Assessing the Utility of Shellfish Sanitation Monitoring Data for Long-Term Estuarine Water Quality Analysis

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ABSTRACT

Regular testing of coastal waters for fecal coliform bacteria by shellfish sanitation programs could provide data to fill large gaps in existing coastal water quality monitoring, but research is needed to understand the opportunities and limitations of using these data for inference of long-term trends. In this study, we analyzed spatiotemporal trends from multidecadal fecal coliform concentration observations collected by a shellfish sanitation program, and assessed the feasibility of using these monitoring data to infer long-term water quality dynamics. We evaluated trends in fecal coliform concentrations for a 20-year period (1999-2021) using data collected from spatially fixed sampling sites (n = 466) in North Carolina (USA). Findings indicated that shellfish sanitation data can be used for long-term water quality inference under relatively stationary management conditions, and that salinity trends can be used to measure the extent of management-driven bias in fecal coliform observations collected in a particular area.

1. INTRODUCTION

Healthy estuarine environments are critical for maintaining ecological stability, coastal economies, and human health standards. In order to maintain and even improve these habitats, metrics of current and past conditions must be evaluated to inform proper management. Water quality measurements can be used to indicate overall estuarine health and can aid in understanding increasing coastal threats such as rising sea levels, increased salinities, and urbanization. Long-term water quality analysis is key for developing target thresholds for future management action as well as assessing the efficacy of past management measures (Cloern et al., 2016). The value of historical observations in advancing understanding of estuarine water quality has been demonstrated by multi-decadal studies of several systems, including the San Francisco Bay area (Beck et al., 2018; Cloern et al., 2016), May River, South Carolina (Souedan et al., 2021), Texas's coastline (Bugica et al., 2020), and the Chesapeake Bay area (Zhang et al., 2018; Harding et al., 2019). Most notably, long-term water quality monitoring in the Chesapeake Bay has led to the identification of climatic and anthropogenic drivers for certain water quality parameters and subsequent evaluation of the effectiveness of past management and restoration efforts (Kemp et al., 2005; Leight et al., 2011; Zhang et al., 2018; Harding et al., 2019).

Datasets used for prior longitudinal water quality studies are commonly a product of governmental agencies developing localized programs, like the Chesapeake Bay Program (Chesapeake Bay Monitoring Program, 2022), in response to increasing population and significant degradation of vital estuarine ecosystems. While national and regional efforts have attempted to provide unbiased, sustained monitoring, these programs currently lack the spatial extent needed to capture coastwide water quality trends. The National Estuarine Research Reserve System (NERRS) is one of the few organizations with dedicated coastal water quality monitoring stations, which are included as part of the NERRS System Wide Monitoring Program (SWMP) that maintains 355 coastal water quality monitoring stations across 29 designated coastal reserves along the USA coastline (National Estuarine Research Reserve System, 2022). Compared to the over 13,500 freshwater monitoring stations maintained by the United States Geological Survey (USGS, 2022), the relatively small number of water quality monitoring stations across coastal and estuarine waters (NOAA Tides & Currents, 2022; US EPA, 2022) are likely not representative of the variations in environmental conditions that we observe across the tens of thousands of miles of shoreline along the United States.

Because of the limited number of unbiased monitoring programs, the ability to use water quality data from regulatory operations presents a potentially valuable resource for assessing long-term estuarine conditions. Regulatory programs differ from monitoring programs by collecting water quality samples to meet regulatory requirements and inform short-term decision-making. For example, in North Carolina (NC), there are four NERRS SWMP monitoring stations and eight coastal stations with water quality data available through the USGS (South Atlantic Water Science Center, North Carolina Office, 2022) and fifty stations from the NC Ambient Monitoring System (Water Quality Portal, 2021), but the NC Division of Marine Fisheries (NCDMF) shellfish sanitation program maintains 1,924 water quality monitoring stations. In fact, state shellfish sanitation programs across the USA collect an abundance of water quality observations, and often have for decades. Shellfish mariculture is highly dependent on water quality monitoring due to the direct influence that ambient conditions have on the safety of shellfish meat consumption. The U.S. Food and Drug Administration's National Shellfish Sanitation Program (NSSP) was developed in 1925 to maintain public safety and human health standards in relation to the consumption of shellfish grown in potentially polluted waters (NSSP, 2019). The implementation of the NSSP has resulted in systematic sampling of water quality for day-to-day fisheries regulation, specifically for Fecal Indicator Bacteria (FIB), a group of bacteria that are commonly used as a proxy measure for harmful pathogen loads in the waterway that could potentially be incorporated into shellfish meat through filter feeding. Thus, fecal coliforms (FC), a type of FIB, and other environmental factors that contribute to FC load and water quality, are regularly measured in shellfish growing waters due to the food safety implications. As a product of this regular testing, fisheries operations have accumulated decades of data with the potential to provide insights on historical trends with wide spatial extents, potentially filling gaps in long-term water quality monitoring capacity.

However, because of the limited resources and industry specific priorities, regulatory data can maintain underlying biases as a result of the sampling methodology used to collect the water quality sample. Often, the collection of a sample can be motivated by day-to-day operational decisions, such as weather, the availability of field technicians, and ease of collection. These operational decisions lead to non-random sampling that provides observations that are not always representative of the system's true dynamics. Engaging regulatory personnel to understand their fisheries management and sampling decisions is necessary to properly analyze the observations collected by shellfish sanitation programs.

For example, the NSSP permits states to employ one of two sampling strategies when collecting regulatory water quality data in shellfish growing waters: adverse pollution condition sampling and systematic random sampling. The adverse pollution condition sampling strategy describes sampling in periods when known contamination events (commonly due to point-source pollution events or rainfall events) have degraded the water quality, and data collected under these conditions capture peak contamination. States must collect "a minimum of five samples... annually under adverse pollution conditions from each sample station in the growing area" (NSSP, 2019) to meet NSSP sampling requirements. In contrast, the systematic random sampling strategy describes the collection of data across "a statistically representative cross section of all meteorological, hydrographic, and/or other pollution events" (NSSP, 2019), resulting in the data collection under varied environment and climactic conditions. For state programs that use systematic random sampling, the NSSP requires samples be collected at least 6 times throughout the year (NSSP, 2019). As a result of the requirements for the conditions under which the two systems of sampling can take place, the resulting data may be biased and impact their utility for use in long-term water quality assessments. With our growing reliance on aquaculture and the expanding value of shellfish production driving the development of fisheries management infrastructure (Azra et al., 2021), long-term datasets available through shellfish sanitation programs will become increasingly valuable. Realizing the potential of regulatory datasets to inform long-term water quality trends is a vital next step for assessing the health of our coastal ecosystems, but research is needed to determine the utility of these data for water quality analyses.

The goal of this study was to utilize shellfish management data to infer long-term spatiotemporal trends in water quality parameters, including FC and salinity, while accounting for variation in routine sampling conditions and environmental landscapes. Study objectives included (1) analyzing spatiotemporal trends from multidecadal fecal coliform concentration observations collected by a shellfish sanitation program, (2) identifying possible management and environmental drivers of fecal coliform trends, and (3) assessing the feasibility of using these monitoring data to infer long-term water quality dynamics. We focused on North Carolina's shellfish waters as a representative study system due to the availability of public, digitized multidecadal data, and the region's rapidly growing population, wide variety of land use characteristics along the coast, presence of the second largest estuarine system in the contiguous USA, and growing shellfish industry. Ultimately, this study demonstrates the application of shellfish management data for long-term water quality trend analysis in estuaries, informs future resource management strategies, and reveals new insights into the functioning of coastal systems.

2. METHODS

2.1 Study Area Description: Shellfish Waters in North Carolina, USA

The study area spanned all marine and estuarine waters in coastal North Carolina, which are subdivided into shellfish growing areas (SGAs) (Figure 1a,b). SGAs are subdivisions of waterways used to support shellfish harvest through delineating administrative boundaries for regulatory purposes. SGAs spatially cover North Carolina's shoreline from Currituck Sound in the north to Brunswick County in the south. These SGAs are named with alphabetic letters (e.g. "A", "B", etc.; Figure 1b), and further categorized through a letter-number system (ex. "A01"). There are 9 SGA letter groups along North Carolina's coast and these groups of SGAs have similar ecological features and approximately correspond to County jurisdictions. This study excludes the northernmost SGA ("I") due to the lack of open shellfish growing areas and discontinuous water quality data. SGAs vary in environmental and managerial conditions across the NC coast. These variations manifest as differences in estuarine type which can be defined using physical measures of area, depth, volume, freshwater flow, and salinity within the estuary (Engle et al., 2007).

SGAs can contain multiple classifications including approved, conditionally approved, restricted, and prohibited areas. Of the 9,208 km^2 of shellfish waters, 5,910 km^2(64.18%) are approved or conditionally approved. Observations collected through routine monitoring programs are used to help establish the classifications within SGAs. The NCDMF Shellfish Sanitation and Recreational Water Quality Section has jurisdiction over the classification of coastal waters for shellfish harvest, and also regulates closures and openings of conditionally approved SGAs. A majority of the NCDMF sampling stations included in this analysis are within the approved and conditionally managed SGAs (Figure 1a).

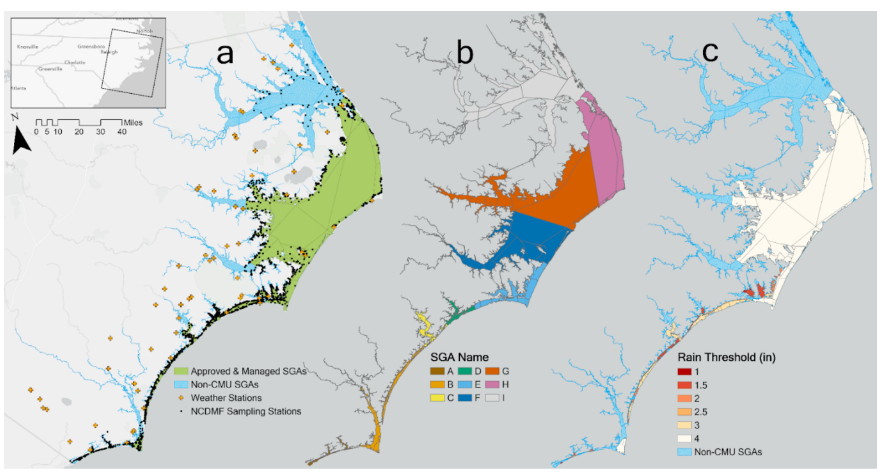

Figure 1 . Leftmost map (a) illustrates NCDMF conditional management units with water quality sampling

stations. Blue waters correspond to waters that are not approved for shellfish growing and harvesting, while the green areas correspond to the conditionally managed shellfish growing waters. The black points indicate the exact locations of the NCDMF water quality sampling stations and the orange crosses indicate the locations of the weather stations used to gather the precipitation time series data. The middle map (b) illustrates the NCDMF SGAs colored by the first letter of their SGA name. The rightmost map (c) illustrates rainfall thresholds (as of July 2021) for NCDMF conditionally managed areas measured in inches. Dark red indicates a rainfall closure threshold of 1 inch where the lightest white indicates areas with a rainfall closure threshold of 4 inches. Study area maps were created in ArcGIS Pro version 2.8.

2.1.1 Sampling strategies

In accordance with the NSSP, NCDMF routinely samples all shellfish growing areas on a regular basis (6 times annually) using the systematic random sampling strategy, meaning samples for each station are collected at randomly scheduled timepoints throughout the year; however, there are some constraints as to when sample collection is permissible. Specifically, samples are only collected during conditions when the SGAs are open for harvest or assumed to be unimpacted by unsafe FC levels, resulting in the subsequent data not capturing peak FC concentrations. Because freshwater input and runoff are tied to increased FC concentrations in estuarine waters, precipitation intensity is used as a management indicator for closures of conditionally approved waterways (NSSP, 2019; Leight et al., 2016). In North Carolina, rainfall closure thresholds in conditionally approved waters range from 1-3 inches of rainfall within a 24-hour period (Figure 1c) and dictate if a managed shellfish area will be closed for harvesting after a meteorological event. The "emergency closure" of additional areas can occur after higher rainfall amounts are noted and, for the purposes of this analysis, those areas will be represented by a 4 inch threshold. Rainfall thresholds are assigned to conditionally managed areas. To reopen closed areas that have been temporarily closed following exceedances of those rainfall thresholds, NCDMF tests the water, and reopening will only occur after samples confirm safe harvest conditions, which is defined by waters that do not exceed a FC density of 14 MPN per 100 mL of sample (NSSP, 2019); sampling for reopening is hereafter referred to as "conditional sampling". NCDMF balances its limited resources with speed of reopening by only conditionally sampling when the organization suspects the FC concentrations will be low enough to support reopening. SGAs A, C, D, E, and the northern half of B are characterized by moderate to low rainfall thresholds (i.e., 1-3 inches) whereas SGAs F, G, H, I, and the southern half of B have high rainfall thresholds (i.e., 4 inches; Figure 1c).

Under systematic random sampling, routine sampling in conditionally approved waters must occur when the SGA is open and not during a temporary closure. Accordingly, this stipulation creates bias in the routine monitoring data. As such, areas with a 1 inch rainfall threshold will close more often than areas with 4 inch thresholds, resulting in more restrictive sampling conditions and more homogeneous water quality samples in the long-term. Furthermore, NCDMF will change rainfall thresholds or exclude stations for areas with persistently poor water quality, resulting in the longest-standing monitoring stations typically being located in growing areas that are known to be productive with relatively good water quality, contributing another source of bias to the data.

2.2 Data Description

To analyze shellfish sanitation program data, water quality observations were compiled for the study area along with additional environmental variables believed to be potential descriptors of FC trends including salinity, precipitation, land use, and sampling station distance to closest shoreline. These variables have been established as relevant to FC concentrations in estuarine waters (Chigbu et al., 2004; Chigbu et al., 2005; Campos et al., 2013; Leight et al., 2016)

2.2.1 Water quality

During each sampling event, a grab sample is collected for FC analysis. To enumerate FC, NCDMF uses a five-tube decimal dilution, method SM 9221 (NSSP, 2019). Data records also include monitoring type to indicate the type of sampling (either conditional or routine), coded SGA designation (i.e., names listed in Figure 1b), station name, date of the sample, tide at the time of sample, salinity, and water temperature. The data are publicly available through NCDMF, and have been reorganized into a normal form database called "ShellBase", which is freely available through the Southeast Coastal Ocean Observing Regional Association's Data Portal (SECOORA, 2022).

In our study, the data were filtered to only include routine samples from stations that had 20 years of continuous water quality sampling data from 1999 to 2021. 466 stations met these conditions. We chose the time period of 1999 to 2021 due to changes in management procedures in the NCDMF in the 1990's. Throughout the 1990's, NCDMF progressively introduced conditional management plans to many of the growing areas. Therefore, by confining the dataset from 1999 to 2021, we are safely ensuring that the conditional management plans have been introduced and that the sampling would be reflective of that. Observations collected through conditional sampling were not considered in the analysis because the varied conditions under which conditional sampling occurs results in skewed FC concentrations and is not as representative of the system as the data collected through routine sampling design.

2.2.2 Salinity

Because data collected through the NCDMF monitoring program may have preferential sampling bias, we sought to analyze additional salinity data collected from an unbiased monitoring program in order to understand how the NCDMF observations compared to an independent dataset. We accessed the North Carolina Division of Water Resources (NCDWR) Water Quality Portal (Water Quality Portal, 2021). The NCDWR dataset consists of 17 monitoring stations that had at least 10 years of salinity data between the years 1999 and 2021 and were within proximity to the NCDMF water quality sampling stations (Figure 2); stations with fewer than 10 years of data or in portions of the estuary that did not overlap with NCDMF stations (e.g., fresh headwaters) were not considered in the analysis. However, data from NCDWR sites in SGAs D and E were still considered despite being relatively distant from NCDMF monitoring locations as these were the only NCDWR sites present in SGAs D and E.

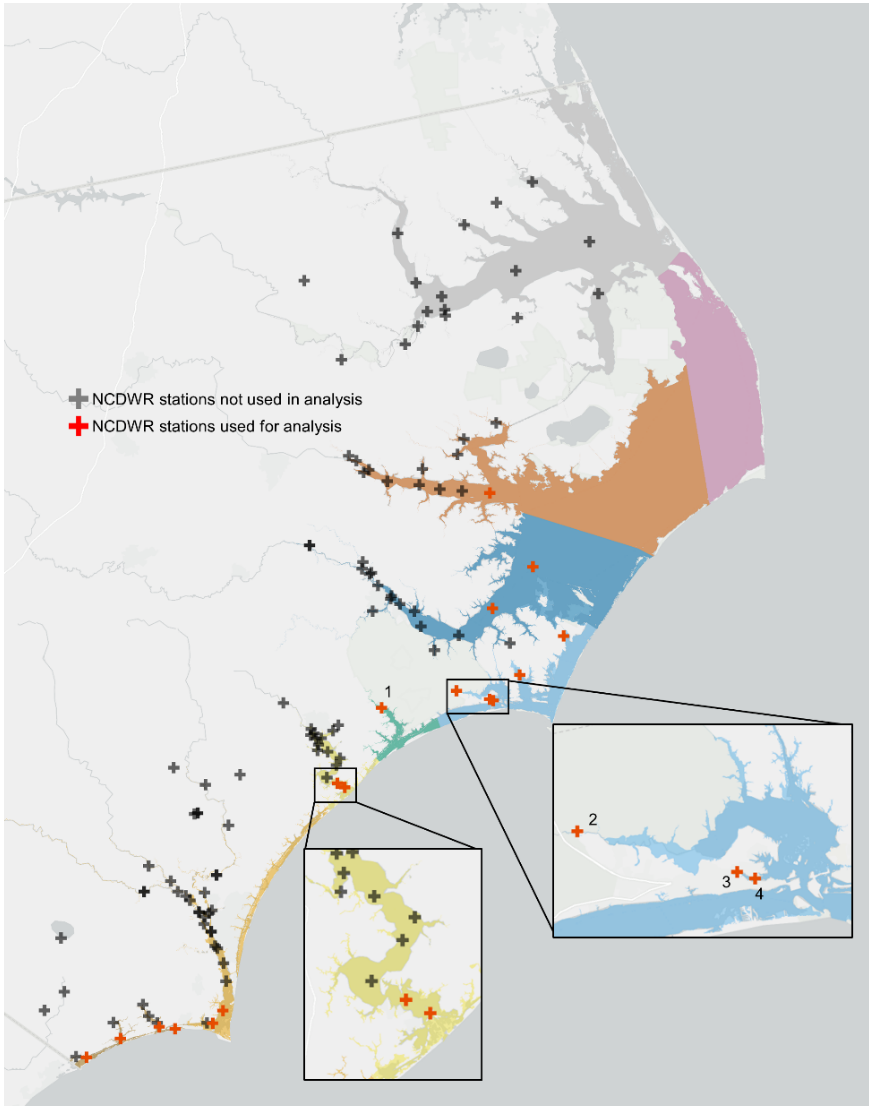

Figure 2 . Map of NCDWR water quality sampling stations along the coast of North Carolina. Numbers 1 – 4 are added to annotate stations referenced in Figure 5. Stations are colored by their use in the analysis. Study area maps were created in ArcGIS Pro version 2.8

2.2.3 Precipitation

The precipitation data was gathered through the North Carolina State Climate Office for weather stations (n = 70) that contained continuous data for 1999 to 2021 (Figure 1a). Precipitation data were related to water quality sampling locations as a function of proximity.

2.2.4 Geographic context

Land Use and Land Cover (LULC) data for North Carolina in 2001 and 2019 were compiled from the National Land Cover Database created by the Multi-Resolution Land Characteristics Consortium (MRLC). LULC categories were consolidated into "Developed", "Barren", "Cultivated", "Vegetated", and "Wetlands" classes (Table S1) and summarized for each coastal watershed, defined by USGS 12 digit Hydrologic Unit Codes (HUC12; Figure 3).

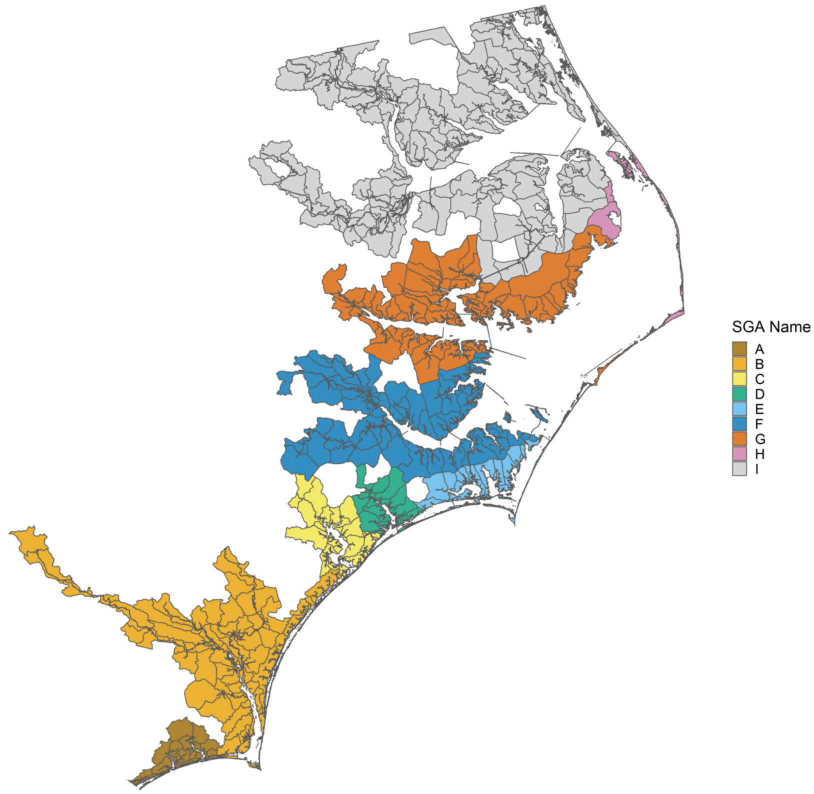

Figure 3. Map of coastal North Carolina HUC12 watershed areas that were used to summarize land use change between 2019 and 2001. The watersheds in this map are colored by the SGA name that the watershed is closest to and therefore associated with. Study area map was created in R version 4.1.0 with the 'sf' version 0.9 package.

Percent changes within each consolidated LULC class for all coastal watershed areas from 2001 to 2019 were calculated in R version 4.1.0. Water quality stations were then related to watersheds based on distance, with stations being assigned to the nearest watershed. Because of the variation in flow volumes of contributing tributaries to these estuaries it is difficult to generalize the exact transport distance of non-point sources of FCs in coastal systems. It is known that FCs are generally sourced from surrounding watersheds and their survivability in the water is dependent on a wide range of environmental factors (Weiskel et al., 1996; Cho et al., 2016; Korajkic et al., 2019). For context, Weiskel et al. (1996) demonstrated that even point source discharges of FC being diluted to near-background levels within 15 meters of the source (Weiskel et al. 1996). Each station's distance to shoreline was also calculated, which was done through nearest feature geoprocessing in R using the estuarine shoreline data layer from the NCDMF Estuarine Shoreline Mapping Project (NC Division of Coastal Management, 2007).

2.3 Data Analysis

The 20-year trends in water quality variables were analyzed to allow for (1) comparison against different environmental variables to understand possible drivers of change and (2) evaluation of spatial variability in water quality trends. Mann-Kendall (MK) testing and Sen Slope Estimators were calculated and applied to annual average FC concentrations and annual average salinity values for the water quality sampling stations, and total annual precipitation for the terrestrial weather stations. Fecal indicator bacteria concentrations are highly variable in space and time, with samples collected consecutively over the span of a few minutes sometimes varying by an order of magnitude over a few minutes (Boehm, 2007). Accordingly, the annual arithmetic mean was selected to represent the central tendency in long-term FC data, with the annual time step selected to average out the effects of seasonal variation. Similarly, using total annual precipitation informed us of the overall freshwater load over the years and allowed us to explore long-term drivers of baseflow FC concentrations. MK testing and Sen Slope Estimation analysis have been used in numerous environmental, hydrological, and water quality studies (Hirsch et al., 1982; Cailas et al., 1986; Hipel et al., 1988; Zetterqvist, 1991; Burn et al., 2002; Meals et al., 2011; Mustapha, 2013) due to their robustness against non-normal data with missing values. Statistical analyses and mapping were conducted in R with the 'trend' package version 1.1.4 (Pohlert, 2020) and the 'sf' package version 0.9 (Pebesma et al., 2022).

2.3.1 Mann-Kendall Trend Test

The MK trend test is a nonparametric test for monotonicity of trends in time series data (Mann, 1945; Kendall, 1975). By ranking the time series observations and measuring the later observations (j) against earlier observations (i), MK testing allows us to understand the monotonicity of an upward or downward trend in a time series. A perfectly monotonic trend consistently increases or decreases. For example, a perfectly increasing monotonic function is never decreasing at any point along the function. Using pairwise comparison of ranked values from all data points, the test statistic (S) is calculated through either adding or subtracting 1 for every value that is larger or smaller than the later value (Equation 1). This results in a test statistic (S) that characterizes the directionality and monotonicity of a trend in a given time series. S is then used to calculate the τ test statistic (Equation 2), which is a measure of correlation that ranges from -1 to +1 with the sign indicating the direction of the value's change over time. This test determines whether there is a significant, monotonic trend in a value over time in either a positive or negative direction.

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \operatorname{sign}(y_j - y_i) \qquad \operatorname{sign}(y_j - y_i) = \begin{cases} 1; \text{if } y_j > y_i \\ 0; \text{if } y_j = y_i \\ -1; \text{if } y_j < y_i \end{cases} \qquad \textit{(Equation 1)}$$

$$\tau = \frac{S}{n(n-1)/2} \qquad \textit{(Equation 2)}$$

In Equations 1 and 2, n is the number of observations in the time series, i is the earlier value in the time series sequence,j is later value in the time series sequence, and $y$represents the measurements at times i or j .

2.3.2 Sen Slope Estimator

While the τ value from the MK test denotes direction and how well a time series fits a monotonic model, the Sen slope estimator (β) determines the rate of change for the resulting MK trends. The resulting β is based on a median of all possible slopes calculated (Equation 3).

$$\beta = median\left(\frac{y_j - y_i}{x_j - x_i}\right) \qquad \textit{(Equation 3)}$$

The Mann-Kendall trend test and Sen slope estimators provided us with FC τ (τ_{FC}), FC β (β_{FC}), salinity τ (τ_{Sal}), salinity β (β_{Sal}), precipitation τ (τ_{Precip}), and precipitation β (β_{Precip}) for each water quality sampling station.

2.3.3 Correlations

Pearson's correlation coefficients were calculated to evaluate the relationships between the FC, salinity, and precipitation trends as well as the LULC class percent change for the consolidated classes and distance to shore.

3. RESULTS

3.1 Coastwide Trends

The results of the MK testing and Sen slope estimation of FC concentrations were mapped alongside the developed land use change for each coastal watershed (Figure 4). Results for all stations including τ values, p-values, and β slopes are documented in Table S1. The spatial distribution of τ_{FC} varied along the coast with defined areas of similar τ_{FC} values. From the most southern coastal area, we observe higher, positive τ_{FC} values for stations down estuary of areas with very high proportions of developed change. Within the south-central portion of the coast, we can see more negative τ_{FC} values. Farther north, in the area characterized by the large, Albemarle-Pamlico sound which spans SGAs F, G, H, and I, there is a mixture of lower positive and lower negative τ_{FC} values. The developed land use change is fairly uniform along a majority of the state's coastline, with higher proportions of developed land change being located in the watersheds associated with SGA C and E and the most intense increase in proportion of developed land being in central watersheds associated with SGA B.

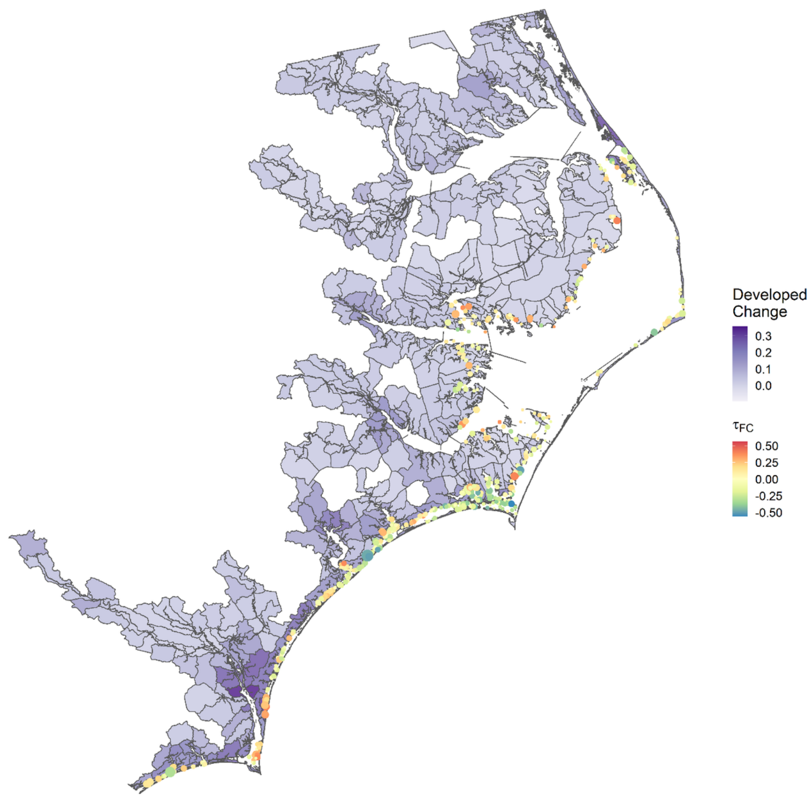

Figure 4 . Map of the 20-year FC trends for all 466 water quality stations (points) along North Carolina's coast alongside the developed change percentage of the HUC12 watershed areas (purple fill). The points representing the water quality station FC trends are colored by the τ_{FC} value with the red and orange colors illustrating the positive trends in FC concentrations while the green and blue colors indicate a negative trend in FC concentrations. These points are also sized by the magnitude of their β_{FC}values (i.e., larger points represent steeper slopes in the FC trends and smaller points represent more gently sloping FC trends). The light purple watershed areas represent smaller proportions of developed land increase whereas dark purple areas represent a more intense increase in developed land proportions from 2001 to 2019; white, unfilled

areas between watersheds correspond to watersheds that do not directly connect to a waterway. This map was created in R version 4.1.0 with 'sf' version 0.9 package.

The Pearson correlation coefficients for the water quality and environmental trends along the full coastal area are summarized in Table 1. These correlations include the relationships between τ_{FC}, β_{FC}, τ_{Sal}, β_{Sal}, τ_{Precip}, β_{Precip}, developed land percentage, barren land percentage, wetlands land percentage, vegetated land percentage, cultivated land percentage, and distance to shore. We observed a clear negative relationship between both τ_{FC} & β_{FC} and τ_{Sal}& β_{Sal} (r values from -0.281 to -0.304). There was a weak positive relationship between β_{FC} and τ_{Precip} & β_{Precip}. The positive relationship between τ_{Sal} & β_{Sal} and all land use classifications with wetlands had the strongest positive coefficients (r = 0.393 and r = 0.401, respectively). Distance to shore was weakly correlated with overall FC trends.

	Wetlands Change	Cultivated Change	Vegetated Change	Barren Change	Developed Change	S
FC τ	0.006	-0.029	0.087	0.062	0.127	0
FC β	-0.072	-0.047	0.017	0.021	0.044	0
Precipitation τ	-0.192	-0.070	-0.023	0.020	0.067	-
Precipitation β	-0.315	-0.149	-0.048	-0.024	0.004	-
Salinity τ	0.393	0.174	0.100	0.119	0.111	0
Salinity β	0.401	0.189	0.078	0.120	0.116	0
Shore Distance	0.187	0.017	0.174	0.087	0.318	1
Developed Change	0.282	-0.005	0.193	0.099	1.000	
Barren Change	0.299	-0.070	0.179	1.000		
Vegetated Change	0.198	-0.004	1.000			
Cultivated Change	-0.082	1.000				
Wetlands Change	1.000					

Table 1. Pearson's correlation matrix representing relationships between water quality trends (FC concentration and salinity) and environmental parameters (LULC change, precipitation, shore distance) for all stations along North Carolina's coast included in the MK trend testing.

When aggregated to annual averages, the NCDWR salinity data is insufficiently short (n = [10, 14] for each station) and limits our ability to apply trend testing. However, the NCDWR salinity time series (Figure 5) largely follow the same trends as the NCDMF salinity data; exceptions include sites in growing areas E and D, where several NCDWR stations were located in the upstream, fresher portions of the estuaries. While we generally selected stations in locations similar to NCDMF stations, these are presented due to lack of stations in the more brackish and marine waters within these areas. Given that the NCDWR data is collected through an unbiased monitoring program, the agreement between the NCDWR and NCDMF salinity observations indicates the NCDMF observations may not be severely affected by sampling bias.

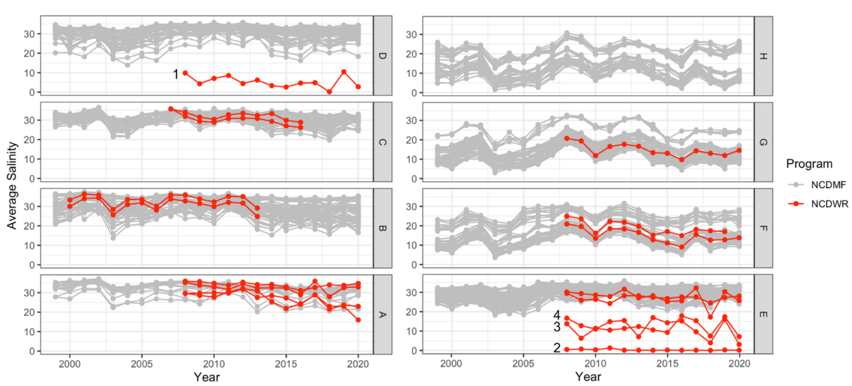

Figure 5. Time series of NCDMF salinity results (grey lines) with the NCDWR salinity results (red lines) from the chosen representative stations along North Carolina's coast. Line graphs are grouped by their SGA area. Locations of NCDWR stations labeled as 1-4 are annotated in Figure 2.

3.2 SGA Specific Trends

To further understand spatial relationships within the results, the data for stations within SGA name groups were pooled. There are 8 total SGA groups (A, B, C, D, E, F, G, H) that were used to aggregate the station results. Correlations between all considered variables were calculated for each SGA (Table 2). From the correlations describing FC trends with LULC changes, we saw strong negative correlations between FC trends and developed land use change in SGA letters C and A despite the weak positive correlation represented by the coefficient calculated from all data (i.e., full coast). Negative correlations between FC trends and wetlands land use change were also observed, being especially strong in southern SGAs (A, B, C, D). β_{Precip} and β_{FC} had a weak positive relationship for most SGAs, with the exception of SGA C. The correlation between $\beta_{\mathrm{Precip}}$and β_{FC} was broken down further for SGA C (Figure S1), which revealed a strong correlation attributed to negative β_{FC} values occurring in conjunction with high β_{Precip} values. Shore distance had a fairly positive to fairly negative relationship with β_{FC} moving from the southern SGAs (A, B, C, D) to the northern SGAs (F, G, H). The relationship between shore distance and β_{FC} is shown in Figure S2. Salinity trends were negatively correlated with β_{FC} along each SGA with the exception of SGA E (Table 2). Due to the ecological importance of salinity when it comes to water quality and predicting FC concentrations (Souedan et al., 2021; Florini et al., 2020; Liu et al., 2012), our understanding of salinity integrating the effects of both land use and precipitation, and the consistent directionality of the correlation coefficients, we analyzed this SGA-specific relationship further (Figure 6).

		β_{FC} and LULC Classes	β_{FC} and LULC Classes	β_{FC} and LULC Classes	β_{FC} and LULC Classes
	Barren	Barren	Cultivated	Developed	Vegetated
H	0.311	0.311	-0.043	0.278	0.255
G	-0.141	-0.141	0.014	-0.006	-0.066
F	0.188	0.188	-0.056	-0.219	-0.105
E	0.003	0.003	-0.207	0.052	0.015
D	0.254	0.254	-0.324	-0.180	-0.500
C	0.040	0.040	0.252	-0.497	-0.006
B	-0.042	-0.042	0.034	0.128	0.167
A	-0.223	-0.223	0.394	-0.355	0.31
Full Coast	0.021	0.021	-0.047	0.044	0.017

Table 2. Pearson's correlation coefficients broken down by SGA letter (rows) for the relationships between β_{FC} and LULC class change, β_{Sal}, β_{Precip}, and shore distance. SGAs are listed in geographic order across the rows from H to A, with H being the northernmost SGA and A being the southernmost.

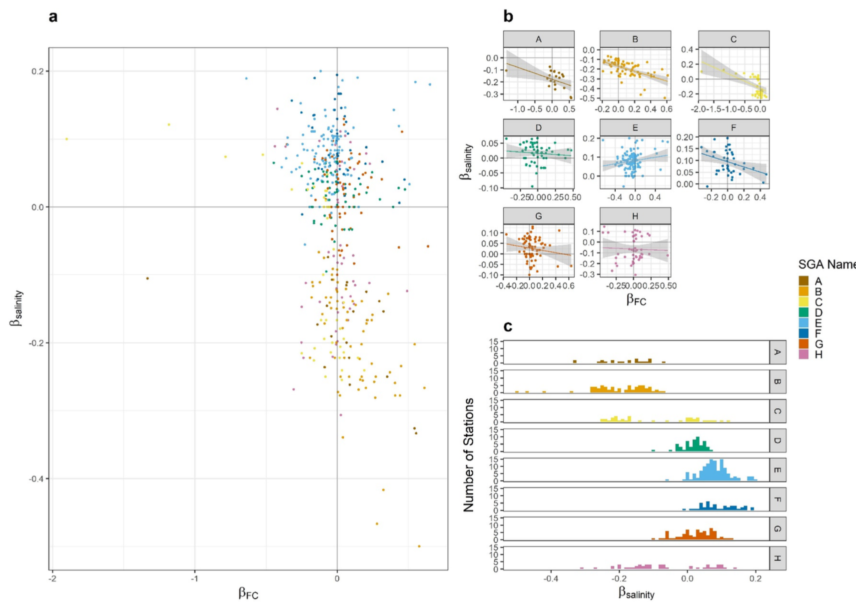

Figure 6. Leftmost scatterplot (a) illustrates the relationships between β_{FC} and β_{Sal} for each station colored by the station's associated SGA letter. The top right scatterplots (b) illustrate β_{FC} and β_{Sal} for each station within the SGA. The lower right histograms (c) show the distributions of β_{Sal} values broken down by SGA letter. Both (b) and (c) further demonstrate the spatial variation in salinity trends and relationships between β_{FC} and β_{Sal} across the coast.

There were weakly correlated, somewhat grouped relationships between β_{FC} and β_{Sal} along the full coast (Figure 6a), with strongly negative correlations in SGA B and somewhat in C, weakly correlated relationships in SGAs D, F, G, and H, and a positive correlation in SGA E (Figure 6b). When broken out by SGA (Figure 6c), the β_{Sal} distribution varied from lower negative values with higher spread in the southern SGAs A, B, and C, to higher positive β_{Sal} values with more narrow spread in the central SGAs D, E, and F. SGAs G and H had lower β_{Sal} values than the more central areas, but higher than the more southern areas (Figure 6c).

3.3 Representative Focal Areas

To explain some of the variation seen within the North Carolina coastal system, we detailed the trends seen under locally specific management and environmental conditions in SGAs B, E, and G (Figure 7). SGAs B, E, and G were selected because they captured different modes of estuarine and management variation, described in more detail in the following sections. More specifically, they represent different classes of estuarine drainage areas as defined by Engle et al. (2007), different shellfish lease distribution, and different levels of developed land change within the surrounding watersheds. Estuary drainage area classifications were originally created by NOAA's Coastal Assessment Framework to incorporate tidal influence into watershed delineation (NOAA, 2003). Engle et al. (2007) uses these areas to reclassify areas through a system that includes area, depth, volume, freshwater flow, and salinity to define an estuary type. This results in a coded class system ranging from 1 to 9 (Engle et al., 2007). By using a variety of different estuarine drainage area classes, we are effectively capturing a variety of physical and hydrological conditions, which enriches the interpretation of our results.

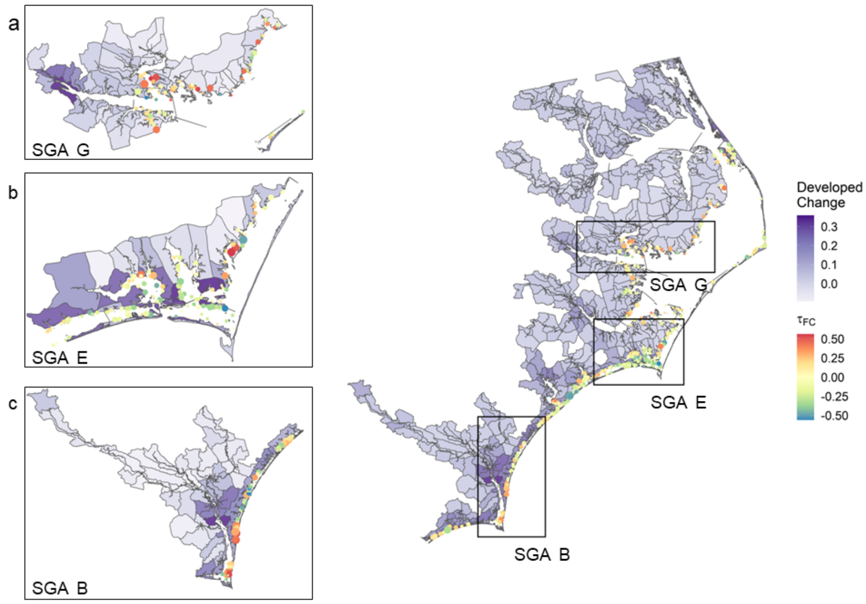

Figure 7. Full map of coastal North Carolina depicting the τ_{FC} values sized by the magnitude of their β_{FC} alongside HUC 12 land areas and their respective percent change in developed land use. SGAs B (a), E (b), and G (c) are used as focal areas to understand how management planning, increase in developed land, and estuary type affect the variation in FC trends. This map was created in R version 4.1.0 with 'sf' version 0.9 package.

Focal Area B

SGA B (Figure 7c) is associated with waters at the mouth of the major river cutting through this region, the Cape Fear River, and lagoonal estuaries. SGA B is a class 6 estuary (Engle et al. 2007) characterized by large area, moderate volume, high freshwater flow, and moderate depth and salinity. This area contains higher rainfall thresholds ranging from 2 to 4 inches indicating that coastal FC concentrations within conditionally approved or approved portions of these growing areas do not respond as intensely to rainfall as compared to other areas of the coast with lower rainfall thresholds. Prior studies have reported poor water quality in SGA B (Alford et al., 2016; NCDEQ, 2022), likely correlated with a high increase in the developed land up-river. Based on the changes in land use observed over the study period, watersheds adjacent to SGA B were associated with increased urbanization along the NC coast (Figure 4). SGA B is also characterized by consistently negative β_{Sal} values indicating decreasing salinity values for the samples that have been taken over the past 20 years (Figure 6c).

FC trends support our prior understanding regarding declining water quality in this region (Figure 7c), as demonstrated by high τ_{FC} and β_{FC} values across most of the study sites. However, some spatial variation in FC trends were observed. The more southern areas within this SGA, associated with the mouth of the Cape Fear River, have higher τ_{FC} values than the lagoonal areas located in the northern portion of the region, which are not generally directly affected by the Cape Fear River. The southern Cape Fear River can experience diverted flow through a man-made waterway under some high tide conditions, resulting in river flows discharging directly into the lagoonal estuaries. This tidal overflow effect might explain the similar trends in β_{FC} from the southern Cape Fear River area to the southern lagoonal estuary area despite being very separate geographies. While there are negative τ_{FC}values in the lagoonal areas, the β_{FC} is higher in the areas where τ_{FC} is positive, meaning that the FC concentrations towards the mouth of the Cape Fear River are increasing at a faster rate than the decreasing, lagoonal FC concentrations.

*Focal Area E*SGA E (Figure 7b) represents a trunk and tributary estuarine system surrounded by moderate urban development in the past 20 years. SGA E contains systems classified as class 2 estuaries (Engle et al., 2007) indicating moderate area with low volume, moderate freshwater flow, and high salinity. SGA E supports a large number of up-estuary shellfish leases within the tributary systems. This area responds strongly to rainfall events in terms of FC load, as demonstrated by the low rainfall thresholds (i.e., 1 inch to 4 inches), with the lowest rainfall thresholds located up-estuary. This watershed, similar to SGA B, experienced a moderate increase in developed land use within the past 20 years (Figure 4). However, the τ_{FC} values in this region were generally negative with a few very positive τ_{FC} values within the river systems, suggesting an improvement in baseflow water quality. This area has also shown more positive β_{Sal} values (Figure 6c), indicating an increase in salinity measured for the samples taken over the past 20 years.

Focal Area G

SGA G (Figure 7c) contains the Pamlico and Pungo Rivers. SGA G is a class 4 estuary (Engle et al., 2007), which is characterized by moderate area, moderate depth, low volume, and high freshwater flow. This area is associated with the back-barrier Albemarle-Pamlico Estuary to the east, which is a class 9 estuary (Engle et al., 2007) indicating very large area, deep bathymetry, high volume, and high salinity environment. This area is also characterized by low increase in surrounding development and relatively fewer shellfish leases, demonstrating that this system is relatively unimpacted by human activity as compared to focal areas B and E. Accordingly, rainfall thresholds within SGA G are all at the highest limit of 4 inches, indicating FC concentrations in these waters are not highly sensitive to precipitation and stormwater runoff. There was a variety of negative and positive τ_{FC} values in this area with a majority of the stations exhibiting negative τ_{FC}values. The β_{Sal} values in this area are also variable, exhibiting a range of both positive and negative trends.

4. DISCUSSION

We found that shellfish sanitation data collected routinely through a systematic random sampling strategy as defined by the National Shellfish Sanitation Program (NSSP) could cautiously be used for long-term water quality trend analysis. By comparing salinity measurements collected by the NCDWR, which maintains an unbiased monitoring program, and NCDMF, which only samples when shellfish waters are open for harvest, we were able to assess whether the sampling constraints imposed on the NCDMF measurements influenced the trend testing results. We found that the NCDMF and NCDWR salinity time series behaved similarly across all SGAs (Figure 5). However, the NCDWR data only spanned 10 years while the NCDMF data spanned 20, and the difference in time series length limits our ability to fully corroborate the NCDMF data using NCDWR observations. Additionally, though not strongly evident in the salinity data analyzed here, the risk for sampling bias to affect routine monitoring data collected by shellfish sanitation programs exists and should always be considered when analyzing their measurements.

We expect sampling bias risk to be greatest in conditionally approved waters with low rainfall thresholds (i.e., 1 to 2 inches), such as SGA E. In contrast, in areas with relatively high rainfall thresholds (e.g., 4 inches), routine FC samples can typically be collected at any time during the year since these waters remain open unless an exceptional event, such as a hurricane or major frontal storm, has occurred. Because waters with high rainfall thresholds largely remain open, the six annual samples are collected under a wider range of environmental conditions, and there is less risk of sampling bias potentially affecting FC trends quantified from the routine monitoring data. For example, SGA G represents an area with high rainfall thresholds (4 inches). These high rainfall thresholds create less restrictive conditions for routine sampling, effectively increasing the variety of conditions captured in the sampling. Accordingly, FC trends determined from shellfish sanitation data from these stations are likely representative of the true improvement or degradation in water quality observed in the system, which also helps to explain why the FC trend results we reported corroborate findings from other studies that have evaluated water quality in this region. In contrast, areas that are more restricted in the time and conditions that routine sampling is able to occur (i.e., areas that are conditionally managed with low rainfall thresholds), such as SGA E, are associated with routine observations that have higher risk of being biased, and there is increased complexity in terms of interpreting these data to infer general water quality trends. Low rainfall thresholds dictate higher rates of closures for even mild meteorological events, which effectively restricts the open times available for routine sampling. However, we demonstrated that the use of an external water quality dataset, in this case for salinity, can be used to assess how sampling bias may have affected measurements collected by shellfish sanitation programs.

Nonpoint source runoff is considered a major contributor to FC loads in estuaries located near developed landscapes (Mallin et al., 2000; Coulliette et al., 2009; Kirby-Smith et al., 2006; Campos et al., 2013). Therefore, the increasing trends we documented in FC concentrations in SGAs B, E, and H align with the known relationship between FC and development. Specifically, the positive correlative relationship between change in developed land cover across a watershed and increasing FC trends was seen in SGAs B, E, and H, while A, C, D, F, and G were associated with negative correlations. Relationships between developed land use change and FC trends could potentially be clarified further by using population density change over watersheds, stormwater management, or differentiating impervious surfaces (Mallin et al., 2000; Carle et al., 2005; Cahoon et al., 2016; Freeman et al., 2019).

The negative correlation between FC and salinity along all SGAs was consistent with established water quality relationships except for a few contradictory results. The inverse relationship between FC and salinity could be a result of the coupled effect of increased freshwater input that comes with increased precipitation (Campos et al., 2013; Coulliette et al., 2009). It is known that FC concentrations increase following runoff after rainfall events, especially in more developed areas (Mallin et al., 2000; Carle et al., 2005; Cahoon et al., 2016; Freeman et al., 2019). These same rainfall events that increase the FC concentrations also decrease salinity, which is illustrated in the inverse relationships reported in this study across each SGA, with the exception of SGA E (Table 2, Figure 6). However, the inverse relationship between FC concentration and salinity trends was often noisy (Figure 6b), with the correlation coefficient between FC concentration and salinity trends being in the range of [-0.147, 0.161] for 5 out of 8 SGAs (Table 2). In the case of SGA E, where a positive correlation between salinity and FC trends was observed, the correlation appears to have been influenced by outlying values (Figure 6b), particularly since most of the β_{Sal} values reflected increases in salinity (Figure 6c) while the β_{FC} values showed there were FC concentrations decreases across most sampling locations (Figure 6a, 6b).

The noisy relationships between FC concentration and salinity trends in our results could be explained by our dataset not capturing short-term FC concentration increases following storm events and instead capturing FC during baseflow conditions. Because the data analyzed in this study was produced from routine systematic random sampling, which is collected when waters are open for harvest to capture baseline fecal coliform loading, the observations will not capture changes in storm-driven FC concentrations. Instead, the measurements may reveal if there is chronic loading in an area (e.g., due to continuously failing septic systems or poorly performing wastewater treatment plants). Therefore, the trends from this analysis are representative of baseflow conditions. Accordingly, had the routine sampling data captured post-storm conditions, we expect stronger correlations between FC and salinity trends would have been observed. Instead, we believe that factors such as increases in tidal flushing (e.g., due to inlet dredging) and changes in baseflow FC loads in these systems play a larger role in explaining the negative relationship between FC and salinity than changes in rain and runoff.

In addition to providing insights on long-term water quality trends, shellfish sanitation data can also be used to assess the efficacy of current management practices. For example, a conditionally managed waterbody with low rainfall thresholds that is still showing a trend towards increasing FC concentrations could indicate a decline in water quality that has not been met with intense enough action by the current management plan. As a result, trends in fecal coliform observations could be used as an "early warning system" to help pinpoint areas where more intense management measures need to be taken. For example, the way in which these data could be used as an "early warning system" is demonstrated by focal SGA B (Figure 7c), where the mouth of the Cape Fear River likely shows increasing FC concentrations due to degradation of water quality that may need to be met with new management actions.

5. CONCLUSIONS

In this study, we assessed the feasibility of utilizing estuarine monitoring data from a representative regulatory program (i.e., shellfish sanitation) to infer long term water quality trends. We used these data to look specifically at the spatial and temporal trends in FC concentrations and identified possible management and environmental drivers of these trends. Our study system, coastal North Carolina, exhibited a variety of trends in both the 20-year FC concentrations and the considered environmental drivers. While the resulting water quality trends and their relationships with environmental factors were complex, there were emergent patterns that we found to offer key insights. In particular, we concluded that shellfish sanitation data collected routinely through a systematic random sampling strategy as defined by the National Shellfish Sanitation Program (NSSP) could be used for long-term water quality trend analysis, and to fill extensive gaps in existing coastal water quality monitoring programs.

Although our results demonstrated opportunities of using shellfish sanitation data for inferring long-term water quality trends, our study was limited by several factors. Firstly, we did not account for tidal circulation due to the major modeling effort that would be required to include tidal circulation and flow patterns at this spatial and temporal scale. Future research should improve upon the methods presented here by including factors that capture the marine flushing of an area such as inlet maintenance or distance to the nearest intracoastal waterway. Secondly, there was a lack of unbiased FC concentration datasets for trend validation, and we relied on findings from prior published studies to "ground truth" FC trends calculated from monitoring data. Regions outside of our study area may not have access to the type of information used to help diagnose the reliability of shellfish sanitation monitoring data for water quality inference. As new monitoring programs are introduced to track changes in marine systems, opportunities to pair sites with existing shellfish sanitation program monitoring locations could help to create data needed to characterize potential sampling bias effects and increase the ability for long-term shellfish sanitation data to be used for water quality analyses. Finally, because of variation in sampling protocols across state programs, shellfish sanitation data are nuanced and challenging to interpret. This study offers context and an approach for confronting nuance in the data. However, directly engaging with shellfish sanitation program managers is essential to accurately interpreting trend results like those presented here, as local expertise provides invaluable insight into the state and function of these estuarine systems and their management.

6. ACKNOWLEDGMENTS

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Supplementary Material

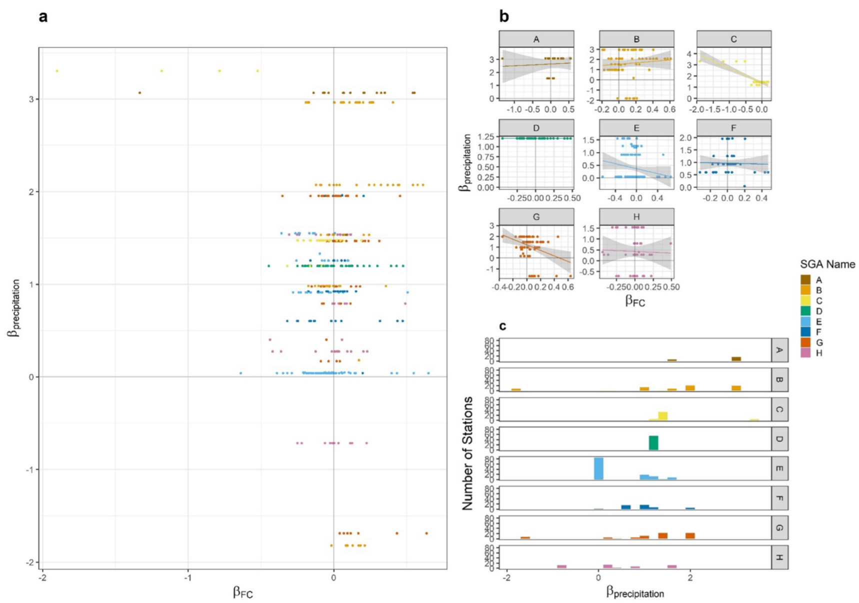

Figure 1: This is a caption

Figure S1. Leftmost scatterplot (a) illustrates the relationships between β_{FC} and $\beta_{precipitation}$ for each station colored by the station's associated SGA letter. The top right scatterplots (b) illustrate β_{FC} and $\beta_{precipitation}$ for each station within the SGA. The lower right histograms (c) show the distributions of $\beta_{precipitation}$ values broken down by SGA letter. Both (b) and (c) further demonstrate the spatial variation in precipitation trends and relationships between β_{FC} and $\beta_{precipitation}$ across the coast.

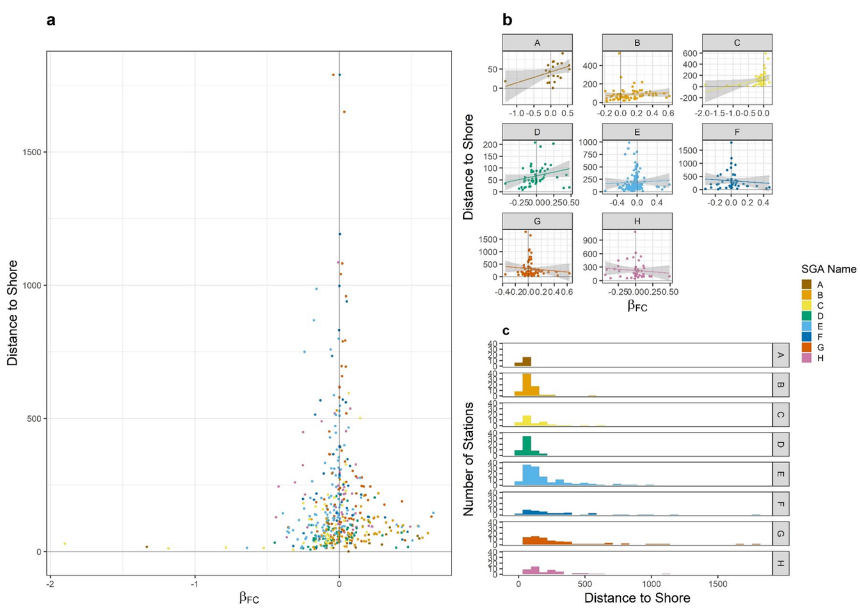

Figure 2: This is a caption

Figure S2. Leftmost scatterplot (a) illustrates the relationships between β_{FC} and distance to shore in meters for each station colored by the station's associated SGA letter. The top right scatterplots (b) illustrate β_{FC} and distance to shore for each station within the SGA. The lower right histograms (c) show the distributions of distance to shore values broken down by SGA letter. Both (b) and (c) further demonstrate the spatial variation in distance to shore and relationships between β_{FC} and distance to shore across the coast.

Table S1. Full results from the Mann-Kendall trend testing and the Sen Slope estimation for each of the NCDMF water quality sampling stations that have 20 years of continuous data between 1999 to 2021.

station	SGA	t_{FC}	b_{FC}	FC p-value
A1-9	A01	0.108225	0.208824	0.498564
A1-36	A01	0.138829	0.542222	0.381855
A1-8	A01	0.125541	0.553333	0.429795
A1-12	A01	0.203463	0.35	0.194595
A1-12	A01	0.203463	0.35	0.194595
A1-35	A01	-0.19957	-0.13889	0.204294
A1-15	A01	-0.09091	-0.07333	0.572782
E4-16-A	E04	0.099567	0.15	0.535024
E7-23	E07	-0.30266	-0.0463	0.054535
D3-55	D03	-0.08225	-0.16952	0.61176
D3-57	D03	-0.2381	-0.19337	0.127837
E7-20	E07	-0.29259	-0.025	0.062317
D3-25	D03	-0.09091	-0.05667	0.572782
D3-26	D03	0.069414	0.050926	0.672195
D3-46	D03	-0.19481	-0.16443	0.214713
E7-21	E07	0.084091	0.004167	0.609859
E7-22	E07	-0.30636	-0.04844	0.051231
D3-45	D03	-0.05206	-0.04333	0.756332
D1-5	D01	-0.29935	-0.4463	0.055083
G1-7	G01	0.134199	0.097778	0.397587
H4-31	H04	0.121154	0.005	0.45891
G1-6	G01	0.090909	0.083333	0.572782
H4-26	H04	0.125541	0.118519	0.429795
G8-12-A	G08	0.047619	0.068421	0.777959
G8-6-A	G08	0.367965	0.115556	0.017854
G8-12-B	G08	-0.02165	-0.03519	0.910196
A2-7	A02	0.038961	0.063725	0.821525
H3-16	H03	-0.05652	-0.008	0.734878
E1-2	E01	0.056277	0.07381	0.73508
E3-35	E03	-0.06941	-0.03277	0.672195
E2-10	E02	-0.21212	-0.20034	0.175896
D3-36	D03	-0.05206	-0.0827	0.756332
E2-11	E02	-0.24783	-0.06042	0.114029
E1-3	E01	-0.02614	-0.01667	0.887715
E6-23	E06	-0.15687	-0.05	0.322972
F6-23	F06	0.056522	0.016667	0.734878
E1-11	E01	-0.16856	-0.03704	0.293383
B2-5	B02	0.286335	0.17037	0.066714
B4-12	B04	-0.02165	-0.01667	0.910196
C1-23-A	C01	-0.07809	-0.045	0.631543
C1-9	C01	-0.15152	-0.09167	0.337695

C1-16	C01	-0.16883	-0.24958	0.283935
E4-14-A	E04	-0.17749	-0.05628	0.259355
E4-11	E04	-0.41649	-0.22727	0.007365
E6-7-C	E06	-0.27273	-0.32	0.080417
E6-8	E06	-0.26898	-0.10198	0.085295
E4-10	E04	-0.14348	-0.04143	0.366498
E6-34	E06	-0.39913	-0.1244	0.010257
E6-17	E06	0.017354	0.007843	0.932558
E6-15	E06	0.038961	0.114286	0.821525
E6-32	E06	-0.31602	-0.20556	0.042331
H3-1-C	H03	0.283854	0.075714	0.070603
H3-7	H03	0.078091	0.033333	0.631543
H3-10	H03	0.108225	0.088095	0.498564
H5-16	H05	0.048037	0.021905	0.777557
H3-18	H03	0.057536	0.002083	0.733719
H3-1	H03	0.404352	0.490476	0.009424
G1-15	G01	0.013101	0	0.954955
E8-52	E08	0.073593	0.036905	0.651869
G5-26-B	G05	-0.21398	-0.08778	0.175093
G5-18-A	G05	-0.11843	-0.03039	0.46228
E6-25	E06	-0.28639	-0.05417	0.070079
E3-37	E03	-0.26522	-0.11	0.090413
E3-2	E03	-0.22078	-0.09964	0.15857
E3-36	E03	-0.39394	-0.19167	0.011155
D3-60	D03	0.194805	0.245	0.214713
E1-9	E01	0.064935	0.102083	0.693012
E3-8	E03	-0.16883	-0.23439	0.283935
G1-17	G01	-0.10412	-0.35278	0.516461
G8-9-B	G08	0.333333	0.433333	0.03211
C2-3	C02	0.168831	0.065	0.283935
D1-9	D01	-0.22078	-0.06771	0.15857
H2-2	H02	0.074568	0.002976	0.651004
G4-7	G04	0.100441	0.006548	0.534374
E8-54	E08	0.194805	0.396875	0.214713
E8-55	E08	-0.06941	-0.02361	0.672195
E8-2	E08	-0.38528	-0.09444	0.013086
D4-1	D04	0.338396	0.205556	0.029848
D4-3	D04	0.108225	0.162619	0.498564
D4-5	D04	-0.02165	-0.01667	0.910196
D4-8-A	D04	0.18778	0.057143	0.235418
D4-6	D04	0.073593	0.057576	0.651869
E6-7	E06	-0.4026	-0.39037	0.009481
E4-24	E04	-0.46855	-0.17619	0.002541
E4-83	E04	-0.41126	-0.28819	0.008035
E6-16	E06	-0.0303	-0.02778	0.865649
E2-3	E02	-0.45653	-0.21021	0.003336
E4-84	E04	-0.15152	-0.09074	0.337695
E3-51	E03	-0.18615	-0.07889	0.236289
E2-1	E02	-0.22078	-0.09643	0.15857
E6-29	E06	-0.33333	-0.18778	0.03211
E4-4	E04	0.056277	0.063725	0.73508

E4-55	E04	0.030303	0.034416	0.865649
E6-48	E06	0.012987	0.011111	0.955026
E6-38	E06	-0.0131	0	0.954943
G8-3	G08	0.099567	0.040741	0.535024
E4-4-A	E04	-0.18615	-0.24074	0.236289
G8-4	G08	0.116883	0.168182	0.463469
G8-9	G08	0.173536	0.093333	0.271264
E6-9	E06	-0.09544	-0.10521	0.553588
E4-35	E04	-0.34199	-0.15824	0.027847
A2-20-A	A02	0.220779	0.325	0.15857
H5-2-A	H05	-0.04396	-0.01333	0.798907
H1-27-B	H01	-0.26407	-0.12273	0.09067
H1-27	H01	-0.2987	-0.25208	0.05518
H2-27	H02	-0.36797	-0.22083	0.017854
H1-24	H01	-0.22078	-0.23733	0.15857
G1-9	G01	0.130722	0.023148	0.412948
H5-3	H05	0.082251	0.031944	0.61176
H5-4	H05	0.086768	0.092333	0.591977
H5-6	H05	-0.21212	-0.42039	0.175896
G1-4	G01	0.078091	0.089583	0.631543
H5-1	H05	-0.13883	-0.1246	0.381855
H4-29	H04	0.134199	0.203333	0.397587
G1-3	G01	-0.07359	-0.07208	0.651869
H4-27	H04	0.194805	0.229167	0.214713
H4-9-B	H04	-0.40435	-0.43788	0.009424
H4-30	H04	0.087148	0.05625	0.591583
F6-25	F06	-0.0303	-0.0599	0.865649
F6-22	F06	-0.02165	-0.00556	0.910196
H1-4	H01	0.082973	0.046667	0.611098
H2-28	H02	-0.1743	-0.00918	0.270882
H2-1	H02	-0.03471	-0.00667	0.843464
H1-7	H01	0.177489	0.117714	0.259355
H1-22-A	H01	-0.21305	-0.03156	0.175552
H1-8	H01	0.125541	0.09381	0.429795
H2-11	H02	-0.15152	-0.06111	0.337695
H2-16	H02	-0.08677	-0.02517	0.591977
H1-10	H01	0.021645	0.040476	0.910196
H2-24	H02	0.261443	0.125	0.095692
H1-11	H01	-0.00433	-0.00556	1
H1-12-E	H01	0.104122	0.113636	0.516461
H2-20	H02	0.052061	0.031296	0.756332
H1-33	H01	-0.16883	-0.30635	0.283935
H2-21	H02	0.212121	0.225238	0.175896
H2-5-B	H02	0.182213	0.112121	0.247446
H3-9	H03	0.004329	0.003704	1
H1-16	H01	0.060738	0.025556	0.713829
H2-33	H02	-0.20346	-0.25	0.194595
H2-9	H02	0.013044	0.001667	0.954991
G1-19	G01	0.026377	0	0.887507
H5-11-A	H05	0.047827	0.035417	0.777787
G1-18	G01	-0.36927	-0.05208	0.018895

H5-9	H05	-0.24675	-0.3629	0.114317
G1-10	G01	0.109174	0.016667	0.497766
G1-11	G01	0.130152	0.047115	0.41332
H5-10-A	H05	0.043384	0.016667	0.799586
G1-7-A	G01	-0.17749	-0.15952	0.259355
H5-8	H05	0.100441	0.022222	0.534265
G1-8	G01	0.108225	0.156667	0.498564
A2-10	A02	0.116883	0.203824	0.463469
A3-10	A03	0.056277	0.087879	0.73508
A3-13	A03	0.064935	0.064583	0.693012
A2-9	A02	-0.05628	-0.06458	0.73508
E4-7	E04	-0.12554	-0.27976	0.429795
E4-8-A	E04	0.264069	0.50625	0.09067
E6-39	E06	-0.04338	-0.01083	0.799586
E4-28	E04	0.056277	0.057407	0.73508
E4-29	E04	0.142857	0.433333	0.36688
E4-8	E04	-0.07359	-0.08095	0.651869
E6-5	E06	-0.16017	-0.17308	0.310046
G8-11	G08	0.112799	0.035	0.480668
E4-3	E04	-0.09957	-0.08526	0.535024
E3-52	E03	-0.11688	-0.09921	0.463469
E2-6	E02	-0.31143	-0.06429	0.047626
E1-1	E01	-0.02603	-0.00476	0.887834
E6-2-C	E06	0.028847	0.001185	0.879734
E6-4	E06	-0.04825	-0.00714	0.777211
E4-56	E04	-0.12554	-0.08294	0.429795
E6-12	E06	-0.15152	-0.17879	0.337695
E6-43	E06	-0.2381	-0.12286	0.127837
C2-2	C02	-0.09586	-0.03533	0.553167
B3-1	B03	0.203463	0.172222	0.194595
B4-11	B04	0.082251	0.083333	0.61176
B3-5	B03	0.186147	0.129167	0.236289
B2-1	B02	0.134199	0.093333	0.397587
B7-65	B07	0.012987	0.037037	0.955026
B7-19	B07	0.078091	0.044444	0.631543
G3-3-C	G03	0.208243	0.198333	0.184896
G3-24	G03	0.100001	0.039744	0.534699
G3-24	G03	0.100001	0.039744	0.534699
G2-4-B	G02	0.043384	0.030556	0.799586
D2-22	D02	-0.20346	-0.11176	0.194595
B9-21	B09	0.168831	0.259524	0.283935
B9-20-A	B09	0.012987	0.05	0.955026
B9-22	B09	0.004329	0.023333	1
B9-23	B09	0.186958	0.405556	0.235916
B9-19	B09	0.238095	0.27037	0.127837
B9-18	B09	0.147506	0.201042	0.351904
B9-20	B09	0.142857	0.15	0.36688
B9-15	B09	0.021739	0.004444	0.910124
B9-14	B09	0.126088	0.161111	0.429428
B9-29	B09	0.242951	0.266667	0.120781
B9-27	B09	0.194805	0.178333	0.214713

B9-28	B09	0.203463	0.171429	0.194595
B9-35	B09	-0.13478	-0.19167	0.397212
B7-33	B07	0	0	1
B7-22	B07	-0.04762	-0.09333	0.777959
A2-6	A02	0.186147	0.294792	0.236289
A3-7	A03	-0.03471	-0.04167	0.843464
E2-7	E02	-0.25995	-0.03472	0.100495
E3-21	E03	-0.44252	-0.35926	0.004384
E7-11	E07	-0.38696	-0.14115	0.013014
E7-13	E07	-0.00871	0	0.977477
E5-24	E05	-0.09171	-0.00455	0.572068
E5-14	E05	-0.07878	-0.00414	0.630909
E7-10	E07	-0.37826	-0.11	0.015226
B7-4	B07	-0.17749	-0.175	0.259355
B7-6	B07	-0.04762	-0.02778	0.777959
G6-15	G06	-0.19089	-0.05167	0.225132
G6-19	G06	-0.20132	-0.03611	0.203453
G6-18-B	G06	-0.12609	-0.08939	0.429428
G6-10	G06	0.138829	0.042063	0.381855
E7-26	E07	-0.15826	-0.005	0.322199
E7-1	E07	-0.13478	-0.02381	0.397212
E1-13	E01	-0.30317	-0.00625	0.063299
E1-12	E01	-0.09423	-0.00357	0.568235
D3-19	D03	0.134199	0.43098	0.397587
E5-23	E05	-0.07196	0	0.668532
D3-20	D03	-0.10823	-0.17778	0.498564
D3-47	D03	-0.13015	-0.08011	0.41332
D1-13	D01	-0.17354	-0.05312	0.271264
D4-11	D04	-0.00868	-0.00159	0.977495
D2-36	D02	0.246753	0.298776	0.114317
D2-13	D02	0.264069	0.392593	0.09067
G5-19	G05	-0.24401	-0.06538	0.120387
G4-9	G04	-0.15618	-0.06952	0.323486
G4-8-B	G04	0.147506	0.118095	0.351904
G3-1-C	G03	0.100001	0.047778	0.534699
D2-32	D02	-0.00435	0	1
D2-20	D02	-0.35065	-0.16272	0.024081
D2-26	D02	-0.2987	-0.17361	0.05518
D2-18	D02	0.116883	0.05	0.463469
D2-23	D02	-0.06074	-0.01246	0.713829
D2-21	D02	-0.12554	-0.08839	0.429795
D1-12	D01	-0.04338	-0.01394	0.799586
D1-15	D01	-0.33988	-0.25	0.029674
C4-11	C04	-0.09091	-0.06976	0.572782
C1-10-A	C01	-0.00868	-0.00278	0.977495
C1-2-A	C01	-0.02165	-0.02444	0.910196
C4-5	C04	-0.35065	-0.09375	0.024081
C2-11	C02	0.035013	0.016667	0.843177
G3-3-A	G03	0.125541	0.214583	0.429795
G3-2	G03	0.092525	0.01	0.571145
G3-18	G03	0.168831	0.238611	0.283935

B6-20	B06	0.047619	0.039286	0.777959
B6-13	B06	0.038961	0.019444	0.821525
G2-7	G02	-0.03896	-0.03611	0.821525
G4-11	G04	0.109174	0.016667	0.497766
G2-6	G02	0.081339	0	0.627123
G3-16	G03	0.012987	0.053333	0.955026
C1-12	C01	-0.23427	-0.15833	0.134892
B5-19	B05	0.212121	0.448148	0.175896
B5-20	B05	0.281385	0.440278	0.071127
C1-20	C01	-0.07809	-0.08333	0.631543
C1-13	C01	-0.14751	-0.16389	0.351904
B5-14	B05	0.393939	0.369444	0.011155
B5-14	B05	0.393939	0.369444	0.011155
B5-16	B05	0.264069	0.407018	0.09067
B5-17	B05	0.238095	0.241667	0.127837
B5-22	B05	0.186147	0.156667	0.236289
B5-23	B05	0.385281	0.54359	0.013086
B5-40	B05	0.246753	0.6125	0.114317
B5-30	B05	0.341991	0.575	0.027847
B5-6	B05	0.324675	0.32381	0.03692
B5-7	B05	0.316017	0.278307	0.042331
C2-44	C02	0.139437	0.033333	0.381476
C4-6	C04	-0.1342	-0.06973	0.397587
C2-9	C02	0.239133	0.144444	0.127534
C2-22	C02	0.100001	0.021212	0.534699
C4-14	C04	-0.29004	-0.24762	0.062735
C2-19	C02	0.069414	0.032222	0.672195
C2-4	C02	0.099567	0.045614	0.535024
B6-10	B06	-0.12554	-0.0875	0.429795
B6-10	B06	-0.12554	-0.0875	0.429795
G2-5	G02	0.105039	0.011905	0.51568
G3-22	G03	0.225597	0.086111	0.150245
G2-4-A	G02	0.069414	0.032143	0.672195
G4-6	G04	0.236338	0.019048	0.134167
G3-10	G03	0.352177	0.145238	0.023969
G2-9	G02	-0.29788	-0.04074	0.060789
G4-1	G04	0.312365	0.154167	0.045193
G4-1-D	G04	0.290043	0.312088	0.062735
G3-11	G03	0.136585	0.00625	0.395829
G4-3	G04	0.333835	0.00625	0.039028
G4-14	G04	-0.32456	-0.05	0.040807
G4-12	G04	0.054484	0	0.753142
G2-3	G02	0.30706	0.02	0.060245
G2-2	G02	-0.00433	-8.33E-04	1
G2-1-B	G02	-0.12554	-0.1039	0.429795
G2-1-A	G02	-0.05628	-0.08333	0.73508
G2-1	G02	0	0	1
A2-12	A02	-0.03896	-0.03667	0.821525
B2-22	B02	0.252729	0.205128	0.107479
A3-16	A03	-0.1342	-0.08944	0.397587
D1-16	D01	-0.24675	-0.09697	0.114317

F6-26	F06	0.069414	0.204464	0.672195
F6-11	F06	-0.21212	-0.25175	0.175896
E8-49	E08	0.064935	0.056122	0.693012
B7-35	B07	-0.08715	-0.03056	0.591583
B7-28-A	B07	-0.20346	-0.12222	0.194595
G6-18-A	G06	0.069414	0.019913	0.672195
F4-9	F04	0.195654	0.061667	0.214349
F2-8	F02	0.362102	0.045667	0.021715
F4-8-A	F04	-0.17208	-0.05	0.311793
F4-12	F04	0.213904	0.023958	0.187786
F2-7	F02	0.29277	0.051852	0.06491
E9-63	E09	-0.20824	-0.08148	0.184896
E9-23-A	E09	0.121475	0.098148	0.446271
E9-13-A	E09	-0.09091	-0.06278	0.572782
E9-14-A	E09	0.039131	0.004762	0.821386
E9-20	E09	0.142857	0.094444	0.36688
E3-44-A	E03	-0.27273	-0.14259	0.080417
E1-18	E01	0.082251	0.052778	0.61176
E5-11	E05	-0.21787	-0.02341	0.166445
E3-20	E03	-0.18615	-0.2641	0.236289
E6-21	E06	0.043384	0.015556	0.799586
E3-41	E03	-0.18615	-0.16806	0.236289
E3-42	E03	-0.21212	-0.24325	0.175896
D3-18	D03	-0.17749	-0.07679	0.259355
E7-6	E07	-0.30736	-0.16	0.048398
E7-8	E07	-0.3731	-0.34861	0.016495
D3-21-B	D03	-0.04762	-0.0197	0.777959
E5-22	E05	-0.44423	-0.03733	0.005244
E7-4	E07	-0.32972	-0.15926	0.034372
E7-17	E07	-0.38501	-0.0369	0.016337
E2-27	E02	-0.20986	-0.00714	0.196029
E9-16	E09	-0.05229	-0.00357	0.756144
E9-43	E09	0.052061	0.009091	0.756332
E9-11	E09	0.012987	0.007407	0.955026
E9-44	E09	-0.07809	-0.08622	0.631543
E8-17	E08	-0.49458	-0.63878	0.001434
E8-48-A	E08	0.333333	0.54881	0.03211
E8-46	E08	-0.33626	-0.11667	0.031791
E8-48	E08	0.082251	0.142857	0.61176
E8-48-B	E08	0.393939	0.650595	0.011155
C4-10	C04	-0.16883	-0.08333	0.283935
B2-7	B02	0.286335	0.211905	0.066714
B7-8	B07	-0.06494	-0.03733	0.693012
B7-20	B07	0.229437	0.357692	0.142569
B7-45	B07	-0.21692	-0.05333	0.166896
B7-49	B07	-0.25273	-0.07889	0.107572
D1-6-A	D01	-0.07359	-0.07381	0.651869
E7-24	E07	-0.09715	-0.00833	0.552108
D1-7	D01	-0.20346	-0.15625	0.194595
D1-6	D01	-0.19481	-0.2	0.214713
D1-17	D01	-0.1	-0.04444	0.534699

D1-8	D01	-0.21692	-0.10417	0.166896
D1-11	D01	-0.01735	-0.00833	0.932558
D1-8-A	D01	-0.22944	-0.17361	0.142569
F6-8-A	F06	-0.27273	-0.31923	0.080417
F6-9-A	F06	-0.20346	-0.16667	0.194595
F6-31	F06	0.008677	0.011458	0.977495
F6-16	F06	-0.1342	-0.15833	0.397587
F6-17	F06	-0.07809	-0.01845	0.631543
F6-18	F06	-0.01735	-0.01667	0.932558
F5-17-B	F05	-0.09091	-0.13631	0.572782
F5-19	F05	0.116883	0.436364	0.463469
F5-15	F05	0.350649	0.472222	0.024081
F5-15	F05	0.350649	0.472222	0.024081
F5-17-A	F05	0.212121	0.316667	0.175896
F3-4	F03	0.125935	0.004545	0.441878
F3-3	F03	0.207848	0.002857	0.207672
F5-7	F05	-0.21692	-0.13095	0.166896
F3-5	F03	0.091305	0.028571	0.572477
F3-2-B	F03	-0.17749	-0.1748	0.259355
F3-1	F03	-0.01751	0	0.93245
F3-2	F03	-0.31373	-0.10758	0.044965
F3-6	F03	-0.21196	-0.04167	0.183027
E6-3	E06	-0.2256	-0.15	0.150245
E6-2	E06	0.030303	0.058333	0.865649
E3-50	E03	-0.02603	-0.02158	0.887834
E3-48	E03	-0.28139	-0.18188	0.071127
E5-25	E05	-0.24401	-0.07048	0.120387
E3-7	E03	-0.1342	-0.13214	0.397587
E7-16	E07	-0.24401	-0.09667	0.120387
E6-20	E06	-0.01735	-0.00267	0.932558
E5-19	E05	-0.15218	-0.02941	0.337311
E3-19	E03	0.043384	0.006667	0.799586
E5-12	E05	-0.38514	-0.05463	0.013942
E7-29	E07	-0.14475	-0.01667	0.365606
E7-2	E07	-0.55844	-0.34902	3.07E-04
E7-18	E07	-0.25384	-0.02745	0.107292
E1-14	E01	-0.27883	-0.05741	0.078813
D3-56	D03	0.021645	0.053846	0.910196
C4-12	C04	-0.04338	-0.07434	0.799586
D1-10	D01	-0.0303	-0.0049	0.865649
F4-10	F04	0.168565	0.009091	0.293383
F2-21	F02	-0.18615	-0.24722	0.236289
F2-8-A	F02	0.127204	0.074026	0.428199
F4-11	F04	-0.09989	-0.00119	0.548234
F2-6	F02	0.048037	0.005882	0.777557
F2-1-B	F02	0.116883	0.09127	0.463469
F2-13	F02	0.100001	0.057143	0.534699
F4-5	F04	-0.20824	-0.16125	0.184896
F2-1-C	F02	0.177489	0.152183	0.259355
F4-4	F04	-0.16576	0	0.322077
F2-1-A	F02	0.229437	0.198333	0.142569

D1-14	D01	-0.49458	-0.21515	0.001434
C4-9-B	C04	-0.48052	-1.18182	0.001924
C4-13	C04	-0.32468	-0.1631	0.03692
B8-10-A	B08	-0.12554	-0.17143	0.429795
G4-7-A	G04	0.295012	0.153333	0.058756
G5-12-C	G05	-0.1342	-0.06889	0.397587
G5-13-A	G05	-0.09091	-0.08	0.572782
G5-12-D	G05	0.234274	0.114815	0.134892
G5-10-A	G05	0.303688	0.180532	0.051603
B7-18	B07	-0.06941	-0.04306	0.672195
B2-19	B02	0.257652	0.0725	0.101313
B2-25	B02	-0.01735	-0.0037	0.932558
G5-26-A	G05	-0.12148	-0.15238	0.446271
C4-4	C04	-0.2381	-0.09286	0.127837
C2-10	C02	0.226584	0.045455	0.149812
C4-1	C04	-0.17354	-0.07007	0.271264
C2-13	C02	-0.18615	-0.11389	0.236289
C2-12	C02	0.134784	0.044872	0.397212
C2-14	C02	0.043384	0.02	0.799586
C1-7	C01	-0.21212	-0.09881	0.175896
C1-8	C01	-0.16017	-0.07821	0.310046
A3-9	A03	0.082251	0.083333	0.61176
A3-9	A03	0.082251	0.083333	0.61176
B2-9	B02	0.359307	0.183333	0.020765
A2-21	A02	0.090909	0.112963	0.572782
B4-29	B04	-0.16087	-0.08889	0.309661
B2-10	B02	0.378264	0.241667	0.015226
A2-18	A02	0.064935	0.068125	0.693012
A2-16	A02	-0.31602	-1.33205	0.042331
B9-3	B09	0.008677	0.002564	0.977495
B9-6	B09	0.073593	0.068627	0.651869
B7-17	B07	-0.22944	-0.10476	0.142569
B8-2-A	B08	-0.15618	-0.12949	0.323486
D2-17	D02	0.090909	0.054762	0.572782
D2-16	D02	-0.12148	-0.07222	0.446271
D2-24	D02	-0.02614	-0.00222	0.887715
B9-4	B09	0.168831	0.107556	0.283935
B9-5	B09	-0.06494	-0.18	0.693012
F4-3-C	F04	0.147506	0.075	0.351904
F4-3-B	F04	0.138829	0.052381	0.381855
F4-2	F04	0.147122	0	0.38704
C4-9-A	C04	-0.39394	-0.78472	0.011155
C4-8	C04	-0.48052	-0.31921	0.001924
C4-9-C	C04	-0.47186	-1.9	0.002324
C2-7	C02	0.378264	0.189815	0.015226
C4-7	C04	-0.20346	-0.07959	0.194595
C4-7	C04	-0.20346	-0.07959	0.194595
C4-9	C04	-0.49784	-0.52308	0.001306
C4-2	C04	-0.14348	-0.05424	0.366498
C4-3	C04	-0.25218	-0.20833	0.107712
B2-20	B02	-0.07391	-0.00625	0.65161

B2-17	B02	-0.15618	-0.04444	0.323486
B8-38	B08	-0.02165	-0.00476	0.910196
B8-5	B08	0.01743	2.98E-04	0.932504
B8-8	B08	0.260304	0.246667	0.096045
B8-9	B08	0.104122	0.061224	0.516461
G5-11	G05	0.095445	0.048333	0.553588
G5-13	G05	0.286388	0.044857	0.069929
F4-1	F04	-0.13665	0	0.429494
E9-22	E09	-0.14751	-0.03542	0.351904
E9-23	E09	-0.08261	-0.03848	0.611477
E9-25	E09	0.060738	0.025	0.713829
E9-36	E09	-0.1128	-0.25714	0.480668
D4-7	D04	0.298701	0.115686	0.05518
D4-27	D04	0.257652	0.085	0.101313
D4-9-A	D04	0.125541	0.126667	0.429795
D2-19	D02	0.160173	0.083651	0.310046
D2-5	D02	0.047619	0.103571	0.777959
D2-7	D02	0.090909	0.120635	0.572782
D2-11	D02	0.281385	0.475397	0.071127
G8-8-B	G08	0.272077	0.637222	0.090684
G1-1	G01	0.273684	0.454167	0.097994
G3-19	G03	-0.03846	-0.00486	0.83224
B2-16	B02	0.017507	0	0.932432
E4-5-A	E04	-0.04211	-0.16066	0.820338