

Quantitative Analysis of New River Estuary Water Quality in the Context of Up-Estuary Concentrated Animal Production

Author

Dan Crownover

Collaborators

Dr. Rachel Noble[PI], Riley Lewis, Lisa Ryder, Denene Blackwood, Tamara Bennett, Thomas Clerkin, Mark Ciesielski, Javier Gallard-Góngora, Steph Smith

Abstract

This project utilized a combination of traditional water quality assessment methods approved by the EPA including Fecal Indicator Bacteria (FIB), Microbial Source Tracking(MST) and a well detailed analysis of environmental and physical, and chemical parameters over a wide array of meteorological conditions(Nobel et al., 2020) to identify biological sources of fecal contamination(humans, hogs and poultry) in the New River Estuary over a year long period. The primary objectives of this research project where to 1) relate FIB and MST marker concentrations to environmental and meteorological parameters such as, antecedent rainfall, total suspended solids (TSS) during both king tide events and a wide collection of dry and wet weather conditions over a four-month period; 2) Development geospatial map showing the distribution of CAFO's in relation to waterways in the White Oak River Basin; 3) To understand the viability of integrating aerial imagery data for digitizing future photos, facilitating a quantitative evaluation of contamination levels within specific areas of the New River Estuary. The objective of the report was to quantify and analyze the extent of contamination in the New River Estuary. The results reveal severe biological contamination of the New River Estuary with Fecal Indicator Bacteria, surpassing both EPA and state guidelines at all sample sites and across all environmental parameters. Urgent and sustained contamination reduction measures are advised to mitigate risks to human, fish, and shellfish life in the Estuary.

1. Introduction

The water quality of estuaries in the United States and around the world is an escalating international concern. Serving as the interface between humans and the sea, estuaries remain at the forefront of marine ecosystem health, offering vital services for a growing global human population. In the US, estuaries such as the New River Estuary (NC), play a crucial role by providing essential habitat for 68% of commercial fish species (NOAA, 2019). However, polluted runoff due to anthropogenic and farmed sources poses a serious threat to numerous

pelagic fish and shellfish species that are commonly utilized for food consumption and economic benefit.

A highly profitable industry in Eastern North Carolina, Concentrated Animal Feeding Operations or CAFOs, comprises farms where animals, primarily poultry and hogs, are kept in tight, narrow pens and raised until they are slaughtered for human consumption. NC is the 2nd largest producer of hogs in the US. Of the more than 2,500 permitted CAFOs in NC, 88.3% are hog operations, predominantly located in southeastern NC(Son et al., 2021). Waste produced by CAFOs is dumped into massive holding lagoons, where it sits, often uncovered, until subsequently sprayed onto local farm fields for fertilizer use (Miralha et al. 2021). Major pollutants from CAFOs waste lagoons include nutrients, sediments, pathogens, heavy metals, hormones, antibiotics, and ammonia, (Mole, 2013; Scanes, 2018; Randad et al., 2019; Kronberg and Ryschawy, 2019) and can wash into waterways during heavy rainfall events. While CAFO waste placed onto fields can be a valuable source of nutrients such as phosphorus (P) and nitrogen (N) and organic carbon for agricultural use (Yan et al., 2017), the excess accumulation of manure can impact the environment, principally water bodies, through eutrophication and hypoxia issues which can result in long-term ecological degradation(Qi et al., 2017).

Tucked at the end of the New River Estuary in Eastern North Carolina, the city of Jacksonville is the commercial hub for Onslow County and home to the country's largest Marine Corps Base Camp: Camp Lejeune (*City of Jacksonville website 2023*, <https://jacksonvillenc.gov/847/About-Jacksonville>). Today, the countryside surrounding the municipality is home to a plethora of agricultural and domestic infrastructure, where historically cheap land and increased demand for poultry and hog farms (FOA 2018) have given rise to an explosion of industrial CAFOs. A study from 2022 showed that over 2 percent of North Carolina's 7,352 swine and poultry factory farms are in or just outside floodplains where regional flooding can contaminate nearby water supplies (Grady et al. 2022). Today, these CAFO lagoons sit at the physical interface between land, domestic and recreational enterprises and are of increasing environmental concern as to the extent of pollution occurring following major rainfall events and king tides in coastal North Carolina.

In 2023, the Coastal Carolina River Watch and UNC Institute for Marine Science began collaborating on a project to quantifiably characterize the extent of potential CAFO and human fecal contamination in the New River Estuary. The primary goal of the research project was to quantify the magnitude of fecal contamination of the New River Estuary from six sample sites, each within close proximity to a CAFO area, over the span of a four month period.

2. Materials and Methods

The selection of sample site locations took into consideration both budgetary constraints related to transportation costs between sites and the geographical proximity to hog spray fields. Each location was meticulously chosen based on geospatial visualization, focusing on the relative proximity to potential sources of fecal contamination. The six sampling sites were numerically designated to correspond with increasing distance to the major estuarine body,

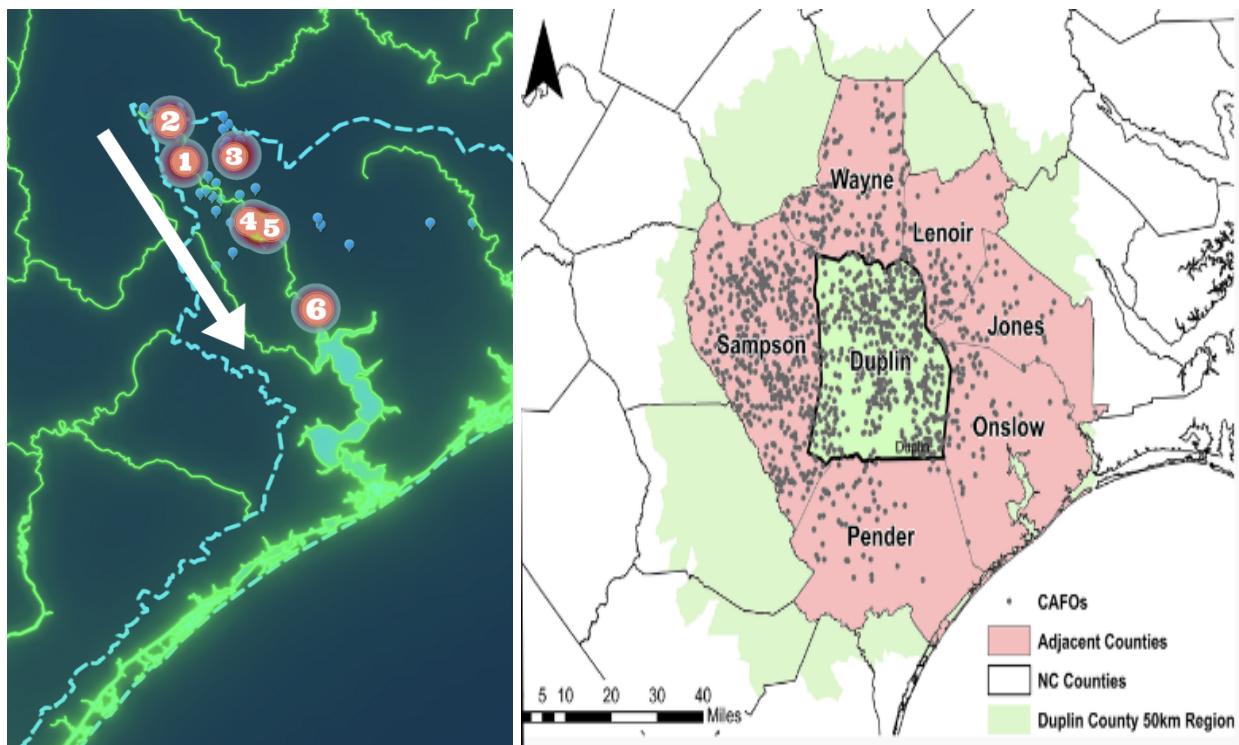
forming a spatial gradient that progresses from ‘up estuary’ to ‘down estuary’ within the New River Estuary itself (see *Figure. 1*).

Samples were collected once a month and after major rainfall events(rainfall>4 inches). Water from each site was collected in 1000mL polypropylene sample bottles, which were labeled for accurate site identification. The physical collection process involved the use of a sterilized bucket attached to a 40m rope. This bucket was lowered into the water column and washed out three consecutive times with the corresponding site water before the fourth sample, constituting the final collection, was transferred, wearing sterile nitrile gloves, into the polypropylene bottle.

Following the termination of sample collection, each bottle was promptly stored on ice at 4°C and processed within six hours of the sampling event. Control blanks were obtained through the process of de-capping and capping a bottle of 1000 ml of deionized water(obtained in the lab) to the open air in the field and placing it immediately into an icing unit for subsequent processing.

A YSI Sosodone was used to collect environmental parameters for water quality at each sample site, including Dissolved Oxygen, Salinity, Temperature(C) and pH.

In lab sample processing was a straightforward process. FIB Enumeration, Total Suspended Solids(TSS) and dPCR were collected using a standardized EPA guideline process.



Site	Latitude	Longitude	Location
NR1	34.91887177	-77.61659611	Beulaville Hwy
NR2	34.9674568	-77.63485565	Al Taylor Road
NR3	34.92771018	-77.54295082	Petersburg Road
NR4	34.84917602	-77.51935214	NW Bridge Road
NR5	34.84324478	-77.50281736	Rhodestown Road
NR6	34.75237303	-77.43302011	Waterfront Park

Figure 1. Map left: showing the sample locations relative to the new river estuary and spray fields in the Jacksonville NC (Crownover et al, ArcGIS Online Story Maps, 2023).

Map right: showing the CAFOs in eastern NC in relation to county and NRE(Battye et al, 2023)

2.1 FIB Enumeration

All samples, *E. coli* and *Enterococci* were enumerated in duplicates using USEPA approved Defined Substrate Technology™ Colilert-18® and Enterolert™ kits combined with the most probable number (MPN) Quantitray/2000© trays at a 1:10 dilution (sample: Deionized water) per manufacturer instructions (*IDEXX Laboratories, Westbrook, ME*).

2.2 IDEXX Analysis:

Colilert-18® and Enterolert™ values were averaged in Microsoft Excel using MPN equations from Hurley and Roscoe (1983). All values were corrected to the unit of MPN/100 mL based on dilution. Samples exceeding the detection limit for IDEXX Quantitray/2000© were assigned the highest value within the averaged limits of detection (24,197 MPN/100 mL); values below the limit of detection were assigned value of 9.0 MPN/100 mL, the lowest value within the averaged limits of detection. The NCDEQ Tier 1 (heavily utilized) standard of 104 ENT MPN/100 mL was applied to place the results of this study into the context of recreational water quality management (USEPA, 2014). Additionally, while NCDEQ does not monitor EC concentrations to manage water quality, EC is a recommended FIB as per the Revised EPA Recreational Water Quality Criteria of 2012. EC results were compared to the statistical threshold value of 320 MPN/100 mL as highlighted by the EPA guidelines (USEPA, 2014). FIB concentrations were log10-transformed to avoid skewness.

2.3 Total Suspended Solids(TSS)

Following the standard operating procedure for applied microbiology and biotechnology laboratory for TSS, we followed the following procedure: filter preparation involved placing filter disks onto a filtration apparatus, followed by rinsing each filter with three successive 20-mL volumes of deionized water. The rinsed filters were placed into a small folded, 5 in 5 in aluminum sheet then dried in an oven set to $104 \pm 1^\circ\text{C}$ for a minimum of 1 hour. Post-drying, the filters were allowed to cool in a desiccator to reach balance temperature. Weighing each filter, in the aluminum sheet, with a precision of 0.0001 mg, we recorded the mass on the bench sheet as the filter tare mass in mg. Subsequently, the filters were stored in a desiccator(for five days) until required for use.

Each filtration was run in duplicate, we used a 500mL magnetic beaker filtration apparatus and a pre-processed filter which was inserted into an area at the base, followed by the application of vacuum for efficient filtration. Ensuring proper seating, the filter was wetted with a small volume of deionized water. Subsequently, the sample underwent rigorous shaking, 250 mL was measured using a graduated cylinder into the filtering beaker. The sample water was suctioned until thorough extraction of TSS was complete(dry filter). To rinse any external TSS residue from the sides of the filtering apparatus, three washes of 20 mL volumes of deionized water were used, allowing complete drainage between washings and extended suction for three

minutes post-filtration ensured through extraction of suspended solids. The filter would then be delicately transferred back into the aluminum sheet and placed into a drying oven set to $104 \pm 1^{\circ}\text{C}$ for a minimum of 2 days. The filter would be re-weighed and collected into a physical and digital data sheet. All statistic analysis were done in Microsoft excel.

TSS measurements in mg/L were determined for each sample site using the mass increase divided by the water volume filtered in the following equation:

$$\text{TSS (mg/L)} = \frac{(A - B)}{V}$$

A = mass of filter + dried residue (mg),
B = mass of filter (tare weight) (mg), and
V = volume of sample filtered (L)

Figure 2. Above equation to find total suspended solids from the sample weights

2.4 dPCR

Total Nucleic Acid Extraction:

Archived samples were removed from -80°C , thawed at room temperature for 10 minutes, and each filter submerged in 800uL of Applied Biosystems™ MagMAX™ Microbiome Lysis Solution (Applied Biosystems™, Waltham, MA) containing 2204 copies of the gyr a gene from a haloalkaliphilic archaeon to assess the extraction recovery efficiency of each sample. Lysed samples were incubated at room temperature for 10 minutes and then bead beaten in a BioSpec Mini BeadBeater (BioSpec Products, Inc., Bartlesville, OK) for 3 mins. After bead beating, samples incubated at room temperature for 10 minutes and then were centrifuged at 14,000 rpm for 2 minutes. Following centrifugation, 400uL of supernatant was transferred to a 96 deep-well plate (95040450, Thermo Fisher, Waltham, MA) for automated total nucleic acid extraction on the KingFisher™ Flex (Thermo Fisher Scientific) with Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation reagents according to manufacturer's kit instruction and protocol. Total nucleic acids were eluted in 100 μL Applied Biosystems™ Elution Solution and transferred into 96-Well ddPCR Plates (12001925, BioRad Laboratories).



Figure 3. Photos above illuminate the tedious process of DNA extraction and digital droplet PCR.

2.5 BioRad Droplet Digital PCR :

Table 1. Primers and probes for the HF183 and Pig2Bac assay [31].

Assay	Oligo ID	Sequence: Primer and Probe 5' → 3'	Concentration	Reference
HF183 TaqMan	HF183	ATCATGAGTTCACAGTGCCG	0.9 μM	Haugland et al. (2010)
	BFDRev	CGTAGGAGTTGGACCGTGT	0.9 μM	
Pig2Bac	Pig2Bac41F	GCATGAATTAGCTTGCTAAATTTGAT	0.9 μM	Mieszkin et al. (2009)
	Pig2Bac163Rm	ACCTCATAACGGTATTAATCCGC	0.9 μM	
	Pig2Bac113MGB	FAM-TCCACGGGATAGCC-BHQ1	0.9 μM	

Equation to calculate gene copies/L:

To calculate **X gene copies/ml**, the machine generated copies/μL (A) is multiplied by the total volume of the duplicated PCR reaction (here, 50 μL) divided by the volume of cDNA in the PCR reaction (here, 10 μL), multiplied by the RNA to cDNA dilution factor (here, 800μL lysate/400μL lysate extracted), multiplied by the total nucleic extraction elution volume (here, 100 μL), divided by the volume filtered (here, 40 μL), multiplied by the mL(here, 100ml) conversion factor.

$$X \frac{\text{copies}}{\text{ml}} = A \frac{\text{copies}}{\mu\text{L}} \times \frac{50\mu\text{L}}{10\mu\text{L template}} \times \frac{800\mu\text{L lysate}}{400\mu\text{L lysate extracted}} \times 100\mu\text{L eluate} \times \frac{100\text{ml}}{100\mu\text{l filtered}}$$

Figure 4. Equation used to calculate gene copies/ml

3. Results

3.1 FIB Enumeration:

Concentrations of *Enterococcus spp.* and *E. coli* in the logarithm (log) exceeded the maximum guidelines for Most Probable Number (MPN) across all sites and parameters throughout the study period (refer to *Figures 5, 6, and 7*).

Eighty-three percent of *Enterococcus spp.* log concentrations sampled over the four-month period exceeded the single sample maximum for *Enterococcus spp.* (refer to *Figure 5*) for the state of North Carolina (NCDEQ, 2021). Fifty percent of the concentrations observed during the four-month period exceeded both the EPA single sample standard (USEPA 2014) for *E. coli* and *Enterococcus spp.* across all sites and all parameters.

Log concentrations for *Enterococcus spp.* per site over each sample event were all above the EPA guidelines of 104 MPN/100L (refer to *Figure 6*). During a major dry spell, sites NR6, NR5, and NR1 fell below the EPA maximum from sample events 9/15/23 to 10/27/23, while the rest (NR2, NR3, NR4) remained above the maximum EPA guideline for the entire study over all sample event dates. Major rainfall events, including tropical storm Idalia, and king tides observed significant peaks in all six sample sites, peaking on 9/1/23 (tropical storm Idalia) and slightly on 10/27/23 (king tide). A very strong increase of *Enterococcus spp.* from sample date 10/27/23 and 11/14/23 occurred, however more data is needed to come to any conclusions for this spike.

Log concentrations for *E. coli* exhibit varied results. Initially, NR1 and NR6 levels are below the maximum EPA guideline. All log concentrations peak on 9/1/23, aligning very closely with the spike for *Enterococcus spp.* and increased rainfall from tropical storm Idalia. From sample event dates 9/1/23 to 10/10/23, concentrations at sites NR1 and NR5 fall below the maximum EPA guideline, while NR2, NR3, NR4, and NR6 remain above the maximum. Subsequently, from sample event dates 10/10 to 11/14/23, there is a substantial decrease across all sites(NR1 through NR6) which all submerge below the EPA max guideline, except for NR3, which remains almost 0.3 log concentration above the EPA max throughout the entire duration of the study.

Under wet conditions compared to dry conditions (refer to *Figure 7*), higher average concentrations of both *E. coli* and *Enterococcus spp.* were observed, likely attributed to wet weather conditions. During major tidal fluctuations(king tide vs. no king tide), slightly higher average concentrations of *Enterococcus spp.* and *E. coli* were observed for king tides compared to no king tides.

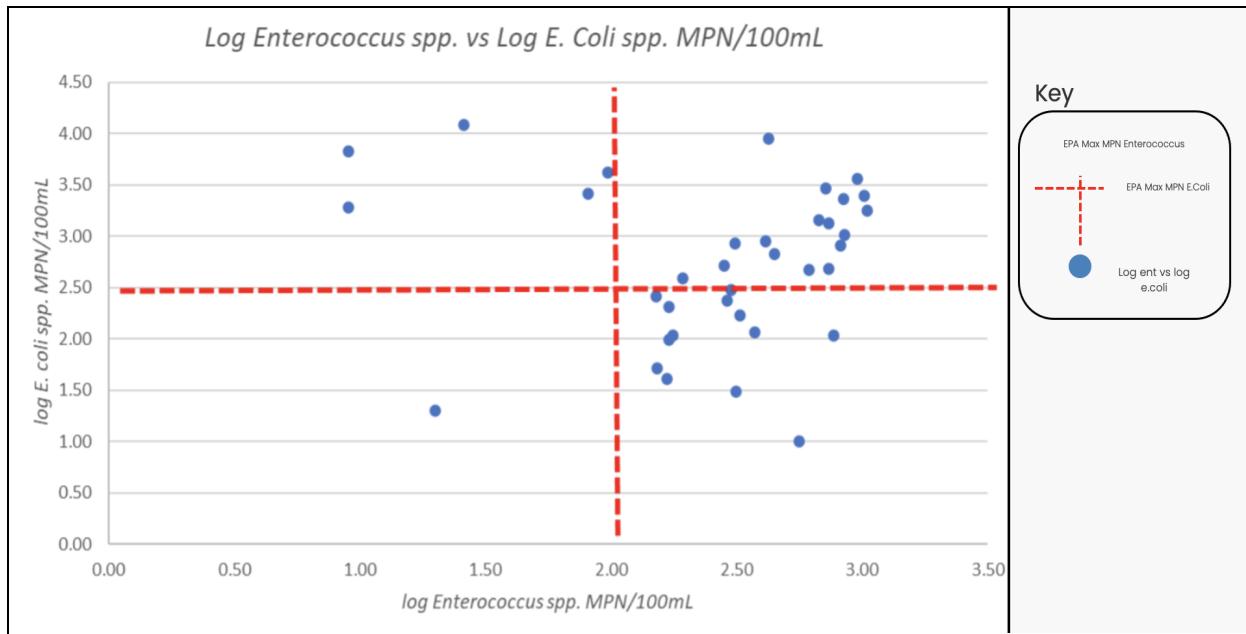


Figure 5. This graph compares the log concentration of *Enterococcus spp.* bacteria to *E. coli*, with the X-axis showing log *Enterococcus spp.* most probable number per 100 milliliters and the Y-axis indicating the log concentration of *E. coli* most probable number per hundred milliliters. A horizontal line at 320 MPN per 100 ml represents the EPA's single sample standard for *E. coli* in ocean water. Additionally, a vertical line at 104 MPN/100ml signifies North Carolina's (and EPA) single sample standard for *Enterococcus spp.* in estuarine water quality management.

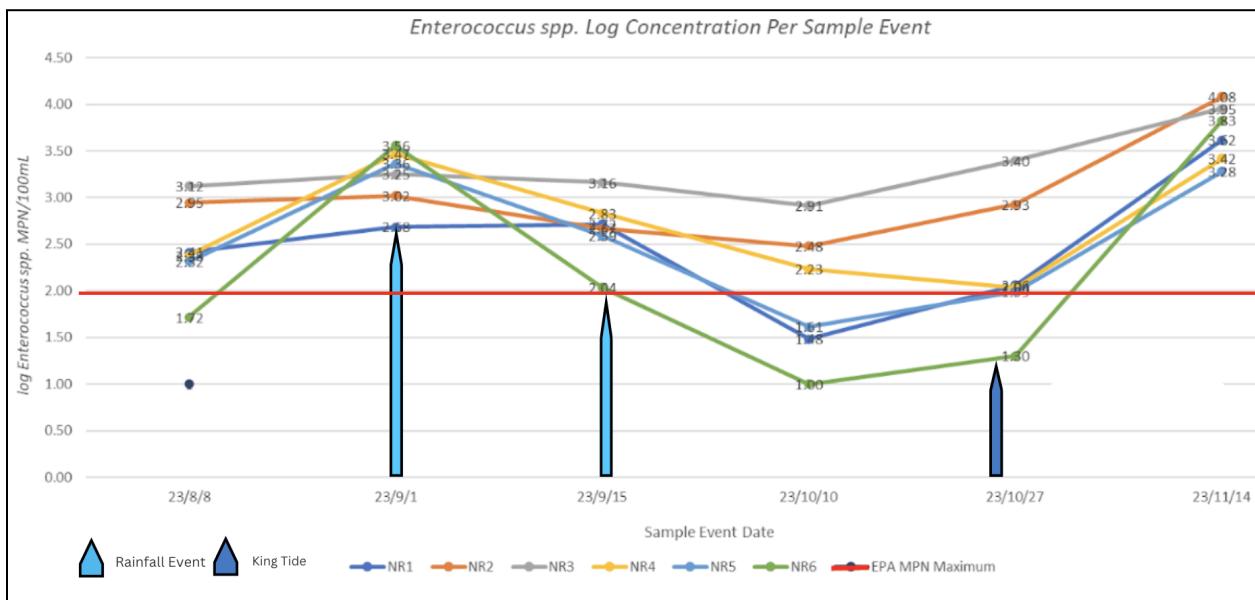


Figure 6. The graphic illustrates the log concentration of *Enterococcus spp.* bacteria over time across different New River Estuary sites. The X-axis represents time by date, while the Y-axis shows the log concentration of *Enterococcus spp.* most probable number (MPN per

hundred milliliters. A red horizontal line indicates the single sample standard for *Enterococcus spp.* fecal indicator bacteria (104 MPN per 100 ml), used by North Carolina to manage recreational water quality along ocean locations. The vertical bars representing major rainfall and tidal events. The data reveals varying spikes in *Enterococcus spp.* concentration throughout the study, with the highest concentrations occurring during major rainfall events.

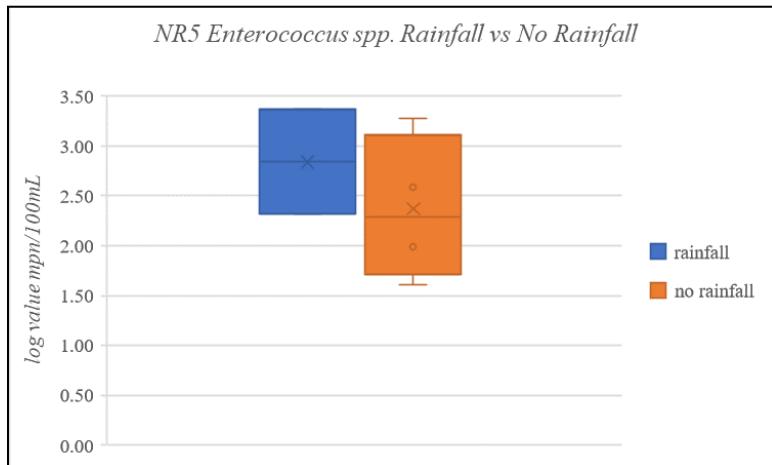


Figure 7. Box and Whisker Plot with rainfall and no rainfall log concentrations of *Enterococcus spp.* at NR5 over the entire 4 month study period. This is representative of the majority of the data for Wet vs Dry for Both *E.coli* and *Enterococcus spp.* across all sites.

3.2 Total Suspended Solids(TSS):

Trends for total suspended solids remained static throughout the study. The bar chart, illustrating the average Total Suspended Solids (TSS) over time per site (refer to *Figure 8*), reveals the pattern of total suspended solids in the New River Estuary from August through November. A notable spike in TSS was observed at NR5 on 8/8/23, while the remaining sites exhibited relatively standard patterns over the 4-month study period.

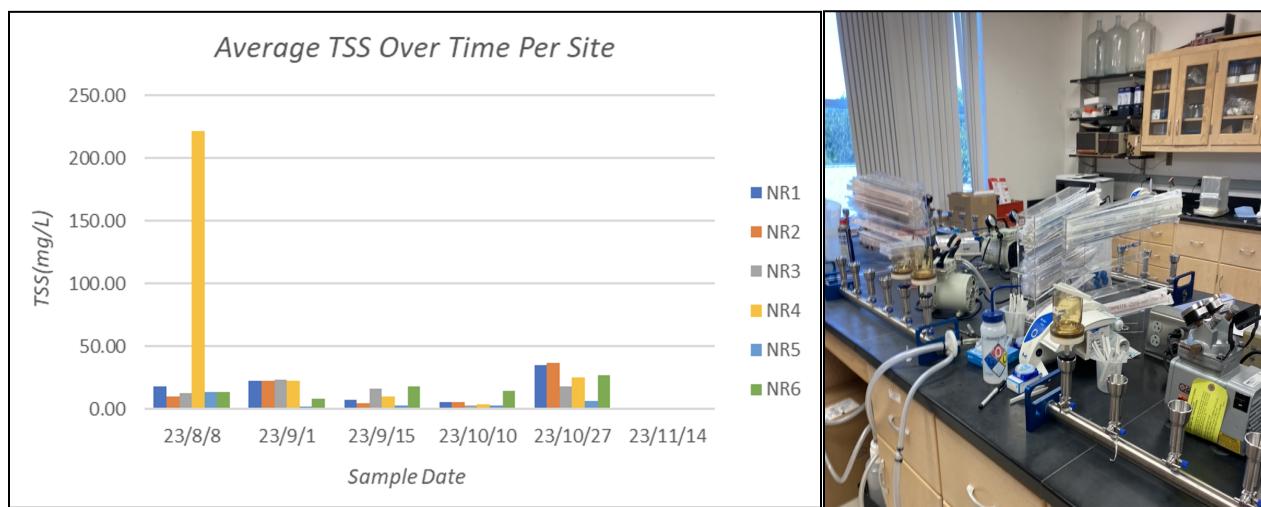


Figure 8. Bar chart displaying average Total Suspended Solids(TSS) for mg/L per sample site over time from August to November, Photo at right is filter process for TSS(Photo Credit: Tamara Bennet)

3.3 dPCR(source marker assessments from HF183 and Pig2Bac)

The HF183 genetic marker exhibited a single detection at sample site NR5 on 8/8/23(refer to *Figure. 9*). A surge in HF183 copies/L were identified across sites NR2 through NR6 on 9/1/23, aligning with the preceding rainfall associated with tropical storm Idalia. On 9/1/23, the highest detections occurred at sample sites NR5 and NR3, coinciding with the elevated levels of *Enterococcus spp.* and *E. coli* observed at these same sites on the same sampling event date. Detections for 9/15/23 had high copies/L for site NR3 and low detection for NR4. On 10/10/23 extremely high detection of NR1 copies/L occurred while sites NR4 and NR5 had low detections. 10/27/23 displayed relatively moderate detections at sites NR4 and NR5.

The Pig2Bac marker consistently remained undetectable/non detect throughout the entire 4-month data collection period. The majority of North Carolina's Concentrated Animal Feeding Operations (CAFOs) are located in Dublin County, approximately 100 miles upstream of Jacksonville and the NRE. Pig2Bac is known for its low sensitivity and susceptibility to degradation once introduced into the environment(McKee et al., 2021). The absence of the Pig2Bac genetic marker in the environment does not necessarily imply its non-existence; instead, it suggests the possibility that the marker may have either 1) degraded in the environment or 2) differed from the specific marker found in North Carolina hogs' fecal material.

The lack of detection underscores the importance of exploring alternative genetic markers that may be more closely related to the hogs inhabiting this specific region of North Carolina. This can be achieved through direct sampling of the lagoons, which would allow for identification of a genetic marker more relevant for accurate future assessment.

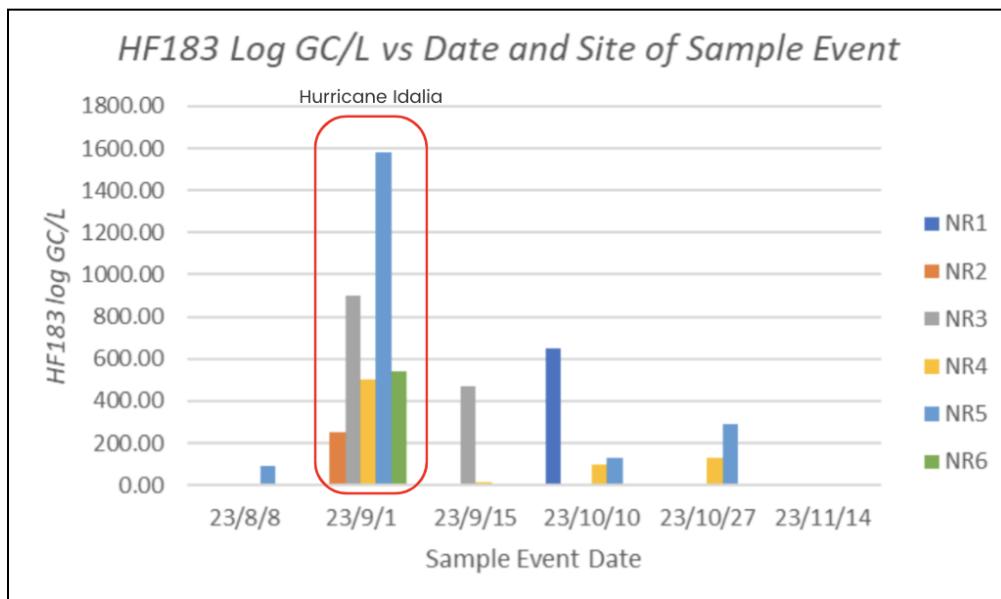


Figure 9. Human marker HF183 per sample site, per sample date, with the peak corresponding to tropical storm Idalia

4. Discussion

The New River Estuary has contaminated water with harmful pathogens, both detrimental to human and shellfish/fish health, well above US EPA standards. FIB log concentrations for both *E.coli* and *Enterococcus spp.* display clearly degraded water quality in all six sampling sites, with all sites exceeding the EPA single sample standard for at least 3 out of 6 sampling events. During a 4-month period, 83% of the samples collected exceeded the *Enterococcus spp.* maximum guideline of 104 MPN/100L, as set by the NCDEQ and US EPA standard. While 50% being above standards for both EPA and NCDEQ guidelines for *Enterococcus spp.* and *E. coli*. This constitutes a clear violation of established water quality guidelines from both state(refer to NCDEQ regulations, 2023) and national regulatory bodies(refer to Clean Water Act, 1972), including the US EPA and NCDEQ, governing the protection of water quality. These findings indicate water quality of the New River Estuary constitutes a major public health risk for both the food consumption(shellfish/fish) and human recreational(swimming/boating) standpoints.

The concentrations of *Enterococcus spp.* bacteria at sample sites NR2, NR3, and NR4 consistently exceeded the EPA(max) well above single sample standard across all parameters, regardless of wet or dry conditions. These sustained contamination levels, unaffected by runoff or wet weather conditions, raise concerns about the possibility of a persistent point source contamination, such as a septic tank leak, industrial discharge ect., in these areas. To confirm this hypothesis, further investigation is warranted, involving additional FIC data and a thorough examination of potential point source pollutants in the vicinity of these sites (NR2, NR3, NR4).

Elevated *E. Coli* levels at these same sites, and especially high at sample site NR3 for both *E. coli* and *Enterococcus spp.*, persisting regardless of changing environmental conditions, emphasizes that a real contamination problem is occurring in these areas and additional investigation and FIC data is needed to identify and address the possible source.

Observing slightly higher average concentrations(see *Figure. 6 and 7*) of *E.coli* and *Enterococcus spp.* during wet weather as compared to dry conditions across almost all sites is consistent with what is expected from surface runoff and indicates streamwater infiltration of runoff is common at the sites, which is all NR1-6, that peaked during rainfall events.

Similarly, the significance of king tide bacterial levels being slightly higher is important to note considering the relative distance the sample sites are to the New River Estuary.

Understanding the total Suspended Solids over time remains a highly useful tool in correlating solids with environmental parameters and FIB input in the water. Although no correlation were established over the 4 month duration of the study, more data is needed for comprehensible analysis.

HF183's high sensitivity to human feces makes it a valuable marker for detecting human fecal contamination(Ahmed et al., [2016](#); Harwood et al., [2014](#); Schiaffino et al., [2020](#)). Tropical Storm Idalia's influence on copies/L identified across almost all sites was evidence that the storm coincided with increased human fecal contamination levels in the estuary. The corresponding peak contamination of bacteria observed over the same time duration provides evidence that contamination from human fecal material was the likely cause. However the distance of each

sample site's proximity to both housing developments and agricultural spray fields containing hog waste makes distinguishing between the nonpoint source(human/hog) of the FIB component more challenging. The highest HF183 concentrations in wet weather did occur at the highest *Enterococcus spp.* and *E. coli* concentrations, suggesting that during wet weather, human fecal matter coincides with the contamination that occurred. However the highest dry weather concentration of FIB did not occur with highest dry weather concentrations of HF183, and many of the highest *Enterococcus* and *E.coli* concentrations had undetectable HF183 concentrations-suggesting that it may have been primarily non-human contamination which occurred during dry weather.

As the HF183 genetic marker showed success for contamination sourcing, the full extent of hog contamination in the NRE is still currently unknown. The Pig2Bac marker remained non detect over the course of the 4 month period. However, this occurrence does not in any way indicate that no contamination exists from hog waste in the waterway. Non detect samples of Pig2Bac marker should not be seen as a pitfall. It is well understood that the genetic marker Pig2Bac is both highly sensitive and degrades rapidly in its environment. With preceding knowledge that the hog waste lagoons sit well over 100 miles upstream of the sample sites, it is not trite to suggest that degradation of the marker had occurred and could explain the non detect results.

The Clean Water Act has curtailed most point-source contributions to fecal contamination in the USA, failing waste-water infrastructure (Habteselassie et al., 2014; Paul et al., 2000), the application of livestock manure to landscapes (Cook et al., 2014; Laurensen & Houlbrooke, 2014), and direct livestock access to streams (O'Callaghan et al., 2018) continue to impair rural and urban streams nationwide(Browning et al., 2023). The observance of malfunctioning on-site septic systems have been found to be a significant contributor to human fecal pollution in rural areas near streams. For example, Verhougstraete et al., (2015) found human fecal contamination in several rural streams in Michigan, and that human fecal contamination increased with the total number of septic systems in the watershed that were within 60 m of a stream. Derose et al., (2020) correspondingly found that most of their sampling sites that were associated with rural residential areas exceeded their state and federal standards for FIB, and they suspected malfunctioning on-site septic systems to be the source. The use of septic systems are highly prevalent in rural eastern North Carolina and the detection of human markers(HF183) in areas without municipal sewage systems may be more indicative of on-site septic system failures.

With the study being a year long project, undermining a modification of the study methods to provide a more accurate and inclusive analysis would be a mistake. The use of poultry and more accurate hog genetic markers remain absolutely essential for true and quantifiable results of non-human contamination that could exist in the Estuary. Simultaneously increasing sample sites to smaller creeks, ditches and tributaries could help better distinguish FIB and MST results between human and nonhuman constituents.

5. Conclusion

Nestled at the crossroads of anthropogenic demand, domestic agriculture, and wildlife utilization, the New River Estuary stands as a pivotal resource to preserve for the posterity of future generations. The quantified extent of contamination in the waterway is staggering and remains a major cause for public concern. Half of all observed concentrations exceed both EPA and state mandated guidelines during the four months. The data generated from this ongoing year-long study will be indispensable for the proactive safeguarding and conservation efforts aimed at preserving the New River Estuary. The comprehensive documentation of future contamination can also serve as compelling evidence for the effective management of nonpoint source pollutants within the Estuary.

With the continued support of Coastal Carolina River Watch and UNC Institute of Marine Science, the betterment of water quality in the New River Estuary is soon to come.

Acknowledgments:

A huge thanks to Tammy Bennett for her pivotal role with lab work, as well as Riley Lewis for leading field sampling and data collections! Also, big thanks to PI Dr. Rachel Noble for her guidance and infinite help over the course of the project! Thanks to all who helped the development of results! Thank you!!

Citations

Ahmed, W., Masters, N., & Toze, S. (2012). Consistency in the host specificity and host sensitivity of the *Bacteroides* HF183 marker for sewage pollution tracking. *Letters in Applied Microbiology*, 55(3), 283-289. <https://doi.org/10.1111/j.1472-765X.2012.03291.x>

Ahmed, W., Hughes, B., & Harwood, V. J. (2016). Current status of marker genes of bacteroides and related taxa for identifying sewage pollution in environmental waters. *Water*, 8(6), 231. <https://doi.org/10.3390/w8060231>

Browning, D. A., Mausbach, W. E., Stookey, C., Nikolai, S. J., Barrow, J., & Townsend, D. E. (2023, May 25). *Validating microbial source tracking markers and assessing the efficacy of culturable *E. coli* and *enterococcus* assays in Ozark streams, USA - water, air, & soil pollution*. SpringerLink. <https://link.springer.com/article/10.1007/s11270-023-06355-z>

Ballesté, E., Bonjoch, X., Belanche, L. A., & Blanch, A. R. (2010). Molecular indicators used in the development of predictive models for microbial source tracking. *Applied and Environmental Microbiology*, 76 (6), 1789-1795. <https://doi.org/10.1128/AEM.02350-09>

Bernhard, A. E., & Field, K. G. (2000). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides*-*Prevotella* genes encoding 16S rRNA. *Applied and Environmental Microbiology*, 66 (10), 4571-4574. <https://doi.org/10.1128/AEM.66.10.4571-4574.2000>

Boehm, A. B., Van De Werfhorst, L. C., Griffith, J. F., Holden, P. A., Jay, J. A., Shanks, O. C., ... & Weisberg, S. B. (2013). Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research*, 47 (19), 6812-6828. <https://doi.org/10.1016/j.watres.2012.12.046>

Boehm, A. B., Soller, J. A., & Shanks, O. C. (2015). Human-associated fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environmental Science & Technology Letters*, 2 (8), 270-275. <https://doi.org/10.1021/acs.estlett.5b00219>

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., ... & Wittwer, C. T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611-622. <https://doi.org/10.1373/clinchem.2008.112797>

Cahoon, L. B., & Hanke, M. H. (2019). Inflow and infiltration in coastal wastewater collection systems: effects of rainfall, temperature, and sea level. *Water Environment Research*, 91 (3), 322-331. <https://doi.org/10.1002/wer.1036>

Cahoon, L. B., Hales, J. C., Carey, E. S., Loucaides, S., Rowland, K. R., Toothman, B. R., ... & Toothman, B. R. (2016). Multiple modes of water quality impairment by fecal contamination in a rapidly developing coastal area: southwest Brunswick County, North Carolina. *Environmental Monitoring and Assessment*, 188 (3), 1-14. <https://doi.org/10.1007/s10661-015-5081-6>

Cao, Y., Hagedorn, C., Shanks, O. C., Wang, D., Ervin, J., Griffith, J., ... & Weisberg, S. B. (2013). Towards establishing a human fecal contamination index in microbial source tracking. *International Journal of Environmental Science and Engineering Research*, 4(3), 46-58.

Abstract As non-point sources of pollution begin to overtake point sources in watersheds, Badgley, B. D., Bonsch, M., Brooks, Y., Bullerjahn, G. S., Evans, A. E., Jarrett, R., Lee, D.-Y., Luszcz, E. C., McKee, B. A., Srinivasan, S., Sowah, R. A., Ahmed, W., Aslan, A., ... Mateo-Sagasta, J. (2022, April 29). *Connecting microbial, nutrient, physiochemical, and land use variables for the evaluation of water quality within mixed use watersheds*. Water Research. <https://www.sciencedirect.com/science/article/pii/S0043135422004791#bib0035>

Cao, Y., Raith, M., & Griffith, J. (2015). Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research*, 70, 337-349. <https://doi.org/10.1016/j.watres.2014.12.008>

Colford, J. M., Wade, T. J., Schiff, K. C., Wright, C. C., Griffith, J. F., Sandhu, S. K., ... & Weisberg, S. B. (2007). Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology*, 18(1), 27-35. <https://doi.org/10.1097/01.ede.0000249425.32990.b9>

Converse, R. R., Blackwood, A. D., Kirs, M., Griffith, J. F., & Noble, R. T. (2009). Rapid QPCR-based assay for fecal *Bacteroides* spp. as a tool for assessing fecal contamination in recreational waters. *Water Research*, 43(18), 4828-4837. <https://doi.org/10.1016/j.watres.2009.06.036>

Converse, R. R., Piehler, M. F., & Noble, R. T. (2011). Contrasts in concentrations and loads of conventional and alternative indicators of fecal contamination in coastal stormwater. *Water Research*, 45 (16), 5229-5240.

North Carolina Beach monitoring program quality assurance project plan. (n.d.-b).
<https://www.deq.nc.gov/marine-fisheries/shellfish-sanitation-and-swimming-advisories/recreational-water-quality/north-carolina-beach-monitoring-program-quality-assurance-project-plan/open>

Ahmed, W., Hamilton, K., Toze, S., Cook, S., Page, D. (2019). A review on microbial contaminants in stormwater runoff and outfalls: potential health risks and mitigation strategies. *Science Total Environment*, 692, 1304-1321.
[\[https://doi.org/10.1016/j.scitotenv.2019.07.055\]](https://doi.org/10.1016/j.scitotenv.2019.07.055)(<https://doi.org/10.1016/j.scitotenv.2019.07.055>)

Ahmed, W., Masters, N., Toze, S. (2012). Consistency in the host specificity and host sensitivity of the *Bacteroides* HF183 marker for sewage pollution tracking. *Letters in Applied Microbiology*, 55, 283–289.
[\[https://doi.org/10.1111/j.1472-765X.2012.03291.x\]](https://doi.org/10.1111/j.1472-765X.2012.03291.x)(<https://doi.org/10.1111/j.1472-765X.2012.03291.x>)

Recreational water quality criteria and methods | US EPA. (n.d.-b).
<https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods>

Applied biosystems. Thermo Fisher Scientific - US. (n.d.).
https://www.thermofisher.com/us/en/home/brands/applied-biosystems.html?gclid=CjwKCAiAmsurBhBvEiwA6e-WPJcK0I_nGf4tpJzR66vD6Vap0CYq-coscfM0KEo6nfUV89VWnMkCRhoCAhkQAvD_BwE&cid=gsd_cbu_sbu_r01_co_cp1447_pjt8158_gsd00000_0se_gaw_bt_awg_gabi_tergaexa&s_kwcid=AL%213652%213%21562996439667%21p%21%21g%21%21applied+biosystems&ef_id=CjwKCAiAmsurBhBvEiwA6e-WPJcK0I_nGf4tpJzR66vD6Vap0CYq-coscfM0KEo6nfUV89VWnMkCRhoCAhkQAvD_BwE%3AG%3As&gad_source=1

Ahmed, W., Stewart, J., Powell, D., Gardner, T. (2008). Evaluation of *Bacteroides* markers for the detection of human fecal pollution. *Letters in Applied Microbiology*, 46(2), 237–242.
[\[https://doi.org/10.1111/j.1472-765X.2007.02287.x\]](https://doi.org/10.1111/j.1472-765X.2007.02287.x)(<https://doi.org/10.1111/j.1472-765X.2007.02287.x>)

DDPCR 96-Well Plates. Bio. (n.d.).
<https://www.bio-rad.com/en-us/life-science/digital-pcr/droplet-digital-pcr-consumables/ddpcr-96-well-plates>

Mieszkin S, Furet JP, Corthier G, Gourmelon M. Estimation of pig fecal contamination in a river catchment by real-time PCR using two pig-specific *Bacteroidales* 16S rRNA genetic markers.

Appl Environ Microbiol. 2009 May;75(10):3045-54. doi: 10.1128/AEM.02343-08. Epub 2009 Mar 27. PMID: 19329663; PMCID: PMC2681621.

Arnold, B.F., Wade, T.J., Benjamin-Chung, J., Schiff, K.C., Griffith, J.F., Dufour, A.P., Weisberg, S.B., Colford, J.M. (2016). Acute gastroenteritis and recreational water: highest burden among young US children. *American Journal of Public Health*, 106 (9), 1690–1697.
[<https://doi.org/10.2105/AJPH.2016.303279>] (<https://doi.org/10.2105/AJPH.2016.303279>)

Aslan, A., Anderson, K.W., Chapman, A. (2018). The Impact of Tides on Microbial Water Quality at an Inland River Beach. *Journal of Environmental Quality*, 47, 1123-1129.

[<https://doi.org/10.2134/jeq2017.12.0499>] (<https://doi.org/10.2134/jeq2017.12.0499>)

Boehm, A.B., & Weisberg, S.B. (2005). Tidal Forcing of Enterococci at Marine Recreational Beaches at Fortnightly and Semidiurnal Frequencies. *Environmental Science & Technology*, 39 (15), 5575–5583. [<https://doi.org/10.1021/es048175m>] (<https://doi.org/10.1021/es048175m>)

City of Jacksonville. (n.d.). About Jacksonville. Retrieved Nov 18, 2023, from <https://jacksonvillenc.gov/847/About-Jacksonville>

Automated statistical analysis of microbial enumeration by dilution series. Academic.oup.com. (n.d.). <https://academic.oup.com/jambio/article-abstract/55/1/159/6729929>