An Integrated Assessment of Water Quality in Town Creek, an Estuary in Beaufort, North Carolina

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
UNC Institute of Marine Science Field Site, Morehead City, NC

Fall 2019 Capstone Report

Carly Dinga, Tom Dix, Abraham Elmir, Elizabeth Farquhar, Kayla Fiser, Christina Hardtle, Lanna Jin, Ella Luebbe, Jess McCoppin, Paxton Oberle, Rachael Rankin, Hayley Russo, Jacob Stain, Eric von Amsberg, Rebecca Williams

Course Instructors: Dr. Johanna Rosman and Dr. Nathan Hall
Field Site Director: Dr. Rachel Noble

UNC INSTITUTE OF MARINE SCIENCES

TABLE OF CONTENTS

INTRODUCTION

Town Creek is an embayment located within the town limits of Beaufort, NC. The waterway provides residents with the opportunity to boat, fish, swim, and engage in other aquatic activities. Directly to the east of Town Creek is an old elementary school site. To the north is a small airport and the site of a future maritime museum where a summer sailing camp takes place. On the south side of Town Creek is a site slated for development into a major tourism hotel. Directly to the southwest is downtown Beaufort, which can also be reached by traveling south down Town Creek to the Beaufort waterfront. The section of Town Creek east of Turner St. bridge is a marsh which extends into residential areas. As of 2018, the town of Beaufort is home to 4,391 residents (*United States Census Survey). Tourism is a growing sector of the local economy, generating approximately $325 million in Carteret County and 3,200 jobs (Carteret County Chamber of Commerce, 2019).

Contamination levels in Town Creek are at risk of rising due to the upcoming development of Interstate 42, the construction of a themed hotel on the embayment shore, as well as the recent construction of the Highway 70 expansion bridge which will attract more visitors to the area. A number of marinas were added to Town Creek, as well as upgrades to some of the existing marinas (Harvey, 2017). As development continues, water quality may deteriorate. The new infrastructure will likely cause an influx of waterway users, underscoring the need to clean water in Town Creek. In addition to development, four stormwater drains from the Town of Beaufort, suspected dumping from three marinas located within Town Creek, as well as inflow from Gallant's Channel, add to the possibility of contamination in the waterway. Although new development may increase runoff to the waterway, the Town of Beaufort has plans to develop and improve existing sewer and stormwater infrastructure. A detailed assessment of contaminant concentrations at Town Creek will give a baseline dataset for interpretation of the overall impacts of the recent and future development in the area, as well as the existing influx of contaminants.

The purpose of this investigation is to inform the town of Beaufort on the current state of water quality in Town Creek and areas of concern within the water system. This project was divided into six categories: (1) spatial analysis and community response, (2) circulation and flushing, (3) nutrients and algae, (4) microbial, (5) marsh filtration, and (6) oyster filtration. Water samples were taken between September 18 and October 16, following Hurricane Dorian that occurred in early September of 2019. The water samples were collected for nutrient concentration, chlorophyll *a* content, and microbial composition. Additionally, by quantifying the residence time, flushing time, and the movement of the water in Town Creek, we were able to determine how the contaminants were transported throughout the water system. Furthermore, we surveyed existing oyster reefs and marsh habitat to quantify the rate of removal of nutrients and bacteria.

Figure 0.1. Map of Town Creek

CHAPTER 1: Stakeholder Survey and Potential Contaminant Mapping

1. INTRODUCTION

A waterway such as Town Creek that is located in a developed area simultaneously affects and is affected by the human community surrounding it. Estuarine environments offer important benefits such as coastal erosion protection, carbon sequestration, and local revenues from tourism and recreation (Barbier et al. 2011). Conversely, human development along the shorelines of an estuary in the form of marinas and housing has been demonstrated to increase observed fecal coliform bacteria (Kirby-Smith & White 2006). As this is just one example in a long list of ways that community waterways can be negatively impacted by human activity, it is clear that the management of such resources are paramount to their continued function.

When considering the management of a public resource, perspective should be placed on the fact that such resources do not exist in a vacuum. Different people, ranging from those that live adjacent to such a feature to those that travel in order to utilize it to those that do business on it daily, will have different opinions on the current and future status of a waterway like Town Creek. Consequently, feedback relating to a resource like Town Creek provided by the public can be beneficial to understanding how the waterway is currently used while also providing valuable input about how it could be used and managed in the future to maximize benefit to the community around it (Nicolson & Mace Jr. 1975). Further, a demonstrated disconnect between watersheds and their stakeholders illustrates a need for more communication between the managers of these waterways and the communities they serve (Giacalone et al. 2010). As federal agencies like the Environmental Protection Agency are now looking for strategies relevant to local communities to address the problem of unsatisfactory water quality and input from local communities is seen as valuable in terms of evaluating perceptions of water quality in a given area, understanding a community's motivations relating to a waterway like Town Creek will be a boon to managers that wish to understand in what ways such a waterway are already perceived to be compromised in addition to the methods in which they may protect the resource for use in the future (Giacalone et al. 2010, Nicolson & Mace Jr. 1975).

As human attitudes about the waterway and spatial distribution of differing levels of human development around the waterway have both been demonstrated to contribute to the ongoing and future prosperity of the waterway, these two concepts lend themselves to examination in relation to Town Creek. To best understand the spatial distribution of possible contaminants of Town Creek, tools for mapping these possible pollutant sources are used to provide an assessment of their distribution in relation to Town Creek. Conversely, future management recommendations for Town Creek will be dependent upon stakeholders' opinions gathered through the distributed surveys. Finally, the use and value of Town Creek (economic, community-related, development, etc.), both currently and what is projected for the future, will be determined through the examination of the results of analysis of both the spatial and survey datasets.

2. METHODS

2.1 Spatial Mapping

The computer software ArcMap 10.6.1 was used to create a map of Town Creek that showed sources of pollution within or near the study site. Datasets were requested from the shellfish sanitation department located in Morehead City that were applied to the map as layers which included various sources of pollution, sampling locations, and locations that shellfish sanitation has determined where shellfish grow. The purpose of using these datasets was to pair them with the data obtained from the survey collection and identify any relationships between spatial patterns in water quality and locations of potential pollutant sources. Among the sources of pollution determined to be threats towards the water quality are animal, dockage, golf courses, storm water, wastewater, and house subdivisions. The locations of these sources are marked on the map to see how close they are from the water that is within the boundaries of Town Creek. Besides sources of pollution, sampling stations, shellfish growing areas, and areas of concern (AOCs) within the town were also marked on the map to support the validity of the data collected as well as emphasize the threat of pollution towards Town Creek. AOCs are locations marked by shellfish sanitation that were determined to be threatened with contamination by the sources of pollution surrounding them. Another layer included was a circle whose center is at the center of the waterbody next to Town Creek and radius is a mile long. A mile was selected to be an appropriate distance to see how many sources of pollution and areas of concerns were located within Town Creek's watershed.

2.2 Stakeholder Survey

In order to gather data pertinent to the community outlook of the Town Creek waterway, in addition to concerns relating to its current and future use, a written survey was drafted in order to gauge individual stakeholders' thoughts on said issues. Surveys followed a similar format regardless of their study area; questions relating to concerns relating to and use of the waterway were preceded by simple demographic questions to aid categorization of respondent data. Response types were for the most part limited to multiple-choice or scale-based options to facilitate ease of data analysis. Aside from the surveys themselves, the enclosed materials included a letter explaining the purpose of the team's research in addition to the required IRB documentation explaining the anonymity of any responses submitted to the team. The methods herein described were reviewed by the UNC Office of Human Research Ethics and determined to be exempt from federal regulations governing research on human subjects.

Of our 95 surveys, 60 were distributed to local residences, while 15 were distributed to Beaufort businesses, and another 20 were distributed at points of public access along the perimeter of Town Creek. Businesses were selected randomly prior to delivery by searching for 'businesses' in Google Maps within a mile of Town Creek. The point of access locations surveyed would include the Cedar Street Park and the public boat ramp adjacent to Town Creek Marina (Figure 1). In order to complete the residential survey in a way that created a diverse sampling of Beaufort's residents, the city limits of the Town of Beaufort were divided into four sections of roughly equal size which were to be sampled in equal measure of 15 surveys per area (Figure 2). Prior to delivery, survey materials were placed in pre-stamped envelopes addressed to IMS.

Delivery of surveys took place on October 30th. Surveys to businesses and subjects at points of access were distributed according to a standard written script written prior to delivery. Residential surveys were placed in between the door and jamb of each individual residence. Upon their arrival, survey responses were entered into Microsoft Excel for analysis. Completed surveys were stored at the Institute of Marine Sciences per IRB regulations in order to prevent a breach of promised anonymity for respondents.

Figure 1. Map of Town Creek with access points displayed.

Figure 2. Map of Town Creek with boundaries displayed of where surveys were distributed.

3. RESULTS

3.1 Spatial Mapping

As presented in the map above, there are multiple counts of sources of pollution that exist within the one-mile circular radius. To be more specific, there are 16 sources of dockage pollution, 44 sources of storm water pollution, seven sources of wastewater pollution, five sources of house subdivision pollution, and zero sources of pollution for both animal and golf courses. Alongside this, all of the water surrounding Town Creek is recognized as being areas where shellfish grow. In terms of AOCs, there were a total of 4 within the one-mile radius. The map above also displays storm water as having the most sources of pollution within Town Creek.

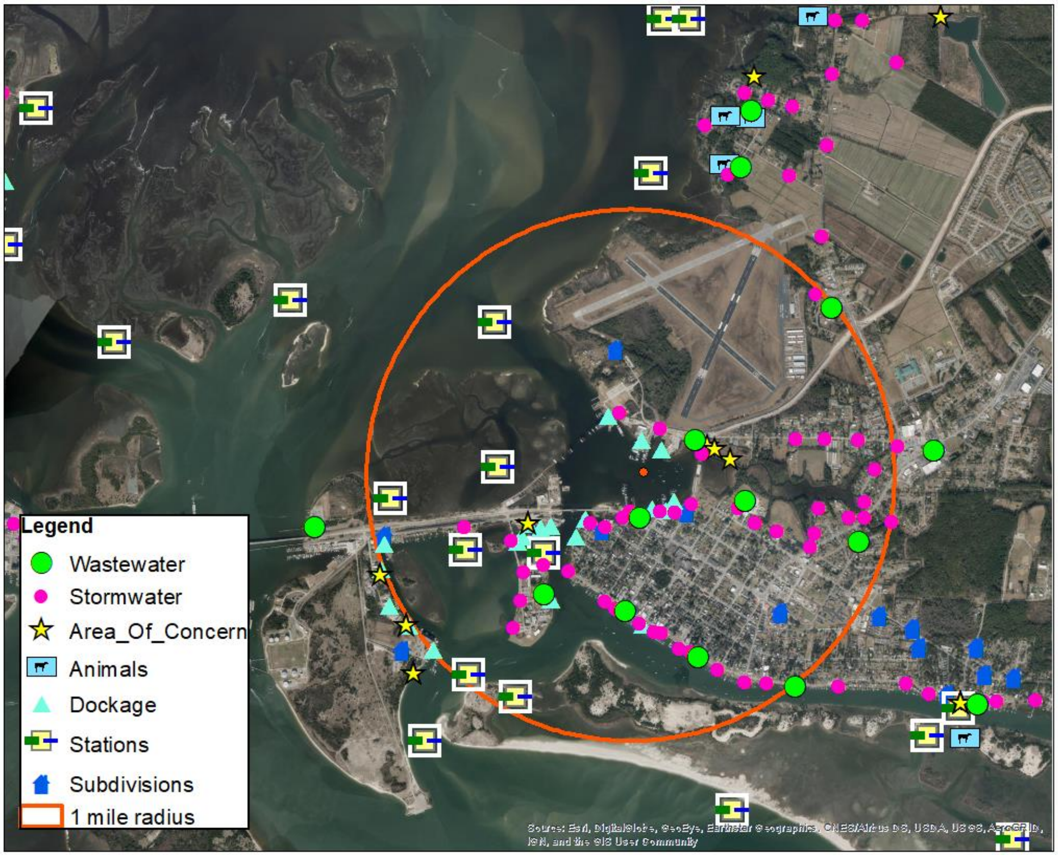


Figure 3. Map of Town Creek with sources of pollution and other layers displayed.

3.2 Stakeholder Survey

Thirty-one of the 95 distributed surveys were returned complete, presenting a response rate of 32.6%. By group, seven of surveys came from point of access users (35% response rate), six came from local business owners (40% response rate), and 18 of the surveys were responded to by local residents (30% response rate). Although response rates from our demarcated regions were not measured, data gathered relating to residents' distance to Town Creek is an acceptable proxy - 10 of the 18 resident respondents lived over a mile away from the waterway (six from one to two miles away, another four from two to three miles away), of the remaining eight, four lived within a half-mile of Town Creek, and the other four were between half a mile and a mile away from the waterway.

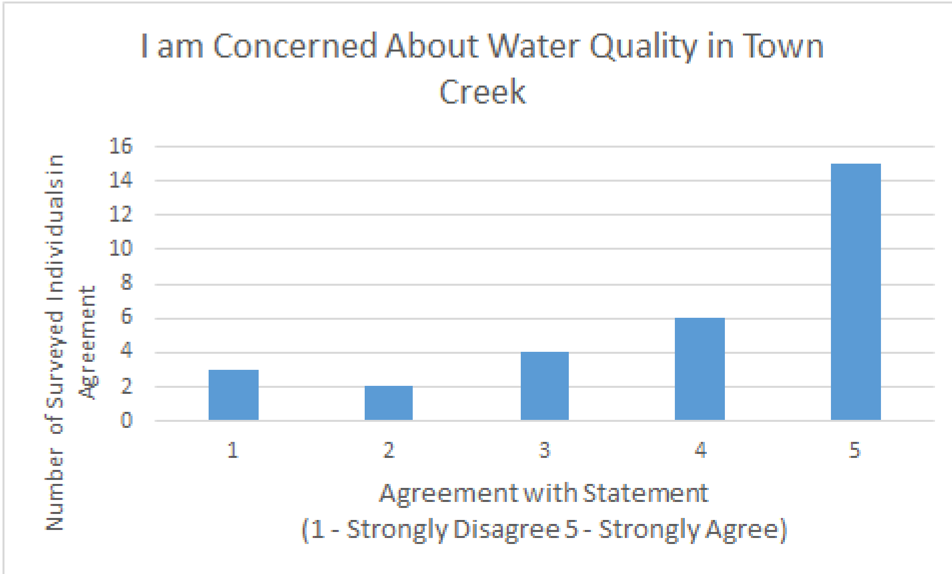


Figure 4. Aggregated responses from all surveyed groups indicating agreement with the statement: “I am concerned about water quality in Town Creek.”

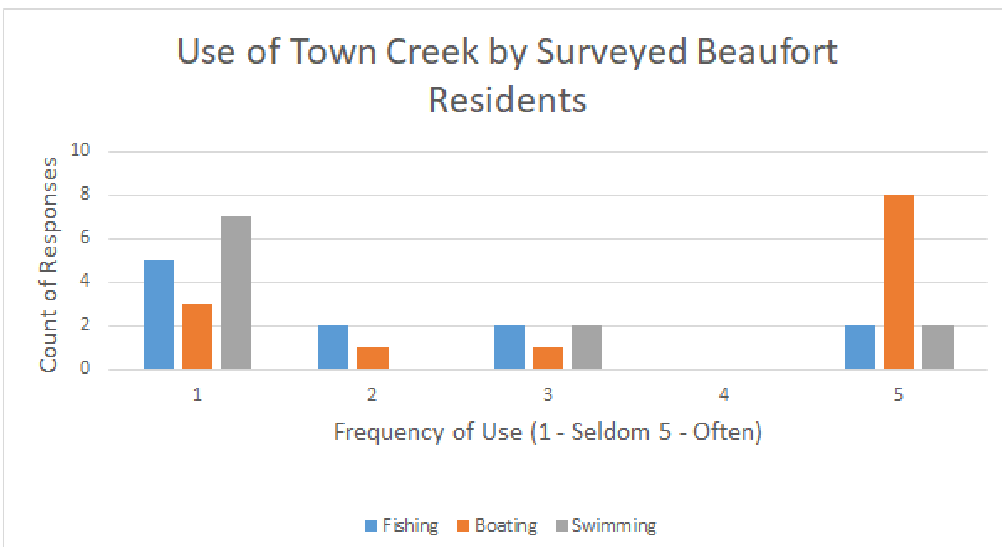


Figure 5. Respondents’ frequency of use of Town Creek for various recreational activities.

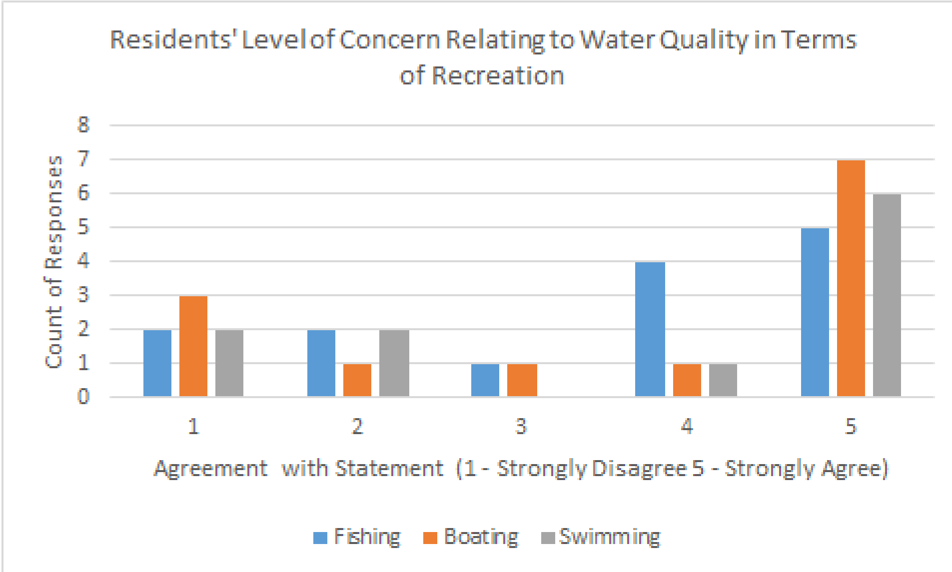


Figure 6. Respondents' level of concern related to water quality in terms of different recreational activities.

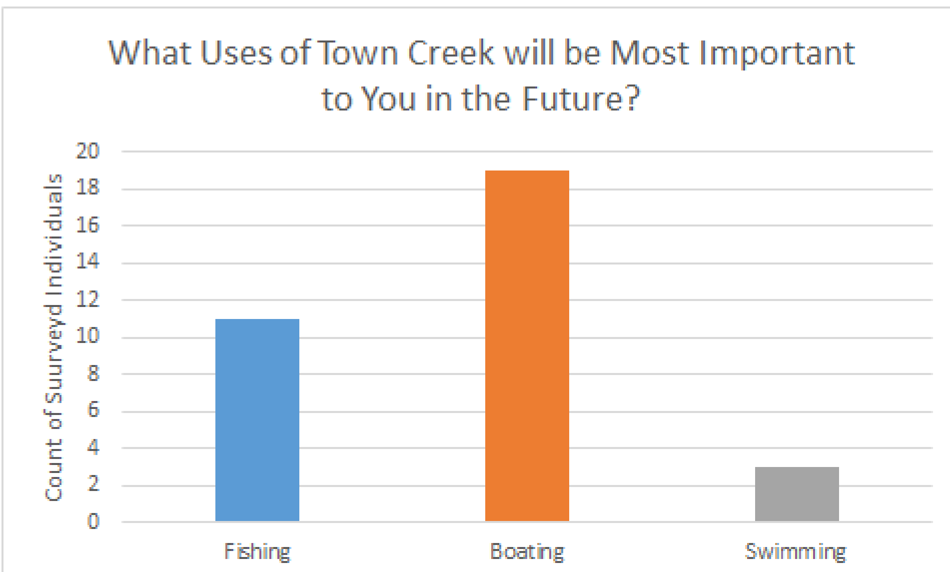


Figure 7. Important uses of Town Creek in the future, as perceived by survey respondents.

Nearly 50% of survey respondents strongly agreed that water quality in Town Creek was concerning to them in some capacity. In terms of future uses of Town Creek, less than 10% of survey respondents considered swimming a means of recreation that is important in the area. A large majority (61.2% overall) of all respondents considered boating to be an important source of future recreation in Town Creek, with 35.4% of all respondents believing fishing would be important to future recreation in the waterway, and another 29% perceiving swimming as an important future use.

The majority of business owners did not feel that their business was negatively impacted by existing environmental regulations - only one business agreed with the statement "I feel my business has been restricted by environmental regulations set by Beaufort". Marinas, which made up one third of businesses who responded to the survey, either agreed or strongly agreed with the same statement. More than two thirds of business respondents use Town creek in some capacity, with the same percentage using Town Creek at least weekly and 50% of respondents using it daily - though it should be noted that two of those three respondents self-identified as marinas on the survey.

Sixty-six percent of public access user respondents have been using the waterway for 10 years or more. Distribution of frequency of use for public access users was two users for daily use, two users for weekly use, and two users monthly use, with one user reporting never using the waterway. Public access users preferred boating and fishing to other forms of recreation in the waterway. Two thirds of these public access respondents were highly concerned with water quality in Town Creek, especially when it came to their level of concern in regards to water quality's effect on fishing.

Two opportunities were given to survey respondents to freely respond with any concerns they may have about Town creek, both related to water quality and more general items. Though there was not necessarily a glaring issue presented by these free responses, the most repeated was the presence of boats in Town Creek, both dilapidated and liveaboard. Something else listed by more than two respondents was a proposal for permanent moorings in Beaufort waterways that are managed by the town itself, which were indicated to be preferable to the current system.

4. DISCUSSION

4.1 Spatial Mapping

When analyzing the results, there are multiple conclusions that can be noticed when viewing the visual layers applied onto the map. The first would be the existence of four AOCs within the one-mile radius and their locations within Town Creek. The fact there are multiple AOCs within Town Creek indicates that the water quality is at risk of being contaminated. There are three of the four AOCs that are located close to one another at the shore, which indicates that the water next to these AOCs are at threat from the nearby sources of stormwater and wastewater pollution. While the last AOC is located near Morehead city, the fact that it is located on the shore, surrounded by sources of dockage and storm water pollution, and is within the one-mile radius gives evidence that it can be a threat towards the water quality of nearby Town Creek.

Another noticeable feature of the pollutant source layers presented on the map was that there were three main sources of pollution that existed heavily in Town Creek and the waterbody surrounding it. These three sources were stormwater, wastewater, and dockage pollution with storm water having the most overwhelming presence in Town Creek. When looking specifically at the location of the sources of storm water pollution, it can be seen how most of the marked locations are clustered with one another and lined up along the coast of the town.

While there is no presence of animal pollution within the one-mile radius, it is worth mentioning that north of the circle there are three sources of animal pollution close to one another that are on the coast of the waterbody connecting to Town Creek. While these three sources are not within the one-mile radius, they are close to the boundary and indicate that there may be a trail of animal pollution runoff that could travel southward and adjacent to Town Creek depending on the direction and force of the tides and wind.

After evaluating the results, it is evident that the water quality within Town Creek may be affected by various sources of pollution. Alongside the multiple recordings of sources of pollution within the one-mile radius, the seven sampling stations that were used by shellfish sanitation to sample the water offers validation that the data collected is justified to the claim that change needs to be made to drastically reduce the presence of pollution existing within the waterway. According to the area of shellfish growth layer on the map, all of the water that surrounds Town Creek is considered capable of growing shellfish, which is pivotal towards the region's economy.

A progressive start to resolving this issue would be to focus on the AOCs close to Town Creek and strategize with the community on how to eliminate the threat of pollutants flowing through the water. Another main action the community can take is finding solutions beside storm water drains to counteract against runoff during storm activity, since storm water pollution had the biggest presence within the one-mile radius (mostly along the coast). The reduction of impervious surfaces in the town could also work well alongside storm drains in reducing sources of pollution. Keeping track of this information can help to improve their sewage system so that runoff during storm activity will not be able to travel down the coast and taint the water of Town Creek. A good example would be the weekly monitoring of the water quality through various government, academic, and community organizations or institution. Specifically, with Town Creek, the tracking of dockage pollution, wastewater, storm water is critical because it allows for the local government to be informed of the current issue and as a result, be forced to enforce laws that will hopefully reduce the presence of these pollutants within the water.

4.2 Stakeholder Survey

Data gathered illustrates that the majority of survey respondents showed at least middling concern for water quality in Town Creek, especially relating to the recreational or business use of boats in the waterway. Although boating and fishing were highly regarded by respondents as future means of recreation on Town Creek, swimming was not, with only 9.6% listing swimming as an important future use of the waterway. Consequently, according to data gathered by this survey, future considerations regarding management of water quality in Town Creek should place a greater focus on the safety of boaters and fishermen that use the waterway as well as the hygiene and health of organisms living within Town Creek. The use of swimming can be interpreted to be a secondary concern at best and so should not be a main focus of any management plans regarding Town Creek.

Free response survey questions wherein respondents were encouraged to explain any concerns they might have relating to Town Creek contained some of the most interesting data collected. For instance, respondent from all three groups mentioned the dilapidated vessels that line the waterway and the issue of marina waste in the area. Additionally, the idea of municipally-managed moorings as a replacement for the current system of unregistered mooring in Beaufort waterways is mentioned on multiple returned surveys in both the business owner and point of access respondent samples. Another concern aired by multiple respondents is the effect of future proposed real estate development on water quality in Town Creek. Due to the repeated nature of these concerns, management plans involving Town Creek could benefit from investigating these concerns and suggestions in greater detail. In this way, Beaufort's management choices affecting the waterway will be the most agreeable to the stakeholders of Town Creek: the residents, local business owners, and users of public access points along its shores.

5. CONCLUSION

Data collected through both spatial and human data survey indicates perceived and observed issues with water quality in Town Creek. There are 72 possible sources of contamination within a one-mile radius of the center of Town Creek and the vast majority of survey respondents exhibited at least moderate concern for water quality in this waterway. Human data collected presents investigable methods of management to the waterway, while spatial data presents possible contaminant sources that may require such management. In this way, managers of the area can ensure they are taking care of existing threats while also addressing the concerns of stakeholders' future.

CHAPTER 2: Circulation

1. INTRODUCTION

The mixing and circulation of both freshwater and saltwater throughout Town Creek is a necessary factor in understanding the flow of contaminants in the waters surrounding Beaufort. The movement of water determines the flushing and residence times of the system (Monsen et al., 2002), which provides information on how long contaminants may be present within Town Creek. Furthermore, examining the various processes that influence the flushing and residence times, such as bathymetry, tides, and wind, also play a crucial role in determining the water quality of the system. Each of these factors have their own effects on the movement of the water but understanding how they interact is a key aspect in understanding how contaminants flow within Town Creek.The flushing time of a system is a comprehensive parameter that represents the amount of time in which a particular body of water is refreshed with a new input of water. This exchange does not detail the physical processes that play a role in the movement of water (Monsen et al., 2002). Because flushing time provides a holistic view of the body of water, it helps bring an understanding of how long a particular contaminant may be present in Town Creek before the system is completely refreshed. This is important because if the concentration of a contaminant in a body of water gets too high before the system is flushed, it could pose serious harm to organisms present in the water, as well as any nearby communities that rely on the water for both economic and tourism based uses (Coulliette & Noble, 2008).

Residence time can be used to understand water circulation on a more localized scale. Rather than looking at the body of water as a whole, residence time describes the time it takes a particular parcel of water in a specified location to exit the system boundaries (Monsen et al., 2002). This plays a crucial role in determining how contaminants flow throughout the system because the residence time can vary depending on a number of internal and external processes that may act on a particular parcel of water. This results in the potential for contaminants present to stay within the boundaries of the system for a prolonged or reduced amount of time. For example, bathymetry can have strong impacts on the circulation of water, and thus the movement of contaminants throughout the body of water. The roughness of the seafloor causes mixing of the water column, which affects the circulation of the water (Kimmerer, 2004).

In addition to the bathymetry of Town Creek, water motion is also affected by tides, freshwater inputs from the Newport River and Town Creek watershed, and wind. The tides bring in dense salt water from the ocean up into the estuary. This results in both a vertical and horizontal salinity gradient, as well as a vertical velocity gradient throughout the water column (Kuo & Nielson, 1987). North Carolina experiences semidiurnal tides (Pietrafesa & Janowitz, 1985), meaning there are two high tides and two low tides of approximately the same height each tidal day (U.S. Department of Commerce & NOAA, 2013). The tides account for a large portion of the current and water level differences (Lentz et al., 2001), meaning that both the flushing and residence times of Town Creek are greatly influenced by the tidal regimes. Furthermore, tides influence water circulation by inducing mixing (Simpson and Hunter, 1974). Mixing causes contaminants to become more evenly distributed throughout the water body.

As the tides enter the estuary, they mix with freshwater from the Newport River. As fresh and saltwater meet, the salt water sinks to the bottom while the freshwater flows overtop due to the fact that freshwater is less dense. This stratification produces both a vertical density and salinity gradient (Kuo & Nielson, 1987). These salinity and density gradients are factors that inhibit vertical mixing within the water column; however, seafloor roughness caused as a result of the bathymetry and the strong tidal regimes moving along the bottom, producing velocity shear, can induce vertical mixing (Sanford et al., 1992). This is indicative of the Newport River estuary, specifically, being tidally dominated.

While the water within the water column is strongly affected by the tides, the surface water is strongly affected by the wind. Stronger winds will result in larger surface waves which will have more drastic effects on the movement of contaminants in the surface current. The wind plays a role in producing velocity shear, but it can also have certain effects on the surface of the water that are different to the effects of the tides and currents.

Our study aimed to understand the correlation between these processes, how the movement of the water influences flushing and residence times, and the circulation of contaminants throughout the Town Creek system. By examining the movement of the water within the system and any potential circulation patterns that may arise, the length of time in which contaminants may be present in the system can be determined. This is important in understanding the overall water quality of Town Creek, as well as any risk that may be posed to both public and ecological health.

2. METHODS

2.1 Bathymetry Survey

Bathymetry data was needed to calculate the volume of water in Town Creek for the flushing time calculation. Because the U.S. Army Corps of Engineers (Army Corps) only had previously collected data on the central area of Town Creek (USACE Hydrographic Surveys, 2017-2019), it was necessary to compile sounding depth data points around the perimeter in order to ascertain a more holistic bathymetry map of Town Creek. Depth measurements and their respective GPS coordinates were recorded using a handheld Garmin GPS 72 H unit and a Vexilar LPS-1 depth sounding instrument. These measurements were combined with the Army Corps data in order to make a 3D bathymetry model for the study area.

2.2 Bathymetry Data Analytical Methods

In order to create the model, the depth measurements we recorded were converted to water levels relative to MLLW to ensure consistency with the existing Army Corps data. Using the depth points and ArcMap 10.5 software, a triangular irregular network (TIN) surface was created of the embayment and used to calculate the volume of the system in reference to MLLW, MHW, and MLW surfaces.

The MLLW volume estimates were then used to calculate flushing time using the tidal prism model equation:

$$T_f = \frac{VT}{(1-b)P} \qquad (1)$$

where T_f represents the flushing time, V the volume of the system relative to MLLW, T the tidal period, P the tidal prism, and b the return flow fraction. To obtain a value for P, the difference between the MHW and MLW volumes was taken. The value of b is unknown, but based on the geometry of the system and the large volume of water that moves through Gallant's channel, we estimated that a tidal cycle returns at most 50% of the water in the harbor. Therefore, we made several calculations to obtain flushing times for 0-50% return flow to account for variability caused by wind or other factors.

By using this equation, four assumptions were made (Monsen et al., 2002):

1. Tides exclusively flush the system, which is consistent with the low river inflow to the Town Creek harbor.

2. The system is well mixed, which tends to lead to an underestimation of the flushing time. We were able to make this assumption based on the lack of strong salinity gradients on days without strong wind that we saw when we made in-field salinity measurements and previous literature (Coulliette et al., 2008).

3. The water body directly outside the system must be large enough to dilute the water exiting the system so its water quality does not change, which was Gallant's Channel in this experiment.

4. The system is at steady state with the sinusoidal wave signal, which was reflected in the regularity of the tidal cycle during these periods (NOAA Tidal Data, 2019).

2.3 Drifter Deployments

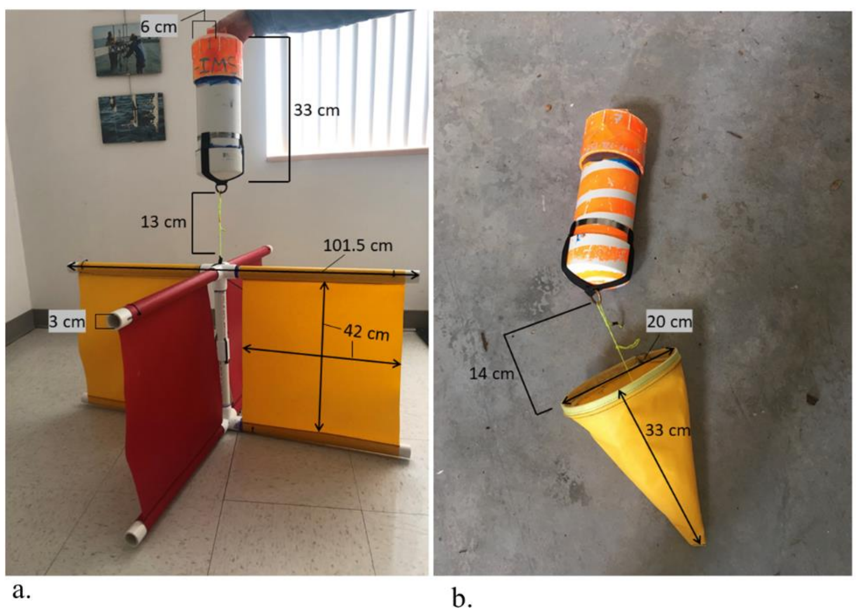


Figure 1. a. Davis Style Drifter with dimensions
b. Cone Style Drifter with dimensions

In order to understand the circulation patterns in Town Creek, we deployed 7 GPS drifters containing Garmin Rino 520 GPS units and Tracfones with GPS capabilities (Alcatel One Touch Pixi Eclipse TFA462C, accuracy +/- 3 m) throughout the study area during periods of ebb and flood tides. The Garmin Rino 520 GPS units sampled every 10 seconds, whereas the Tracfones sampled every minute. Drifter releases were conducted on four days: two during ebb tides and two during flood tides. Tide and weather conditions during testing days 1-4 are shown in Figures 3-6.

We used 6 cone-style drifters and 1 Davis-style drifter. The Davis drifter is less affected by surface currents caused by wind, so we were able to compare the influence of the wind between the two types by releasing a cone style drifter at the same place and time as the Davis drifter (Balfour, 2012). This is because the Davis drifter has a larger drag area ratio on its submerged portion that outweighs the drag of the wind on its floating potion. All drifter releases were approximately 20 minutes long, unless a drifter approached a hazard (boat, docks, getting caught on buoys, etc.) during its deployment and had to be picked up early. Drifters were released in a row across the entrance of Town Creek and in a row down the middle of the harbor (Figures 3-6). Four drifter releases were done on each testing day.

2.4 Drifter Data Analytical Methods

The GPS data was edited to exclude any data collected outside of a deployment for each unit. Their paths were then mapped using ArcMap 10.5 software. Using the starting/ending points and the total drifting time for each trial, geodesic lines were calculated to show the overall direction of the drifters. Along each path line, vectors proportional to the velocity of the drifters were drawn in order to show how the speed of the water varied spatially during that tidal period.

In order to estimate the residence time throughout Town Creek, we categorized the drifter data based on whether the tide was incoming or outgoing and used the equation:

$$T_R = \frac{D}{S} \quad (2)$$

where *D* represents the horizontal distance from the starting point of each drifter release to the entrance of Town Creek harbor (approximately the longitude of -76.667° W), and *S* represents the magnitude of the velocity of each drifter during that release. In estimating the residence time this way, we made the following assumptions:

1. For incoming tide, the residence time represents the amount of time it would take to get to its starting point from the entrance. For an outgoing tide, the residence time represents the amount of time it takes for the drifter to exit Town Creek harbor.
2. The current and the speed of the drifters were constant during the deployments.
3. Water parcels at the bottom of the water column act the same as those on top.

Because we released a cone style drifter with the Davis drifter at the same time and place, we were able to compare their paths and how much the wind may have affected the movement of the cone-style drifters. Using the end GPS coordinates from the paths of the joint Davis and Cone drifter releases, geodesic lines were created on Arcmap 10.5 to show the vectors that represent the difference in velocities for the two drifters' types. We standardized the vectors by ensuring we used the position of the drifters at the point in which they spent the same amount of time drifting. Additionally, the speed at which the two drifters separated was plotted against wind speed for that day and is used in conjunction with the difference vectors to determine how the cone drifters may have been affected by wind conditions. Using this information, we can assess how accurate our cone drifter data is and how representative it is of the overall water circulation in Town Creek.

3. RESULTS

3.1 Bathymetry

On average, the depth of Town Creek is approximately 7.9 ft deep in reference to the MLLW datum. However, there are about 9,100 m^2 of space located in the middle of the harbor that are less than 4.8 feet deep, which represents around 51% of the total study area (Figure 2). This shallow middle section likely causes water to flow around it, forcing the water into the deeper channels surrounding the marinas. These areas around the marinas and perimeter of the harbor were, on average, around 9-11 feet deep, as a result of the dredging that has occurred. The area around the outflow of Town Creek into the harbor is very shallow, ranging from 0.47-1.7 feet deep.

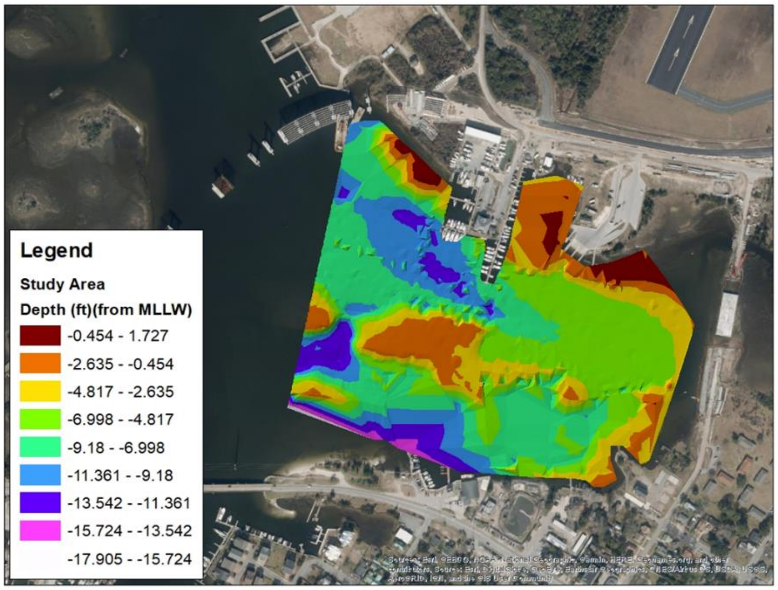


Figure 2. TIN surface of the bathymetry of Town Creek.

3.2 Flushing Time

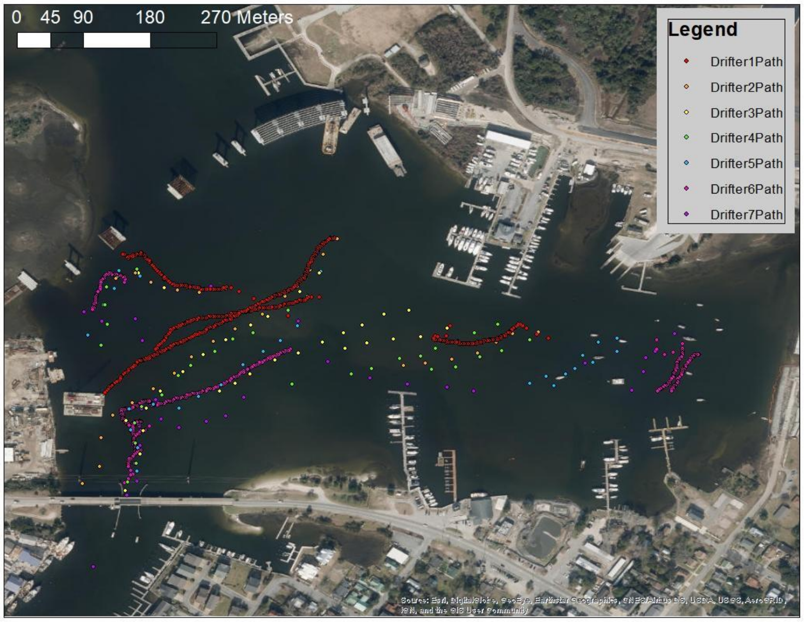
Flushing times based on the tidal prism method (Eq. 1) are summarized in Table 1. We report flushing times for a range of different *b* values since we estimated *b* to vary from 10-50% depending on wind and other conditions. As the amount of water that returns to the embayment in a tidal cycle increases, so does the flushing time. The volume of the embayment relative to MLLW, MHW, and MLW was estimated as 3.60 E5, 5.84 E5, and 4.16 E5 m^3, respectively. The flushing times summarized in Table 1 were estimated relative to MLLW.

Table 1. Raw flushing times for the Town Creek study area, estimated using different values of the return flow parameter *b*.

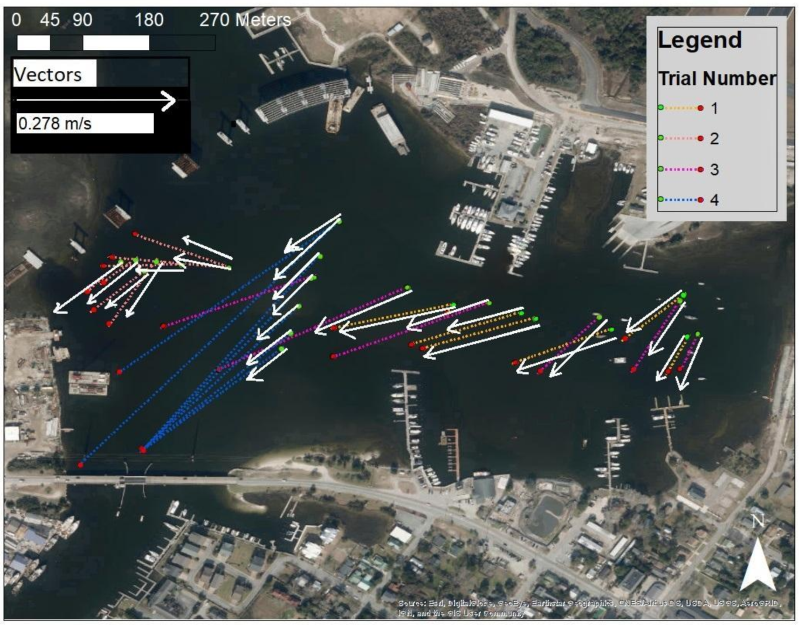
b	Flushing time (hr)
0.0	25.8
0.1	28.7
0.2	32.3
0.3	36.9
0.4	43.1
0.5	51.7

3.3 Water Movement

The path of the drifters serves as a proxy for the movement of the water. As the tide went out, the drifters tended to go west, towards Gallant's Channel (Figure 3 - 6). However, as the tide came in, they did not tend to go in a consistent direction. The mapping analysis done on ArcMap 10.5 for each day of deployments suggests that there is a major zone of mixing occurring where the entrance of Town Creek meets Gallant's channel, as the drifter vectors tend to vary in velocity the most in that area. This was also where a visible tidal front sometimes occurred. The water moves on average 3.46 times faster at the entrance of Town Creek than the water at the East side based on the lengths of the vectors in those areas. Additionally, by using the average length of the vectors and the wind speeds for each day (Figures 3 - 6), we estimated that for every 1 m/s increase in wind speed, the drifters moved 0.122 m/s faster. This indicates that the movement of water accelerates during times of high wind conditions.

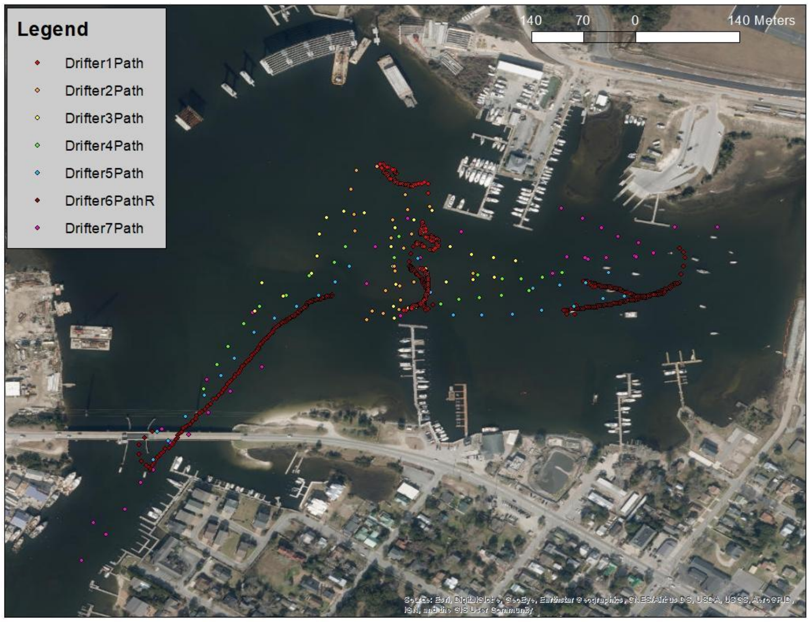


a.

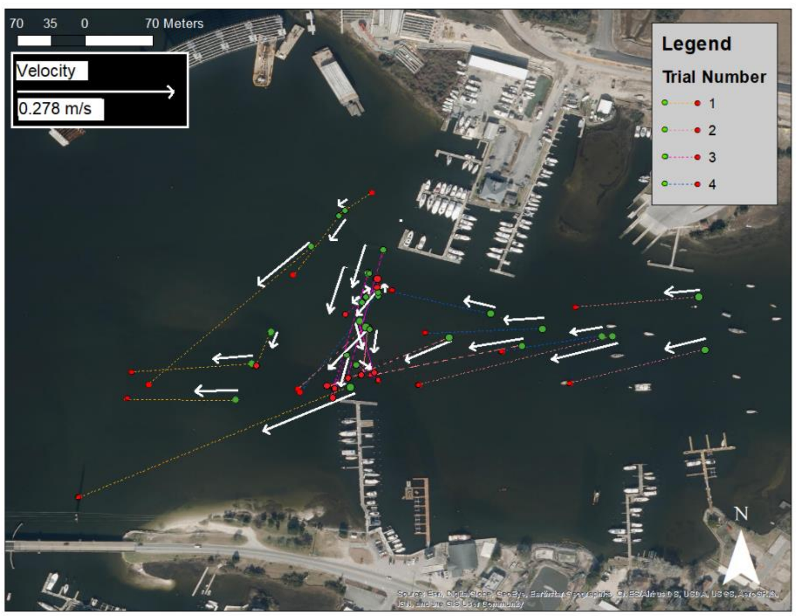


b.

Figure 3. Drifter data for the incoming tide on 9/18/19. The wind at the time of sampling was 5.88 m/s NE and the tidal range that day was 3.17 feet. All wind data was collected from NOAA station at the Duke Marine Lab.
a. The raw paths of the drifters.
b. The overall direction of each drifter and its velocity vectors. The white vectors represent the velocity vectors; a longer vector indicates a higher speed.

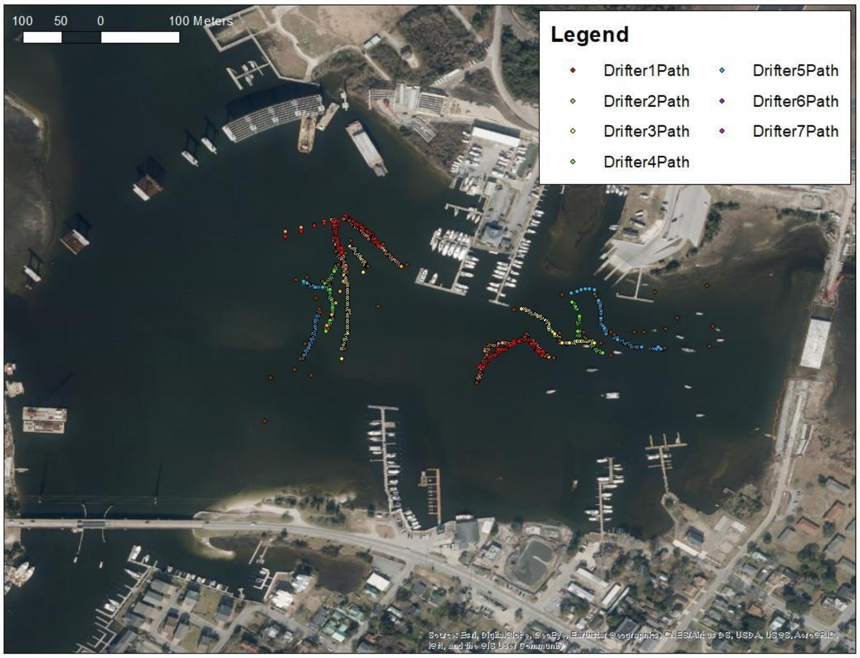


a.

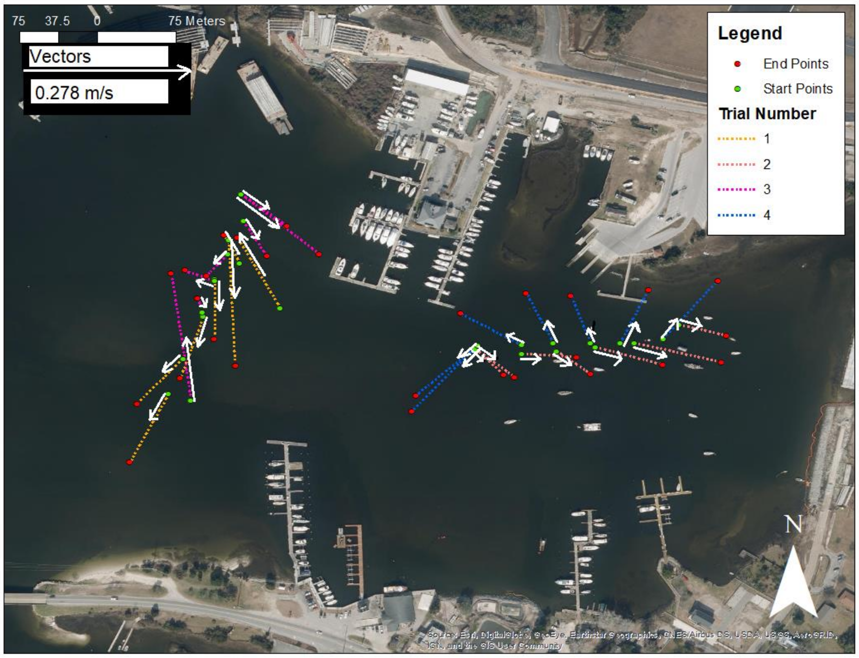


b.

Figure 4. Drifter data for the outgoing tide on 9/25/19. The wind at the time of sampling was 1.69 m/s N and the tidal range was 3.15 feet.
a. The raw paths of the drifters.
b. The overall direction of each drifter and its velocity vectors.

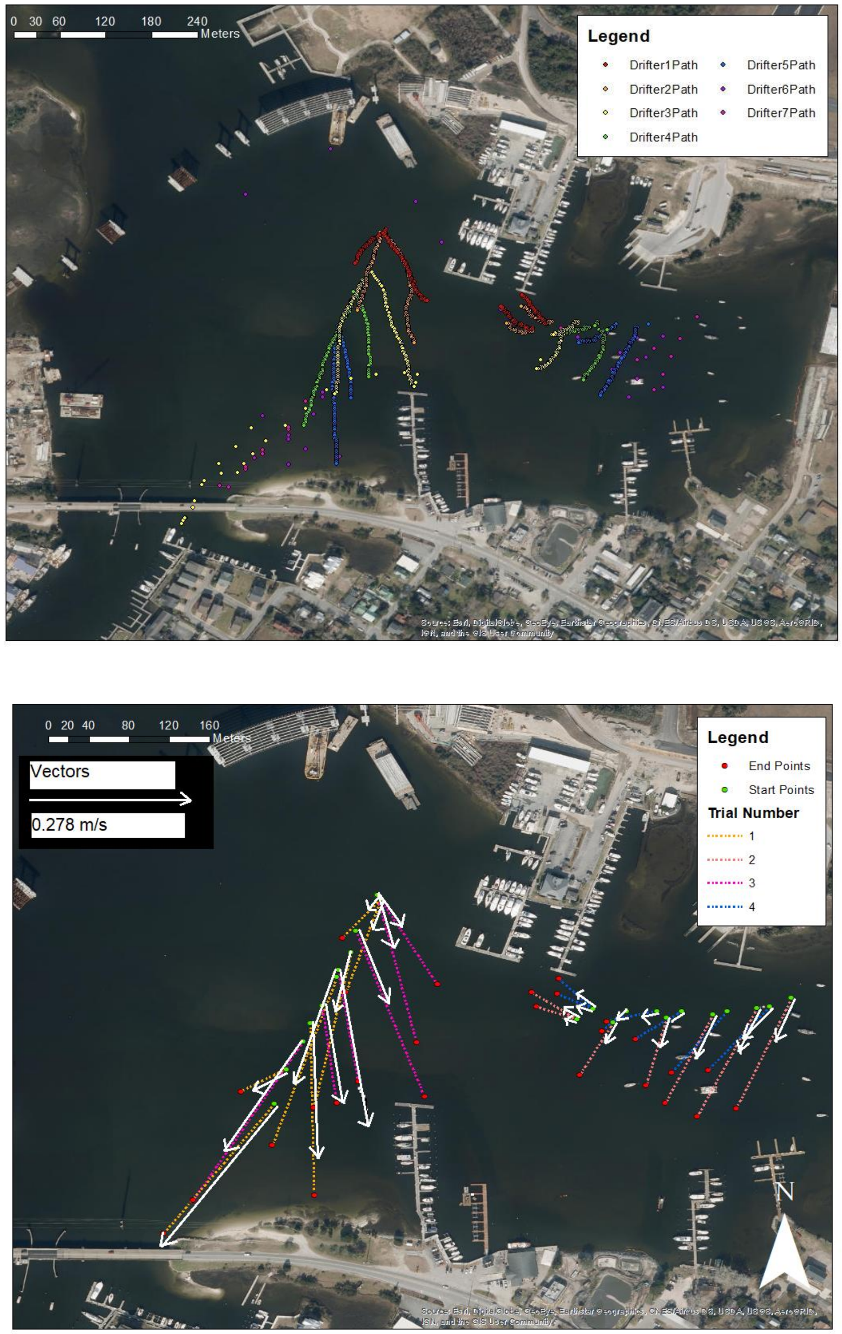


a.



b.

Figure 5. Drifter data for the incoming tide on 10/2/19. The wind at the time of sampling was 0.78 m/s N and the tidal range that day was 4.10 feet.
a. The raw paths of the drifters.
b. The overall direction of each drifter and its velocity vectors.

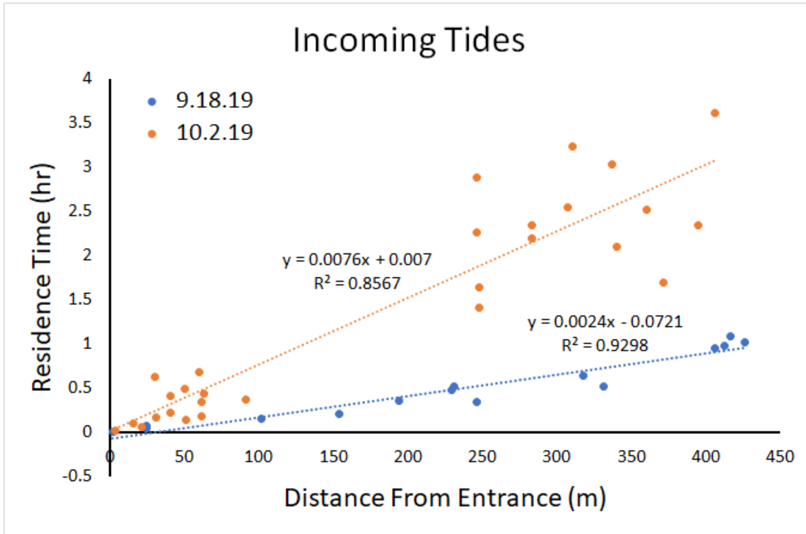


a.

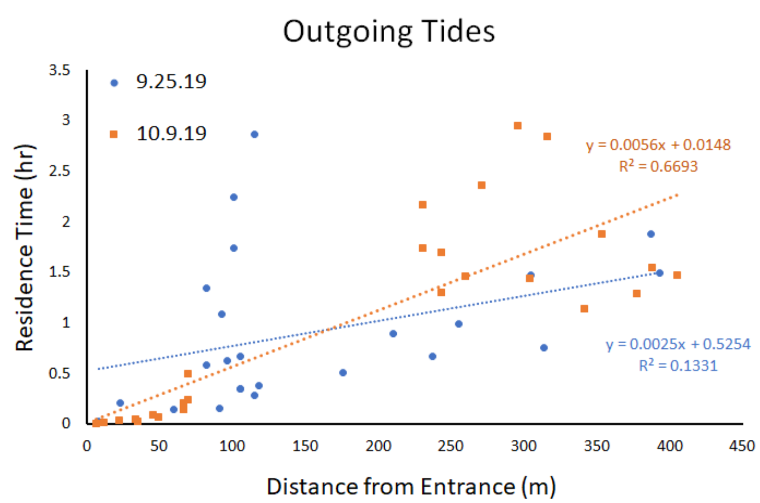
b.

Figure 6. The drifter vectors for the outgoing tide on 10/9/19. The wind at the time of sampling was 7.55 m/s N and the tidal range was 2.84 feet.
a. The raw paths of the drifters.
b. The overall direction of each drifter and its velocity vectors.

3.4 Residence Time



a.



b.

Figure 7. Residence time over increasing distance from the entrance of Town Creek. Different sampling days for each tide are represented by orange and blue, and the dashed line shows the regression line for each day. 10/2/19 and 10/9/19 were days with weaker winds conditions compared to the stronger winds on 9/18/19 and 9/25/19.

a. The residence times for the testing days with incoming tides.

b. The residence times for the testing days with outgoing tides.

As the distance of a drifter's starting point increased from the entrance, so did its residence time. Since most drifter releases showed water moving out of the embayment regardless of the tidal direction, the residence time refers to the estimate of time it takes for a water parcel to leave the system (Monsen et al., 2002). The average slope of each linear regression line for both panels, a and b, in Figure 7 is approximately equal. This suggests that a water parcel will take a similar amount of time to leave the system regardless of what direction the tide is going. For the outgoing tide, there was an anomaly where the drifters released about 100 m from the entrance had the highest residence time. This is likely due to the drifters getting caught in a tidal front across the entrance and moving along the front rather than out of the entrance. Furthermore, our residence time calculations did not account for the movement of bottom currents and may be estimated to be faster than the true value. This is because we measured the surface currents, which are likely moving faster than the bottom currents due to the strong influence of the wind. Overall, the average residence times for incoming and outgoing tides were 0.92 hours and 0.98 hours, respectively.

3.5 Cone vs Davis style drifter differences

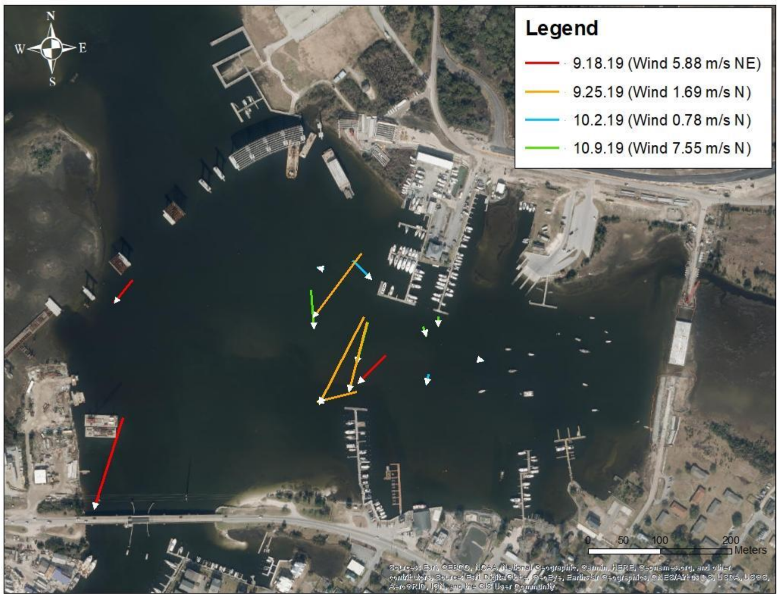


Figure 8. Vectors showing the difference in velocities of the Davis and cone drifter. A longer vector indicates the drifters separated at a higher speed. The drifter positions used for vector calculations were standardized to be in reference to the same time spent in the water.

Generally, the vectors point southward, indicating that the northern winds may have pushed the cone drifters significantly. The vectors from 9/18/19, a day with high wind speed, are longer, suggesting that the cone drifters were strongly affected by the surface currents (Figure 8). On 10/9/19, there were high wind conditions but short vectors. This shows that the wind was so strong that day that it affected the Davis and cone drifters similarly. On 9/25/19, a day with low wind speeds, the vectors were long, indicating that the wind that did occur had a strong effect the two drifters' separation.

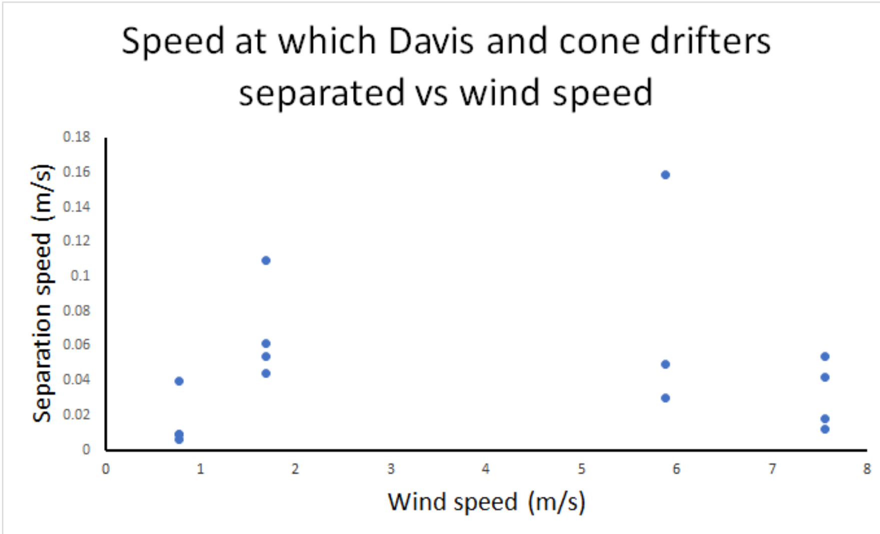


Figure 9. The speed at which the drifters separated for each joint deployment plotted against the wind speed for that day.

No strong correlation is shown in Figure 9; however, in low and high wind speed conditions, the separation speed was low. This suggests that the wind speed was high enough to completely drive the currents pushing the drifters in the same direction. At mid wind speeds, the highest separation speed occurs, indicating that these conditions may lead to the most skewed paths for cone drifters.

4. DISCUSSION

4.1 Main Implications of Drifter Movement

Because the Newport River Estuary is tidally dominated, the drifters generally tend to move in the direction of the tides - either incoming or outgoing, with the exception of 9/18/2019 due to the strong northeasterly winds. This means that any contaminants present in the water can be expected to flow in a similar trend based on the tidal regime. The tidal regimes remain predominantly constant; however, the wind speed and direction changes drastically depending on a variety of outside factors, such as approaching fronts and large-scale weather patterns. If the wind speed is high and the direction is opposite that of the tides, then the surface waters may move in the direction of the wind while the bottom water continues to move in the direction of the tide, as seen on 9/18/2019 in Figure 3b. Because of this, it is important to consider both the tides and the winds when determining the movement of contaminants throughout the system.

Since the cone drifters floated on the surface and had a smaller drag area ratio , they were strongly impacted by the wind and tended to stray from the general trend following the tidal regime on days where the wind speed was high and the wind direction varied from the tidal flow. Because the Davis drifter was suspended deeper in the water column, its movement was more impacted by the currents and tidal flow. It is essential to take into account the potential for wind to greatly affect the movement of the surface waters when determining the circulation of contaminants because any matter that is suspended in the water column may circulate in a different pattern compared to matter in the surface waters.

4.2 Factors Influencing Flushing and Residence Times

The flushing time was calculated based on upper and lower bounds established by the return flow parameter *b*. Due to the fact that b is generally unknown in a system because of the varying processes, such as wind, affecting the water body, it was difficult to estimate b as a single value. Because of this, flushing times were calculated using a range of *b* values (0.0 - 0.5) to accommodate the variability that may occur in the system as a result of wind and weather factors. The residence times were calculated based on upper and lower bounds established by the distance from which the drifters were deployed from the entrance of Town Creek. This produced considerable variability among residence times because each drifter was deployed at a different distance from the entrance to Town Creek and on each testing day, the water was moving differently due to various processes, such as the wind and the currents.

It is important to have upper and lower bounds for both residence and flushing times because factors such as tides, wind, and bathymetry can affect how long the water stays in the system. For example, contaminants that enter the water at any location within Town Creek will experience a longer residence time if they are present during an incoming tide because the water will push them further upstream into the creek. When the tide begins to go back out again, it will take longer for the contaminants to leave the embayment since they are further up the creek from where they initially entered the system. In comparison to the 2.5 hr residence time of a water parcel 400 m away from the entrance (Figure 7b), the lower bound 25.8 hr flushing time (Table 1) for the entire embayment seems very high. This high flushing time value accounts for the semidiurnal tidal range, as well as the occurrence of spring and neap tides that may result in a larger or smaller volume of water entering Town Creek.

Although our calculated flushing time does not account for wind, it can also have large effects on the actual flushing and residence times since its speed and direction can force the surface waters to circulate differently from the water in the rest of the column. This can make the residence and flushing times longer if the winds are pushing the surface waters further up Town Creek or shorter if the winds are pushing the surface waters out of Town Creek. Figures 3b shows the drifters moving outside of the embayment at incoming tide. This was likely caused by the 5.88 m/s winds coming out of the northeast, pushing the surface waters in a southwesterly direction, despite the fact that the tide was incoming and the water in the rest of the column should have been moving into Town Creek. Figure 7a shows that the residence times for the drifters were very low. This shows how large of an effect the wind can have on the residence time. Similarly, the flushing time is also greatly affected by the scale of the wind effects which is large and encompasses the entirety of Town Creek.

The residence time, specifically, can be affected by the bathymetry. Depending on the path a water parcel takes from its position within Town Creek, it may encounter depth variations or structures on the seafloor that force the water to move in a different direction. Additionally, water parcels in different locations will flow in the path of least resistance based on bathymetry. This path will vary for each parcel of water. This makes it difficult to understand the exact flow of contaminants because contaminants that are present lower in the water column will encounter more bathymetry effects than surface waters.

4.3 Confounding Factors

By comparing the difference in velocities between the Davis-style and cone-style drifters with the velocity of the wind that day (Figure 9), we were able to conclude that the wind considerably skewed the paths of the cone-style drifters. Therefore, our results largely focus on the movement of the surface currents of Town Creek, which is not entirely representative of the bottom currents occurring in the harbor. However, because Town Creek harbor is a small, shallow embayment, its circulation is presumably dominated by the wind and tides regardless.

5. CONCLUSION

- The overall circulation of the water suggests that any contaminants that make their way into Town Creek are flushed in 52 hours at the most, and 25 hours at the least. This flushing time does not take wind into account, but varies because of the range of return flow factor values used.
- The circulation patterns are affected by the tides, wind, and bathymetry, making it difficult to predict the flow path of contaminants. However, using the information we gathered, we estimate that contaminants are likely to linger along the perimeter and the back of the harbor.
- Surface water parcels containing contaminants can be expected to leave the system in approximately 1 hour or less. The closer a water parcel is to Gallant's Channel, the quicker it is expected to leave the system.

CHAPTER 3: Nutrients and Algae

1. INTRODUCTION

Anthropogenic disturbances aid the mobilization of nutrient elements like phosphorus and nitrogen in hydrologic systems. Consequently, augmented concentrations of these elements via localized point and nonpoint pollutant sources have hastened the transfer of nitrogen and phosphorus into coastal waters which stimulates organic matter production in the coastal environment (Cloern 2001). While phosphorus is the leading limiting element in freshwater primary production (Cloern 2001), nitrogen is the principal limiting element of algal primary production in marine ecosystems (Gowen et al., 1992). Therefore, since Town Creek is a saltwater-dominated system, the quantity of algal biomass is largely dependent on the input of nitrogen (Gowen et al., 1992). Autotrophic organisms like phytoplankton are integral in the creation and transfer of organic carbon throughout aquatic food webs and thus bear the effects of changes to the watershed. In Town Creek, the significant point and nonpoint sources of pollution to consider are five small-scale farms, discharge from docks and boat ramps, and numerous wastewater discharge facilities that run through nearby residential neighborhoods (Day et al., 2017). Lawn fertilization, pet waste, leaky septic systems among other factors in residential areas all contribute to pollutant runoff (Day et al., 2017). These sources of nutrient influx coupled with the appropriate abiotic conditions can trigger eutrophication (Gilbert, 2016).

Physical properties, like residence time, flow rate, nutrient availability, and light, can all influence phytoplankton primary production (Cloern, 2001). A combination of high residence time, nutrient concentration, light level, and low turbulence make up the most desirable conditions for algal blooms. Algae growth is maximized in the summer since water temperatures are higher. While calm waters yield more favorable conditions for algal blooms, alternating between droughts and large storm events also accelerate eutrophication and facilitate the expansion of harmful algal blooms across the freshwater-to-marine continuum (Paerl et al., 2018). Algal blooms exist naturally; however, excessive growth can degrade coastal ecosystems which generates a more suitable environment for harmful, toxin-producing algal species to proliferate (Paerl et al., 2018). Essentially, the ability of harmful algal bloom (HAB) species to maintain bloom conditions is dependent on their assimilative capacity (Bricker et al., 2008), or the phytoplankton's ability to access and uptake nutrients, competition with other phytoplankton species, and mortality via grazing (Hall et al., 2008).

In addition to altering estuarine and marine processes, HABs pose risks to humans and coastal-reliant sectors of the economy. Decreased oxygen availability due to an increase in HAB concentration can cause a rise in fish kills and bloom-stimulating nutrients from the sediment surface (Paerl et al., 2018). Further nutrient release from the sediments can restrict sunlight for aquatic vegetation (Bricker et al., 1999) thus propagating a positive-feedback loop, maintaining good algal bloom conditions (Paerl et al., 2018). This eutrophic environment puts human health at risk with toxin-contaminated shellfish consumption, or free toxins in the water or air (Bricker et al. 1999). Furthermore, the occurrence of harmful algal blooms can have devastating consequences on tourism, fishing, health, and commercial industries, thereby jeopardizing regional economic vitality.

Due to the myriad of adverse effects on humans and the estuarine environment, we wanted to conduct an ecosystem-specific study that quantifies the algal and nutrient concentrations of Town Creek and assess its sensitivity to an influx of either. By measuring nutrient and chlorophyll *a*, which are both indicators of the health of an estuarine environment, and comparing it with state water quality parameters, we can conclude how and to what extent Town Creek is at risk of harboring a harmful algal bloom. Residence time and flow rate are also important factors for the assessment of Town Creek's water quality and will be quantified and discussion in Chapter Two of this report. This information can be used to plan future usage and regulation of the Town Creek watershed.

2. METHODS

2.1 Sample Sites

We sampled at 11 different sites around Town Creek. Sites G1 and G2 are to the north of the Beaufort high-rise bridge and in the channel where the old drawbridge was, respectively, to measure the gradient from higher up the estuary going toward the ocean, and to understand the characteristics of the water entering Town Creek from up and downstream (Figure 2.1). Site M2 is the water outside Town Creek Marina while Site M1 is between two marinas on the south side of the embayment. Site C2 is located in the channel between the two marina points and Site C1 is located closer to the Turner Street bridge. Site C3 samples were taken while standing on the north side of the Turner Street bridge facing Gallant's Channel. We sampled at four different storm water outfalls. SW1 is located outside the Discovery Dive scuba company and SW3 is located further east on the waterside of the Turner Street bridge. SW2 is situated on the north side of the bridge facing the marsh while SW4 is further west next to Town Creek Marina.

Figure 2.1. Map of Town Creek with the seven identified sampling sites (G1, G2, C1, C2, C3, M1, M2, SW1-S, SW2-N, SW3-S, and SW4-N).

Table 2.1. Sampling site information.

Site	Key	Latitude	Longitude	Access point	Surface (T)	Bottom (B)
G1	Gallants Channel	34°43'39.59"N	76°40'6.86"W	water	X	X
G2	Gallants Channel	34°43'21.31"N	76°40'9.60"W	water	X	X
C1	Channel	34°43'27.95"N	76°39'44.37"W	water	X	X
C2	Channel	34°43’27.89”N	76°39’54.18”W	water	X	X
M1	Marina	34°43'22.45”N	76°39'53.17"W	water	X	X
M2	Marina	34°43'31.87"N	76°39'52.53"W	land	X	X
SW1	Stormwater Drain-S	34°43'20.50"N	76°39'48.94"W	land	X	
SW2	Stormwater Drain-N	34°43'33.00"N	76°39'37.13"W	land	X	
SW3	Stormwater Drain-S	34°43'21.94"N	76°39'41.75"W	land	X	
SW4	Stormwater Drain-N	34°43'35.60"N	76°39'48.73"W	land	X	
C3	Channel	34°43’31”89”N	76°39’39.29”W	land	X	

Table 2.2. Sampling Dates and Relative Tides.

Date	*Tide*
18-Sep	*High*
25-Sep	*Low*
2-Oct	*High*
9-Oct	*Low*
14-Oct	*N/A (stormwater only)*
10/16 #1	*High*
10/16 #2	*Low*

2.2 Field Measurements

We sampled water at the surface and at 0.5 meters above the sediment surface at the sites indicated in Table 1.1. Stormwater sites and site C3 were only sampled at the surface. A surface grab was believed to be representative of the entire water column because of the shallow site depths. We collected the samples over three high tides and three low tides between September 18th and October 16th with an additional sample taken at the four stormwater sites on October 14th after a rain event (Table 1.2). We collected surface water samples in 500 mL plastic bottles that were stored in a cooler of ice until processing in the laboratory at UNC Institute of Marine Sciences two hours later. The bottom water samples were collected using a Van Dorn water sampler. Each bottle was rinsed with sample water once before collecting the final sample. Prior to the first sampling and after each sampling trip, all sampling bottles were cleaned with 3 DI rinses followed by 0.1 M HCl acid wash and stored containing approximately 50 mL of 0.1M HCl.

We conducted three laboratory tests: measuring nutrient concentrations with an auto-analyzer, chlorophyll *a* quantity as a measure of phytoplankton biomass using a fluorometer and quantifying and characterizing phytoplankton species using microscopy.

2.3 Chlorophyll a Analysis

To analyze chlorophyll *a*, the 50 mL samples were filtered using Whatman 25 mm GF/F filters. With the chlorophyll facing upwards, the filters were folded in half. To absorb additional water, the filters were dried with a paper towel and rolled out with a metal cylindrical weight. The filters were wrapped in aluminum foil and placed in a -20℃ freezer until frozen. Each of the filters was then unfolded, transferred into individual plastic test tubes with 5 mL of 90% acetone until the next day. The following day, the acetone mixture was measured for chl *a* content using the Turner Trilogy Fluorometer which was calibrated beforehand.

2.4 Nutrient Analysis

To measure nitrate+, nitrite (NOx), phosphate, and total dissolved nitrogen, 50 mL aliquots were filtered using Whatman 25 mm GF/F filters and transferred into 50 mL plastic test tubes in a -20℃ freezer (Peierls et al., 2012). The frozen samples were tested for NOx, PO4, and ammonium concentrations using a Lachat QuikChem 8000 auto-analyzer (Lachat, Milwaukee, WI, USA; Lachat QuikChem methods 31-107-04-1-C, 31-107-06-1-B, and 31-115- 01-3-C). The detection levels for NO_x, NH_4, PO_4, and total dissolved nitrogen were 0.48 µg/L, 1.46 µg/L, 1.14 µg/L, and 18.20 µg/L, respectively.

2.5 Species Identification via Microscopy

For each site, we filled a 15 mL vial with water collected from the 500 mL bottles. We immediately added three drops of Lugol's iodine to each vial to ensure the microbial composition did not change to remain representative of the site at the date and time of collection. Using the Utermöhl method, three settling chambers were set up 24 hours prior to viewing the slides with a Leica DMIRB inverted microscope at 400x magnification with phase contrast (Utermöhl, 1958). We counted until we reached either 50 fields or 100 or 400 total specimens per sample, depending on the abundance of that particular species, to measure phytoplankton diversity and quantity. We analyzed surface samples from C1 (near Turner Street bridge) for all five dates, including an additional C2 surface sample on September 25. The organisms partially inside the field of view grid were only recorded if they were located above or left of the grid. Any organisms partially positioned right or below the grid were not counted.

3. RESULTS

3.1 Chlorophyll a Analysis

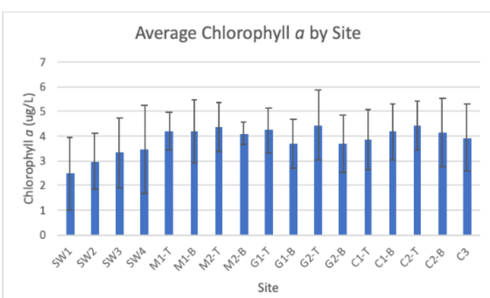


Figure 3.1. Average chlorophyll *a* concentration over all five sampling dates (September 18 - October 16).

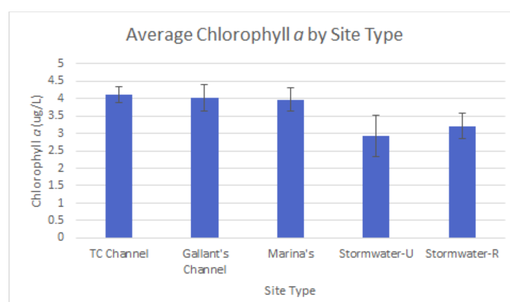


Figure 3.2. Average chlorophyll *a* concentration across all sampling days by site type.

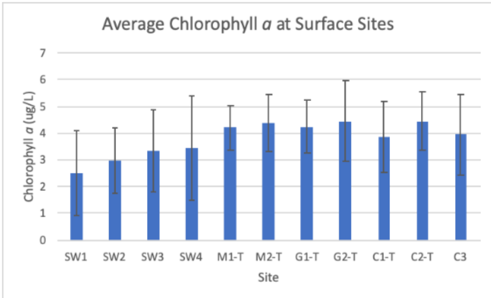


Figure 3.3. Average chlorophyll *a* content over all five sampling dates at surface sample sites.

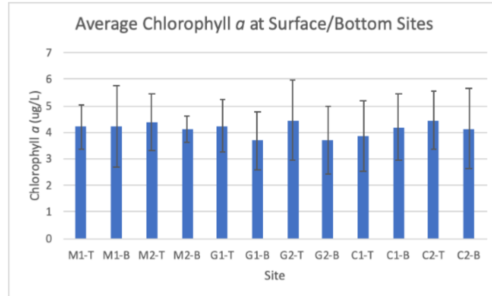


Figure 3.4. Average chlorophyll *a* content over all five sampling dates at surface and bottom of in-channel sites.

All of the site-specific average chlorophyll *a* values fell within the range of 2 to 5 µg/L, while the full range of chlorophyll *a* values fell within the range of 0.8 to 7.5 µg/L (Figure 3.1). The G2T site had the highest average chlorophyll *a* concentration at 4.6 µg/L while the SW1 site had the lowest at 2.5 µg/L. In-channel sites (C, G, M) showed higher average chlorophyll *a* concentrations across all sampling sites when compared to stormwater sites (Figure 3.2) Variation of chlorophyll *a* was also seen between surface sites (Figure 3.3) with a pattern similar to that shown by the total site range (Figure 3.1). Chlorophyll *a* levels within surface samples were slightly higher than bottom samples (Figure 3.4).

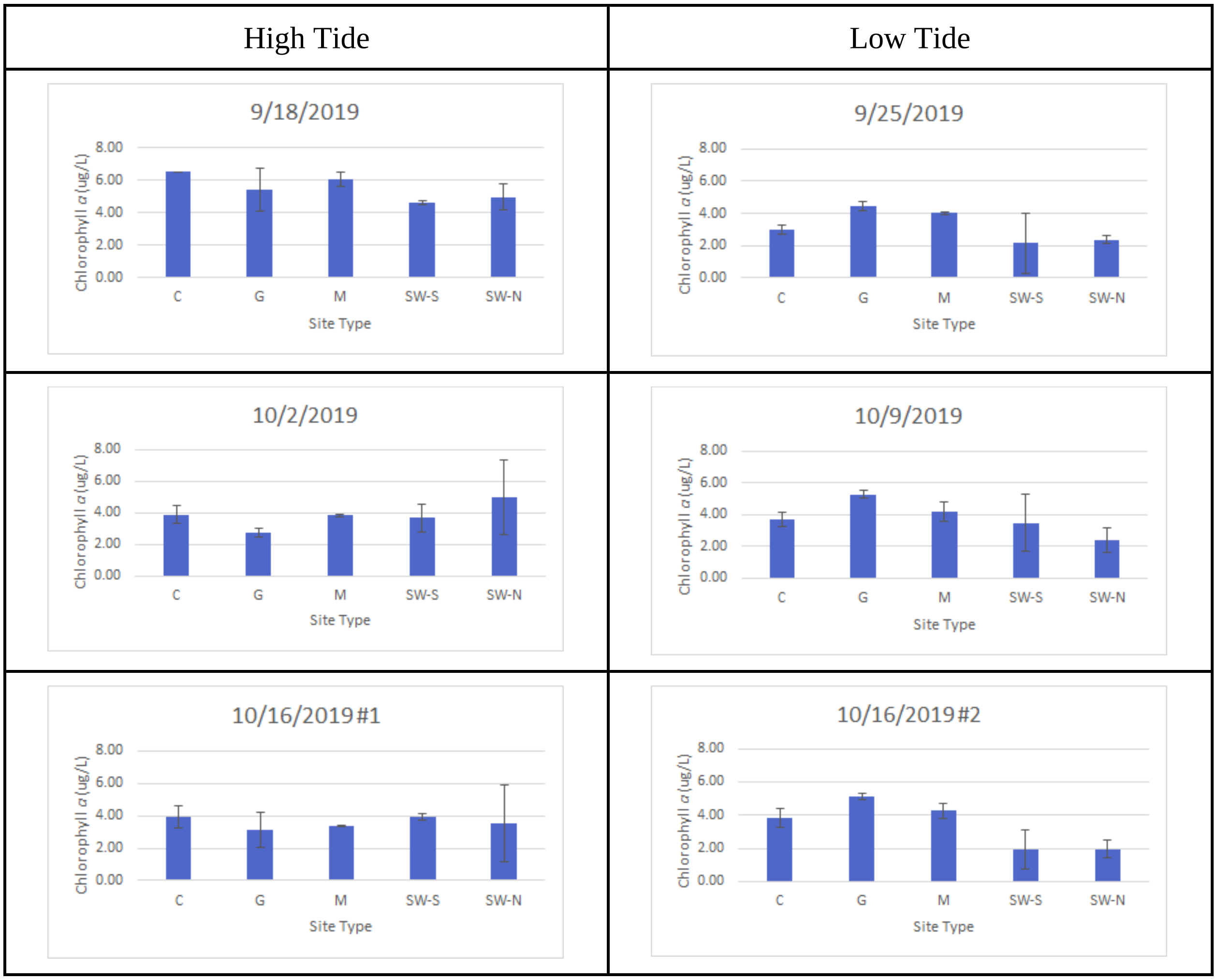


Figure 3.5. Chlorophyll *a* data aggregated by site for each sampling date.

Table 3.1. Results from ANOVA Statistical Test - Chlorophyll *a* by Date/Site Type.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	12.85	4.00	3.21	3.56	0.017	2.69
Columns	33.90	5.00	6.78	7.52	0.00011	2.53
Interaction	31.16	20.00	1.56	1.73	0.086	1.93
Within	27.07	30.00	0.90			
Total	104.99	59.00				

The chlorophyll *a* data was aggregated by site type (C, G, M, SW-S, and SW-N) for each of the five sampling dates. The stormwater north and south sites showed consistently lower chlorophyll *a* concentrations than in-channel sites (C, G, M). An ANOVA test was performed on the chlorophyll *a* data using site type and date as factors with individual sites as replicates (Table 3.1). Results indicated a significant variation by site type (p= 0.017) and date (p= 0.00011), but not the interaction between site and date (p= 0.086) (Table 3.1).

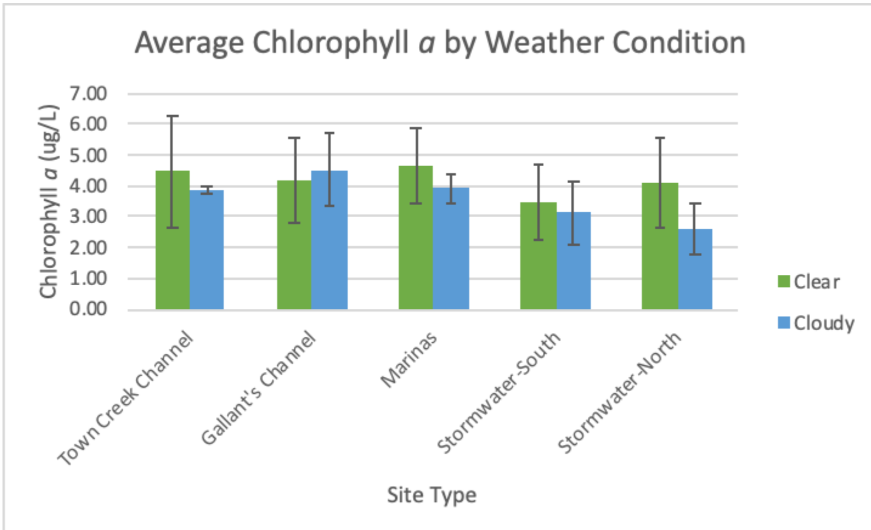


Figure 3.6. Chlorophyll *a* averages at each site type aggregated by weather conditions at the time of sampling.

Table 3.2. Results from ANOVA Statistical Test - Chlorophyll *a* by Weather Condition/Site Type.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	1.79	1	1.79	1.36	0.26	4.49
Columns	5.92	3	1.97	1.51	0.25	3.24
Interaction	2.61	3	0.87	0.66	0.59	3.24
Within	20.96	16	1.31			
Total	31.28	23				

We investigated the effect of the weather, but there was no statistically significant effect. Overall, in-channel sites (C, G, M) contained higher concentrations of chlorophyll *a* than stormwater sites (SW-S, SW-N). An ANOVA analysis was performed using site type and weather condition as factors. Results indicated no significant difference in chlorophyll *a* concentration between site types or when comparing clear and cloudy weather conditions. (Table 3.2).

3.2 Nutrient Analysis

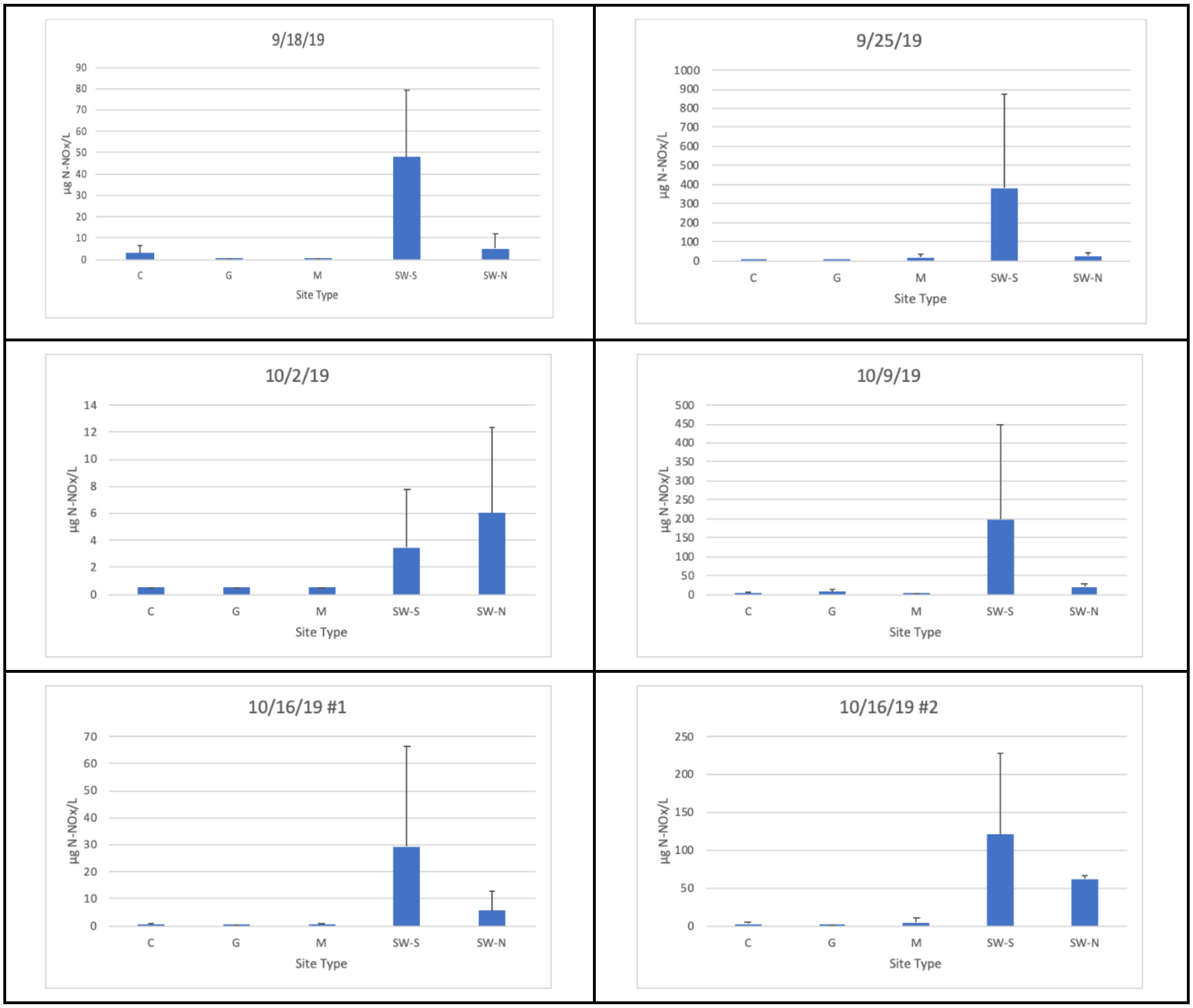


Figure 3.7. The nutrient NOx concentration aggregate by site for each date.

Table 3.3. Results from ANOVA Statistical Test - NOx by Date/Site Type.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	148942.81	4.00	37235.70	3.53	0.02	2.69
Columns	49371.47	5.00	9874.29	0.94	0.47	2.53
Interaction	157901.91	20.00	7895.10	0.75	0.75	1.93
Within	316607.39	30.00	10553.58			
Total	672823.57	59.00				

The average NOx concentrations were aggregated by site type (C, G, M, SW-S, and SW-N) for each of the five sampling dates. An ANOVA test was run to compare the site types over all sampling dates and found that there is a significant variation by site (p=0.02) (Table 3.3).

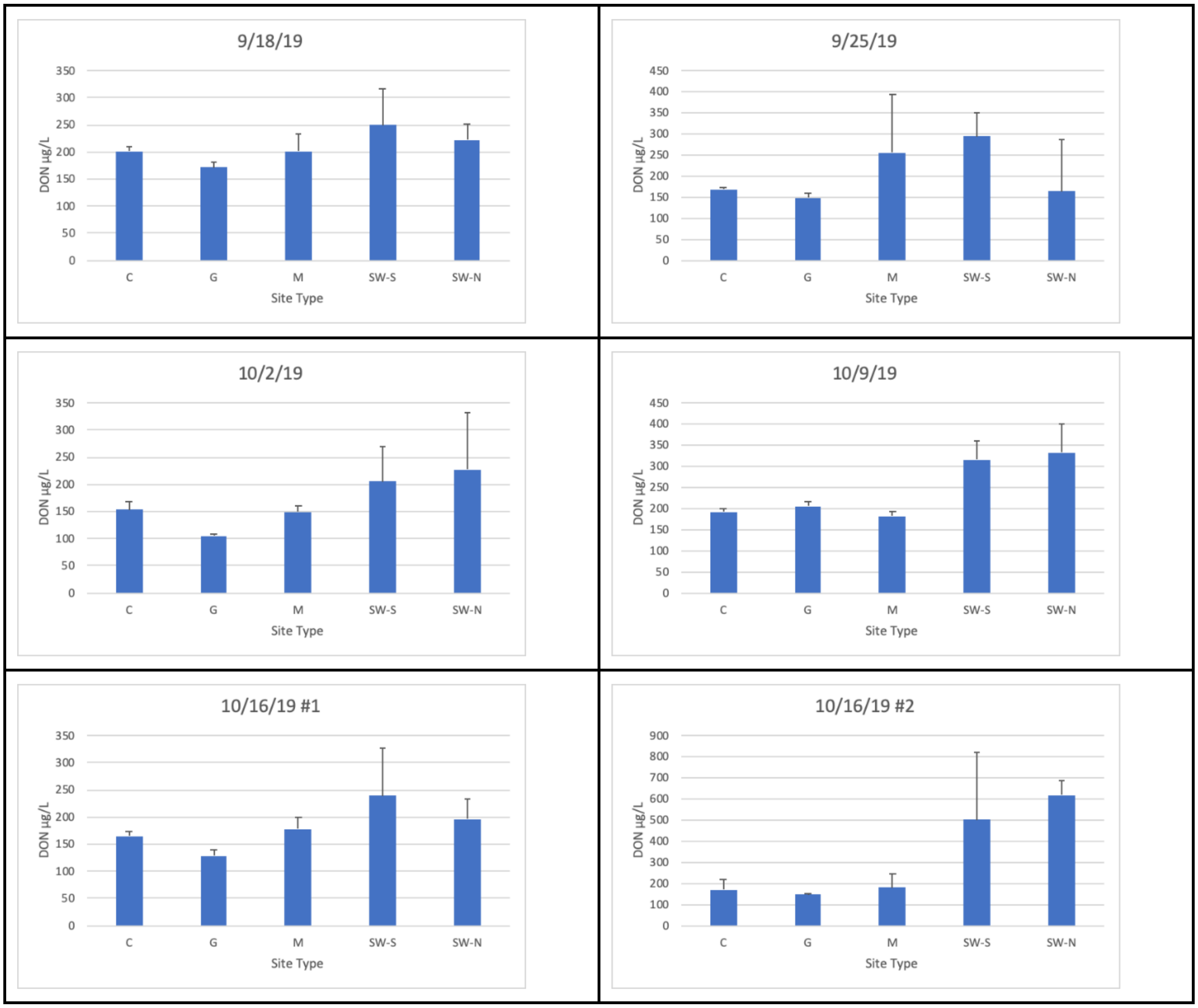


Figure 3.8. DON data aggregated by site for each sampling date.

Table 3.4. Results from ANOVA Statistical Test - DON by Date/Site Type.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	234428.03	4.00	58607.01	9.20	5.63E-05	2.69
Columns	156521.62	5.00	31304.32	4.92	0.0021	2.53
Interaction	269506.46	20.00	13475.32	2.12	0.031	1.93
Within	191050.32	30.00	6368.34			
Total	851506.44	59.00				

Average dissolved organic nitrogen (DON) analyses indicate a higher average DON concentration at Stormwater North and South sites when compared to in-channel (C, G, M) sites. An ANOVA test was performed on the average DON concentration values using site type and date as factors with individual sites as variates. Results of the ANOVA analysis indicates that there is significant variation by both site type and date, as well as within the interaction of the two factors.

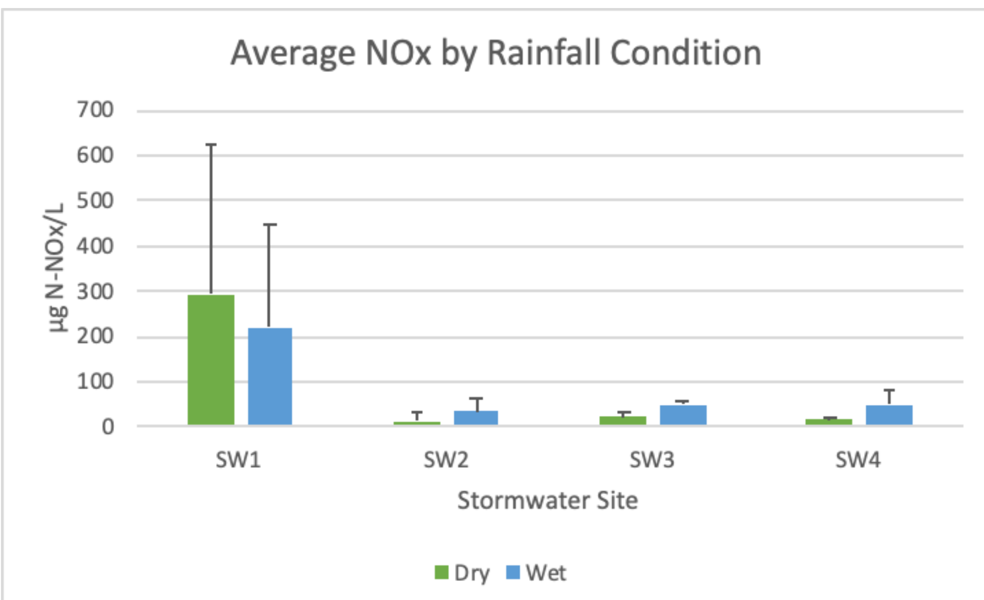


Figure 3.9. NOx concentrations at each stormwater site, averaged over all dry and all wet sampling days.

Table 3.5. Results from ANOVA Statistical Test - NOx by Weather Condition/Site Type.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.29	1	6.29	0.00047	0.98	5.99
Within Groups	80685.39	6	13447.56			
Total	80691.68	7				

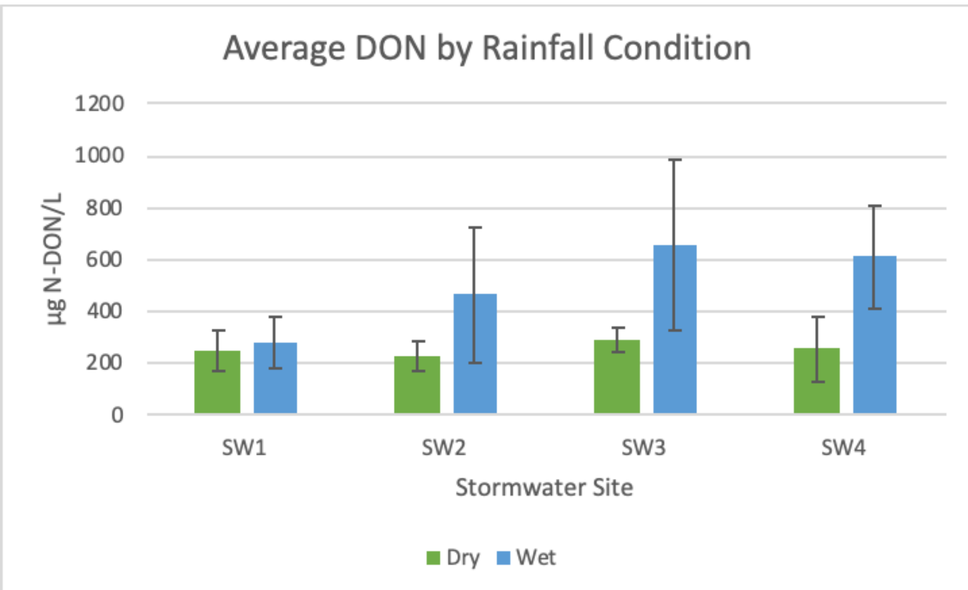


Figure 3.10. DON concentrations at each stormwater site, averaged over all dry and all wet sampling days.

Table 3.6. Results from ANOVA Statistical Test - DON by Weather Condition/Site Type.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	123930.257	1	123930.257	8.21	0.029	5.99
Within Groups	90605.45	6	15100.91			
Total	214535.711	7				

Average NOx concentrations were higher at SW1 than other stormwater sites. Average NOx concentrations found were higher on sampling days that experienced precipitation (10/14/19, 10/16/19 #1-2) compared to those that did not (9/18/19, 9/25/19, 10/2/19, 10/9/19) at stormwater sites 2-4. A single-factor ANOVA was used to evaluate significance in variation by presence/absence of a rainfall event. Results showed no significant difference in average NOx concentration by rainfall condition (Table 3.5).

Average dissolved organic nitrogen (DON) concentrations were consistently highest at site SW-3. Sampling days that experienced rainfall (10/14/19, 10/16/19 #1-2) showed considerably higher DON concentrations than sampling days that had no precipitation. A single-factor ANOVA was used to evaluate significance in variation by presence/absence of a rainfall event. Results showed a significant difference in average DON concentration by rainfall event. (Table 3.6) Figure 3.10 shows a notable difference in DON levels at stormwater sites when comparing sampling days that experienced precipitation to those that did not.

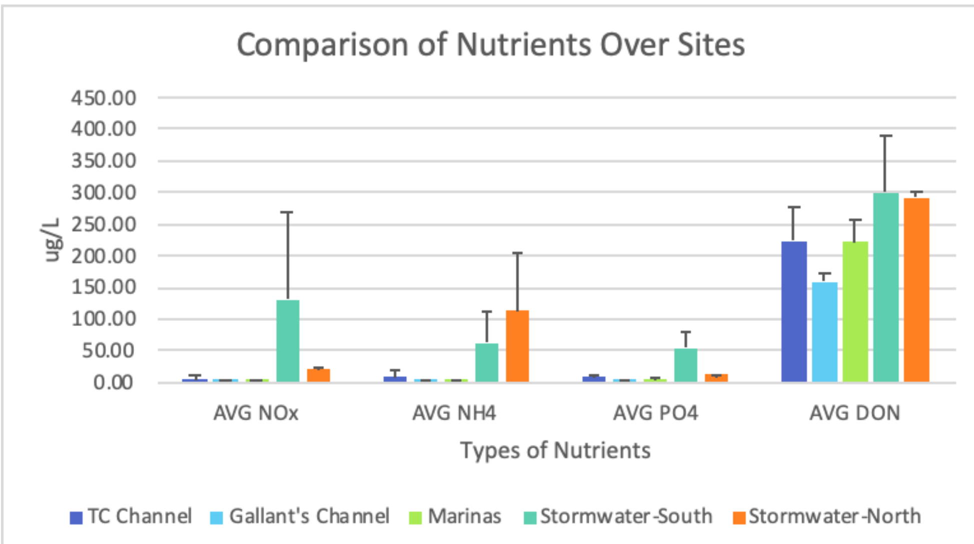


Figure 3.11. Average of the four nutrients tested for including nitrate/nitrite (NOx), ammonium (NH4), phosphate (PO4), and dissolved organic nitrogen (DON).

The average of the four main nutrients tested for in this study consistently displayed higher concentrations of nutrients at the stormwater sites. At most locations in the Town Creek and in Gallants Channel, nutrient concentrations were below the detection limits. DON was the only nutrient present at a concentration above detection limits at all sites and was lowest at the Gallant Channel sites.

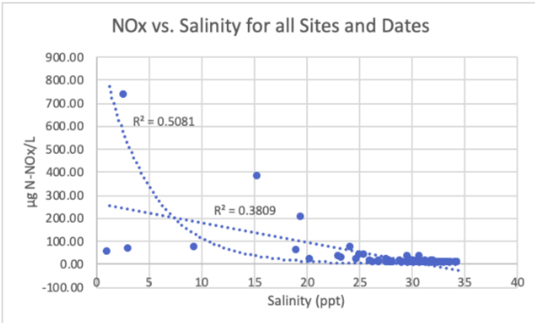


Figure 3.12.NOx concentration (ug/L) plotted against Salinity (ppt) for all sampling sites and dates.

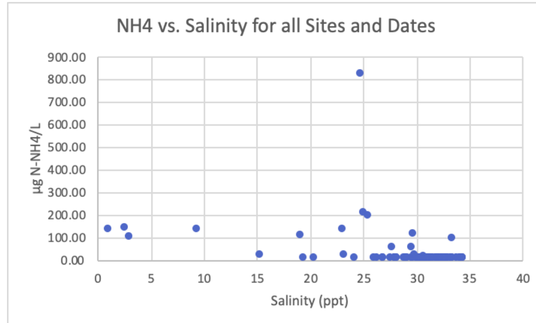


Figure 3.13. NH4 concentration (ug/L) plotted against Salinity (ppt) for all sampling sites and dates.

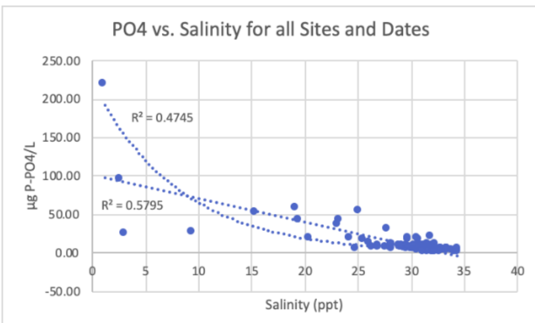


Figure 3.14. PO4 concentration (ug/L) plotted against Salinity (ppt) for all sampling sites and dates.

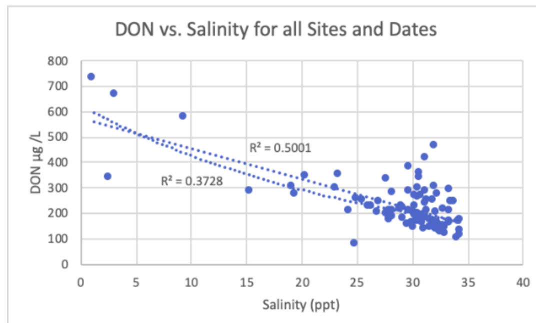


Figure 3.15. DON concentration (ug/L) plotted against Salinity (ppt) for all sampling sites and dates.

When plotted against salinity (ppt) at each site, NOx concentrations show an exponential relationship (r^2= 0.5081) as opposed to a linear relationship (r^2= 0.3809). Phosphate (PO4) and Dissolved Organic Nitrogen (DON), however, show a stronger linear relationship with r^2 values of r^2= 0.5795 and r^2= 0.5001 respectively. When plotted against salinity (ppt) at all sites, NH4 appears to have no linear or exponential relationship with salinity.

3.3 *Species Identification via Microscopy*

Table 3.7 : Cell counts (cell/mL) of phytoplankton in Town Creek channel sites over all dates.

Site	Date	Tide	Pennate	Centric	Karlodi-nium	Pseudonitzchia	Unidentified Dinoflagellate	Prorocentrum minimum	Chlamydomonas
C1-T	9/18	high	11680	68800	0	0	0	0	160
C1-T	9/25	low	448	304	0	0	0	0	0
C2-T	9/25	low	3632	16032	16	0	0	32	0
C1-T	10/2	high	7264	44448	64	128	160	0	0
C1-T	10/9 (1)	low	4800	61091	0	1163	0	146	146
C1-T	10/16 (2)	low	2954	53046	0	861	0	123	739

Phytoplankton abundance by cell type at representative surface Town Creek channel (C) sites across all sampling dates are shown in Table 3.7. Centric diatoms were found to have the highest abundance at all sites with Pennate diatoms also found in high abundance. Other phytoplankton genera; Karlodinium, Pseudonitzchia, Prorocentrum, and Chlamydomonas, were found in low abundance at half the sites with variation by date and genus. Karlodinium, Pseudonitzchia, and Prorocentrum are all considered potential HAB species. Unidentified dinoflagellates were also found at the C1-T site on 10/2/19, but were not seen at the other Town Creek channel site (C2-T) or within samples from other sampling dates.

4. DISCUSSION

A low abundance of chlorophyll *a* was found at all 11 sampling sites in Town Creek. In North Carolina, the state standard for chlorophyll *a* is 40 µg/L (NC DEQ, 2014). Chlorophyll *a* at higher concentrations is typically characterized by an algal bloom visible as a thin, green scum layer. All chlorophyll *a* values measured in Town Creek were less than 7 µg/L, far below the NC state standard. The chlorophyll *a* levels were consistently low over all five weeks of sampling. Low chlorophyll levels could be due to NOx concentrations measured at or near the lower detection limit at most sites. Because N is the limiting nutrient for phytoplankton growth, low NOx abundance would limit chlorophyll *a* concentrations (Piehler et al., 2004). Of all the sites, stormwater drainage sites showed higher levels of nutrients compared to the rest of Town Creek. The low level of chlorophyll *a* and NOx could be attributed to the short flushing time in Town

Creek, 25.8 to 51.7 hours, resulting in a nutrient residence time too short for an algal bloom to occur (Phlips et al., 2012).

When compared to DON, non-stormwater inorganic nitrogen concentrations are low. Phosphate, which is also known to act as a limiting nutrient in some estuarine systems, follows a similar pattern to NOx and NH4. Overall Stormwater drains showed consistently higher nutrient concentrations than other site types, suggesting that they could be the point of influx for nutrients into the system (Figure 3.11). Significantly lower nutrient concentrations in the channel are likely due to the fast residence time, which flushes the incoming nutrients from the system

Results from stormwater drains indicate higher concentrations of nutrients than Gallant's Channel and Town Creek. The non-stormwater sites likely experience lower nutrient concentrations when compared to stormwater sites due to dilution by incoming saltwater. The oceanic saltwater flowing into the system contains very little nutrients, therefore most nutrient content is due to terrestrial influence (Yang et. al, 2008). When the freshwater containing nutrients from runoff is diluted by saltwater, those sites with higher saltwater contents show diminished nutrient content when compared to less-diluted sites. NOx concentrations when plotted against salinity (ppt) at all sites shows an exponential relationship with an r^2 value of r^2= 0.5081 (Figure 3.12). An exponential relationship indicates that the removal of nutrients is likely due to uptake by organisms in Town Creek and surrounding water bodies, rather than decreased concentrations due to dilution. Phosphate and dissolved organic nitrogen, however, show indication of a linear relationship with salinity concentrations at each site. When plotted against salinity (ppt), phosphate (PO4) shows an r^2 value of r^2= 0.5795 and dissolved organic nitrogen shows an r^2 value of r^2= 0.5001 (Figures 3.14 and 3.15). The linear relationship suggests that the removal of the nutrients PO4 and DON occurs on average via dilution rather than uptake across all sites. Phosphate and dissolved organic nitrogen show some evidence of an exponential relationship, indicating the influence of uptake (r^2= 0.4745 and r^2= 0.3728) respectively, however the linear relationship indicating the effect of dilution appears to be stronger (Figures 3.14 and 3.15). NOx concentrations when plotted against salinity (ppt) also show evidence of a weak linear relationship (r^2= 0. 3809), however the higher correlation to an exponential relationship indicates that the influence of uptake is stronger on NOx concentrations as a whole. Ammonium (NH4) does not show evidence of a linear or exponential relationship, indicating that concentrations of ammonium in and around Town Creek are only minimally affected by uptake and dilution and instead appear at consistent concentrations across all salinity levels measured (Figure 3.13).

The nutrient levels found in Town Creek during periods with no rainfall indicate a continuous source of nutrients flowing into the water body. While nutrient levels are low, sites M1, C1, and C3, the sites closest to the stormwater outflows, display nutrient levels higher than the surrounding sites. All other sites recorded nutrient levels at or near the detection limit. The lack of relationship between NOx concentrations and rainfall events suggests that some outside source has a higher influence on the contents of stormwater drainage outfall NOx concentrations than rainfall events. This, combined with visual confirmation of constant flow at each outfall, suggests that water flowing out of the storm drains is not sourced exclusively by stormwater drainage. Rainfall events increased the presence of DON in samples. This relationship is most noticeable at stormwater site 4 with the DON concentration range of error for rainfall events existing exclusively above the DON concentration range of error for non-rainfall events (Figure 3.10). Error bars on (Figure 3.10) indicate a difference based on an error range of one standard deviation. Although rainfall events increase the nutrient load to Town Creek, it is not reflected in the overall nutrient load in the channel due to the fast residence time flushing the system

The nutrient levels within Town Creek are higher than those found in Gallant's Channel. The combination of elevated nutrient levels and the dominant phytoplankton community, however, will likely not result in a harmful algal bloom within Town Creek. Although there is a high abundance of diatoms, the most populous genera, centric and pennate diatoms, do not produce toxic secondary metabolites. Gong et al. (2017) establishes an algal bloom of large dinoflagellates at 1200 cells/mL while NCDEQ quantifies a bloom as 10,000 cells/mL of a medium-sized dinoflagellate. Although almost all observed centric and pennate diatom cell densities exceed both of these parameters, these concentrations are not seen as an algal bloom because the dominant cells are too small for the abundance to cause bloom effects (Table 3.7). Knowing that the flushing time of the water body is at most two days and that the average chlorophyll *a* concentration in Gallant's Channel is 4 µg/L, we used the exponential growth rate equation A = Pe^rt to calculate the net growth rate shown in dominant diatom species in this watershed. The net growth rate, accounting for grazing mortality is 0.36 (Hall et al., 2008). The net phytoplankton in Gallant's Channel is therefore approximately 8.22 µg/L during the two days that water spends on average in Town Creek. This estimate is a net growth rate and is unrealistic as assumptions used included maximum residence time and the largest apparent growth rate for phytoplankton local to this area. Generally, HAB taxa grow at a slower rate than diatoms (Smayda, 1997). Increasing the nutrient content in this system would therefore have little effect on chlorophyll abundance because phytoplankton would likely experience slower growth and be flushed quickly from the system; however other species of algae, like macroalgae, could be stimulated by nutrient loading.

5. CONCLUSIONS

Nutrient and chlorophyll *a* levels are significant to understanding ecosystem health. In terms of nutrient concentrations over the five sampling dates, we can conclude that there are higher concentrations near the stormwater drains in comparison to the other sampling sites in Town Creek and Gallant's Channel. This could potentially be due to excess nonpoint pollution from runoff but more information would be needed to understand what water is flowing out of the pipes and where it is coming from. In addition, low levels of chlorophyll *a* were observed throughout the study area and no harmful algal blooms were found. Neither the chlorophyll nor the nutrient levels exceed state regulations. Given how quickly the estuary flushes, it seems unlikely that HAB-induced problems would result from direct nutrient loading in Town Creek.

CHAPTER 4: Fecal Contamination and Emerging Pathogens

1. INTRODUCTION

Town Creek hosts a variety of recreational activities, such as SCUBA diving and sailing clubs, in addition to maintaining economic relevance through businesses like marinas. Town Creek is home to several marinas and stormwater outfall pipes which can be sources for various microbial contaminants such as fecal indicator bacteria (FIB) and also provide resources that accelerate the growth of *Vibrio* sp. While not all of the bacteria in these categories are harmful, they are indicators of clinically relevant pathogens and can provide information regarding the risks associated with a specific site (Recreational Water Quality Criteria, 2012). Assessment of these risks is valuable due to the various uses of the waterway that increase the likelihood of people coming into contact with the water. Infections from relevant groups of bacteria are transmitted through direct contact of contaminated water with open wounds or ingestion of water. Considering the activities that take place in Town Creek that range from boating, sailing, and SCUBA diving, water quality monitoring is increasingly pertinent.

Multiple factors influence the water quality of Town Creek, including the physical forces of this system that account for a large portion of changes in bacterial concentrations in the water. Flushing, circulation, tidal influence, anthropogenic factors, and weather all influence the microbial abundances in this system. Fluctuations in environmental parameters including salinity, temperature, and suspended solids result from these processes and can cause variable bacteria abundance in the area.

1.1. Fecal indicator bacteria

Fecal indicator bacteria, *E. coli* and *Enterococci*, are often used as proxies to assess water-associated health risks as there is a demonstrated relationship between heightened concentrations of these bacteria with gastrointestinal and respiratory illness following exposure to the water (Lee et al., 2006). These bacteria do not naturally occur in the estuary and are therefore indicative of upstream sources (Fries et. al, 2008). Concentrations of FIB in surface waters can increase dramatically following a storm event due to runoff from impervious surfaces (Stumpf, 2010). In North Carolina, *E. coli* and *Enterococci* levels are used to monitor recreational water quality due to the combination of cost-effective testing and a strong correlation of these indicators to relevant diseases. FIB are typically quantified using a most probable number (MPN) per 100 mL of sample. Thresholds set by the Environmental Protection Agency define unsafe levels of *E. coli* and *Enterococci* to be in excess of 320 MPN/100 mL and 104 MPN/100 mL, respectively (USEPA, 1986; Fries et al., 2006). While these concentrations are deemed hazardous for recreational use, lower levels of FIB also pose a public health risk.

1.2. Vibrio species

Another important factor when considering the overall microbial water quality of Town Creek is *Vibrio* spp. concentrations. *Vibrio* spp. are found naturally in estuarine systems meaning that minimal levels of these bacteria are expected (Fries et al., 2008). However, increased concentrations of *Vibrio* spp. heighten the risk for bacterial wound infections resulting from exposure to contaminated water. Ecologically, *Vibrio* spp. play important roles in ecosystem function and organismal population dynamics, as they regulate processes such as nitrogen fixation and chitin degradation (Froelich et al., 2019). Therefore, the monitoring of *Vibrio* spp. is integral to the preservation of the Town Creek ecosystem due to the role this species plays in regulating important natural cycles. Furthermore, Town Creek houses an abundance of oysters located in sites that are closed for shellfish harvesting due to FIB concentrations that exceed limits set by the NC Division of Water Quality (Henderson, 2002). Shellfish are filter feeders with the capacity to concentrate waterborne contaminants. An increased abundance of *V. parahaemolyticus* and *V. vulnificus*, bacteria that infiltrate shellfish, corresponds with an elevated risk of bacterial infection for individuals consuming these shellfish (Froelich et al, 2017). If the FIB concentrations declined enough to reopen the waters to shellfish harvesting, having baseline data for the *Vibrio* spp. concentrations would provide a more inclusive understanding of the risks associated with the consumption of Town Creek oysters.

The goals of this project were to identify and quantify strains of bacteria in Town Creek. Both FIB and *Vibrio* spp. are relevant to human health and the risks associated with exposure to contaminated water bodies. Testing at both low and high tide as well as during a variety of weather conditions provided a comprehensive understanding of concentrations and possible sources of contaminants. Additionally, sampling at a variety of sites including storm drains and marinas helped isolate possible sources for microbial contamination. Finally, this study aimed to correlate environmental parameters like salinity, temperature, and total suspended solids to the concentrations of bacteria.

2. METHODS

To assess the water quality of Town Creek from a microbial perspective, four groups of bacteria were monitored: total coliforms, *E. coli, Enterococci,* and *Vibrio* spp.

2.1. Field methods

To assess concentrations of *Vibrio* spp. and FIB in the water of Town Creek in Beaufort, the microbial water quality group collected 1-L samples of water from 10 sites, along with a Field Blank for reference (Fig. 2.1). The field blank was transported and processed identically to the samples. The samples were taken from sites across Town Creek: three in Gallant's Channel (G1, G2, G3), two at nearby marinas (M1, M2), one near the Turner St. bridge (C1), and at four stormwater outfalls (SW1, SW2, SW3, SW4) (Fig. 2.1). The sites in Gallants Channel were chosen as representatives for circulation in Town Creek as water enters this area during an incoming tide and exits during an outgoing tide. The sites near the marinas were chosen due to their proximity to potential illegal dumping activity. The bridge site was chosen because of the location close to stormwater outfalls and ability to represent the main input of freshwater runoff to Town Creek. The stormwater sites were chosen because they are outfalls for stormwater drains across the town of Beaufort, North Carolina. Samples were taken from the aforementioned sites at low tide on 25 September 2019, 9 October 2019, and 16 October 2019. Samples were taken at high tide on 18 September 2019, 2 October 2019, and 16 October 2019. Additionally, samples were collected from the stormwater outfalls after a rain event on 14 October 2019.

Figure 2.1 Aerial imagery of Town Creek in Beaufort, North Carolina. Locations of sampling sites are denoted by pins, along with site marker labels.

The sites located in Town Creek were accessed via boat while a separate team collected storm drain samples from the shore. The samples collected by boat were obtained with gloved hands and the bottles were rinsed three times before the sample was collected and placed into the cooler. The samples collected from land used the same methods during high tide, but during low tide a scoop that held the sample bottle on the end of a one-meter PVC pipe was used for easier site access. The field blank was transported on the boat, and transferred *in situ* between sites G1 and G2. The field blank bottle was rinsed once with deionized water and then filled with the remaining deionized water. The purpose of the blank was to account for any background contamination that may occur due to conditions during sampling or transport. During each sampling date, the field blank was taken at the same time and location and processed using the same methods that were applied to the samples. Coincident with each sample collected by boat, a YSI 6600 multiparameter data sonde was used to measure depth profiles of turbidity, water temperature, salinity, dissolved oxygen, and pH. At stormwater sites collected from shore, salinity and surface temperature data were measured with a refractometer and thermometer.

2.2. Lab methods

After collection, samples were immediately taken to the lab to be processed within six hours for concentrations of total coliforms, *E. coli*, *Vibrio* spp., *Enterococci*, and total suspended solids (TSS).

2.2.1. Total Coliforms, E. coli, and Enterococci

Total coliforms, *E. coli,* and *Enterococci* concentrations were determined through defined substrate technology using IDEXX Quanti® -Tray tests. A 1:10 mL dilution was created for each sample using 10 mL of raw sample and 90 mL of deionized water. Colilert-18® media packets were added to test for *E. coli* and total coliforms while Enterolert™ media packets were added to test for *Enterococci* to the dilution and the bottles were inverted until the media was fully dissolved. The mixtures were poured into IDEXX Quanti® -Tray trays and sealed using a Quanti® -Tray Sealer Plus. Colilert-18® and Enterolert™ trays were incubated at 35 and 41°C, respectively, for 18-22 hours. Wells on the Colilert-18® IDEXX Quanti®-Tray that were yellow tested positive for coliforms. A positive test for *E. coli* was indicated by the fluorescence of yellow wells under a black light. For each bacterium, the most probable number (MPN) was calculated using the IDEXX MPN calculator and then multiplied by 10 to account for the dilution. The MPN quantifies the most likely number of bacteria present per 100 mL of sample water. In the absence of a positive test, a value of 9 was used to indicate concentrations below detection limits. When all the wells tested positive, an MPN value of 2006 was used to indicate the counts were too numerous.

IDEXX Quanti® -Tray tests used trays with either 51 large wells or 49 large wells and 48 small wells. The choice between these was due to availability of materials. The only difference between the trays is the scale on which the MPN is calculated.

2.2.2. Vibrio species

Selective membrane filtration methods were used to quantify the *Vibrio* spp. concentrations in the samples. CHROMagar, a highly selective media, was prepared according to the manufacturer's guidelines. The color of the colony indicates the species of *Vibrio* present. *V. parahaemolyticus* corresponded with mauve colonies, *V. vulnificus* with turquoise colonies, and *V. alginolyticus* with clear/white colonies (Kaysner & DePaolo, 2004). Four total plates were prepared per sample, duplicates for two dilutions. Dilution A consisted of 25 mL of raw sample added to 25 mL of PBS (Phosphate Buffered Saline), while dilution B was the undiluted raw sample. Using a manifold, 5 mL of each dilution was filtered through .45 μm membrane filters. Through this method, the dilution A plates contained 2.5 mL of sample and those of dilution B contained 5 mL.

The plates were then incubated at 37 °C for 24 hours. After 24 hours had elapsed, *Vibrio* spp. colonies were counted and recorded based on color. The counts were later transformed into colony forming units (CFU) per 100 mL by multiplying the counts per plate by 20 for dilution B or 40 for dilution A to scale the filtered volume up to one liter. This value indicates the most likely number of CFU within 100 mL of sample water.

2.2.3. Total suspended solids (TSS)

For TSS, we used glass microfiber filters that were folded in tin foil and dried out in an oven at 55 °C for at least a week prior to filtration. The filters were then weighed inside the foil to determine initial mass. For the first week, we filtered 100 mL of raw sample twice to provide replicates for each sample. For the remainder of processing, we filtered 200 mL of raw sample for each site. After filtering, the filters were placed back in the foil and into the oven at 55 °C for a week. After a week, the filters were weighed again to determine change in mass. The drying process ensured that the only change in mass would be due to the suspended solids in the sample, not any excess moisture. TSS was measured as the final dry weight of the sediment containing filter minus the original weight of the filter. Then, this number was multiplied by 5000 to convert the TSS concentration to mg/L.

3. RESULTS

3.1. E. coli and Enterococci

During high tide, the range of *E. coli* concentrations was 0.954 to 3.302 MPN/100 mL on a log 10 scale. Low tide *E. coli* concentrations were similar to those of high tide, with values ranging from 0.954 to 3.788 log 10 MPN/100 mL. On the other hand, *Enterococci* concentrations relative to high and low tide were not entirely consistent with one another, ranging from 0.954 to 2.505 and 0.954 to 4.019 log 10 MPN, respectively (Figure 3.1.1). The most significant antecedent rainfall occurred prior to sampling on 10/14 (2.16 inches) and 16 October 2019 (3.36 inches) (Figure 3.1.2). These rain events corresponded to increases in FIB concentrations by approximately one order of magnitude (Figure 3.1.1).

Specific to the stormwater outfall sites, the concentrations of *Enterococci* and *E. coli* were approximately one order of magnitude higher during low tide than high tide (Figure 3.1.1). *Enterococci* concentrations relative to stormwater outfalls ranged from 0.954 to 2.772 log 10 MPN/100 mL at low tide and 0.954 to 2.656 log 10 MPN/100 mL at high tide. Similarly, *E. coli* values for outfall sites ranged from 0.954 to 2.656 log 10 MPN/ 100 mL at low tide and from 0.954 to 3.302 log 10 MPN/ 100 mL at high tide. The exception to this pattern occurred across all stormwater outfall sites on 16 October 2019 during high tide, as samples collected during this period contained *E. coli* concentrations ranging from 0.954 to 1.875 log 10 MPN/100 mL and *Enterococci* concentrations ranging from 0.954 to 1.875 log 10 MPN/100 mL. Samples collected from the other sites (C1, M1, M2, G1, G2, G3) on 16 October 2019 during high tide contained comparatively higher FIB concentrations than those collected from the stormwater outfall sites. For these sites during this sampling period (10/16 H), this is evidenced by a range of 1.939 to 3.302 log 10 MPN/100 mL for *E. coli* and 1.875 to 3.302 log 10 MPN/100 mL *Enterococci* (Figure 3.1.1).

Concentrations of *E. coli* and *Enterococci* were higher (1.5 to 2 orders of magnitude) near the stormwater outfalls compared to the sites located in the channel, which contained concentrations ranging from 0.954 to 3.302 log 10 MPN/100 mL (Figure 3.1.1). However, at the channel and marina sites, *Enterococci* and *E. coli* levels were generally in compliance with NC water quality standards for *Enterococci* at 104 MPN/100mL, respectively (Figure 3.1.3). For two sampling events, *Enterococci* levels at site M2 exceeded the threshold by up to one order of magnitude while *E. coli* levels remained below the threshold. At sites M2 and C1 MPN values range from 0.25 to 1 order of magnitude higher than the threshold value. The highest frequencies of exceedance occurred at sites C1 and M2. FIB levels at sites G1, G2, and G3 were the lowest of all sites and never exceeded the recreational water quality limits. Stormwater outfalls were not included in this comparison as they are not regulated by the same standards.

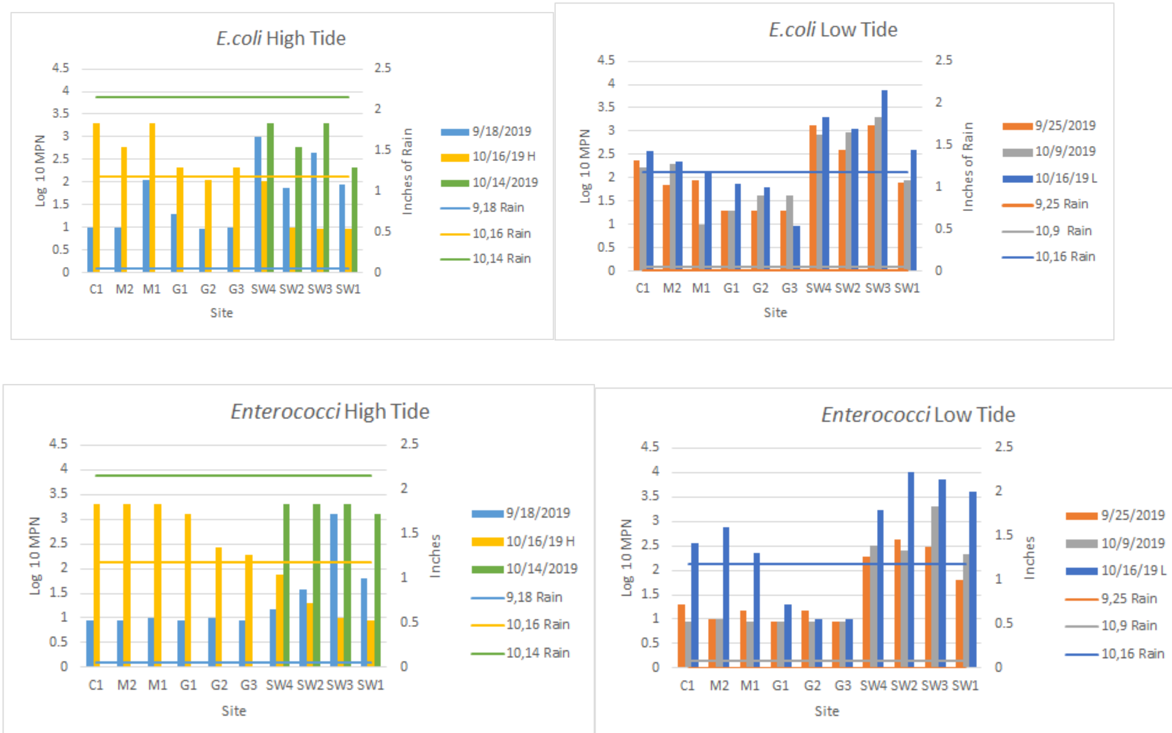


Figure 3.1.1. FIB concentrations for each sampling date categorized by tidal influence. Horizontal lines are plotted on a secondary axis to indicate the amount of rain during the 24-hour period preceding sampling.

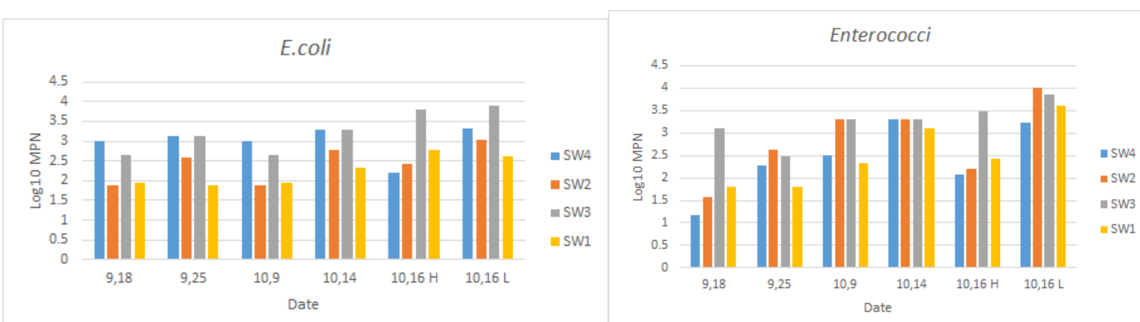


Figure 3.1.2. FIB concentrations organized by sampling date. The blue dots above columns indicate a sampling event with antecedent rainfall. 10/14 and 10/16 experienced 24-hour antecedent rainfall.

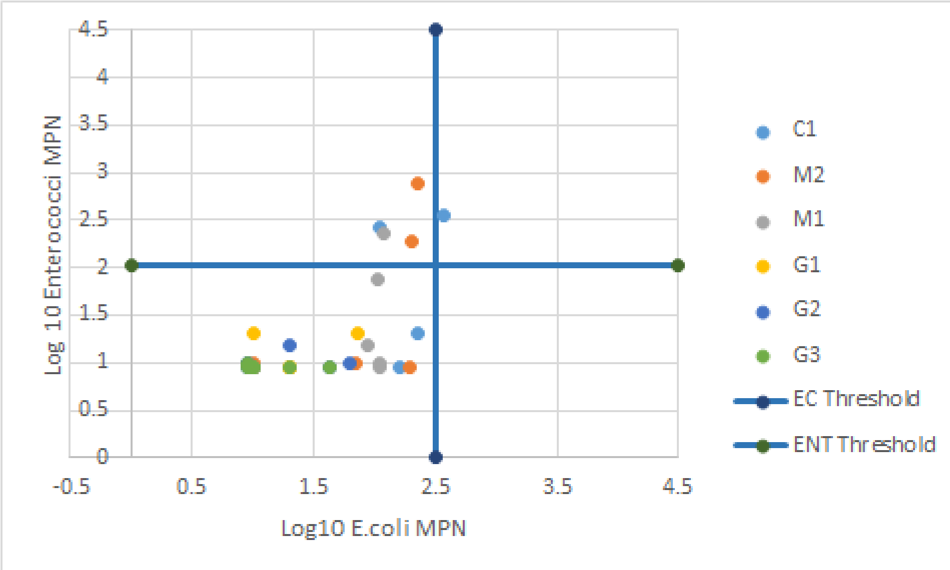


Figure 3.1.3. Enterococci and E. coli plotted in relation to Recreational Water Standards. Solid blue lines indicate the levels of FIB that exceed these standards.

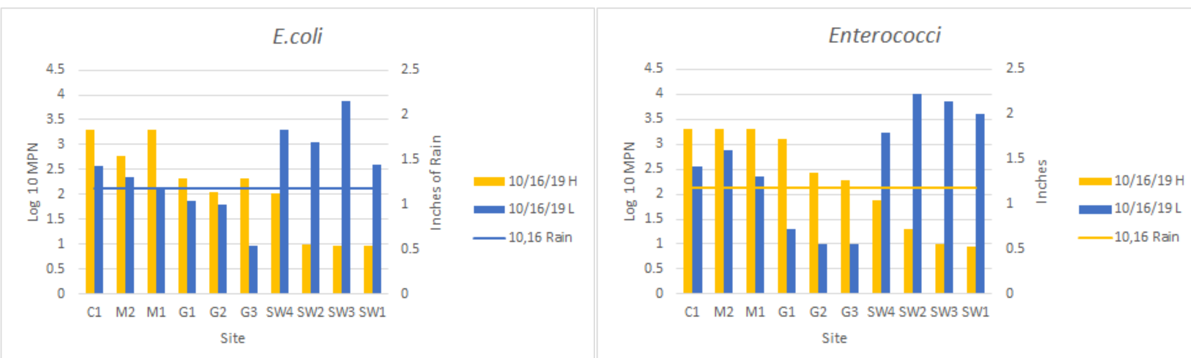


Figure 3.1.4 Juxtaposition of FIB concentrations for 10/16 during high and low tides. Horizontal lines indicate the rainfall 24 hours prior to sampling.

3.2. Vibrio Species

Vibrio concentrations remained relatively consistent from week to week with concentrations typically falling between 2.5 and 3 log10 CFU/100 mL. The sites in the channel exhibited concentrations within these bounds regardless of time in the tidal cycle, but the storm water outfall sites exhibited concentrations ranging from 3 to 3.48 log values per 100mL during low tide (Figure 3.2.1.). This pattern was consistent across all three types of *Vibrio* spp.. On average, the mean concentration for *V. parahaemolyticus* was .586 orders of magnitude higher at the storm water outfall sites than in the channel. Following this trend, the concentrations for *V. vulnificus* and *V. alginolyticus* were .381 and .423 orders of magnitude higher at the storm drains relative to the channel, respectively (Figure 3.2.1.).

Tidal influence proved to be minimal as *Vibrio* spp. concentrations did not change significantly with respect to the tidal cycle (High tide: 9/18, 10,2, and 10/16) (Figure 3.2.1). The exception to this pattern occurred at the storm drain sites on 16 October 2019 during low tide, likely due to a significant rainfall event that extended over multiple days prior to 16 October 2019.

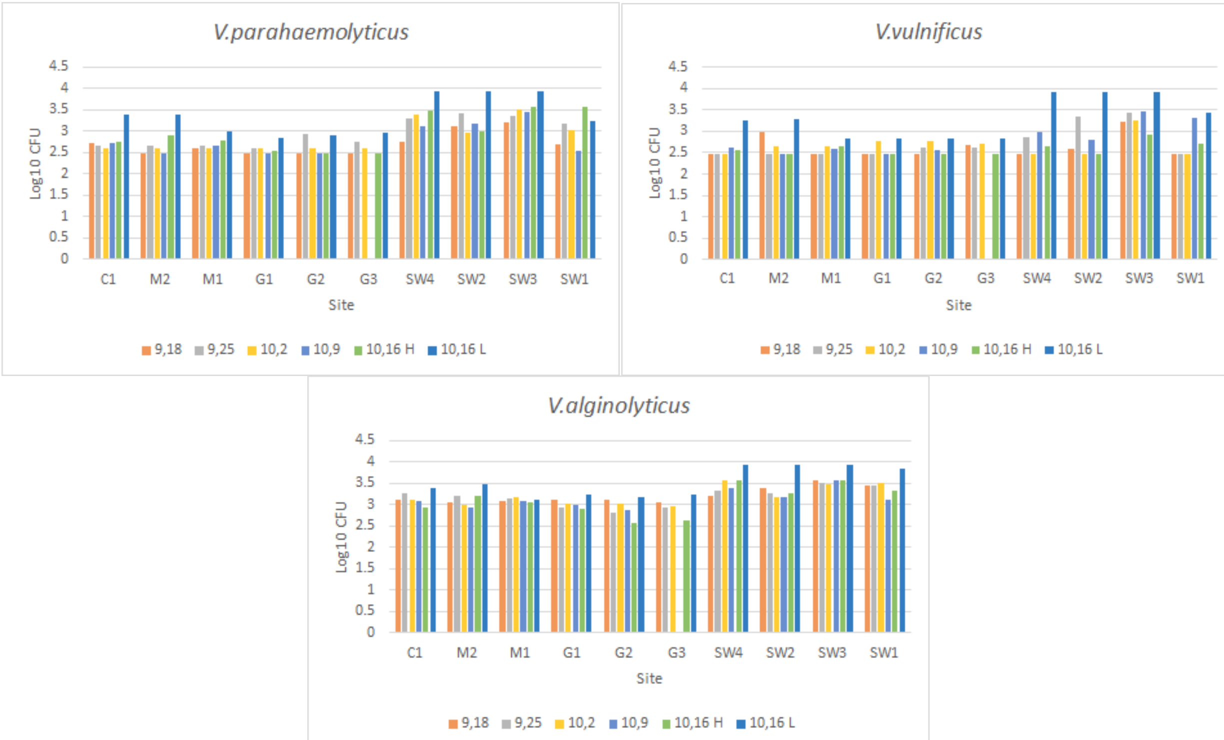


Figure 3.2.1. Shows a time lapse of Vibrio spp. abundance across sampling period. Color bars refer to dates (e.g. 9,18 designates results from sampling on September 18), whereas H and L refer to high tide and low tide sampling for 10/16, respectively (e.g. 10,16 H designates that the green bars correspond to high tide sampling on 10/16). Other instances of high tide are not labelled with H and L as 10/16 is the only day for which double-sampling occurred (High tide dates: 9/18, 10,2, and 10/16).

Weak association was observed between environmental parameters (temperature, salinity, and turbidity) and *Vibrio* spp. populations. A weak (R^2 values: 0.109 to .163) negative correlation was seen between salinity and all three *Vibrio* spp. concentrations. Salinity ranges were higher than the optimal range for the studied *Vibrio* spp. populations, ranging from 23-34 ppt. A slightly stronger relationship was exhibited with temperature. Correlation coefficients for *Vibrio* spp. with temperature ranged from .023 to .224 with *V. vulnificus* exhibiting the weakest relationship. Relationships between all three types of *Vibrio* spp. and TSS were not statistically significant (Figure 3.2.2).

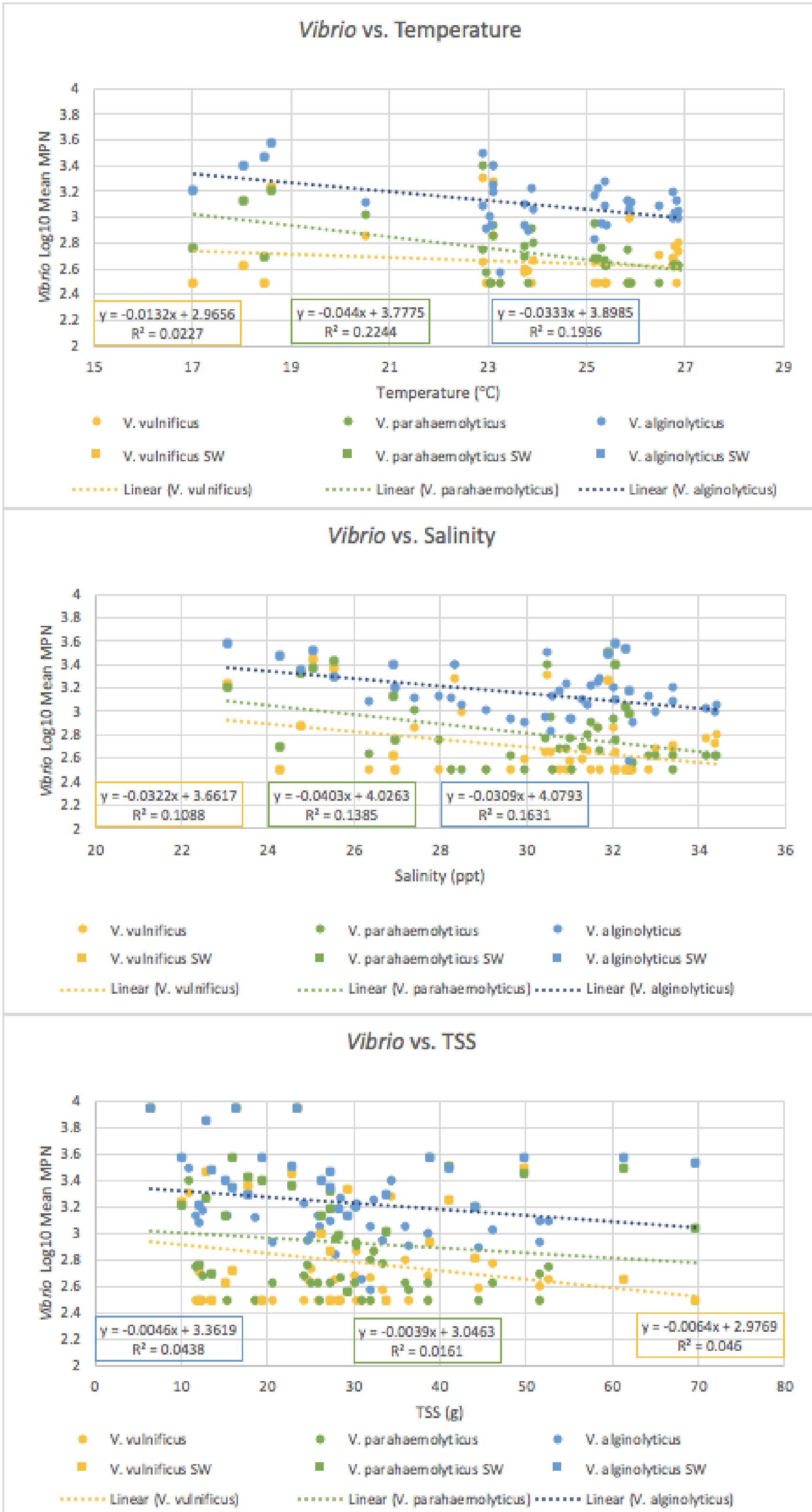


Figure 3.2.2. Relationship between Vibrio spp. abundance and the environmental parameters: temperature, salinity, and TSS. SW designates that a point corresponds to a stormwater site.

The data also exhibit a positive correlation between concentrations of different Vibrio strains (Figure 3.2.3). A strong association exists between *V.parahaemolyticus*, *V.vulnificus*, and *V.alginolyticus* (Figure 3.2.3.).

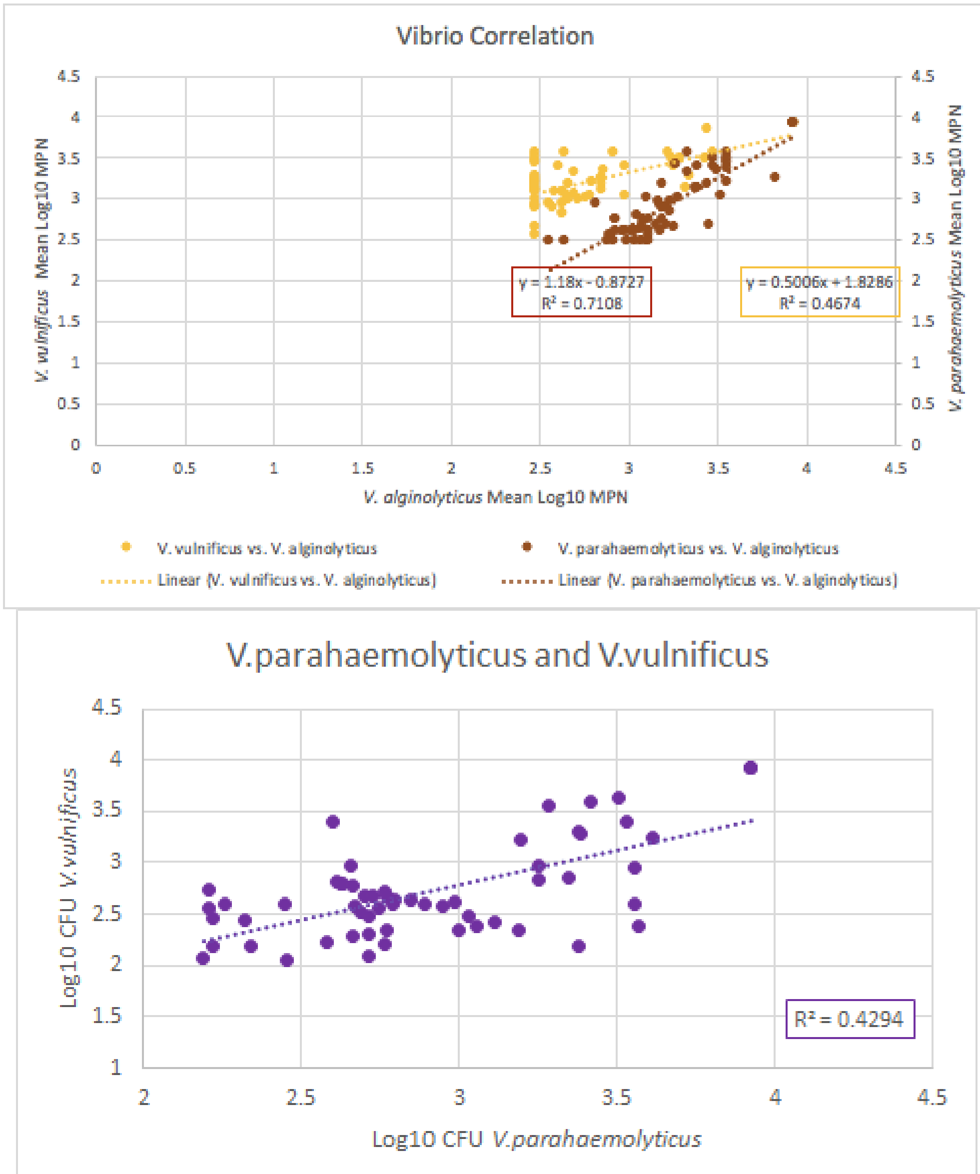
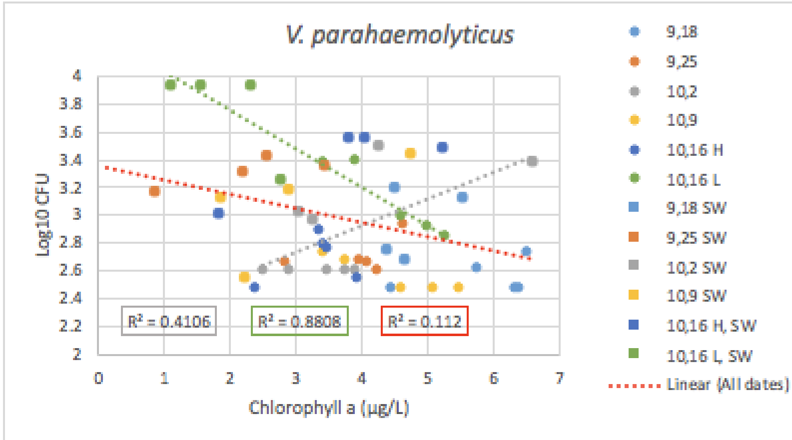
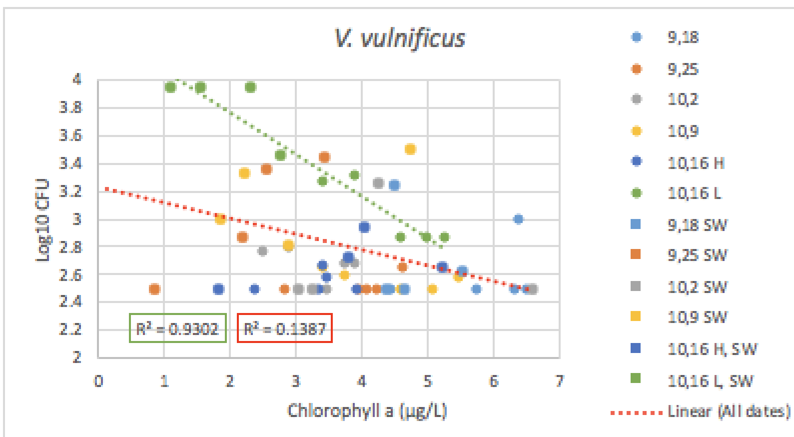


Figure 3.2.3. Correlation between V. vulnificus, V. parahaemolyticus, and V. alginolyticus. Equations and R^2 values are displayed to quantify the relationship.

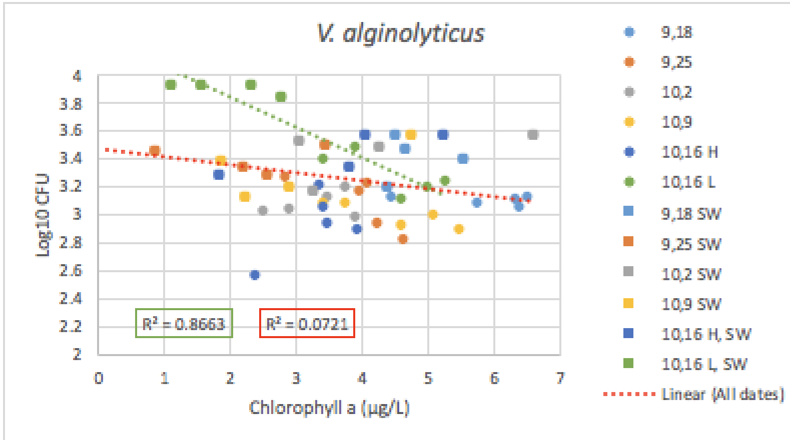
Significant relationships were exhibited between Chlorophyll *a* and *Vibrio* spp. only on 10/16 following a rain event. Correlations were not consistent over time as they were negative as often (or more often in the case of *V.vulnificus*) as they were positive. The strength of the association did not depend on tidal cycle however *V.parahaemolyticus* and *V.alginolyticus* displayed stronger correlation than *V.vulnificus* (Figure 3.2.4).



Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.1032	.3051	.4106	.0949	.2772	.8808
p	.3655	.0978	.0459	.3864	.1179	.000058



Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.0051	.3051	.0051	.1547	.2467	.9302
p	.8446	.0978	.8446	.2608	.1442	<.00001

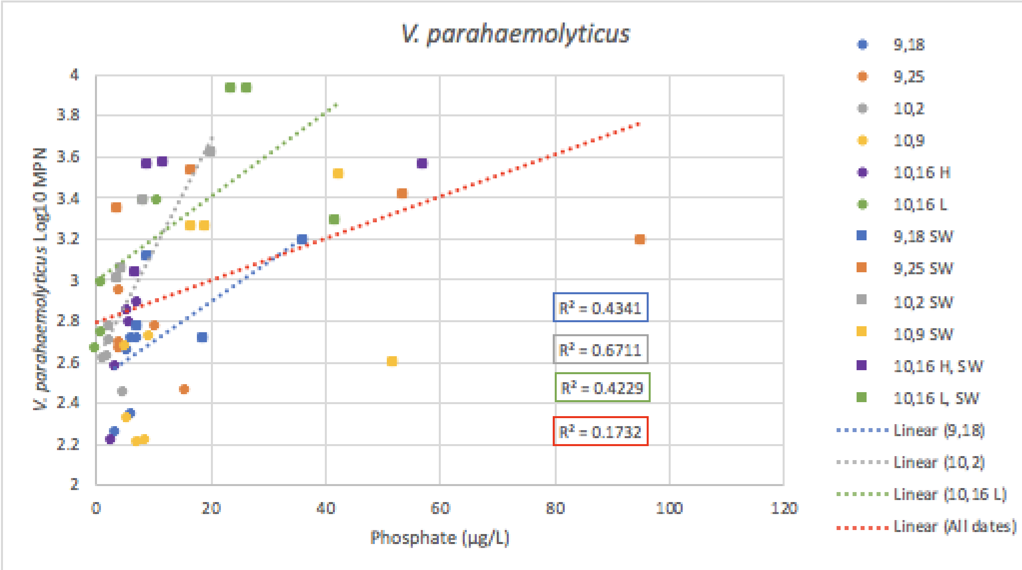


Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.3250	.5252	.3608	.1460	.2696	.8663
p	.8530	.0177	.6007	.2759	.1241	.000092

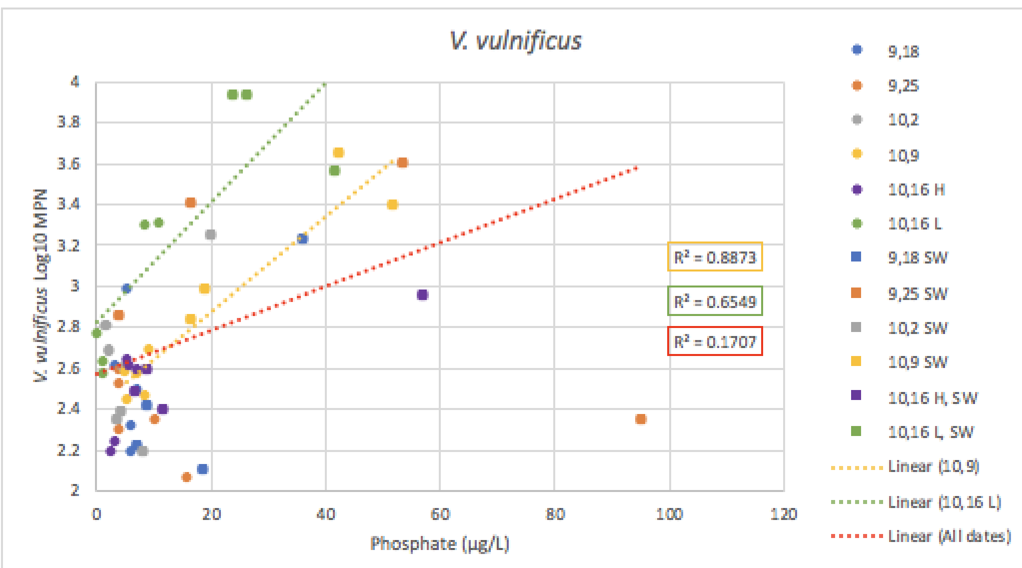
Figure 3.2.4. Shows relationship between each type of Vibrio with Chlorophyll a. Data are plotted based on sampling date. SW designates that a point corresponds to a stormwater site. Values in the table that are highlighted green indicate statistically significant results (p<.05).

Significant relationships occurred between *Vibrio* spp. and nutrient content. This association is supported by the positive correlation in the comparison between *Vibrio* spp. and TDN or Phosphorus. Both high and low tide on 16 October 2019 showed consistently high correlation coefficients. Again, tidal cycles did not influence the relationship between these two variables (Figure 3.2.5.).

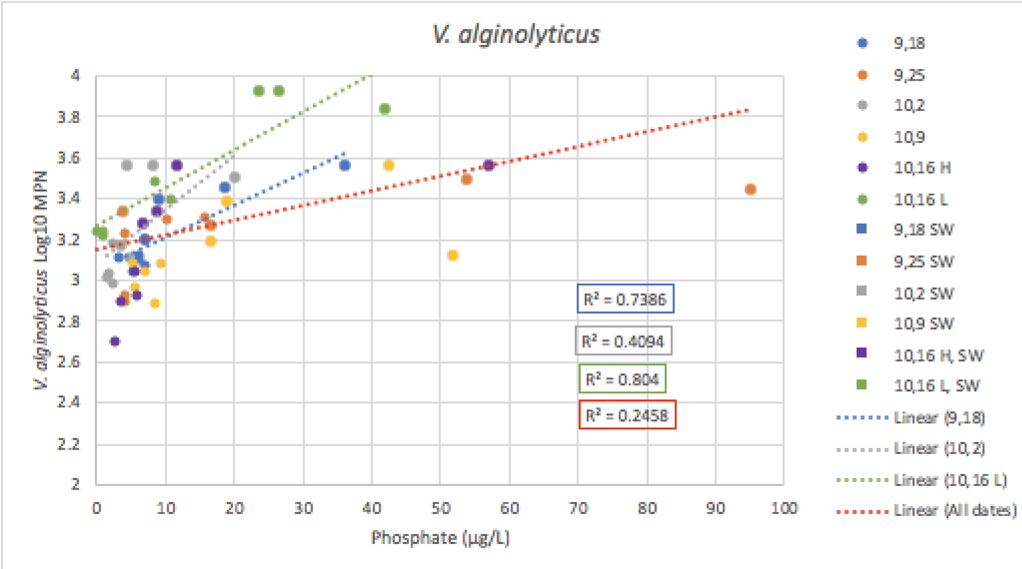
The correlation coefficients for *Vibrio* and Total Dissolved Nitrogen (TDN) range from 0.0229 to 0.8985, demonstrating variability across dates and times. *V. alginolyticus*, showed the highest average correlation with TDN while *V. vulnificus* showed the lowest average correlation (Figure 3.2.6.).



Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.4341	.1424	.6711	.2152	.3195	.4229
p	.0383	.2823	.0037	.1769	.0886	.0418

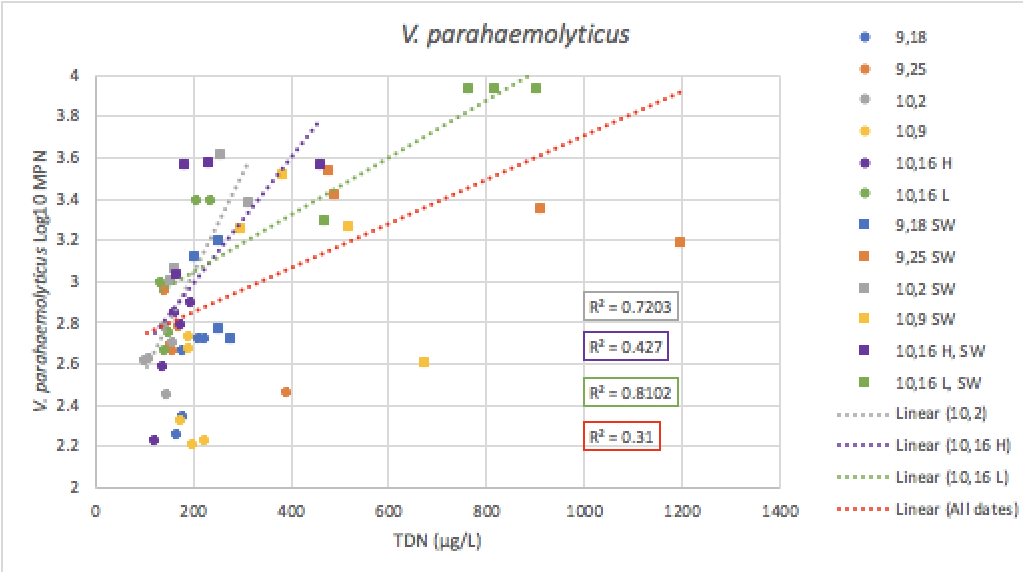


Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.2315	.0168	.2288	.8873	.5450	.6549
p	.1592	.7212	.1620	.000046	.0148	.0046

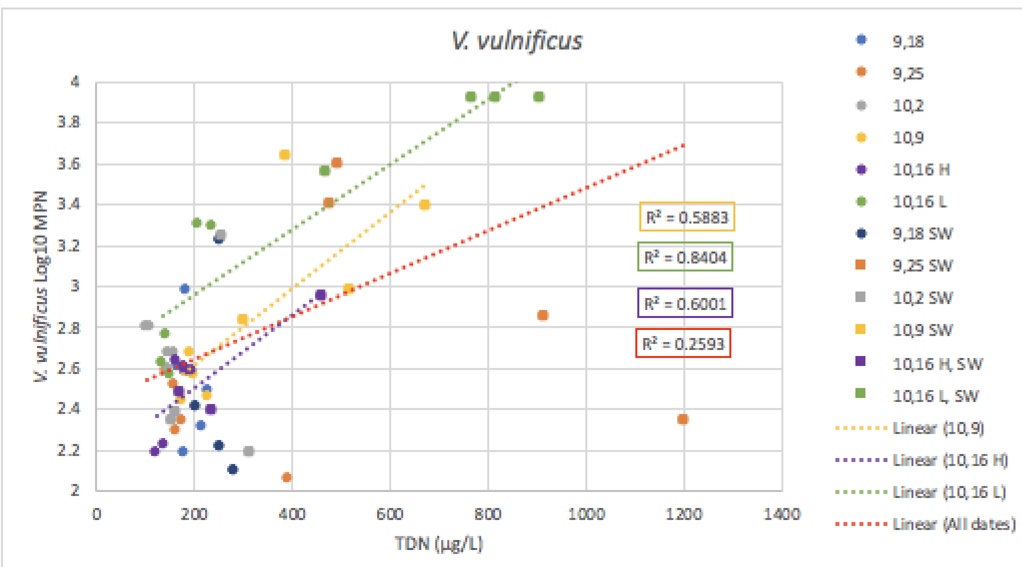


Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.7386	.3811	.4094	.3336	.3819	.8040
p	.0014	.0572	.0463	.0804	.0569	.0004

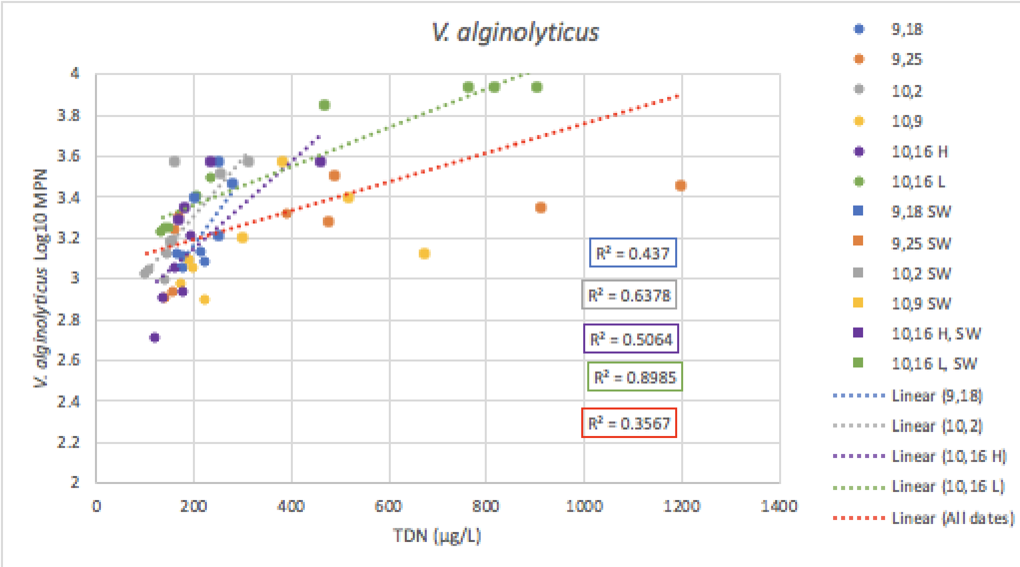
Figure 3.2.5. Vibrio spp. and phosphate concentrations. Values highlighted in green indicate statistically significant results (p<.05).



Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.3066	.3040	.7203	.1650	.4270	.8102
p	.0968	.0985	.0019	.2441	.0404	.0004



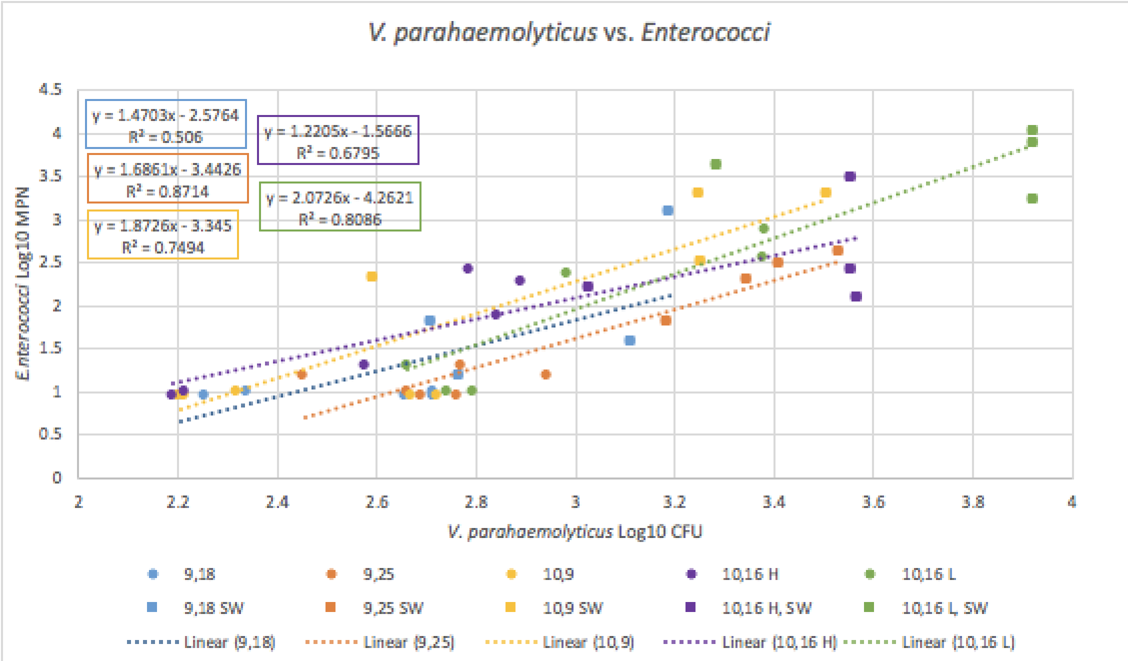
Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.0255	.0169	.0229	.5883	.6001	.8404
p	.6594	.7204	.6765	.0096	.0085	.0002

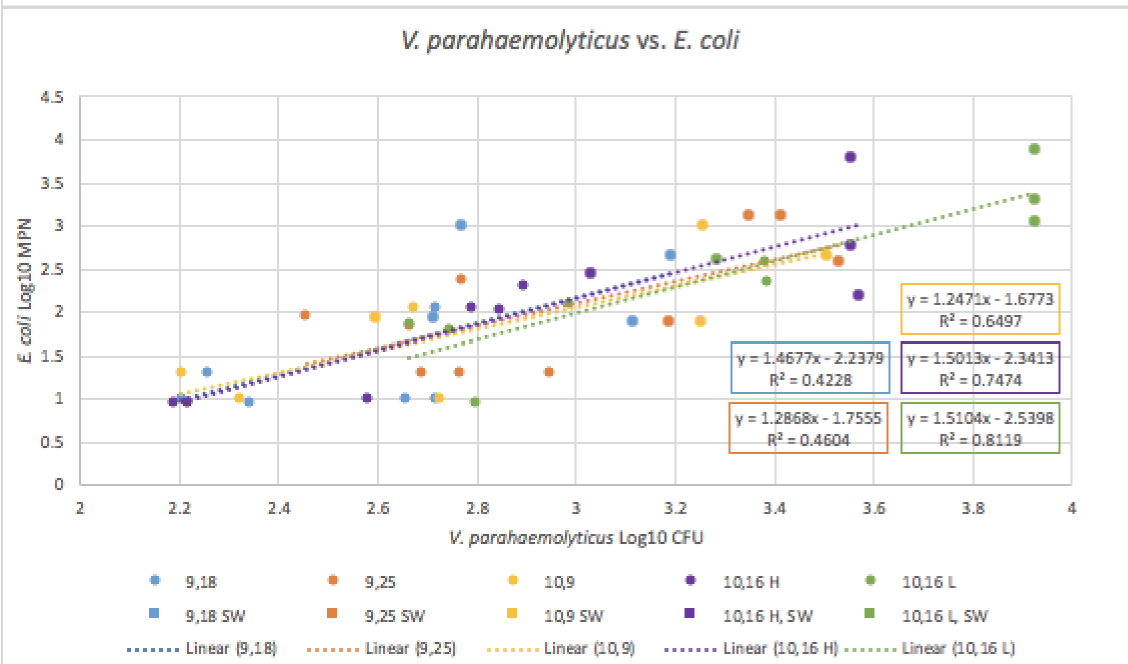


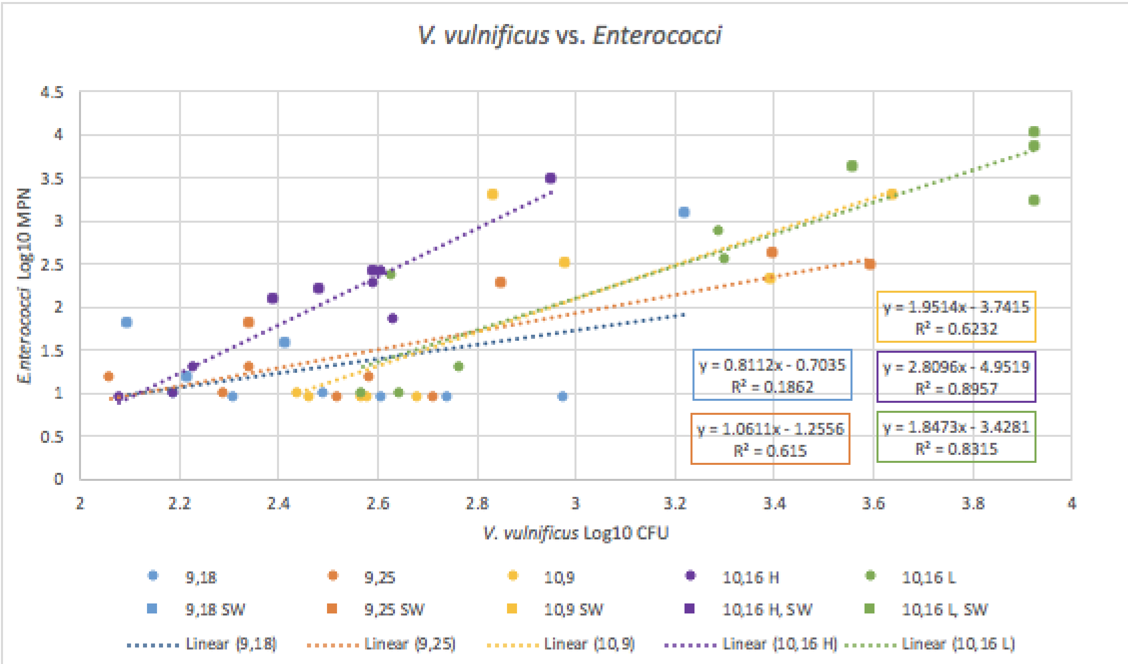
Date	9/18	9/25	10/2	10/9	10/16H	10/16L
R^2	.437	.3966	.6378	.2362	.5064	.8985
p	.0374	.0510	.0056	.1544	.0210	.00003

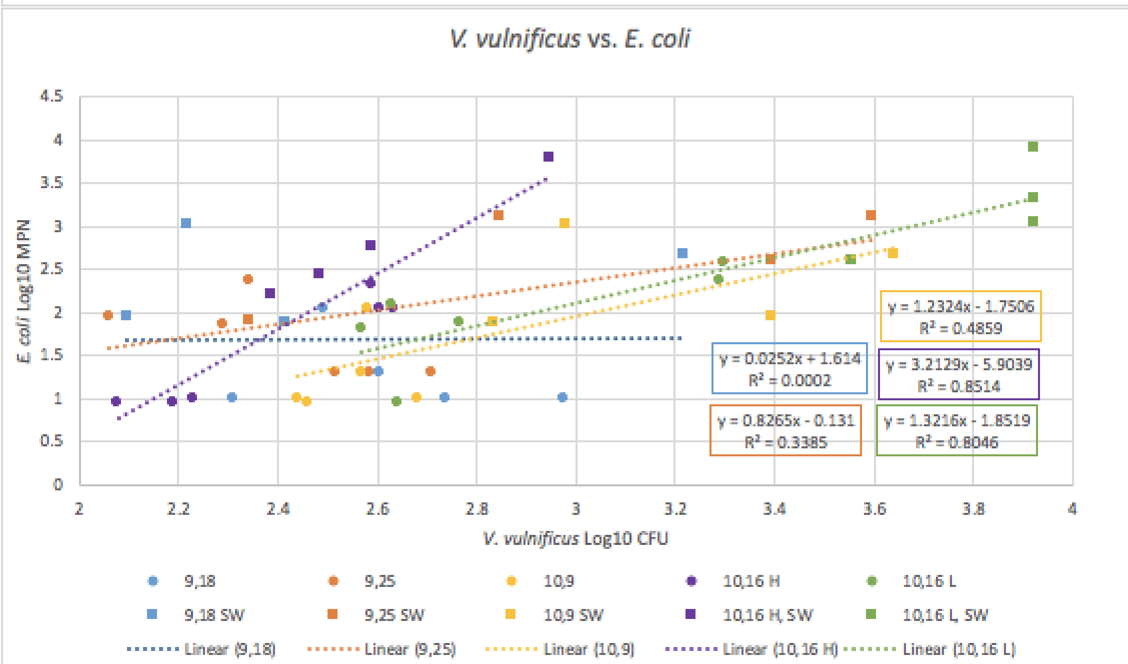
Figure 3.2.6. Vibrio spp. and TDN concentrations. Values highlighted in green indicate statistically significant results (p<.05).

The correlation coefficients for the comparison between *Vibrio* spp. and FIB range from .0002 to .8985, proving to be inconsistently significant. A strong relationship existed across all three types of *Vibrio* spp. with higher correlation typically occurring between *Vibrio* and *E.coli* rather than *Enterococci* (Figure 3.2.7.).


V. parahaemolyticus vs. Enterococci
y = 1.4703x - 2.5764
R² = 0.506
y = 1.2205x - 1.5666
R² = 0.6795
y = 1.6861x - 3.4426
R² = 0.8714
y = 2.0726x - 4.2621
R² = 0.8086
y = 1.8726x - 3.345
R² = 0.7494
Enterococci Log10 MPN
V. parahaemolyticus Log10 CFU
9,18
9,25
10,9
10,16 H
10,16 L
9,18 SW
9,25 SW
10,9 SW
10,16 H, SW
10,16 L, SW
Linear (9,18)
Linear (9,25)
Linear (10,9)
Linear (10,16 H)
Linear (10,16 L)


V. parahaemolyticus vs. E. coli
E. coli Log10 MPN
V. parahaemolyticus Log10 CFU
y = 1.2471x - 1.6773
R² = 0.6497
y = 1.4677x - 2.2379
R² = 0.4228
y = 1.5013x - 2.3413
R² = 0.7474
y = 1.2868x - 1.7555
R² = 0.4604
y = 1.5104x - 2.5398
R² = 0.8119
9,18
9,25
10,9
10,16 H
10,16 L
9,18 SW
9,25 SW
10,9 SW
10,16 H, SW
10,16 L, SW
Linear (9,18)
Linear (9,25)
Linear (10,9)
Linear (10,16 H)
Linear (10,16 L)


V. vulnificus vs. Enterococci
Enterococci Log10 MPN
V. vulnificus Log10 CFU
y = 0.8112x - 0.7035
R² = 0.1862
y = 1.0611x - 1.2556
R² = 0.615
y = 1.9514x - 3.7415
R² = 0.6232
y = 2.8096x - 4.9519
R² = 0.8957
y = 1.8473x - 3.4281
R² = 0.8315
9,18
9,25
10,9
10,16 H
10,16 L
9,18 SW
9,25 SW
10,9 SW
10,16 H, SW
10,16 L, SW
Linear (9,18)
Linear (9,25)
Linear (10,9)
Linear (10,16 H)
Linear (10,16 L)


V. vulnificus vs. E. coli
E. coli Log10 MPN
V. vulnificus Log10 CFU
y = 0.0252x + 1.614
R² = 0.0002
y = 0.8265x - 0.131
R² = 0.3385
y = 1.2324x - 1.7506
R² = 0.4859
y = 3.2129x - 5.9039
R² = 0.8514
y = 1.3216x - 1.8519
R² = 0.8046
9,18
9,25
10,9
10,16 H
10,16 L
9,18 SW
9,25 SW
10,9 SW
10,16 H, SW
10,16 L, SW
Linear (9,18)
Linear (9,25)
Linear (10,9)
Linear (10,16 H)
Linear (10,16 L)

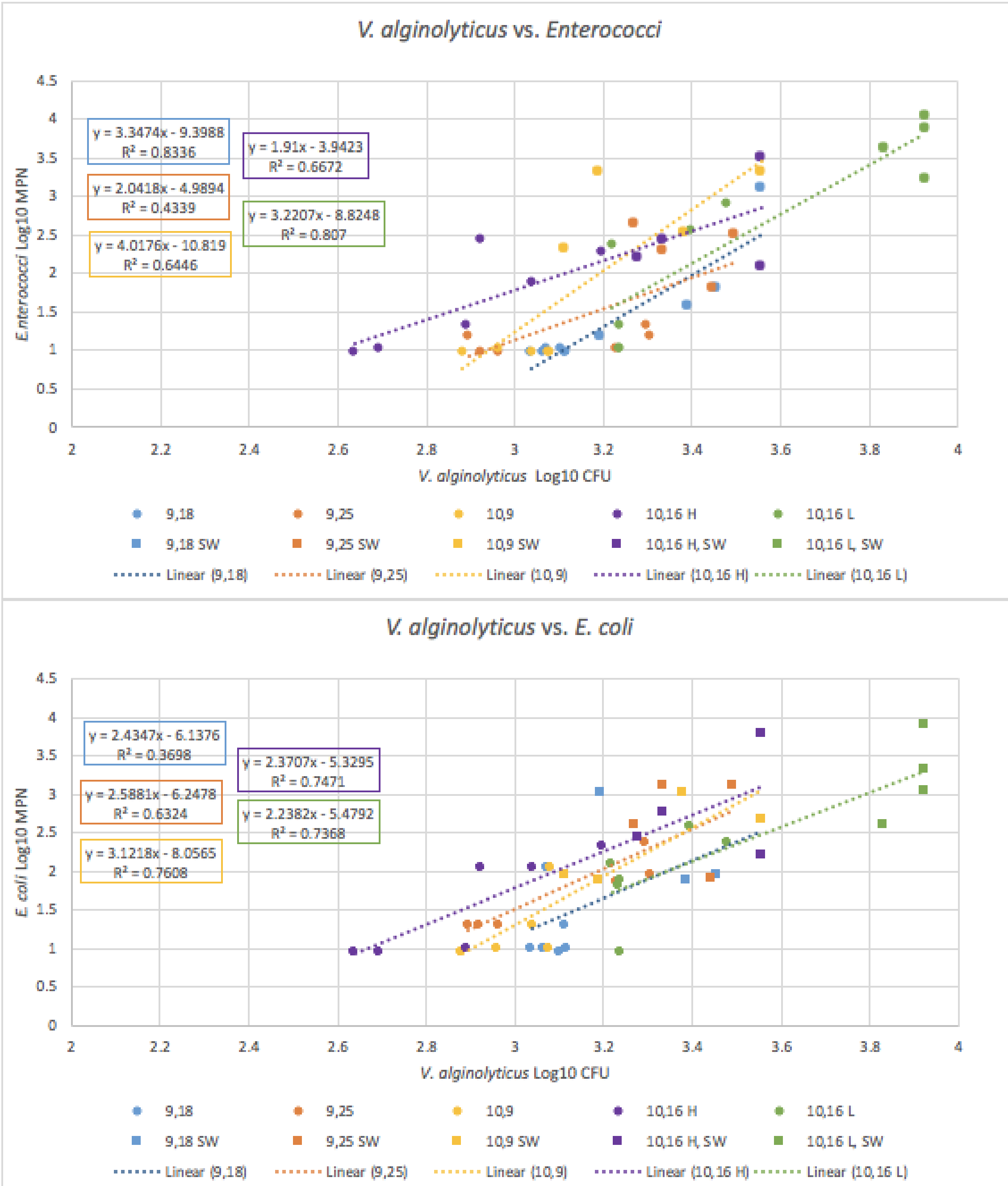


Figure 3.2.7. Vibrio concentrations versus E. coli and Enterococci concentrations over the course of sampling. An outlier corresponding to Site 9, SW 3, from 10/16 low-tide sampling was excluded as it did not align with other data trends.

4. DISCUSSION

Low tide sampling was expected to result in higher concentrations of both *Vibrio* spp. and FIB due to minimal effects of dilution from the tides and uninhibited outflow from storm water outfalls (Fries et al., 2008). High tide sampling was expected to yield lower concentrations of *Vibrio* spp. and FIB and indicate whether or not the bacteria experienced disruptions from tidal changes.

4.1. E. coli, and Enterococci

E. coli, and *Enterococci* concentrations were higher during low tide and storm events, at the stormwater sites (SW1, SW2, SW3, SW4) and in the channel of Town Creek (C1). While the stormwater sites contained consistently high numbers, these sites are held to a different water quality standard, which is why the increased levels of *Enterococci* at sites M1, M2, and C1 are important. Due to the highest concentrations of bacteria occurring at low tide and at the stormwater outfalls, it is apparent that the stormwater outfalls convey the majority of the FIB contamination. Since stormwater outfalls are considered nonpoint sources of contamination, the only conclusion that can be drawn concerning the origin of the bacteria is that the bacteria did not originate in Town Creek, but rather from various locations in the town of Beaufort. This contamination reaches Town Creek through runoff, drainage or seepage. Even though FIB concentrations were elevated following rain events on 14 October 2019 and 16 October 2019, the base flow exiting the outfall pipe still introduced significant levels of FIB (Figure 3.1.1). The group did not expect levels of FIB to exceed 360 MPN/100 mL in dry weather conditions. Due to the transport mechanisms for FIB relying mainly on runoff, the presence of FIB in the system without antecedent rainfall is indicative of a problem within the stormwater transport system (Noble et al., 2003).

Although there was a pattern suggesting that stormwater outfalls convey the majority of FIB to the Town Creek system, there are some exceptions in the data. High tide in Town Creek indicated what was present in the water column and low tide indicated where the bacteria was coming from. Sampling on 16 October 2019 was conducted during both high and low tides, providing the opportunity to compare the influence of tides on concentrations of FIB. The group expected that FIB levels would be elevated on 16 October 2019 due to the rainfall coincident with sampling however, sampling conducted at high tide resulted in FIB concentrations at the stormwater sites that were 2 orders of magnitude lower than levels at low tide (3.1.4). Stormwater concentrations were also 1 order of magnitude lower at the stormwater sites than at the channel sites, a pattern that contradicts what normally occurs (Figure 3.1.4). This difference could be accredited to the ability of FIB to grow in estuarine systems or resuspension that occurs as a result of rainfall or high winds (Parker et al., 2010). These possibilities designate reasons why FIB are not ideal indicators for water quality. FIB that grow in estuarine systems provide inflated estimates for the number of contaminants that can be attributed to human sources. Additionally, FIB that adsorb to sediment and settle on the seabed of the Town Creek water system would persist in the environment undetected when only surface samples were collected.

While the base flow through the stormwater outfalls proved significant to the system, there were elevated levels of FIB following rainfall events (Figure 3.1.3). Considering that runoff mediates the transport of FIB, this increase is not surprising. The greatest difference in FIB between dry and wet weather occurred at site SW1 (up to half an order of magnitude for *E.coli* and 1 order of magnitude for *Enterococci*) (Figure 3.1.3).

In contrast to the stormwater outfalls, sites located farther in the channel of Town Creek exhibited relatively low concentrations of FIB (Figure 3.1.1). The USEPA has set a threshold value of 320 MPN for *E. coli* and 104 MPN for *Enterococci* to determine when recreational water advisories should be issued. The majority of sites exhibited agreement in terms of recreational water standards, meaning that if *E. coli* levels complied with the standards, so did

Enterococci and vice versa (Figure 3.1.2). This represents a trend of acceptable water quality in Town Creek based on the criteria for FIB on a non-weather impacted day. After rainfall events, elevated levels were observed because of increased drainage from stormwater outfalls. However, sites C1, M1, and M2 demonstrated occasional exceedance of these values which calls for continued monitoring (Figure 3.1.3). With the exception of site C1, violation of recreational water standards only occurred with regard to *Enterococci* concentrations (Figure 3.1.3). Site C1 is located near Turner St. Bridge, near two stormwater outfalls and between three marinas, possibly explaining the high levels of both *Enterococci* and *E. coli.* One of the goals of our project was to monitor marinas for increased levels of FIB, which can be attributed to faulty pump out stations or dumping of on-board waste, however, we are unable to prove any true correlation to these factors considering we did not observe these activities.

The low counts of these bacteria throughout the sites in Gallants Channel (G1, G2, G3), indicate efficient flushing or circulation since the concentration of bacteria is consistently lower than concentrations at the stormwater outfalls. Gallants Channel sites never exceed the MPN standard for either *E. coli* or *Enterococci* (Figure 3.1.3).

4.2. Vibrio spp.

The highest counts of *Vibrio* spp. occurred in samples taken from the stormwater outfalls (SW1, SW2, SW3, SW4) (Figure 3.2.8). Since *Vibrio* spp. occur naturally in the estuary, the conditions of the environment around the outfalls could be conducive for growth, and therefore responsible for the high counts seen at these sites. Tidal influence did not play a significant role in the concentrations of *Vibrio* spp. throughout the sampling period, as CFU/100mL typically ranged from 2.5 to 3 orders of magnitude, regardless of tidal cycle. The higher concentrations at the stormwater outfalls suggest that runoff from the surrounding area is supplying nutrients and substrate that facilitate elevated growth rates of *Vibrio* spp., such as phytoplankton (Hsieh et al., 2007). Furthermore, *Vibrio spp.* are naturally occurring in estuarine systems, which introduces a confounding factor when attempting to characterize the overall water quality in terms of *Vibrio* spp.

A weak correlation was observed among all strains of *Vibrio* with respect to TSS, but a stronger correlation existed with temperature (Figure 3.2.2). The lack of correlation with TSS was unexpected as suspended solids provide a substrate on which *Vibrio* tend to aggregate (Fries et al., 2008). Interestingly, not all strains showed the same degree of relation as *V. parahaemolyticus* and *V. alginolyticus* were more tightly correlated with temperature than *V. vulnificus.* These results agree with a decadal study by Froelich et al. that cites temperature being a leading driver of *Vibrio* spp. populations, while his study shows positive correlation, and this one does not, it is still indicative of a connection between the two. The negative correlation in this study was an unexpected result as *Vibrio* spp. concentrations are expected to increase with the temperature. *Vibrio* are associated with higher water temperatures, causing an anticipated positive correlation as observed by Froelich et al. (2017).

Salinity is also thought to be a major factor in determining *Vibrio* concentration (Froelich et al., 2019). Our results agree with this, though weak correlation was displayed (Figure 3.2.2). The weak correlation is possibly due to the relatively static state of Town Creek's water motion in comparison to Gallants Channel, as this relationship is indicative of lesser tidal influence throughout Town Creek. All three strains of *Vibrio* were similarly correlated with salinity. Typical acceptable salinities for *Vibrio* typically reach a maximum of 27.56% in estuarine environments, and observable decreases in *Vibrio* spp. populations are seen at salinities of ~30 ppt (Froelich et al., 2019) (Kaspar and Tamplin, 1993). The salinities in Town Creek ranged from 23-35% which are in excess of the previously determined maximum value in the study on the Neuse River Estuary. *V. parahaemolyticus* has an established salinity range of 10-30 ppt while *V. vulnificus* is typically restricted to environments with lower salt content (5-15 ppt). Our results show higher than expected *Vibrio* abundances according to the established preferred environments either due to inherent capabilities or through population-specific adaptations of the *Vibrio* in Town Creek.

Because all three species of *Vibrio* spp. are persisting in similar salinity and temperature ranges, another explanation for the higher than average *Vibrio* spp. levels exists in the correlation between Chlorophyll *a* and *Vibrio* spp. (Figure 3.2.3). Measuring Chlorophyll *a* provides a proxy for phytoplankton in the system and can therefore be used as an indicator of *Vibrio* spp. (Hsieh et al., 2007). *Vibrio* frequently attach to particles, phytoplankton in particular, due to the numerous sugars and compounds available for assimilation (Hsieh et al., 2007). This possibility is further supported by the high nutrient levels in the samples (Figure 3.2.6 and Figure 3.2.5.). High levels of nutrients support greater phytoplankton growth, and therefore, provide *Vibrio* spp. with a food source, as well as a substrate to grow on, the exception to this being the inverse proportions of chlorophyll a to *Vibrio* spp.. An unexpectedly weak connection was observed between *Vibrio* spp. abundance and Chlorophyll *a* however, there were stronger positive correlations among *Vibrio* spp. and nutrient contents (Figure 3.2.5 and 3.2.6). The connection to nutrients could also explain the heightened abundance of *Vibrio* spp. on 16 October after the rain event. Rain results in runoff that carries nutrients, like TDN and phosphate, into the estuary and causes phytoplankton to increase and subsequently, *Vibrio* spp. abundance. Rainfall could also lower the salinity of Town Creek to create a more hospitable environment for the *Vibrio* spp., allowing their growth rate to increase.

Along the same lines, the group expected to see a higher correlation between TSS and *Vibrio* spp. as suspended solids provide these bacteria with a surface on which to aggregate. All three types of *Vibrio* spp. exhibited a weak relationship with TSS, which was an unexpected result and indicates that another factor is driving the abundance of *Vibrio* spp. (Figure 3.2.2). *Vibrio* spp. are able to live suspended in the water column as well as in association with sediment and other particles. The lack of connection between these two variables suggests that the *Vibrio* spp. in Town Creek are predominantly located in the water column rather than the sediment.

4.3 Comparison between Vibrio spp. and FIB

A comparison of *Vibrio* spp. and FIB revealed highly variable relationships (R^2 values ranging from 0.0105 to 0.7479) (Figure 3.2.7). The correlation coefficients for sampling date 2 October 2019 consistently stayed below 0.5, indicating a poor relationship between FIB and *Vibrio* spp. A clear pattern emerges among the samples taken on 18 September 2019, 2 October 2019, and 16 October 2019 H as correlation coefficients were reported in excess of .7 among all relationships with *E. coli*; however, low correlation (R^2 < 0.1) was reported for relationships with *Enterococci* (Figure 3.2.7). With higher amounts of suspended solids, higher counts of both FIB and *Vibrio* spp. were expected due to the tendency of these bacteria to adhere to the sediment (Lee et al., 2006). In terms of TSS, FIB and *Vibrio* spp. are expected to behave similarly (Lee et al., 2006) (Fries et al., 2008). High correlation was not expected between FIB and *Vibrio* spp. as recent studies demonstrate that these bacteria typically do not behave similarly, but rather act independently (Fries et al., 2008). The exhibited correlation could be due to high levels of sediment in the water column, as this is the one variable that might result in similar behavior among the bacteria. *Vibrio vulnificus* showed the lowest correlation of the three species with FIB, specifically the lowest for *Enterococci* (Figure 3.2.7). The other *Vibrio* spp. demonstrated overall positive correlations between the species and FIB, which was interesting since they are thought to act independently. In this case, since we see some correlation between *Vibrio* spp. and FIB, the ambient conditions of the water may be conducive for consequential and parallel growth between the two.

Notably, the procedures utilized are only capable of indicating the abundance of bacteria in the water, not isolating the pathogens. The proportion of the bacteria that are pathogenic is unknown, as quantification would require additional laboratory processing. Furthermore, the general population is not likely to contract associated illnesses and infections due to *Vibrio* spp. Individuals who are over the age of 40, immunocompromised, diabetic, and are male are more susceptible to infections caused by the potential presence of pathogens. *Vibrio* spp. are found naturally in estuarine systems. However; increased abundances of *Vibrio* heighten the risk for bacterial wound infections as there is a greater likelihood of coming into contact with a pathogenic strain (Fries et al., 2008).

5. CONCLUSION

TSS, *Vibrio* spp., and FIB concentrations were consistently higher in samples collected during low tide and comparatively lower in samples collected during high tide. Geographically, the storm water outfall sites had the highest concentrations, followed by the sites located in Town Creek, including those surrounding marinas. The sites in Gallants Channel yielded lower concentrations throughout the course of sampling and rarely exceeded recreational water quality standards for FIB. These findings suggest the possibility that high bacterial concentrations are entering Town Creek via the stormwater outfall pipes, are resultant of nonpoint sources of contamination, and are being diluted and pushed into the channel by tidal fluctuations. Therefore, continued monitoring is necessary to ensure that these remain compliant with state standards, especially given the high levels of FIB stemming from the stormwater outfalls (sites SW1, SW2, SW3, SW4).

Overall, the physical factors influencing water motion in Town Creek caused a distribution change in the concentrations of bacteria throughout sampling. The FIB concentrations, even diluted by the water in the Town Creek system, still exceeded state standards for a significant amount of time during the sampling window.

CHAPTER 5: Filtration by Marshes

1. INTRODUCTION

It is well understood that marsh habitats provide many valuable ecosystem services (Barbier et al., 2011). These services include regulating biogeochemical cycles and other processes which not only promote ecosystem health, but also have direct and indirect benefits to humans such as clean air, water and soil, and nutrient regulation (de Groot, 2002). The service that was most important to our group was nutrient regulation. Reducing the nutrients present in coastal waters is important as excess nutrients cause a number or detrimental outcomes including eutrophication. Eutrophication refers to the proliferation of phytoplankton and algae due to the presence of excess nutrients. The increasing frequency of these events has led to more low oxygen or hypoxic zones in the water, harmful algal blooms, and finally food web disruption (Paerl et al., 2018). Town Creek has fringing marsh habitat that exists in patches around its perimeter. The two components of marshes that were the focus of this project were the common marsh grass, *Spartina alterniflora*, and denitrification that occurs in marsh sediment.

Our group was interested in understanding the role that the marsh plays in improving the water quality of Town Creek. More specifically, we wanted to explore how marsh habitats can remove and sequester pollutants. We focused on nitrogen (N) and phosphorus (P) as our pollutants. Our primary focus was to survey how effective existing marsh habitats can be in removing pollutants from the water. To do this, we surveyed the area and density of *Spartina alterniflora* in Town Creek because *Spartina* takes up and stores these nutrients in plant tissues, removing them from the water. The other focus was bacterial nitrogen cycling in marsh sediment. Specifically, the goal was to understand the extent that sediment dwelling bacteria conduct denitrification. This was achieved by continuous flow experiments with intact sediment cores.

1.1 Spartina alterniflora

Spartina is nearly ubiquitous in temperate estuary systems such as those found in North Carolina (USDA, 2019). Town Creek, a tidal stream located in Beaufort, NC, is no exception. Spartina alterniflora grows in dense patches along the periphery of the basin of Town Creek. In salt marshes dominated by Spartina alterniflora, nutrient uptake and production can be influenced directly by nutrient availability. Nitrogen (N) applications increase overall yield and tissue concentrations of N (Osgood and Zieman, 1993). While phosphorus can limit growth rates in Spartina alterniflora, N is often accepted as the limiting nutrient in Spartina dominated salt marshes (Osgood and Zieman, 1993).

Spartina dominated salt marshes such as that in Town Creek improve water quality by taking up nutrients like nitrogen and phosphorus by storing, processing, and assimilating these elements in their biomass thereby removing these nutrients from the water (Hill et at., 2018).

1.2 Nitrogen cycling

Nitrogen cycling is a biogeochemical process and ecosystem service that is associated with estuarine habitats (Gutiérrez and Jones, 2006) (Kunu et al., 2003). Nitrogen is particularly important as N limits primary production in most coastal waters (Paerl et al., 1997). In sediment, simplified nitrogen cycle "begins" with atmospheric nitrogen in the form of N_2 gas. N_2 is fixed into ammonia (NH_3) by nitrogen fixing bacteria before the ammonia is converted under aerobic conditions into nitrites and nitrates (NO_2^- and NO_3^-) by nitrifying bacteria. Finally denitrifying prokaryotes convert nitrate to N_2 in anaerobic conditions. This process is referred to as denitrification (DNF). The N_2 escapes into the atmosphere as gas, removing nitrogen that was once in the water flowing over the sediment. This cycle is made more complex by the fact that nitrogen fixing bacteria need aerobic conditions, while, denitrifying bacteria need anaerobic conditions (Brush, 2008). The amount of oxygen needed by these bacteria is known as sediment oxygen demand, or SOD, and has been used to predict denitrification (DNF) rates (Cornwell et al., 1999). In systems like Town Creek, the nitrogen source could come from nitrite and nitrate contained within lawn fertilizer or wastewater in runoff. During heavy rainfall events, water can carry the nutrients from the lawns and streets of Beaufort into Town Creek. When nutrients are present in high concentrations under appropriate conditions, denitrification can transform and remove nitrogen from the aquatic system into the atmosphere improving water quality (Seitzinger, 1999).

1.3 Phosphorus Cycling

Similar to Nitrogen, Phosphorus (P) can be a limiting nutrient (Filippelli, 2002). P is cycled through a variety of methods because of the lack of new P availability in the ecosystem (Filippelli, 2002). P is most common in bedrock and soils making it less bioavailable than other more common elements (Ruttenburg, 2003). When P is released through the geochemical processes of weathering through erosion or soil development, the proportion of P that is both released and bioavailable, is low (Filippelli, 2002). After weathering, P is first available in organic matter in the soil, then phosphatase is released by plant roots and microbes to extract inorganic P as a useable form (Filippelli, 2002). This deposition becomes a source of fuel for microbial communities (Ruttenburg, 2003). For plants, this can become a site for roots to uptake high concentrations of P from the soil (Filippelli, 2002). A common cause of high P concentrations in water comes from the use of P containing fertilizers used in agriculture (Paerl et al., 2016).

In the aquatic environment, the exchange of P relies heavily on phytoplankton and bacteria. The bacteria release bioavailable P through the decomposition of organic matter, and phytoplankton consume P as they grow (Sundby et al, 2003). A P-rich layer of sediment and soil exists dynamically as new and old sediment is deposited and overturned by the water (Sundby et al, 2003). Aquatic vascular plants such as marsh grasses in estuaries, are able to transport P from deep sediment through uptake processes and into the water column. Once the plants decompose, the P is utilized by phytoplankton and microbes keeping P cycling through the system. (McRoy et al, 2003).

Spartina Alterniflora accounts for the majority of marsh grass on the southeastern coast of the United States and is considered to be one of the dominating factors in phosphorus cycling through uptake from sediments and release through bacterial degradation (Reimold, 2003). The cycling of phosphorus in estuarine ecosystems is important considering the limited phosphorus inputs. Town Creek has Spartina Alterniflora salt marshes, which help cycle P within the marsh and surrounding ecosystems.

2. METHODS

2.1 Spartina alterniflora Survey

Smooth cordgrass (*Spartina alterniflora*) was collected by hand from the marsh on the perimeter of Town Creek in Beaufort, NC. In total, 9 locations were selected for a quadrat survey. The quadrats themselves were 20.32cm x 20.32cm, .0413m^2 squares made of PVC piping. The sampling sites in Town Creek were, ‘Kayak’, a site west of the Beaufort public boat ramp, ‘Public Access’, a site east of the public boat ramp, and ‘Bridge’, a site adjacent to the Turner Street bridge (Figure 1).

Figure 1. An aerial image from Google Earth of Town Creek. The ocean, Gallant’s Channel and the marsh on the other side of Turner Street Bridge (upstream marsh) represent some of the areas where water enters Town Creek. The site in blue loosely represents the Kayak site, the site in green loosely represents the Public Access site, while the site in red loosely represents the Bridge site. The stars indicate the areas where quadrats were haphazardly taken. The color of the star corresponds with the color outlining the site where the survey was conducted.

A kayak was used to access all of the sites. At each site three quadrats were placed haphazardly to ensure no bias. Within each quadrat, the number of shoots were counted at their base. This number was used to calculate the density of the marsh in shoots per unit area of the quadrat. The area of one quadrat was then scaled to represent 1m^2 of marsh. Once the count was completed, 3 randomly selected shoots from within that quadrat were cut at their base and removed from its place in the marsh and stored with the other samples from that site. The sample helped to estimate the total biomass of that area, and later nitrogen and phosphorus content. The shoot density of the marsh east of Turner Street Bridge (upstream marsh) was not measured. As a result, the value for the average mass of *Spartina* g*m^{-2} was applied to the upstream portion of the marsh in any calculations.

2.2 Analysis of Total Nitrogen and Phosphorus Present from Spartina alterniflora Biomass

Once collected the samples of Spartina alterniflora were cut into smaller pieces and stored in foil. The samples were dried at 60°C for 2 weeks using a drying oven. After they were dried, the samples' dry weights were measured with a scale, yielding the aboveground biomass. First, by dividing the shoot count by the area of the quadrat, the number of shoots per 1m^2 or shoot density was found. The product of the average aboveground mass of an individual shoot and shoot density gave the aboveground mass of Spartina per 1m^2 area of marsh. These values were different for each of the three survey areas as the masses and densities varied between sites. The mass of Spartina in each 1m^2 area was multiplied by the total area of each marsh taken from the calculation of marsh area (Section 2.4) yielding the aboveground biomass of Spartina within each area of marsh.

Darby and Turner (2008) related the aboveground biomass (dry weight) to belowground productivity (biomass) of Spartina, this value is known as the root to shoot ratio. In their study they found an average root to shoot dry weight ratio of 2.6:1. Therefore the mass of the belowground biomass was calculated by taking each marsh area's aboveground biomass and multiplying it by 2.6. The product represents the estimate for belowground biomass of Spartina present in each marsh area. The aboveground and belowground biomasses were summed to find the total biomass of each marsh area.

The total amount of nitrogen and phosphorus present in one square meter of Spartina dry weight came from literature values. Values from Osgood and Zieman (1993) were utilized to relate the dry mass of Spartina to the mass of nitrogen and phosphorus present. The total percent biomass that contained N and P in their study varied depending on tissue type. Among all their sites tested in the study, the average mass of nitrogen relative to the total dry weight of leaf tissue was 1.532$\mp$ 0.073%. The average mass of nitrogen relative to overall dry weight contained in stem tissue was 0.845$\mp$ .065%. Combined the overall estimate of nitrogen content in aboveground biomass was 1.189$\mp$ .069% of the overall dry weight in Spartina. The nitrogen content of belowground biomass was assumed to be the same as that of the aboveground biomass. The same assumption was made for phosphorus. The average mass of phosphorus relative to the dry weight of the leaf from the same study by Osgood and Zieman was .1967 $\mp$.0467. Phosphorus contained in the stem tissue was .1017$\mp$.0133% of the total dry weight. Combined the overall estimate of phosphorus content in aboveground biomass was .1492$\mp$ .0300% of the overall dry weight in Spartina.

The rate that *Spartina alterniflora* takes up nitrogen is variable, depending on factors such as salinity and temperature (Linthurst and Seneca, 1981). The rate of growth was measured in each of the following papers as the total change in biomass (dry weight) or net primary productivity. Dame and Kenny (1986) and Darby and Turner (2008) created tables detailing rates of net primary productivity. Rates from studies conducted in North Carolina, South Carolina, and Georgia reported the annual net primary productivity with values ranging from 1110- 7620 g*m^{-2}*year^{-1} with a mean value of 4383 g*m^{-2}*year^{-1}. Using this value as a proxy for Spartina growth rate in Town Creek, the average growth rate of all biomass (both below and aboveground biomass) was calculated to be 365 g*m^{-2}*month^{-1}.

The same conversion of total biomass to mass of mass of nitrogen used above was applied to this rate. The product of the average growth rate and the average mass of nitrogen relative to the total dry weight yielded the rate of nitrogen accumulation, 4.34 ∓.25 g*m^{-2}*month $^{-1}$. The total amount of phosphorus present in one meter of marsh also came from values found in Osgood and Zieman (1993). The mass of phosphorus in a sample of Spartina only accounts for 0.15 ∓.03% of the total plant biomass. Therefore, the rate of accumulation of P was calculated to be .05∓.11 g*m^{-2}*month^{-1}.

2.3 Marsh Sediment Nitrogen Fluxes

Three sediment cores were collected at three locations in Town Creek. The three sites where a quadrat survey was taken (Section 2.1) correspond roughly with the areas where the cores were taken. Triplicate cores were taken from each location for a total of nine cores. Once collected, the cores were stored in insulated coolers to keep their biotic processes as stable as possible before transport to the lab.

Continuous flow experiments with intact sediment cores were used to determine the fluxes of nutrients and dissolved gases (Lavrentyev et al., 2000; Gardner et al., 2006; McCarthy et al., 2007). Nine intact sediment cores (6.4 cm diameter and approx. 17 cm high) and overlying water (~400 ml per core) were collected by hand from each habitat two hours after low tide on Tuesday September 24th, 2019. Cores included only sediment; however, roots and rhizomes of vegetation along with other debris like shells and glass were often contained within the cores. Additionally, 40 L of Town Creek water was collected as a reservoir for the continuous flow incubations. The continuous flow system was incubated in an environmental chamber at 23 °C to mimic the conditions observed when collecting the cores. The system was also incubated under dark conditions to prevent changes in gas concentrations due to autotrophic microbial activity. Each core was capped with a Plexiglas top with two O-rings to maintain an air- and watertight seal. Each cap contained two ports plumbed with Tygon tubing, one for inflow and one for outflow to create a well-mixed water column above the sediment within each core. Water column volume was maintained at approximately 400 ml. Inflow water from the reservoir was aerated and unfiltered water was passed over cores at a flow rate of 1 mL per minute. Cores were pre-incubated for a period of no less than 18 hours prior to sampling to allow the system to reach equilibrium (Eyre et al. 2002). Following pre-incubation, 5 mL samples were collected from the inflow and outflow of each core at 18, 24, 36 and 48-hour increments. After 36 hours, the reservoir water was spiked with an addition of 30 µMol/L nitrate. Cores after this time point were considered spiked. The goal of this spike was to assess how the cores respond to elevated levels of bioavailable nitrogen. Membrane inlet mass spectrometry (MIMS) was used to measure concentration of dissolved gasses (N2, O2 and Ar) in both inflowing and outflowing water. Since there were multiple sediment cores per sample site, the inflow concentration of water entering the core was measured from the reservoir water, which bypassed the cores and flowed directly into the sample vials. Measuring inflow concentrations from the bypass line also accounted for any changes in water chemistry resulting from pump or tubing effects.

2.4 Calculation of Marsh Area of Town Creek

Using Google Earth Pro, an outline was created of each marsh area we sampled. An outline of the upstream marsh area was created, but this area was not sampled. The outlines were made using the polygon tool to calculate the area of each patch of marsh in Town Creek. Taking the sum of these areas gave a combined area of marsh habitat in Town Creek.

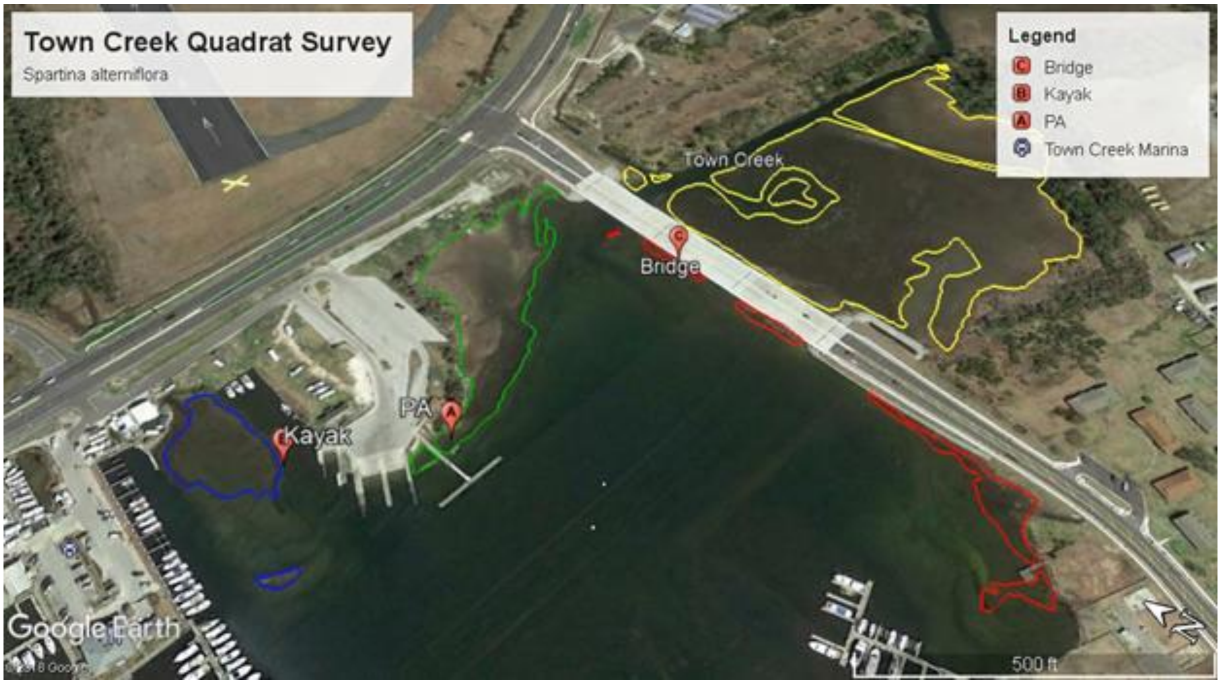


Figure 2. The image of Town Creek from Google Earth Pro that was used in calculating the marsh area. The outlined areas were surveyed on foot to improve the accuracy of the borders for the marsh area.

3. RESULTS

3.1 Spartina alterniflora Survey

The average shoot density of *Spartina alterniflora* at the Kayak site was 533 shoots/m^2. Similar results were found at the Public Access site and the Bridge site where shoot densities were 436 shoots/m^2 and 412 shoots/m^2 respectively. The average shoot density across the measured marsh segments in Town Creek was 460 shoots/m^2 (Table 1). The average dry weight of each shoot randomly collected from all sites was 6.343 g. The average aboveground biomass across the three sampled marshes was 2847∓1716 g/m^2, with biomass ranging from 1135.9 g/m^2 at the Public Access Site to 4569.6 g* m^2 at the Bridge site. The upstream area of Town Creek is 32,085m^2 with an estimated 9.14E+07g of *Spartina* biomass.

Table 1. Each value was broken down by site then each site was averaged to create the average biomass contained in the creek. The total mass represents the biomass (dry weight) of Spartina alterniflora including all marsh areas within Town Creek.

Site	***Shoot Density (Shoot Count/m^2)***	***AVG Single Shoot Biomass (g)***	***AVG aboveground mass (g/m^2)***	***Area of Marsh (m^2)***	***Total Mass (g)***
Kayak	532.82	5.325	2837.249	2,399	6.81E+06
Public Access	435.94	2.606	1135.917	7,016	7.97E+06
Bridge	411.72	11.099	4569.565	1,807	8.26E+06
Upstream	-N/A-	-N/A-	-N/A-	32,085	9.14E+07
Average	**460.16∓64.077**	**6.343∓ 4.337**	**2847.577∓1716.847**	**-**	**-**
Total	**-**	**-**	**-**	**43,307**	**1.23E+08**

3.2 Nitrogen and Phosphorus Removal via Spartina Growth

The aboveground biomass of Spartina alterniflora in Town Creek was estimated to be 1.233E+08g. Using the relationship from Darby and Turner (2008), belowground biomass was estimated to be 3.206E+08g. Therefore, the estimated total Spartina biomass in Town Creek was 4.440E+08g. Using the concentration of N found in Osgood and Zieman (1993) the estimated total nitrogen content of Spartina in Town Creek is 5.279E+06∓ 3.652E+03g. P concentrations from Osgood and Zieman 1993 aided in the calculation that estimated the phosphorus stored in Spartina across Town Creek is 6.622E+04 ∓ 1.987E+01.These values represent the estimated size of nitrogen and phosphorus sink that *Spartina* represent in Town Creek.

The rate of primary production suggested from the methods for Town Creek was $365 g*m^{-2}*month^{-1}$. The same conversion of total biomass to mass of nitrogen and phosphorus was applied to this rate yielding the rate of nitrogen accumulation as $4.34 \mp .25$ $g*m^{-2}*month^{-1}$ and the rate of phosphorus accumulation through assimilation into biomass as $0.0545 \mp .110$ $g*m^{-2}*month^{-1}$. The rate of nitrogen uptake in all Spartina alterniflora in Town Creek is estimated to be between 177,193.8551 and 199,079.5112 $g*month^{-1}$. The rate of phosphorus uptake in Spartina alterniflora is estimated to be between 2312.491 and 2407.4158 $g*month^{-1}$. To take the tidal cycle into account these rates were divided by 2 as the *Spartina* cannot remove nutrients from water it does not have access too. Therefore the final removal rate for N via Spartina uptake was estimated to be or 2.914 - 3.275 $kg*day^{-1}$ while P removal rates were estimated to be 0.038-0.040 $kg*day^{-1}$.

3.3 Sediment Nitrogen Flux

The nitrogen fluxes from the sediment cores taken in Town Creek represent nutrients that were once in the water escaping into the atmosphere as gaseous forms of nitrogen. The data for each location is recorded in Figure 3.

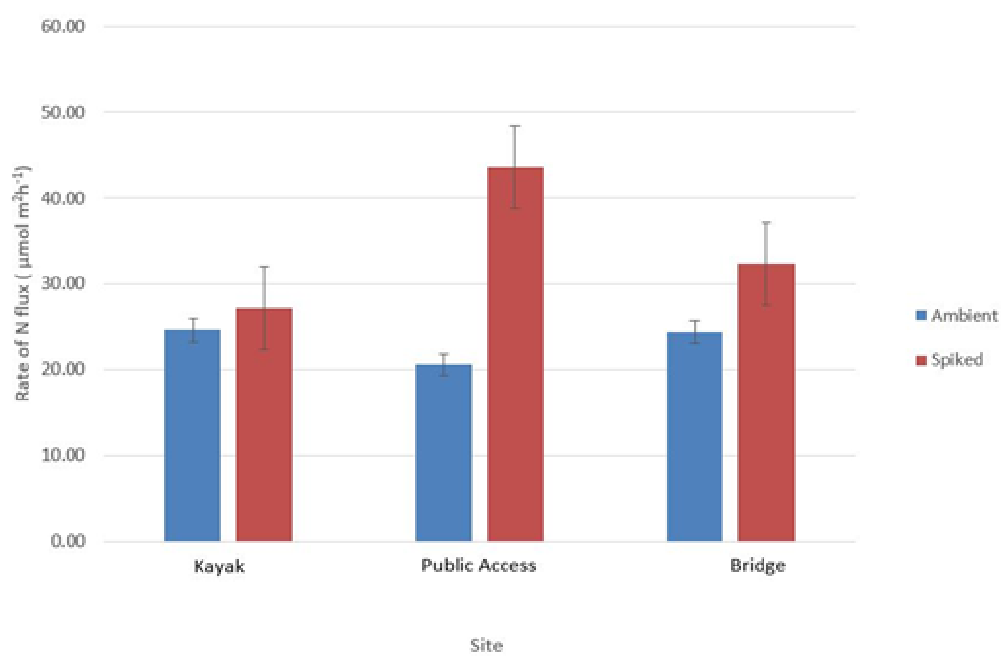


Figure 3. Average rates of denitrification for each sample site. The ambient DNF rate at each site is represented by the blue bars while the red bars represent the rate of DNF after the addition of a 30 μMol/L NO_3^- spike.

The average N flux from the sediment across all sites was 23.17 μMol N m^2h^{-1} during ambient conditions. The positive nitrogen flux indicates that denitrification was occurring at a greater rate than nitrogen fixing. The fluxes yielded an average rate of denitrification which indicates that 10.89 kg of nitrogen can be removed from town Creek via denitrification each month.

After spiking, the sediment cores exhibited elevated levels of DNF across all sites. Comparing the DNF rates during ambient conditions to the DNF rates in the spiked cores increase in DNF rates by 2.63 µMol m^2h^{-1} or 10.70% at the Kayak site, a 23 µMol m^2h^{-1} or 111.86% increase at the Public Access site, and an 8.03 or 32.98% increase at the Bridge site. An average increase in DNF rates of 48.43%. The largest change in rates of DNF came from the cores collected at the Public Access site which exhibited a change in N flux of 23.00 µMol m^2h^{-1}, this value corresponds to 10.18 kg of N a month. The average flux of nitrogen for the spiked cores was, 34.39 µMol m^2h^{-1}, and represents the average rate of DNF in 1m^2 of marsh under high nutrient conditions that may occur following stormwater runoff. DNF occurs in anoxic sediment so the rates of oxygen exchange were measured. However, the effect that oxygen concentration has on the rate of DNF was not necessary to examine further. This is because the purpose of performing the flow through experiment was to establish values indicating how nitrogen is removed from the water during every day current marsh conditions.

4. DISCUSSION

4.1 The Value of Spartina

To put the estimated N and P removal rates into perspective we attempted to simulate what would occur when a typical estuarine N load was "applied" to the marshes of Town Creek. The nutrient load of Town Creek was not measured. However, given the total volume of town creek and some nutrient concentrations, we could assess how effective *Spartina alterniflora* biomass is at removing nutrients.

The volume of Town Creek was estimated to contain 582,853 m^3 of water at high tide. A typical NOx (nitrate and nitrite) concentration in a North Carolina estuary is 10µgN L^{-1}. The sum of this concentration and the total volume of Town Creek results in an estimated stock of N in Town Creek of 58,285.3g. Utilizing the estimates for the capacity of N removal through uptake in *Spartina alterniflora*, it would take 8.7-9.8 days for that mass of nitrogen to be removed. The uptake by Spartina assumes that the water and marsh are constantly interacting, which is not the case. To correct for this, we assumed the marsh was only flooded half the time. This involved dividing the rates of N and P removal by 2. The corrected values indicate that it would take an estimated 17.4-19.6 days to remove this load via Spartina uptake alone.

To estimate the P load the N load was divided by 16. This value was selected as it complies with the Redfield Ratio which is a trend found in marine phytoplankton and dissolved nutrient pools that relates relative concentrations of carbon to nitrogen to phosphorus in a ratio of 106:16:1 (Nature Geoscience, 2014). This gave an estimated P load of 3.64 kg of phosphate. Phosphate was selected as it is a common component of chemical fertilizers (Speight, 2017). Utilizing the estimates for the capacity of P removal through uptake in Spartina alterniflora, it would take 45.5 to 47.9 days for that mass of nitrogen to be removed. The same assumptions for nitrogen removal were also made of phosphorus removal, therefore the corrected values indicate that it would take an estimated 91.0 to 95.8 days to remove this load via Spartina uptake alone. This value may serve as an underestimate, due to the fact that P made up such a small fraction of the mass relative to all biomass of *Spartina* in the literature we used for the calculation.

Assumptions limited the accuracy of our data however overall trends could be seen despite the variations in values. First, belowground biomass was not sampled, so no baseline of belowground biomass was observed firsthand. This meant that literature values were used to approximate the belowground biomass, introducing larger uncertainty. Depending on the time of year root and rhizome biomass can make up a much larger fraction of total biomass. In the winter, for example, shoot biomass rapidly decreases while root biomass remains more constant (Darby and Turner, 2008). The growth rate is another highly variable rate that was not measured in the survey.

Despite the many assumptions that needed to be made, the trend emerged that *Spartina* could remove nutrients present in water within Town Creek. Furthermore, the time scales that P, and N especially, can be removed by *Spartina* represent how valuable the marsh is to the city of Beaufort. Losing this habitat could not only lead to loss of a coastal ecosystem it could also lead to water quality being a larger issue for Town Creek and the Town of Beaufort. This would occur because the filter that is the marsh would no longer be as effective and the load of N would not be filtered from the water. Leading to increased loading of N and P into Town Creek where tides and flushing could carry these nutrients away, representing a problem for another local water body.

4.2 The Role of Sediment

Based upon the sediment nitrogen flux, our group was able to determine the rate that nitrogen leaves the sediment under ambient conditions. This proves that nitrogen, in the form of nutrients like NO_3^- and NO_2^-, can enter the sediment and leave in another form, most commonly leaving as N_2O or N_2. The nitrate addition simulated the effects that a large storm may have on the marsh of Town Creek. When a storm event occurs, nutrients from the land are flushed into the estuarine system (Paerl et al., 1997). The results of our experiment indicate that under storm conditions denitrification rates increase. This is supported in our data as an increase in nutrient concentrations due to the spiked water was matched with increased DNF rates. The increase of DNF rates of marsh sediment after additions of nutrients indicates a capacity of marsh sediment to respond to excess nutrients. In effect, the sediment microbes can process nutrients at a greater rate when those nutrients are at a greater concentration in the water.

If the same load of 58,285.3g of nitrate from Section 4.1 was input into the water that feeds into Town Creek, based on the estimated rates of nitrogen removal, DNF would remove .51 kg of nitrogen a day. Therefore, it would take roughly 114 days for that mass of nitrogen to be removed via DNF alone. With *Spartina* and sediment DNF both removing N it would take an estimated 15.4-17.0 days for 58.3kg of nitrate to be removed from the water. The addition of DNF increased the rate of N removal by 13.5% to 15.0%.

One of the largest limitations to the study was the lack of a control for the tidal cycle. Cores were incubated with water constantly covering the sediment. When water constantly covers the core, it interacts with the sediment microbes that carry out denitrification. In a natural system, a marsh is not always covered. Depending on the marsh location, sediments can be without water for the majority of the tidal cycles. When this occurs, there can be no nitrogen drawn from the water as there is no water to draw N from. Due to this limitation, DNF values represent a marsh core always covered by water, not necessarily a marsh system in the environment. Another limitation stems from the way the cores were incubated. Cores were incubated in the dark to prevent phytoplankton and other photosynthetic microbes from changing oxygen and nitrogen concentrations in any of the waters. In the marsh environment, the water above a sediment core is subject to changes in dissolved gas and nutrient concentrations. These changes can occur due to photosynthetic microbes drawing in CO2 and releasing O2. Additionally, microbial respiration can also lead to changes in dissolved gas concentrations in the sediment (Goreau et al., 1980). To account for these changes, it was assumed that DNF only occurred when the marsh was flooded and at night. This is not true in nature but for our purposes it gave a conservative estimate for the potential of N removal in the marshes of Town Creek.

A similar study was conducted in the Neuse River Estuary in 2005 by Fear et al. They recorded similar values for nitrogen flux rates. In their study rates of DNF ranged from 0 to 275 µMol m^2h^{-1}. They maintained that the variability in rates of nitrogen flux was natural as estuarine environments have factors that are constantly changing. They compared their DNF rates with 8 other studies and found similar ranges of values. Compared to our study, their range of rates was much greater. Our rates of DNF ranged from 15.4 ∓ 1.92 µMol N m^2h^{-1} to 105.5 ∓ 30.38 µMol N m^2h^{-1} compared to their range of 0 to 275 µMol N m^2h^{-1}. A potential method of increasing rates of DNF was put forth by Smyth et al. (2015). In their paper they proposed that rates of DNF were greater for sediment with close proximity to oyster reef habitat. In their work they found that the Pamlico Sound Oyster Sanctuary program had a statistically significant effect on denitrification ($n=18$ $p=.0100$). They found that oyster reef associated nitrogen removal was 2 to 4 times greater than non-reef removal (Smyth et al., 2015). Their data indicates that oysters and marshes benefit one another, and their ecosystem services are magnified when combined. If more oysters persisted in Town Creek, we could see an increase in DNF rates. This increase to DNF rates would mean that nitrogen loads would be removed faster. Furthermore, more oysters could have the effect of removing even more N and P independent of their effect of denitrification.

5. CONCLUSION

- It was estimated that marsh habitats constitute $43{,}307m^2$ of Town Creek.
- It was calculated that *Spartina alterniflora* can remove a typical nitrogen load from the water within 17.7-20.0 days.
- It was calculated that that *Spartina alterniflora* can remove a typical phosphorus load through assimilation into biomass in 91-95.8 days.
- Changes were measured in dissolved gas concentrations that yielded average DNF rates indicting that sediment denitrifying bacteria can remove a typical N load within 114 days.
- Changes were measured in dissolved gas concentrations in sediment cores indicating cores spiked with NO_3^- increased the rate of denitrification by 48% when compared to ambient cores. This finding indicates that less time may be needed for denitrification to remove nitrogen when nutrient concentrations within the water are high.
- Finally, we displayed that uptake of N by sediments and N and P in *Spartina alterniflora* can help remove pollutants from the water, improving the water quality of Town Creek.

CHAPTER 6: Filtration by Oysters

1. INTRODUCTION

Oysters are filter feeders, which means they feed by drawing in water to gather food particles. Oyster filtration is an important ecosystem service that can improve water quality. When a system is overloaded with nutrients or particulate organic matter from runoff or untreated sewage, it can lead to eutrophication, algal blooms, and increased levels of harmful bacteria (Anderson et al., 2002; Chudoba et al., 2013).

Oysters such as Crassostrea Virginica – the Eastern Oyster - can improve water quality by removing particulate matter from the water column. Removing particles creates clearer water by decreasing total suspended solids, which, in excess, can reduce light penetration and thus inhibit nutrient uptake by benthic primary producers (Hughes et al., 2015). Suspended solids are also vectors for groups of microorganisms, like phytoplankton and bacteria, which, by themselves, may be too small for efficient filtration by oysters (Dame et al., 1984). Uptake of phytoplankton and bacteria controls microorganism populations, preventing harmful algal blooms (Coen et al., 2007). Also, removal of fecal contamination bacteria makes recreational use of waterways safer by reducing the risk of waterborne infection (Froelich and Noble, 2014).

After removing these materials, oysters deposit them as feces or pseudofeces onto the benthos, which provides a carbon source for denitrifying bacteria to thrive (Smyth et al., 2015). In addition, oyster reefs provide habitats for a variety of marine organisms who also contribute to the cycling of the above nutrients. Motile invertebrates like worms and crabs use reefs as shelter from predators and foraging grounds, while sedentary invertebrates like sponges and tunicates use reefs as a place to root and filter-feed. These organisms also contribute to nutrient cycling at oyster reefs (Coen et al., 2007; Grabowski and Peterson, 2007). Digested nitrogen from filtered microorganisms and decaying matter is released as ammonium, which is a food source for microorganisms to feed on and recycle the nitrogen (Dame et al., 1985).

Town Creek's shores and dockside structures are covered with Eastern Oyster reefs. Knowing the amount of time it takes for those oysters to filter through the water in Town Creek will help us understand the role they play in the area's microbial and chemical water quality. We first determined the quantity of oysters that there, and then using literature values for filtration rates, calculated the turnover time for the volume of Town Creek.

2. METHODS

To determine how fast oysters in Town Creek filter water and what substances in the water oysters filter out, a quadrat survey, a length assay and a filtration experiment were conducted. An equation from Ehrich and Harris (2015) was used to determine the per-oyster filtration rate by considering the average oyster length and average dry weight of an individual oysters that were measured in this study. Then, using the total area of oyster reef, the density of oysters on reefs, the total number of oysters and the volume of the water in Town Creek, the per-oyster filtration rate was scaled up to the entirety of Town Creek, as described below. To understand what substances in the water oysters filter and quantify the filtration rates for the different substances, a tank experiment was conducted. Water samples taken before and after oyster filtration were analyzed to measure levels of chlorophyll *a*, total dissolved nitrogen, *Escherichia coli*, *Enterococcus*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*.

2.1 Determining oyster reef area

A visual survey of Town Creek was conducted to locate areas with oyster reefs. Oyster reefs were found on shallow mudflats near the shore (horizontal reefs) and attached to vertical structures (vertical reefs). Google Earth Pro was used to map and quantify the total area of oyster reefs in 2009 and 2019 (Figure 1). To determine the area of horizontal reefs, the ruler tool was used to select points on the satellite image of Town Creek to form a polygon around the oyster reefs, and the software quantified the highlighted area.

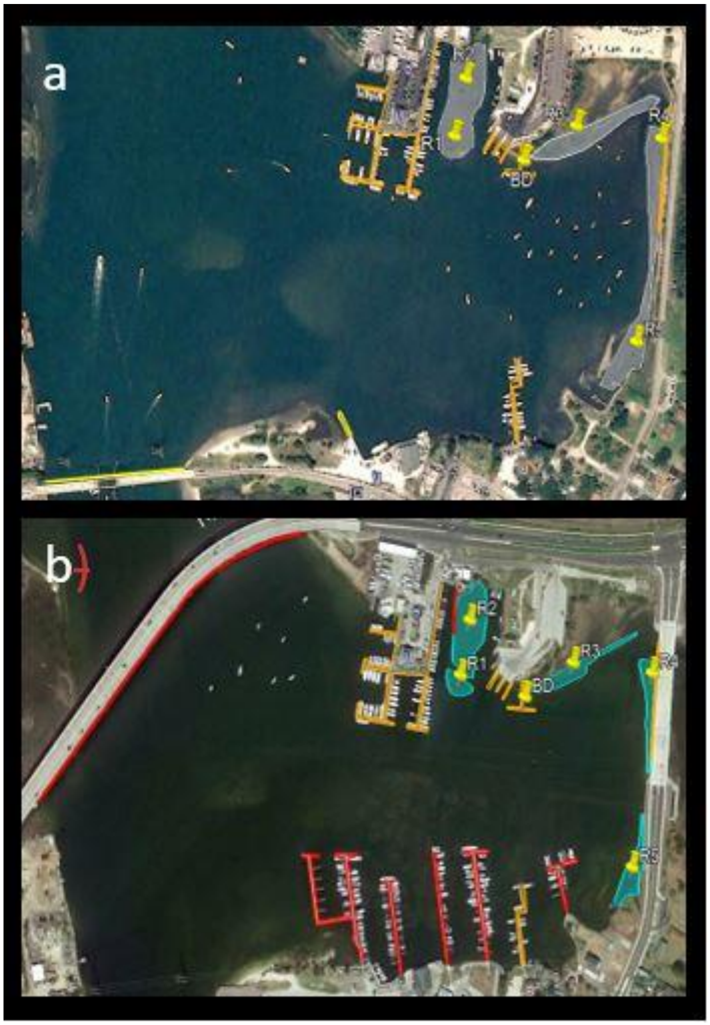


Figure 1. a) October 2009 map of Town Creek oyster reefs. Horizontal reefs are highlighted in white. Yellow lines indicate vertical reefs only present in 2009. Orange lines indicate vertical reefs present in both 2009 and 2019. b) March 2019 map of oyster reefs. Horizontal reefs are highlighted in teal (R1-R5). Red lines indicate vertical reefs only present in 2019. Orange lines indicate vertical reefs present in both 2009 and 2019.

To determine the area of vertical reefs, Google Earth Pro was used to find the total length of structures with oysters. By using the ruler tool, lines were drawn along the perimeter of vertical reef structures and the software computed the lengths. In visual surveys, oysters were found on dock and bridge pilings and on a metal retaining wall. The entire length of the retaining wall was covered with oysters on both sides, so the length was doubled. However, the pilings do not occur along the entire length of the bridge or docks. It was estimated that, if the circumference of the cylindrical pilings were unrolled, the pilings would make up 1/5 of the total length of the vertical structure (Figure 2). A meter stick was used to measure the height of the region covered by oysters on eight individual pilings (four from Turner Street Bridge and four from the boat dock), and the heights were averaged. The length and height were multiplied to determine the total area of vertical reefs in Town Creek.

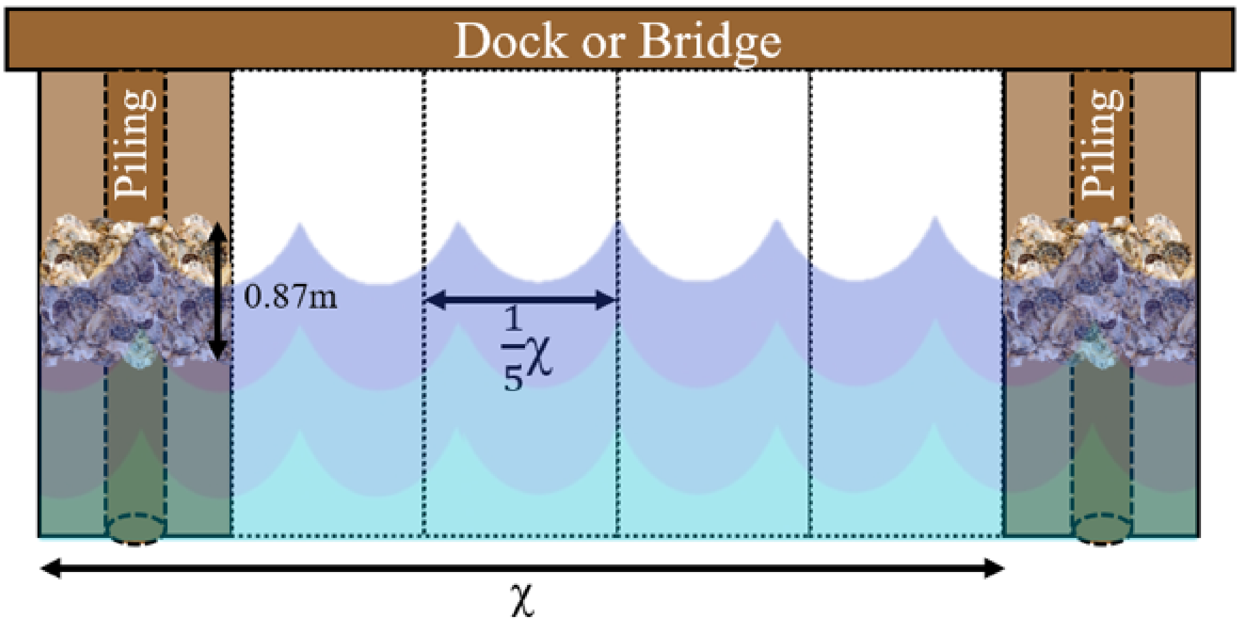


Figure 2. Schematic diagram showing how oyster area was estimated for dock and bridge pilings. The unrolled length of each piling is approximately 1/5 the length of the dock or bridge. The average range of oysters growing on pilings extends 0.87 m.

2.2 Determining oyster reef density

To determine the density of the oyster reefs in Town Creek, a quadrat survey was conducted at three locations. Location 1 (34°43'31.59"N, 76°39'50.43"W) is a reef near the public boat dock access. Location 2 (34°43'29.23"N, 76°39'40.02"W) is a reef along the marsh edge at Turner Street Bridge. Location 3 (34°43'33.79"N, 76°39'50.20"W) is a metal retaining wall in Town Creek Marina (Figure 3). The locations were selected because they contained many oysters and encompassed the variety of substrates on which oysters grow. The survey was conducted at low tide to ensure maximum exposed reef area.

Figure 3. Map of quadrat survey locations. L1) reef without marsh. L2) reef along marsh. L3) vertical reef.

Location 1 (L1) is a large oyster reef that sits upon hardened mud. It is located between the public boat access and Town Creek Marina. There is a small patch of marsh at the center of the reef, but most of the oysters don't come into physical contact with the marsh grass. Two 20 m transects were run at this location. Both transects were run from east to west, starting near the dock and extending out into the creek. Transect 1 (T1) was run along the reef crest or the highest elevation of the reef. Transect 2 (T2) was run along the water's edge, or the lowest elevation of the reef (Figure 4). The two transects were laid to determine if reef elevation impacted oyster density. A tape measure was used to measure the stride-length of the surveyor, which was 0.80 m. Every five strides (4 m), a 0.33 m by 0.33 m PVC plastic quadrat was placed, and the number of live oysters was counted down to the anoxic layer. Five quadrats were surveyed on each transect, totaling ten quadrats.

Location 2 (L2) is a linear oyster reef that runs along the marsh edge on the west side of Turner Street Bridge. The reef has a lower profile than L1 and is embedded in thick mud. A transect (T3) was ran, starting at the reef on the north end of the bridge toward the south end of the bridge. Ten quadrats were placed every ten strides (8 m), starting with x = 0 m, for a total of 72 m. The number of live oysters in each quadrat was counted down to the anoxic layer.

Location 3 (L3) is a metal retaining wall that separates the slips at Town Creek Marina from the public access boat docks. The wall is very rusty and extends about 1.5 m out of the water at low tide. Three random quadrats were placed vertically against the wall and the number of live oysters were counted.

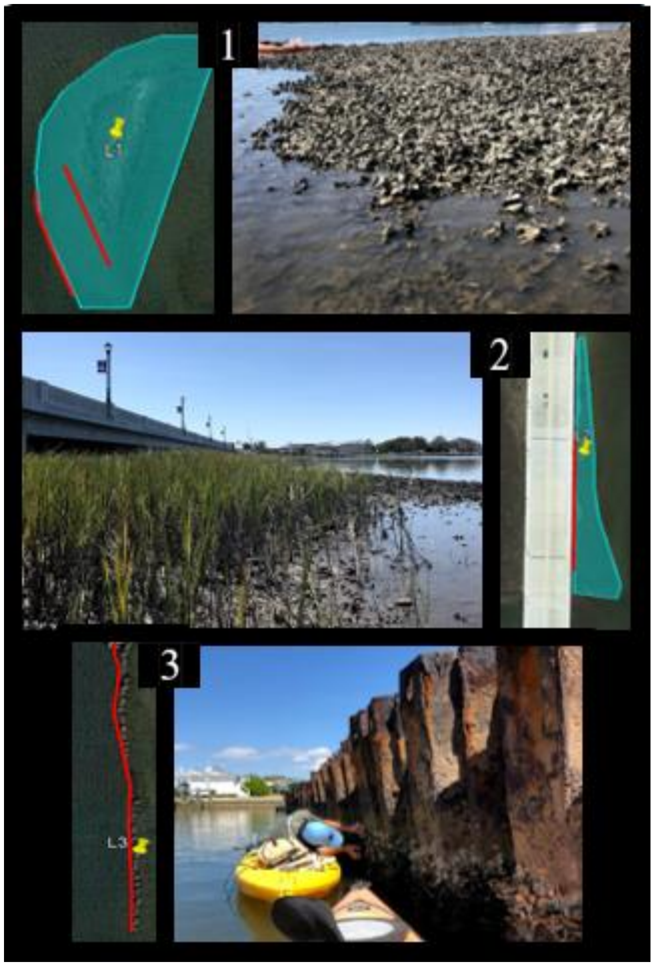


Figure 4. Maps and images of transects and locations for quadrat survey.

2.3 Estimating average oyster length

A length assay was conducted to determine the average length of the oysters in Town Creek. This value is necessary to estimate the filtration and clearance rates of the oysters. A total of 300 oysters were collected from three sites to obtain a complete profile of oyster lengths in the area. One hundred oysters were selected at random from each site – the public boat dock (BD), a reef with no marsh (R1) and a reef along marsh (R4) (Figure 1). The oysters were measured from hinge to lip using calipers with accuracy to 1 mm.

2.4 Estimating filtration rates and clearance times

The total filtration capacity and clearance rates of oysters in Town Creek were estimated using data collected from this study and literature values. The area of the three types of reefs (marsh, no marsh and vertical structure) estimated from Google Earth Pro and the reef densities found from the quadrat survey were used to determine the total number of oysters in Town Creek according to equation 1:

$$N = [(A_2 + A_3 + A_4 + A_5)d_2] + [A_1 d_1] + [A_{VS} d_3] \quad (1)$$

where N is total number of oysters in Town Creek, A_n is the area of reef n, d_n is the oyster density of location n, and A_{vs} is the area of vertical structures. Area is measured in m^2 and density is measured in number of oysters per m^2.

The average individual oyster length was converted into dry weight using an allometric relationship from Southworth et al. (2010) shown in equation 2:

$$DW_{(i)} = (9.6318 \times 10^{-6}) L_{(i)}^{2.743} \quad (2)$$

where $DW_{(i)}$ is dry weight of an individual oyster and $L_{(i)}$ is individual oyster shell length (hinge to lip). Dry weight is measured in g and oyster length is measured in mm.

The number of oysters in Town Creek was multiplied by the dry weight of an individual oyster to calculate the total dry weight of oysters in Town Creek. Then, the total dry weight of oysters was multiplied by several oyster filtration rates gathered from literature (Ehrich and Harris, 2015) to determine a range of estimates for the total filtration capacity of Town Creek oysters.

Because oysters occupy the entire tidal range (~0.8 m), it was estimated that the oysters spend approximately half the time underwater, so they are only able to filter water for 12 hours per day. Thus, the filtration rates were divided by two to account for the time oysters are out of the water.

To determine clearance times, the water volume of Town Creek was divided by the total corrected filtration, as seen in equation 3:

$$C = \frac{V}{FR_{(t,c)}} \quad (3)$$

where C is the clearance time, V is the water volume of Town Creek (449,192.5 m^3), and $FR_{(t,c)}$ is the total corrected filtration rate. Clearance rate is measured in days, volume is measured in m^3 and filtration rate is measured in m^3 H_2O day^{-1}.

Oyster filtration rates and clearance times were also estimated for the 2009 oyster reef data under the assumption that oyster density and length and water volume were the same as measured in 2019.

Temperature, salinity and the amount of total suspended solids in the water impact the filtration capacity of oysters. An equation from Ehrich and Harris (2015) was applied to the best estimate filtration rate (0.17 m^3 H_2O g DW^{-1} day^{-1}) to account for these factors, as seen in equation 4:

$$FR_{(i,c)} = 0.17\, W^{0.67}\, f(T)\, f(S) f(TSS) \quad (4)$$

where $FR_{(i,c)}$ is the corrected filtration rate of an individual and W is dry weight of an individual. Filtration rate is measured in m^3 H_2O $individual^{-1}$ day^{-1}, dry weight is measured in g, temperature is measured in °C, salinity is measured in ppt, and TSS is measured in mg L^{-1}.

The environmental factor equations from Ehrich and Harris (2015) are defined as:

$$f(T) = e^{(-0.006*(T-27)^2}$$

$$f(S) = 1 \; when \; S > 12 \; ppt$$

$$f(TSS) = 1 \; when \; 4 \leq TSS \geq 25 \; mg \; L^{-1}$$

The corrected filtration rate was multiplied by the total number of oysters in Town Creek to find the total corrected filtration rate.

A second method was also used to determine the filtration rate of Town Creek oysters. An allometric relationship from Ehrich and Harris (2015) relates oyster length to the maximum per-oyster filtration rate shown in equation 5:

$$FR_{\max(i)} = \frac{{L_{(i)}}^{0.96} \times T^{0.95}}{2.95} \qquad (5)$$

where $FR_{max(i)}$ is maximum filtration rate of an individual, $L_{(i)}$ is shell length of an individual (hinge to lip) and T is average water temperature (24.14°C). Filtration rate is measured in mL H_2O individual^{-1} min^{-1}, length is measured in cm and temperature is measured in °C. The total number of oysters was multiplied by this per-oyster filtration rate to find the total filtration capacity of Town Creek oysters. The filtration rate was then divided by two to account for the time the oysters were out of the water.

2.5 Determining substances filtered by oysters

A tank experiment was conducted to determine the substances oysters in Town Creek filter. Ten gallons of water was collected from two sites (BD and L2) and the water was well mixed to ensure homogenization. Three 2.5-gallon glass aquariums were each filled with 8 L of water. From each tank, 1.2 L of water was removed prior to the start of the experiment and was used to determine the initial characteristics of the water. A total of six inches of oysters collected from Town Creek were placed into each of the tanks. Tank 1 contained two 3-inch oysters. Tank 2 contained three 2-inch oysters. Tank 3 contained six one-inch oysters. The oysters were allowed to filter the remaining 6.8 L of water for one hour. At the conclusion of the experiment, another 1.2 L of water was removed to determine the final characteristics of the water. The initial and final water samples were analyzed to determine the amount of chlorophyll *a*, total dissolved nitrogen, *E. coli*, *Enterococcus*, and *Vibrio* present in the water. While the experiment was ongoing, water temperature (22.13° C) and salinity (26 ppt) were measured, as these factors can impact oyster filtration rate. Because the results from the tank experiment were not consistent with the literature search conducted, it was not significant to calculate uptake rates for the substances.

3. RESULTS

3.1 Oyster reef area

Using satellite imagery from Google Earth Pro, the total area of 2019 oyster reefs was found to be 9,890 m^2 (Table 1). Over 90 percent of 2019 oyster reef area exists as horizontal reefs, which make up 9,160 m^2 of the coverage. Most of the 2019 horizontal reef (R2, R3, R4, R5) occurs along marsh, combining to 8,454 m^2. The remainder of the 2019 horizontal reef (R1) stands independent of marsh and contributes 705 m^2 to the total. Oysters that grow on vertical structures were also considered. The length of structures on which oysters grow (i.e. the horizontal distance along docks and bridges) was measured to be 3,650 m. The average height of the region covered by oysters on the pilings extending into the water was found to be 0.87 m.

Table 1. Length and area of oyster reefs.

	2019	2009
	Length (m)	
Total vertical structures	3,650	1,810
Vertical structures with oysters	838	470
Average height of oyster reefs	0.87	0.87*
	Area (m^2)	
Total vertical reef	730	409
Reef along marsh	8,454	13,564
Reef without marsh	705	
Total horizontal reef	9,160	13,564
Total oyster reef	**9,890**	**13,973**

*average height of oyster reef is assumed to be the same as measured in 2019

Assuming the pilings cover 1/5 of the total length of the bridge or dock, the total area of 2019 vertical reefs was calculated to be 730 m^2 (Table 1). The total area of 2009 oyster reefs (13,973 m^2) was larger than the total area in 2019. Even though the area of vertical reefs was almost fifty percent smaller in 2009 than 2019, the area of horizontal reefs in 2009 was much larger than that in 2019, resulting in a larger total reef area in 2009 (Table 1).

3.2 Oyster reef density

Density of live oysters was highest on vertical structures (L3), with an average of 543 oysters per m^2. The oyster density was next highest at reefs not adjacent to marshes (L1), with an average of 485 oysters per m^2. The densities from the two transects at L1 were not significantly different, so the data was pooled. Oyster reefs along marsh (L2) had the lowest density, with 225 oysters per m^2 (Figure 5).

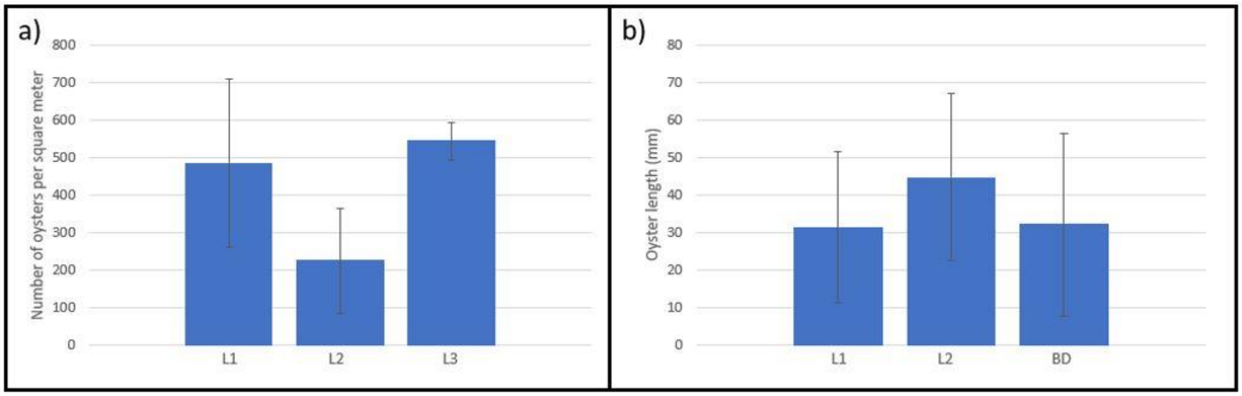


Figure 5. a) distribution of oyster density across sites (locations 1-3). b) distribution of oyster lengths across sites (locations 1-2 and boat dock). Error bars represent one standard deviation.

3.3 Average oyster length

The average oyster length did not vary greatly among the three locations (L1, L2 and BD). The average oyster length was found to be 31 mm at L1, 45 mm at L2, and 32 mm at the boat dock (Figure 5). The lengths were not significantly different, so the average length (36 mm) was used for later calculations.

3.4 Filtration rates and clearance times

Filtration rates, measured in m^3 H_2O per gram of dry weight per day, from literature were multiplied by the total dry weight of oysters to calculate the total filtration rate of Town Creek oysters. Clearance time – the amount of time it would take the oysters to filter the entire volume of water in Town Creek – was also calculated. The filtration rates for 2019 ranged from 2,400 to 160,000 m^3 H_2O day^{-1}. The clearance times for 2019 ranged from 2.7 days to 190 days. Based on Ehrich and Harris's (2015) corrected best estimate filtration rate (0.17 m^3 H_2O g DW^{-1} day^{-1}), the best estimate for total filtration rate is 67,000 m^3 H_2O day^{-1} and a clearance time of 6.6 days.

The filtration rates were higher with the 2009 data and thus yielded shorter clearance times. The calculated filtration rates for 2009 ranged from 2,900 to 200,000 m^3 H_2O day^{-1}. The clearance times for 2009 ranged from 2.2 days to 150 days. Based on Ehrich and Harris's (2015) corrected best estimate filtration rate (0.17 m^3 H_2O g DW^{-1} day^{-1}), the best estimate for total filtration rate is 50,000 m^3 H_2O day^{-1} and a clearance time of 5.4 days. (Table 2).

Table 2. Filtration rates and clearance times.

		2019		2009	
Literature source	Filtration rate range (m^3 H_2O g DW^{-1} day^{-1})	Total filtration rate (m^3 H_2O day^{-1})	Clearance time (days)	*Total filtration rate (m^3 H_2O day^{-1})	*Clearance time (days)
Ehrich and Harris (2015)	0.17	40,000	11	50,000	9.0
Ehrich and Harris (2015) corrected for T, S, TSS	0.17	67,000	6.6	84,000	5.4
Barrera-Escorcia et al. (2012)	0.02 – 0.07	4,700 – 17,000	27 – 95	5,900 – 21,000	22 – 76
Comeau et al. (2008)	0.01 – 0.12	2,400 – 28,000	16 – 190	2,900 – 35,000	13 – 150
Gerritsen et al. (1994)	0.24	57,000	7.9	71,000	6.4
Grizzle et al. (2008)	0 – 0.48	110,000	3.9	140,000	3.2
Langefoss and Maurer (1975)	0.13 – 0.40	31,000 – 95,000	4.7 – 15	38,000 – 120,000	3.8 – 12
Loosanoff (1958)	0 – 0.39	93,000	4.9	110,000	3.9
Loosanoff and Nomejko (1946)	0.32 – 0.46	76,000 – 110,000	4.1 – 5.9	94,000 – 140,000	3.3 – 4.8
Newell et al. (2005)	0 – 0.46	110,000	4.1	140,000	3.3
Newell and Koch (2004)	0.04 – 0.46	9,500 – 110,000	4.1 – 47	12,000 – 140,000	3.3 – 38
Palmer (1980)	0 – 0.26	62,000	7.3	77,000	5.9
Riisgard (1988)	0.33 – 0.69	78,000 – 160,000	2.7 – 5.7	97,000 – 200,000	2.2 – 4.6
Ehrich and Harris (2015)	(Equation 3)	45,000	9.9	56,000	8.0

*calculations based on assumption that oyster density and length were the same as those measured in 2019. Values highlighted in blue are the best estimate values.

3.5 Substances filtered by oysters

The parameters measured during the tank experiment did not exhibit consistent change from the initial to final water samples across the three tanks (Table 3). Chlorophyll *a* increased in tanks 1 and 2 and decreased in tank 3. Total dissolved nitrogen increased in all three tanks. *E. coli* increased in tank 1 and decreased in tanks 2 and 3. *Enterococcus* increased in tanks 2 and 3 and decreased in tank 1. *V. parahaemolyticus* increased in tank 1 and decreased in tanks 2 and 3. *V. vulnificus* increased in tank 3 and decreased in tanks 1 and 2. *V. alginolyticus* increased in tank 2, had no change in tank 3, and decreased in tank 1.

Table 3. Nutrient and microbial makeup of Town Creek water before and after oyster filtration experiment. Tank 1 contained two, 3-inch oysters. Tank 2 contained three, 2-inch oysters. Tank 3 contained six, 1-inch oysters. Units: MPN = most probable number; CFU = colony forming units.

	chlorophyll *a* (µg/L)			Total Dissolved Nitrogen (µg/L)			*E. coli* (MPN/100mL)			*Enterococcus* (MPN/100mL)		
Tank	Initial	Final	Difference	Initial	Final	Difference	Initial	Final	Difference	Initial	Final	Difference
1	3.47	4.27	0.79	422	477	55	2.61	2.68	0.071	2.49	2.43	-0.053
2	2.90	4.09	1.19	351	804	453	2.82	2.80	-0.024	2.40	2.49	0.081
3	4.35	3.66	-0.70	354	366	12	2.73	2.68	-0.046	2.38	2.49	0.11
*Mean	3.92			296			2.06			1.83		
*Range	0.85-7.52			103-1290			0.95-3.89			0.95-4.02		
	V. parahaemolyticus (CFU)			*V. vulnificus* (CFU)			*V. alginolyticus* (CFU)					
	Initial	Final	Difference	Initial	Final	Difference	Initial	Final	Difference			
1	3.41	3.51	0.099	3.33	3.20	-0.14	3.56	3.45	-0.10			
2	**3.58**	3.48	-0.097	3.26	3.13	-0.14	3.50	3.56	0.051			
3	3.24	3.19	-0.050	3.12	3.27	0.15	3.56	3.56	0.00			
*Mean	2.86			2.73			3.24					
*Range	2.20-3.53			2.06-3.92			2.89-3.56					

*mean and range values are from in situ measurements collected by the nutrient and microbial groups. Numbers in bold fall outside of in situ range.

4. DISCUSSION

4.1 Oyster reef area

Although oyster reefs only make up a small percentage of the area of Town Creek, they filter large quantities of water (Table 2). Over the past decade, oyster reef area has decreased from 4.38% to 3.10% of the total area of Town Creek. The decrease in horizontal reef area between 2009 and 2019 is a consequence of increasing human activity. The population increase of Beaufort residents and tourists has likely contributed to an increase in runoff. Greater runoff into Town Creek can lead to more pollutants entering the water body which can be harmful to oyster growth. More runoff can also increase the amount of sediment entering the creek, leading to oyster reefs being buried (NOAA, 2019).

Although the total and horizontal reef area has shrunk over the past ten years, the vertical reef area has increased almost twofold (Table 1). This increase is due to the expansion of marinas and bridges in Town Creek. In 2009, Town Creek Marina, the Discovery Diving dock, the public boat access docks, Turner Street Bridge, and the low-rise bridge connecting Morehead City and Beaufort were the only structures available on which vertical reefs could grow. Between 2009 and 2019, Homer Smith Marina and Town Creek Harbor Homes Marina were built and contributed a total of five large docks to the water body. Furthermore, the construction of the high-rise Highway 70 bridge in 2017 provided lots of new area for vertical reefs to grow.

Since oyster area and total filtration rate are related linearly, the decrease in oyster reef area over the past decade resulted in the decrease in the filtration capacity of Town Creek oysters (Ehrich and Harris, 2015). The compounding impacts of decreased oyster area and increased urbanization suggest that there is lower potential for oyster filtration to remove particulate matter and improve water quality in Town Creek.

4.2 Oyster reef density

Oyster reefs were found to be, on average, the densest on vertical structures; however, vertical structures only make up 730 m^2 of the 2019 total reef area (Table 1). Because oyster density is highest on vertical structures, the quickest way to increase the number of oysters in Town Creek would be to increase the area of vertical structures. Although promoting oyster growth on vertical structures would have the most immediate effect on increasing oyster abundance, the additional vertical structures – which are most likely docks and bridges – would result in increased boat and car activity and therefore increase pollution entering Town Creek. The alternative of increasing the area of vertical reefs would be to increase the area of horizontal reefs. To do this, parts of Town Creek would need to be partially filled in with sediment to create more intertidal area on which oysters can grow (Fodrie et al. 2015). However, filling in parts of the periphery of Town Creek would result in less space available for development and marinas. Thus, there are tradeoffs to be considered when planning to increase oyster abundance in Town Creek.

4.3 Average oyster length

Eastern oysters typically range from two to five inches in length or 50.8 – 127 mm (Eastern Oyster *Crassostrea virginica*, n.d.). The average length of oysters in Town Creek (36 mm) is lower than the expected range. This is likely due to the timing of sampling and oyster spawning. Eastern oysters spawn in early summer and the length assay was conducted in early October (Eastern Oyster *Crassostrea virginica*, n.d.). Because it takes Eastern oysters in North Carolina around 12 to 36 months to reach market size (3 inches or 76.2 mm), it's reasonable that the newest cohort of oysters have not had enough time to grow significantly and are bringing down the average length (Wallace, R. K., 2001). This theory was supported by numerous oysters measuring less than 10 mm in length attached to larger oysters that were collected.

4.4 Filtration rates and clearance times

Based on the total filtration rate, the oysters in Town Creek are not filtering a substantial amount of water (Table 2). The best estimate for the 2019 clearance time is 11 days. However, the amount of time water remained in Town Creek was much smaller. The flushing time ranges from 25.8 to 51.7 hours, and the residence time is approximately three to four hours in the back area of Town Creek near Turner Street Bridge. Therefore, it takes significantly longer for the entirety of oysters in Town Creek to filter the water than the time the water occupies Town Creek.

Even though the current oyster filtration capacity does not impact a significant portion of Town Creek water, the addition of oyster reef area can result in a significant impact on water quality. If the total surface area of Town Creek was covered with oysters, the best estimate for clearance times is 0.4 days or 9.6 hours. These clearance times are comparable to the flushing and residence times, indicating that this number of oysters would filter significant volumes of water in Town Creek. Therefore, creating more oyster reefs is predicted to be a worthwhile strategy to increase water filtration and improve water quality.

4.5 Substances filtered by oysters

When the *in situ* measurements gathered by the nutrient and microbial groups were compared to the initial sample measurements, they corresponded well across all parameters. All initial values from all tanks fell between the *in situ* ranges for chlorophyll *a*, total dissolved nitrogen, E coli, enterococcus, *V. vulnificus* and *V. alginolyticus*. The initial *V. parahaemolyticus* for tank 2 was only slightly higher than the uppermost range from *in situ* measurements. Since the parameters measured from initial water samples largely matched the ranges measured from the nutrient and microbial groups, it can be concluded that the initial water samples were representative of the water in Town Creek.

Therefore, it is likely that the unexpected results came from an error occurring after the oysters were added. There are two reasonable scenarios that would explain the results. First, after the oysters were collected, they sat out of water for approximately two hours prior to the experiment and were not put in water before the start of the experiment to reacclimate. Second, the initial water samples were taken before the oysters were placed in the tanks. The oyster shells could have had nutrients or bacteria attached to them, resulting in increased parameter measurements at the final timepoint. The results from this experiment were not consistent with findings in literature, so conclusions were drawn in respect to information from published, peer reviewed reports.

Oysters are known to take up bacteria including *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus*, *E. coli*, and *Enterococcus*, as well as chlorophyll *a* (a proxy for phytoplankton), and other forms of particulate nitrogen (Chae et al., 2009; Reyes–Velázquez et al., 2010; Love et al., 2010). After digestion, they release dissolved nitrogen in the form of ammonium (Dame et al., 1985). Increased ammonium can lead to increased levels of phytoplankton, chlorophyll *a*, and benthic plants downstream of an oyster reef, as they use ammonium for growth. Because of this, it is thought that oysters play an important role in nitrogen cycling. Oysters regulate phytoplankton populations via a feedback loop of culling phytoplankton and then releasing dissolved nitrogen for them to regrow, but benthic plants uptake some dissolved nitrogen as well and thus prevent overgrowth of phytoplankton.

In Dame et al. (1985), it is found that dense oyster reefs take up particulate nitrogen at about 7 mmol m^{-2} hr^{-1}, and release ammonium at about 5.5 mmol m^{-2} hr^{-1} (Asmus and Asmus, 1991). Uptake and release rates vary based on reef density, as different reefs sampled in Dame et al. (1984) had found rates ranging from 17 to 4112 μg m^{-2} hr^{-1}. Because oyster reef density was not provided in these studies, the uptake and release rates of these substances couldn't be confirmed for Town Creek oysters. However, the reef in Dame et al. (1985) was Bly Creek, South Carolina, which is relatively close in climate and therefore may be a comparable intertidal system. It is then likely that Town Creek oyster reefs have a similar nitrogen net removal rate of 1.5 mmol m^{-2} hr^{-1}.

Oysters do not filter out 100% of particles they encounter, which is due in part to variation in particle size. Greater particle size correlates with greater removal efficiency (Riisgard, 1988). C. virginica gills trap particles 5-7 μm in diameter for digestion, and directly deposit larger particles into sediment as pseudofeces. Efficiency of filtration drastically decreases for particles smaller than 5 μm, such as unattached *V. vulnificus* individuals, which *C. virginica* filter with only ~16% efficiency (Ward and Shumway, 2004). Microorganisms may be attached to larger sediment and nutrient particles however, which can increase filtration efficiency. In Irvine et al. (2002) it was shown that TSS levels positively correlate with fecal coliform levels, indicating that suspended solids harbor microorganisms. With the high levels of TSS in Town

Creek found in the water quality studies earlier in this chapter, we know that oyster microbial filtration efficiency in Town Creek will be quite high, so the removal rate for bacteria should not deviate far from the filtration rate.

5. CONCLUSION

- Oyster reefs currently cover 3.1% of the surface area of the study area in Town Creek. Horizontal reefs (oysters on shallow mudflats near the shore) make up 9,160 m^2 and vertical reefs (oysters attached to vertical structures) make up 730 m^2. It's estimated that there are a total of 2.64 million live oysters in Town Creek.
- In the past decade, oyster counts have decreased by almost 20%. Since October 2009, horizontal reef area has decreased, but vertical reef area has increased due to the construction of new docks and the high-rise Highway 70 bridge.
- Currently, the oysters in Town Creek are filtering at a rate of 2,400 to 160,000 m^3 H_2O day^{-1}, with a best estimate of 67,000 m^3 H_2O day^{-1}. Clearance times range from 2.7 to 190 days, with a best estimate of 6.6 days.
- Eastern oysters have been shown to filter out bacteria, including *V. vulnificus, V. parahaemolyticus, V. alginolyticus, E. coli, and Enterococcus*, as well as chlorophyll *a* and contribute to nitrogen cycling.
- Because flushing times (25.8 to 51.7 hours) and residence times (one hour) are much lower than the calculated oyster clearance times (6.6 days), the current oysters in Town Creek are not filtering a significant amount of water or substances in the water.

SYNTHESIS

To assess water quality in Town Creek, we mapped the spatial distribution of possible contamination sources, analyzed water movement and circulation, determined the nutrient and microbial content of water, and quantified the filtration capacity of oyster reefs and marshes. Additionally, we distributed surveys to community stakeholders and found the public perceives Town Creek as contaminated and unclean. Because the public uses Town Creek for boating, fishing, and swimming the most, their perception of the waterway is likely centered around these activities. Elevated microbial and nutrient abundances found in this study warrant the public's concern for water quality, at least in certain areas of Town Creek.

Three microbial testing sites (C1, M2, and M1) contained levels of *Enterococci* in excess of North Carolina state standards for 33%, 33%, and 15% of sampling dates respectively. However, because the flushing time varied between only 25-52 hours, Town Creek can be classified as a well flushed system, meaning that microbial populations do not have adequate time to incubate and accumulate in the system.

Similarly, Town Creek did not have any chlorophyll *a* nor nutrient levels that exceeded state regulations, which is indicative of adequate ecosystem health. Again, the short residence and flushing times experienced by the system suggest that Town Creek is unlikely to be sensitive to nutrient enrichment and experience algal blooms.

Our study indicated that the area closest to Turner St. Bridge has a higher residence time. This section of Town Creek was also found to contain the highest microbial and nutrient concentrations due to the more numerous stormwater outfalls in this area, which introduce bacteria and nutrients into the system. These findings indicate that the 44 stormwater outfalls within a mile of Town Creek could be a significant source of contamination.

Additionally, both FIB and nutrient concentrations consistently higher following rain events; however, outflow from the stormwater outfalls was present even during dry weather. The localization of elevated contaminant levels near stormwater outfalls indicate that the underlying problem is within the stormwater transport system, rather than marina dumping. However, it is difficult to exclude marinas as a source of contamination since signals from dumping could be diluted due to the high flushing of Town Creek.

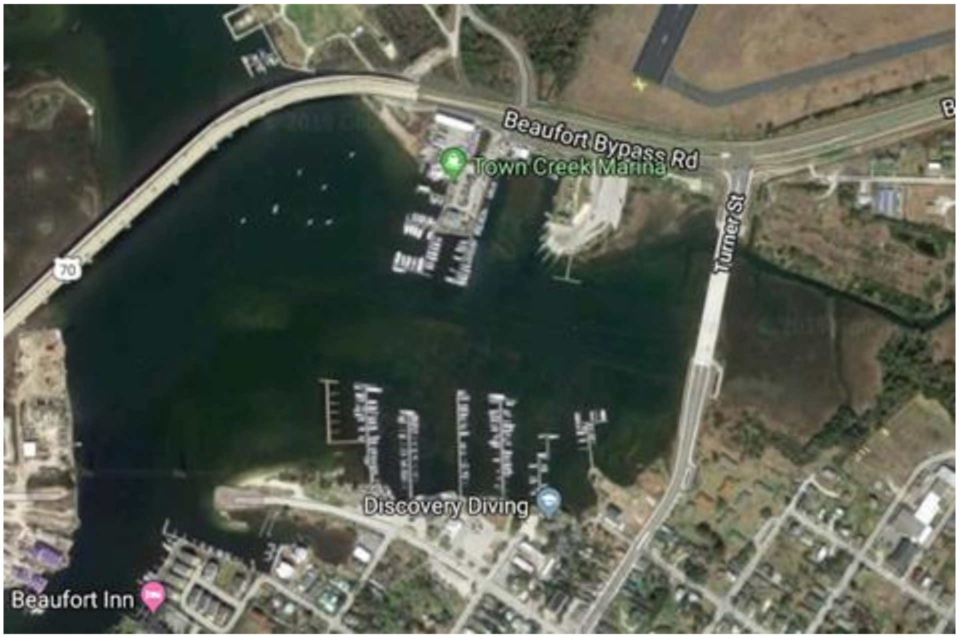
Although there are marsh and oyster reefs within Town Creek, their ability to improve water quality is hindered by the short flushing time, which was around 1-3 days. Oyster clearance time was calculated to be approximately 6.7 days, indicating that the oysters are filtering water, but not a significant volume. Furthermore, the marsh was estimated to remove the total phosphorus load in ~91 days and the nitrogen load in ~19 days with a denitrification rate of 23.17 $\mu mol*m^2*h^{-1}$. Because these times are longer than the flushing and residence times, it is likely the marsh does not have enough time to reduce N-P loading significantly before more loading occurs. To increase the extent of water filtration from these organisms, oyster reef and marsh area would need to be substantially expanded.

As the tourism and the population of Beaufort increases, consistent water quality monitoring in Town Creek will become increasingly important, due to frequent use by the community. Because our data suggests that stormwater outfalls contribute the majority of microbial and nutrient content, it is especially necessary to monitor water quality following rainfall events to assess the public health risks associated with recreational activities. Although nutrient levels were below the standard set by the NC DEQ, they should still be closely monitored since projected land development in the surrounding area may lead to increased runoff. Additionally, it is our recommendation that the town evaluates the condition of the stormwater outfall system since there is a continuous baseflow even during dry weather conditions.

APPENDIX

1. Business Survey

Please refer to this map of the Town Creek waterway and answer the following questions.



1. How long has this business been operating at its current location?

<1 year	3-5 years	10-20 years
1-2 years	6-10 years	20 or more years

2. How does your business use Town Creek?

Fishing Swimming Boating

Other – Please Explain

____

3. How often does your business use Town Creek, on average?

most days	1-3 times per week	1-3 times per month
1-3 times per year	never	Other. Please describe ____________

4. What specific location(s) of the waterway are directly used by your business? Please refer to the included map above and mark the area or areas used frequently by your business.

Answer Scale for Questions 5 and 6 (Choose the answer that best fits)

Strongly Disagree Neutral Strongly Agree

5. I feel my business has been restricted by environmental regulations.

1	2	3	4	5

6. Water quality in Town Creek is a concern of my business.

1	2	3	4	5

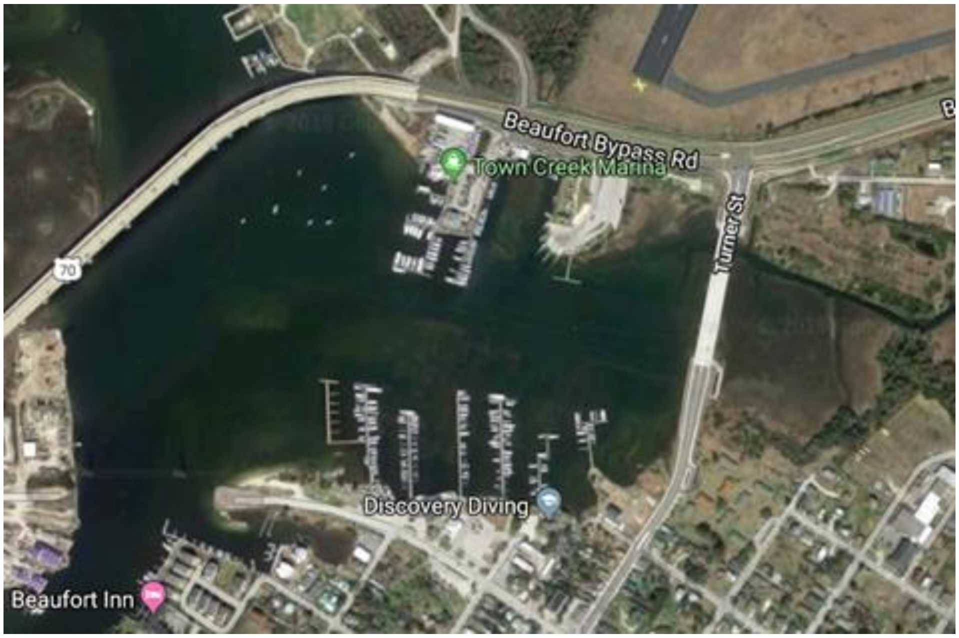
7. Are there any specific concerns you have relating to water quality in Town Creek?
Yes No
If yes, please explain:
____
____
____

8. Are there any other concerns you have relating to Town Creek?
Yes No
If yes, please explain:
____
____

9. What uses of Town Creek will be most important to you and your business in the future?
Fishing Swimming Boating
Other – Please explain
____

2. Resident Survey

Please refer to this included map of the Town Creek waterway and answer the following questions.



1. How long have you been a resident of Beaufort?

<1 year 3-5 years 10-20 years

1-2 years 6-10 years 20 or more years

What is your home's approximate distance to Town Creek?

<0.25 miles 0.25-0.5 miles 0.5-1 miles

1-2 miles 2-3 miles 3 or more miles

2. How often do you use Town Creek, on average?

most days 1-3 times per week 1-3 times per month

1-3 times per year never Other. Please explain ____________

3. How long have you been using Town Creek?

<1 year 3-5 years 10-20 years

1-2 years 6-10 years 20 or more years

4. What specific location(s) of the waterway do you use most often? Please refer to the included map above and mark the area or areas you use most frequently.

Answer Scale for Questions 5 and 6 (Choose the answer that best fits)

Disagree Neutral Agree

5. I am concerned about water quality in Town Creek.

1	2	3	4	5

6. Water quality is a concern of mine when using Town Creek for the following uses:

Fishing:

1	2	3	4	5

Boating:

1	2	3	4	5

Swimming:

1	2	3	4	5

Other: please explain ____

1	2	3	4	5

Answer Scale for Question 7 (Choose the answer that best fits)

Seldom Sometimes Most of the time

7. I use Town Creek for the following uses:

Fishing:

1	2	3	4	5

Boating:

1	2	3	4	5

Swimming:

1	2	3	4	5

Other: please explain ____

1	2	3	4	5

8. Are there any specific concerns you have relating to water quality in Town Creek?

Yes No

If yes, please explain:

____

____

____

9. Are there any other concerns you have relating to Town Creek?

Yes No

If yes, please explain:

____

____

____

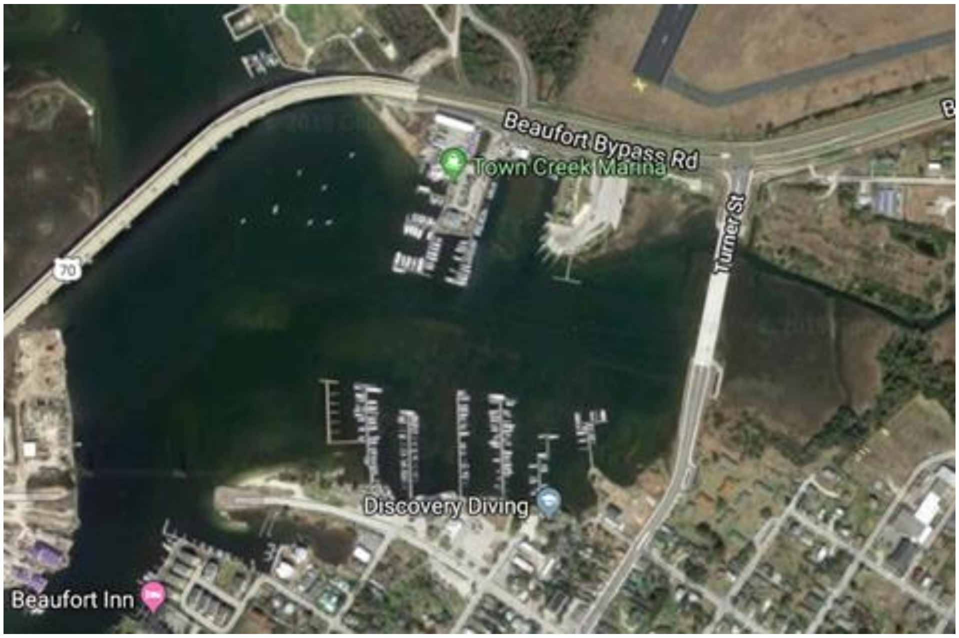
10. What uses of Town Creek will be most important to you in the future?

Fishing Swimming Boating

Other – please explain ____

3. Point of Access Survey

Please refer to this map of the Town Creek waterway and answer the following questions.



1. Are you a resident of Beaufort? Yes No

If yes, how many years have you lived here?

<1 year 3-5 years 10-20 years

1-2 years 6-10 years 20 or more years

If yes, what is your home's approximate distance to Town Creek?

<0.25 miles 0.25-0.5 miles 0.5-1 miles

1-2 miles 2-3 miles 3 or more miles

2. How often do you use Town Creek for recreation, on average?

most days 1-3 times per week 1-3 times per month

1-3 times per year never Other. Please describe _____________

3. How long have you been using Town Creek?

<1 year 3-5 years 10-20 years

1-2 years 6-10 years 20 or more years

4. What specific location(s) of the waterway do you use most often? Please refer to the included map above and mark the area or areas you use most frequently.

Answer Scale for Question 5 (Choose the answer that best fits)

Seldom Sometimes Most of the time

5. I use Town Creek for the following uses:

Fishing:

1	2	3	4	5

Boating:

1	2	3	4	5

Swimming:

1	2	3	4	5

Other: Please Explain ____

1	2	3	4	5

Answer Scale for Questions 6 and 7 (Choose the answer that best fits)

Disagree Neutral Agree

6. I am concerned about water quality in Town Creek.

1	2	3	4	5

7. Water quality is a concern of mine when using Town Creek for the following uses:

Fishing:

1	2	3	4	5

Boating:

1	2	3	4	5

Swimming:

1	2	3	4	5

Other: Please Explain ____

1	2	3	4	5

8. Are there any specific concerns you have relating to water quality in Town Creek?

Yes No

If yes, please explain:

____
____
____

9. Are there any other concerns you have relating to Town Creek?

Yes No

If yes, please explain:

____
____
____

10. What uses of Town Creek will be most important to you in the future?

Fishing Swimming Boating
Other – Please Explain

____

WORKS CITED

Altman, D. (2000, January). A Review of Experimentwise Type I Error: Implications for Univariate Post Hoc and for Multivariate Testing.. Dallas, TX: US Department of Education.

Anderson DM, Gilbert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:4 704-726

Balfour, Adam. (2012) "Design, Construction and Analysis of a Surface Drifter Fleet for Use in Coastal Waters." University of North Carolina at the Institute of Marine Sciences, 10 Dec.

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011, May 1). The value of estuarine and coastal ecosystem services. Retrieved November 15, 2019, from https://esajournals.onlinelibrary.wiley.com/doi/full/10.1890/10-1510.1.

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*(2), 169–193. doi: 10.1890/10-1510.1

Bricker et al. (2007). Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change. NOAA Technical Memorandum NOS NCCOS Coastal Ocean Program Decision Analysis Series 26. Silver Spring, MD. 328 pp.

Brush, G. S. (2008, October 16). Historical Land Use, Nitrogen, and Coastal Eutrophication: A Paleoecological Perspective. Retrieved November 15, 2019, from https://link.springer.com/article/10.1007/s12237-008-9106-z.

Carteret County Chamber of Commerce. (2019). Tourism. Retrieved November 27, 3019, from https://nccoastchamber.com/about-us/our-communities/tourism/.

Chae MJ, Cheney D, Su YC (2009) Temperature effects on the depuration of Vibrio parahaemolyticus and Vibrio vulnificus from the American oyster (Crassostrea virginica). J Food Sci 74(2):M62-6

Chudoba EA, Mallin MA, Cahoon LB, Skrabal SA (2013) Stimulation of fecal bacteria in ambient waters by experimental inputs of organic and inorganic phosphorus. Water Research 47:10 3455-3466

Cloern, James. E. (2001, January). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, *210*, 223-253. Retrieved from: https://www.researchgate.net/publication/235938844_Our_Evolving_Conceptual_Model_of_the_Coastal_Eutrophication_Problem. doi:10.3354/meps210222

Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, Powers SP, Tolley SG (2007) Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341: 303– 307

Cornwell, J., Kana, T., & Kemp, W. (1999, January). Denitrification in coastal ecosystems: Methods, environmental controls, and ecosystem level controls, a review. Retrieved November 15, 2019, from https://www.researchgate.net/publication/226422940_Denitrification_in_coastal_ecosystems_Methods_environmental_controls_and_ecosystem_level_controls_a_review.

Coulliette, A. D., & Noble, R. T. (2008). Impacts of rainfall on the water quality of the newport river estuary (eastern North Carolina, USA). Journal of Water and Health, 6(4), 473-482. doi:http://dx.doi.org/10.2166/wh.2008.136

Dame, R., & Kenny, P. (1986). Variability of Spartina alterniflora primary production in the euhaline North Inlet estuary. *Marine Ecology Progress Series*, *32*, 71–80. doi: 10.3354/meps032071

Dame RF, Spurrier JD, Wolaver TG (1989) Carbon, nitrogen, and phosphorus processing by an oyster reef. Marine Ecology Progress Series 54: 249-256

Dame RF, Wolaver TG, Libes SM (1985) The Summer Uptake and Release of Nitrogen by an Intertidal Oyster Reef. Netherlands Jour. of Sea Research 19 (¾): 265-268

Dame RF, Zingmark RG, Haskin E (1984) Oyster Reefs as Processors of Estuarine Materials. J. Exp. Mar. Biol. Ecol. 83: 239-247

Darby, F. A., & Turner, R. E. (2008, February 16). Below- and Aboveground Biomass of Spartina alterniflora: Response to Nutrient Addition in a Louisiana Salt Marsh. Retrieved November 15, 2019, from https://link.springer.com/article/10.1007/s12237-008-9037-8.

Day et al. (2017, June) Beaufort Watersheds Restoration Plan. Town of Beaufort, Eastern Carolina Council, & North Carolina Coastal Federation. Retrieved from: https://files.nc.gov/ncdeq/Water%20Quality/Planning/NPU/319/WatershedMGTPlans_9element/Beaufort%20Watersheds%20Restoration%20Plan.pdf

de Groot, R. S., Wilson, M. A., & Boumans, R. M. J. (2002, May 9). A typology for the classification, description and valuation of ecosystem functions, goods and services. Retrieved November 15, 2019, from https://www.sciencedirect.com/science/article/pii/S0921800902000897.

Donaghay, Percy L. & Osborn, Thomas R. (1997, July). Towards a Theory of Biological-Physical Control of Harmful Algal Bloom Dynamics and Impacts. *Limnology and Oceanography*, 42(5): 1283-1296. Doi: https://doi.org/10.4319/lo.1997.42.5_part_2.1283

Eastern Oyster *Crassostrea virginica*. (n.d.). Retrieved November 14, 2019, from https://www.chesapeakebay.net/S=0/fieldguide/critter/eastern_oyster.

Ehrich MK, Harris LA (2015) A review of existing eastern oyster filtration rate models. Ecological Modelling. 297: 201-212

Eighty years of Redfield. Nature Geosci 7, 849 (2014) doi:10.1038/ngeo2319 https://www.nature.com/articles/ngeo2319#citeas

Ensign, S., Currin, C., Piehler, M., & Tobias, C. (2016, December 31). A method for using shoreline morphology to predict suspended sediment concentration in tidal creeks. Retrieved November 15, 2019, from https://ui.adsabs.harvard.edu/abs/2017Geomo.276..280E/abstract.

Ensign, S. H., & Currin, C. (2016, December 17). Geomorphic Implications of Particle Movement by Water Surface Tension in a Salt Marsh. Retrieved November 15, 2019, from https://link.springer.com/article/10.1007/s13157-016-0862-4.

Fear, J. M., Thompson, S. P., Gallo, T. E., & Paerl, H. W. (2005, August). Denitrification rates measured along a salinity gradient in the eutrophic Neuse River estuary, North Carolina, USA. Retrieved November 15, 2019, from https://link.springer.com/article/10.1007/BF02696071.

Fensin, Elizabeth E. (2006). Impact of Tropical Storms and Drought on the Dinoflagellates Karlodinium micrum and Prorocentrum minimum in Estuarine Rivers of North Carolina, USA, *African Journal of Marine Science*, 28(2): 277-281. doi: https://doi.org/10.2989/18142320609504162

Filippelli, G. (2002). *The Global Phosphorus Cycle* (1st ed., Vol. 48). doi: https://doi-org.libproxy.lib.unc.edu/10.2138/rmg.2002.48.10

Fries, J., Characklis, G., & Noble, R. T. (2006, October). Journal of Environmental Engineering. Retrieved from https://ascelibrary.org/doi/full/10.1061/(ASCE)0733-9372(2006)132:10(1338).

Fries, J. S., Characklis, G. W., & Noble, R. T. (2008). Sediment–water exchange of Vibrio sp. and fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. *Water Research*, *42*(4-5), 941–950. doi: 10.1016/j.watres.2007.09.006

Froelich, B. A., et al. (2017). "Differences in abundances of total Vibrio spp., V. vulnificus, and V. parahaemolyticus in clams and oysters in North Carolina." Applied and environmental microbiology 83.2e02265-16.

Froelich BA, Noble RT (2014) Factors affecting the uptake and retention of *Vibrio vulnificus* in oysters. Appl Environ Microbiol 80: e02042-14. doi:10.1128/AEM.02042-14

Fulford R.S., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., Luckenbach, M. (2007). Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Mar. Ecol. Prog. Ser. 336: 43–61.

Giacalone, K., Mobley, C., Sawyer, C., Witte, J., & Eidson, G. (2010). Survey Says: Implications of a Public Perception Survey on Stormwater Education Programming. *Journal of Contemporary Water Research & Education*, *146*(1), 92–102. doi: 10.1111/j.1936-704x.2010.00395.x

Gilbert P.M. (2016). Algal Blooms. In: Kennish M.J. (ed) Encyclopedia of Estuaries. Encyclopedia of Earth Sciences Series. Springer, Dordrecht.

Gong et al. (2017). Molecular Insights into a Dinoflagellate Bloom. *International Society for Microbial Ecology,* 11: 439-452. doi: 10.1038/ismej.2016.129

Goreau, Thomas J., et al. "Production of NO2- and N2O by Nitrifying Bacteria at Reduced Concentrations of Oxygen." *Applied and Environmental Microbiology*, American Society for Microbiology, 1 Sept. 1980, aem.asm.org/content/40/3/526.short.

Gowen, R., Tett, P., & Jones, K. (1992). Predicting marine eutrophication: The yield of chlorophyll from nitrogen in Scottish coastal waters. *Marine Ecology Progress Series, 85*(1/2), 153-161. Retrieved from www.jstor.org/stable/24829930

Grabowski JH, Peterson CH (2007) Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds) Ecosystem engineers: concepts, theory and applications. Elsevier-Academic Press, Amsterdam, p 281–298

Hall et al. (2008). Environmental Factors Contributing to the Development and Demise of a Toxic Dinoflagellate (Karlodinium veneficum) Bloom in a Shallow, Eutrophic, Lagoonal Estuary. *Estuaries and Coasts,* 31: 402-418. doi: 10.1007/s12237-008-9035-x

Henderson, C. (2002). "Shellfish Closures: Signs of Troubled Waters." Coastwatch, Sea Grant North Carolina , 2002, ncseagrant.ncsu.edu/coastwatch/previous-issues/2002-2/spring-2002/shellfish-closures-troubledwaters/. Retrieved November 11, 2019.

Hill, T., Sommer, N., Kanaskie, C., Santos, E., & Oczkowski, A. (2018). Nitrogen uptake and allocation estimates for Spartina alterniflora and Distichlis spicata. Retrieved November 15, 2019, from 10.1016/j.jembe.2018.07.006.

Hsieh, J. L., Fries, J. S., & Noble, R. T. (2007, July 1). VIBRIO AND PHYTOPLANKTON DYNAMICS DURING THE SUMMER OF 2004 IN A EUTROPHYING ESTUARY. Retrieved from https://esajournals.onlinelibrary.wiley.com/doi/epdf/10.1890/05-1274.1.

Hughes AO, Davies-Colley RJ, Elliott AH (2015). Measurement of light attenuation extends the application of suspended sediment monitoring in rivers. International Association of Hydrological Sciences. Publ. 367. doi:10.5194/piahs-367-170-2015

Irvine KN, Somogye EL, Pettibone GW (2002) Turbidity, Suspended Solids, and Bacteria Relationships in the Buffalo River Watershed. Middle States Geographer 35: 42-51

Jorge L., J., & G., C. (2006, March 1). Physical Ecosystem Engineers as Agents of Biogeochemical Heterogeneity. Retrieved November 15, 2019, from https://academic.oup.com/bioscience/article/56/3/227/333076.

K. R. Reddy, R. H. Kadlec, E. Flaig & P. M. Gale (1999): Phosphorus Retention in Streams and Wetlands: A Review, Critical Reviews in Environmental Science and Technology, 29:1, 83-146

Kaspar, C., & Tamplin, M. (1993, August 1). Effects of temperature and salinity on the survival of Vibrio vulnificus in seawater and shellfish. Retrieved from https://aem.asm.org/content/59/8/2425.short.

Kaysner, C. A & DePaolo, A. (2004), "Laboratory Methods - BAM: Vibrio. " US Food and Drug Administration Home Page, Center for Food Safety and Applied Nutrition, www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm070830.htm.

Kimmerer, Wim J. (2004) "Open Water Processes of the San Francisco Estuary: From Physical Forcing to Biological Responses." San Francisco Estuary and Watershed Science, vol. 2, no. 1, 2004, doi:10.15447/sfews.2004v2iss1art1.

Kirby-Smith, W., & White, N. (2006). Bacterial contamination associated with estuarine shoreline development. *Journal of Applied Microbiology*, *100*(4), 648–657. doi: 10.1111/j.1365-2672.2005.02797.x

Kunu, T. M., Sullivan, M. B., Cornwell, J. C., & Groxzkowski, K. M. (2003, December 22). Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. Retrieved November 15, 2019, from https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.1998.43.2.0334.

Kuo, Albert Y., and Bruce J. Neilson. (1987) "Hypoxia and Salinity in Virginia Estuaries." Estuaries, vol. 10, no. 4, 1987, p. 277., doi:10.2307/1351884.

Lee, C. M., Lin, T. Y., Lin, C.-C., Kohbodi, G. A., Bhatt, A., Lee, R., & Jay, J. A. (2006). Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. *Water Research*, *40*(14), 2593–2602. doi: 10.1016/j.watres.2006.04.032

Lentz, S., Carr, M., & Herbers, T. H. C. (2001). Barotropic tides on the North Carolina shelf. Journal of Physical Oceanography, 31(7), 1843– 1859. https://doi.org/10.1175/1520-0485(2001)031<1843:BTOTNC>2.0.CO;2

Linthurst, R. A., & Seneca, E. D. (1981, March). Aeration, nitrogen and salinity as determinants of Spartina alterniflora Loisel. Growth response. Retrieved November 15, 2019, from https://link.springer.com/article/10.2307/1351542.

Love DC, Lovelace GL, Sobsey MD (2010) Removal of Escherichia coli, Enterococcus fecalis, coliphage MS2, poliovirus, and hepatitis A virus from oysters (Crassostrea virginica) and hard shell clams (Mercinaria mercinaria) by depuration. Int J Food Microbiol 143(3):211-7

McRoy, C. P., Barsdate, R. J., & Nebert, M. (2003, December 22). Phosphorus Cycling in an Eel Grass (Zostera Marina L.) Ecosystem. Retrieved from https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.1972.17.1.0058.

Monsen, N. E., Cloern, J. E., Lucas, L. V., & Monismith, S. G. (2002). A comment on the use of flushing time, residence time, and age as transport time scales. Limnology and oceanography, 47(5), 1545-1553.

Newell RIE, Langdon CJ (1996) Mechanisms and physiology of larval and adult feeding. V.S. Kennedy, R.I.E. Newell, A. Eble (Eds.), The Eastern Oyster Crassostrea virginica, Maryland Sea Grant College, College Park, MD (1996), pp. 185-230

Nicolson, J. A., & Mace, A. C. (1975). Water Quality Perception By Users: Can It Supplement Objective Water Quality Measures? *Journal of the American Water Resources Association*, *11*(6), 1197–1207. doi: 10.1111/j.1752-1688.1975.tb01842.x

NOAA. (October 2019). Tidal Data. Retrieved November 3, 2019, from https://tidesandcurrents.noaa.gov/noaatidepredictions.html?id=8656483.

NOAA. (2019, August 16). Oyster Reef Habitat. Retrieved from https://www.fisheries.noaa.gov/national/habitat-conservation/oyster-reef-habitat.

Noble, R. T., Weisberg, S., McGee, C., H., J., Vainik, P., Leecaster, M., & Orozco-Borbon, V. (2003, March 1). Storm effects on regional beach water quality along the southern California shoreline. Retrieved from https://iwaponline.com/jwh/article/1/1/23/1788/Storm-effects-on-regional-beach-water-quality.

North Carolina Department of Environmental Quality. (n.d.). NCDWR Algal Bloom Map. Retrieved December 10, 2019, from https://ncdenr.maps.arcgis.com/apps/webappviewer/index.html?id=58e9afca8b724b3f82cc81a8b825f83e.

Osgood, D., & Zieman, J. (1993, December). Factors Controlling Aboveground Spartina alterniflora (Smooth Cordgrass) Tissue Element Composition and Production in Different-Age Barrier Island Marshes. Retrieved November 15, 2019, from https://www.jstor.org/stable/pdf/1352440.pdf?refreqid=excelsior:6ef76a3ee3446ffd38f47716f615efec.

Paerl, H., Otten, T., & Kudela, R. (2018). Mitigating the Expansion of Harmful Algal Blooms Across the Freshwater-to-Marine Continuum. *Environmental Science and Technology*, 52(10), 5519-5529. https://doi-org.libproxy.lib.unc.edu/10.1021/acs.est.7b05950

Paerl, H. (1997). Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as “new” nitrogen and other nutrient sources. Retrieved November 15, 2019, from https://pdfs.semanticscholar.org/4118/d963b708051d9c1775eb65c1308f19319116.pdf.

Parker, J. K., Mcintyre, D., & Noble, R. T. (2010). Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. *Water Research*, *44*(14), 4186–4194. doi: 10.1016/j.watres.2010.05.018

Patrick, W., & Delaune, R. (1976). Nitrogen and phosphorus utilization by Spartina alterniflora in a salt marsh in Barataria Bay, Louisiana. *Estuarine and Coastal Marine Science*, *4*(1), 59–64. doi: 10.1016/0302-3524(76)90006-2

Peierls, B., Hall, N., & Paerl, H. (2012). Non-monotonic Responses of Phytoplankton Biomass Accumulation to Hydrologic Variability: A Comparison of Two Coastal Plain North Carolina Estuaries. *Estuaries and Coasts.* doi:10.1007/s12237-012-9547-2

Phlips, et al., (2012). Climatic Influences on Autochthonous and Allochthonous Phytoplankton Blooms in a Subtropical Estuary, St. Lucie Estuary, Florida, USA. *Estuaries and Coasts*, *35*(1), 335-352.

Piehler, M., et al. (2004). Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. *Estuarine, Coastal, and Shelf Science*, 61(2), 197-209. https://doi.org/10.1016/j.ecss.2004.05.001

Pietrafesa, L.J., and Janowitz, (1985) G.S. Physical oceanographic processes affecting larval transport around and through North Carolina inlets. United States: N. p., Web.

Pruss, A. (1998). Review of epidemiological studies on health effects from exposure to recreational water. *International Journal of Epidemiology*, *27*(1), 1–9. doi: 10.1093/ije/27.1.1

Recreational Water Quality Criteria, Recreational Water Quality Criteria (2012). Retrieved from https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf

Reimold, R. J. (2003, December 22). The Movement of Phosphorus through the Salt Marsh Cord Grass, Spartina Alterniflora Loisel. Retrieved from https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.1972.17.4.0606.

Reyes–Velázquez C, Castañeda–Chávez MR, Landeros–Sánchez C, Galaviz–Villa I, Lango–Reynoso F, Minguez–Rodríguez MM, Nikolskii–Gavrilov I (2010) Pathogenic vibrios in the oyster Crassostrea virginica in the lagoon system of Mandinga, Veracruz, Mexico. Hydrobiology 20(3)

Riisgard, H.U., 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Mar. Ecol. Prog. Ser. 45 (3), 217–223.

Ruttenberg, K. (2003, November 30). The Global Phosphorus Cycle. Retrieved from https://ui.adsabs.harvard.edu/abs/2003TrGeo...8..585R/abstract.

Sanford, Lawrence P., et al. (1992) “Model for Estimating Tidal Flushing of Small Embayments.” Journal of Waterway, Port, Coastal, and Ocean Engineering, vol. 118, no. 6, pp. 635–654., doi:10.1061/(asce)0733-950x(1992)118:6(635).

Seitzinger, S. P. (2003, December 22). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Retrieved November 15, 2019, from https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.1988.33.4part2.0702.

Simpson, J. H., and J. R. Hunter. (1974): Fronts in the Irish Sea. Nature, 250, 404–406.

Smayda, Theodore J. (1997). Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography*, 42(5), 1137-1153.

Smyth, A. R., Piehler, M. F., & Grabowski, J. H. (2015, April 30). Habitat context influences nitrogen removal by restored oyster reefs. Retrieved November 15, 2019, from https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.12435.

Southworth, M., Harding, J. M., Wesson, J. A., & Mann, R. (2010). Oyster (Crassostrea virginica, Gmelin 1791) Population Dynamics on Public Reefs in the Great Wicomico River, Virginia, USA. *Journal of Shellfish Research*, *29*(2), 271–290. doi: 10.2983/035.029.0202

Speight, James G. “Environmental Inorganic Chemistry for Engineers.” ScienceDirect, 2017, www.sciencedirect.com/book/9780128498910/environmental-inorganic-chemistry-for-engineers?via=ihub.

Stumpf, C. H., Piehler, M. F., Thompson, S., & Noble, R. T. (2010). Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: Hydrographic patterns and terrestrial runoff relationships. *Water Research*, *44*(16), 4704–4715. doi: 10.1016/j.watres.2010.07.004

Sundby, B., Gobeil, C., Silverberg, N., & Alfonso, M. (2003, December 22). The phosphorus cycle in coastal marine sediments. Retrieved from https://aslopubs.onlinelibrary.wiley.com/doi/epdf/10.4319/lo.1992.37.6.1129.

U.S. Army Corp. (n.d.). (2017-2019). USACE Hydrographic Surveys. Retrieved October 2019, from https://www.arcgis.com/apps/opsdashboard/index.html#/4b8f2ba307684cf597617bf1b6d2f85d.

U.S. Department of Commerce & NOAA. (2013) “Types and Causes of Tidal Cycles - Tides and Water Levels: NOAA’s National Ocean Service Education.” oceanservice.noaa.gov/education/tutorial_tides/tides07_cycles.html.

U.S. Environmental Protection Agency (USEPA). (1986). Bacteriological Ambient Water Quality Criteria for Marine and Fresh Recreational Waters. EPA 440/5-84-002. EPA Office of Water. PB-86-158-045.

USDA. (2019). USDA Plants Database. *USDA Plants Database*. USDA. Retrieved from https://plants.usda.gov/core/profile?symbol=SPAL

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Wallace, R. K. (2001). *Cultivating the Eastern Oyster, Crassostrea virginica*. *Cultivating the Eastern Oyster, Crassostrea virginica*. Southern Regional Aquaculture Center. Retrieved from https://www.agmrc.org/media/cms/9008383432fs_28E8EB79FF3AA.pdf

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300:83–130.

Water Levels: NOAA's National Ocean Service Education.” Types and Causes of Tidal Cycles - Tides and Water Levels: NOAA's National Ocean Service Education. oceanservice.noaa.gov/education/tutorial_tides/tides07_cycles.html.

Water Quality Assessment Process (2014). North Carolina Department of Environmental Quality. Retrieved from: https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-quality-data-assessment

Wiltsie et al. (2018). Algal Blooms and Cyanotoxins in Jordan Lake, North Carolina. Toxins, 10(2), 92. http://dx.doi.org.libproxy.lib.unc.edu/10.3390/toxins10020092

Yang et. al. (2008). Purification of nitrate-rich agricultural runoff by hydroponic system. *Bioresource Technology*, 99(17), 8049-8053. Doi: https://doi.org/10.1016/j.biortech.2008.03.04