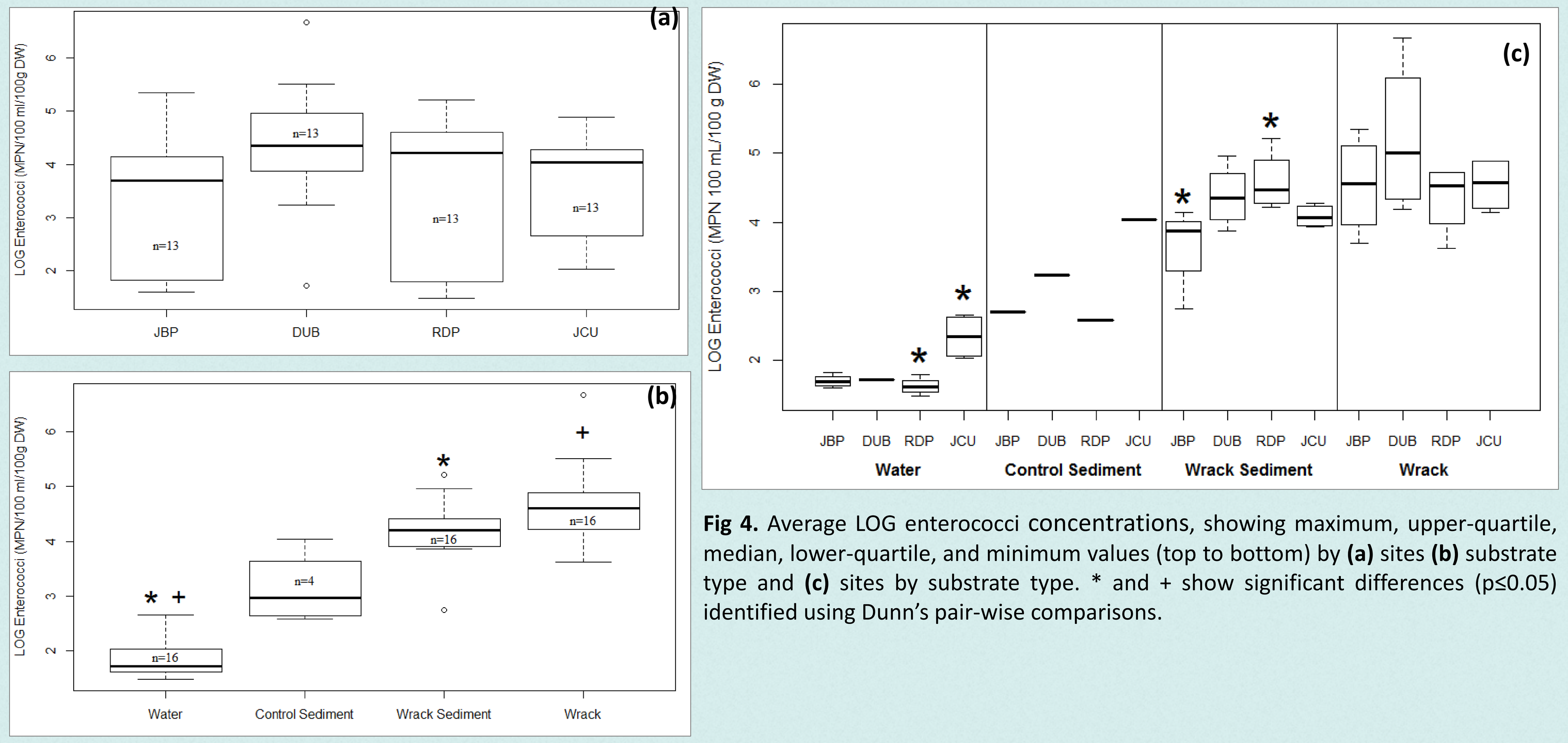

Fig 4. Average LOG enterococci concentrations, showing maximum, upper-quartile, median, lower-quartile, and minimum values (top to bottom) by **(a)** sites **(b)** substrate type and **(c)** sites by substrate type. * and + show significant differences (p≤0.05) identified using Dunn's pair-wise comparisons.

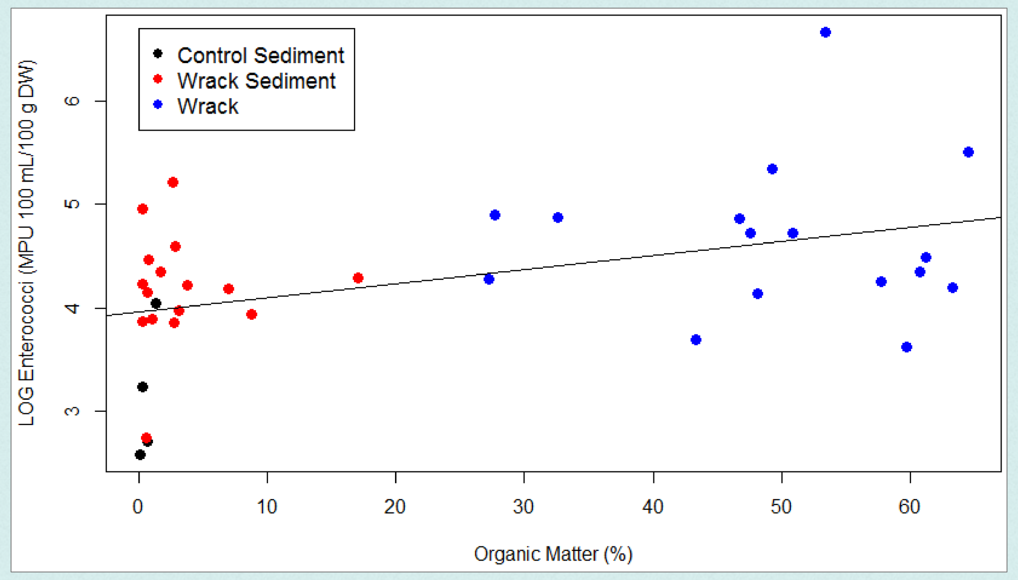

Fig 5. Positive relationship between LOG enterococci concentration and organic matter. Data includes organic matter and enterococci concentrations from: control sediments, sediments underneath wrack, and wrack. Single regression across substrate types shown (n=36, p ≤ 0.001, Pearson's R=0.48) .

Conclusions

- These studies of a subtropical Florida estuary support previous findings along temperate coasts. In study 1, we demonstrated that enterococci concentrations decrease with increasing distance from wrack (Fig.2). We also established that wrack, and the sediments underneath wrack, harbor significantly higher enterococci concentrations than corresponding surface waters (Figs. 3, 4b, and 4c). In study 2 we established that the variability in enterococci concentrations were minimally affected by location (Fig. 4a), but strongly driven by substrate type; with the greatest enterococci concentrations associated with wrack and sediments overlain by wrack (Fig. 4b).
- Our results support the idea that microbial abundance may be regulated by the availability of organic carbon (Shiaris et al 1987). Even though we noted a significant positive relationship between enterococci concentrations and organic matter (Fig 5), this relationship is likely to depend upon the characteristics of both the sediments and wrack (e.g., particle size, lability of carbon). These results, coupled with field observations, suggest that estuarine sites with stagnant conditions and significant accumulations of highly available organic carbon (e.g., excessive mangrove detritus and easily resuspended muck) may experience chronic, elevated enterococci concentrations.
- Future research should quantify the spatial and temporal occurrences of high microbial populations in natural systems. We are in desperate need of a mechanistic understanding of the drivers of high microbial concentrations in relatively unimpacted natural systems (e.g., systems not experiencing sewage contamination), and more work is needed to quantify the virulence of enterococci populations under various environmental conditions.

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Total Organic Carbon (TOC) in Surface Waters - Supplemental Info Handout

Trends

- Historical surface water samples (2012-2017) have been regularly collected monthly-quarterly at water quality monitoring stations throughout the Loxahatchee River Estuary (LRE).
- Samples are outsourced to Pace Analytical Laboratories and processed according to ASTM D5904 - 02 for Total Organic Carbon (TOC).
- Initial regression pattern between LOG Enterococci concentrations and TOC suggested a minimum threshold.
- Based on this, a quantile regression was used for further analysis and visualized using the 'Quantreg' package using R (Koenker 2012)

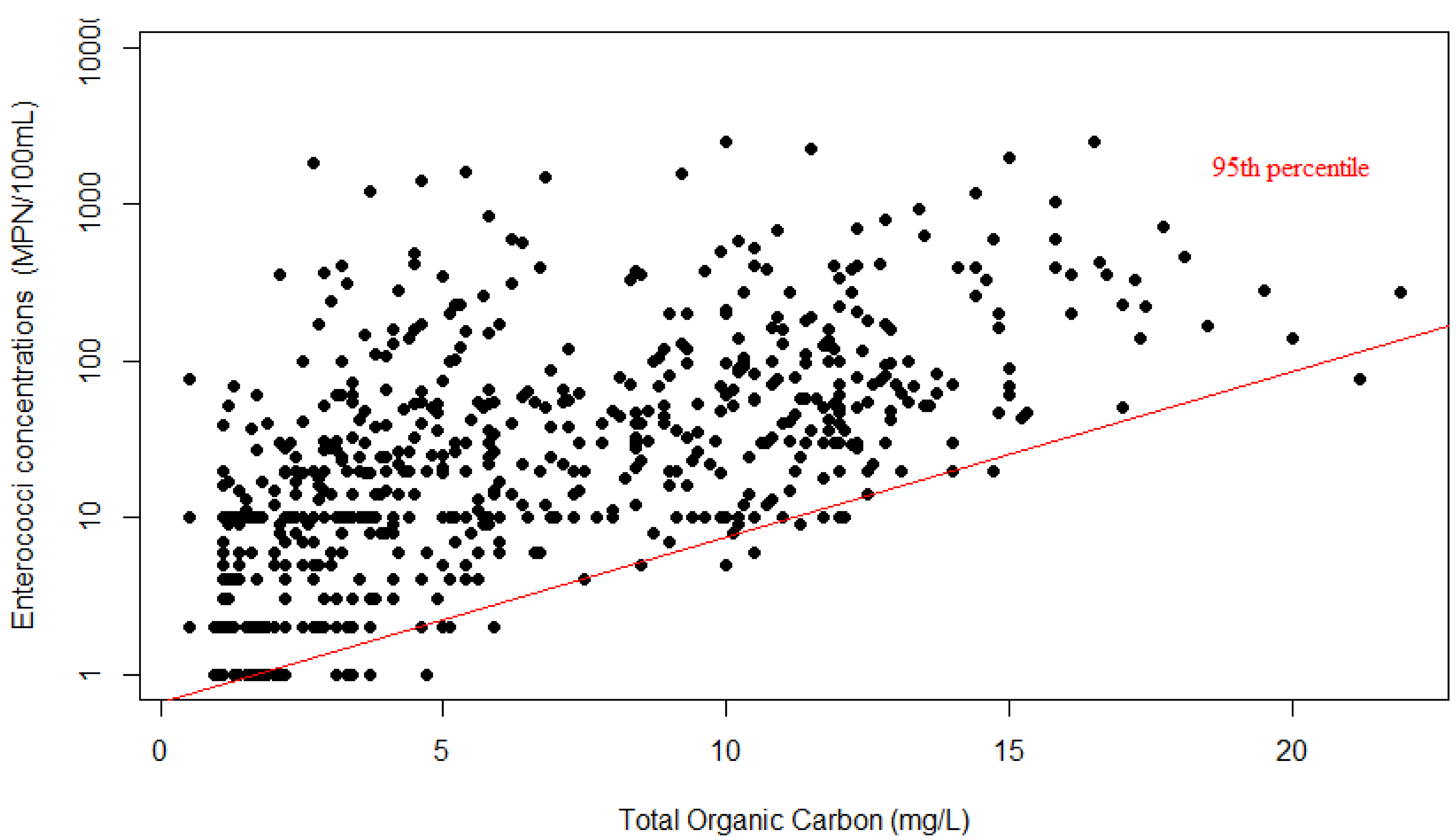

Fig 6. Historical (2011-2017) surface water (< 0.3m) data throughout the entire Loxahatchee River Estuary Watershed (n=669). Relationship between enterococci concentration and Total Organic Carbon shown. Red line indicates Quantile Regression Model based on the LOG enterococci concentration values. The critical threshold of the regression was fitted at the 5th percentile (Ʈ=0.5); only 5% of data occurs below this critical threshold (model program, see Koenker 2012).

- This work is preliminary, but using a 5th quantile regression model we noted a minimum threshold ('factor ceiling thresholds' using 95th Percentiles see: Thomson et al 1994, Pratt et. al. 2014):
  - Lower TOC = greater range of variation & lower minimum LOG enteric bacteria (i.e., greater chance of measuring lower Enterococci concentrations).
  - Higher TOC = greater minimum threshold of LOG enteric bacteria (e.g., if TOC ≥ 15 mg/L than we can be fairly certain our samples will not meet FL state water quality criteria 71 MPN/100mL)
- Future research should quantify the spatial and temporal occurrences of high microbial populations in natural systems. We are in desperate need of a mechanistic understanding of the drivers of high microbial concentrations in relatively unimpacted natural systems (e.g., systems not experiencing sewage contamination), and more work is needed to quantify the virulence of enterococci concentrations under various environmental conditions.

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Additional Trends Observed in Surface water samples