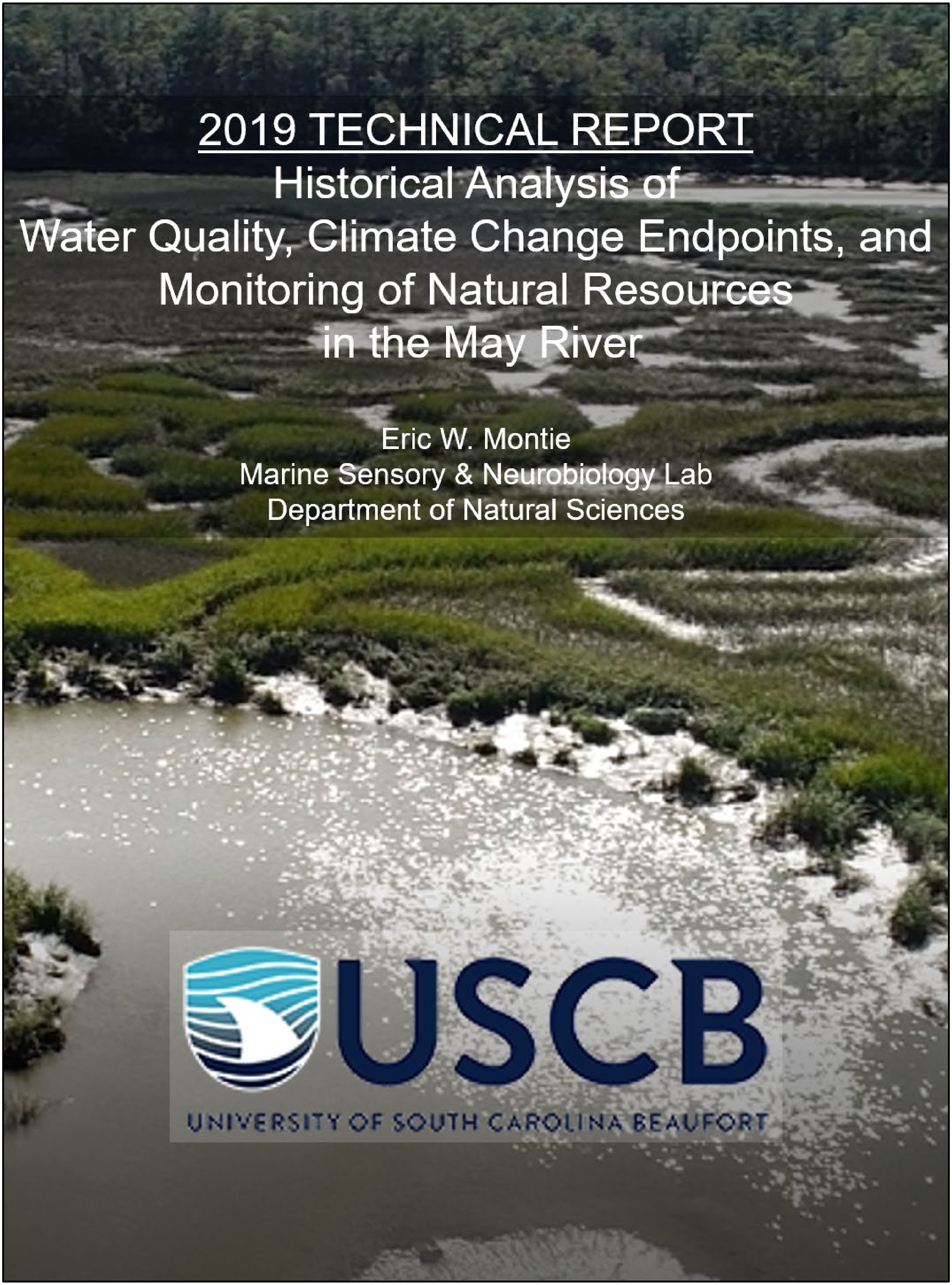

2019 TECHNICAL REPORT
Historical Analysis of Water Quality, Climate Change Endpoints, and Monitoring of Natural Resources in the May River

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USCB
UNIVERSITY OF SOUTH CAROLINA BEAUFORT

2019 TECHNICAL REPORT

Historical Analysis of Water Quality and Climate Change Endpoints and Monitoring of Natural Resources in the May River – A Pilot Study for Other Watersheds in Beaufort County

Project Duration:
11/19/18 – 11/19/19

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1. INTRODUCTION

The May River provides a wonderful setting and natural resources for residents and tourists to enjoy that live and visit the Town of Bluffton, South Carolina. From oyster and blue crab harvesting to fishing for spotted seatrout and red drum to observing bottlenose dolphins – natural resources are at the heart of Bluffton. As in most coastal towns and cities in South Carolina, the population of Bluffton has increased dramatically from approximately 794 residents in 1990 to 21,085 residents in 2017. This change equated to a 2,696% population growth rate in just 17 years (Fig. 1; US Census Bureau, 2018). The associated expansion of housing, roads, commercial infrastructure, and increased recreational use of the May River have resulted in an increased risk to the health of the estuary and its natural resources (Fig. 2).

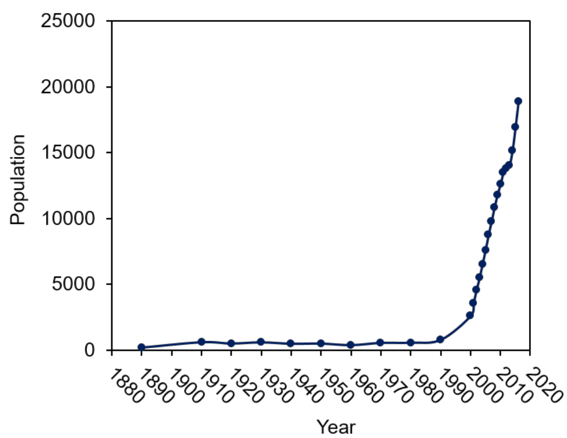

Fig. 1. Population of Bluffton between 1890 and 2016.

In 2018, we proposed to the Town of Bluffton / Beaufort County a five-part plan to analyze existing historical datasets and initiate long-term monitoring programs to define baselines and assess changes in water quality and natural resources. Shifts in baselines may be associated with human activities, climate change, or a combination of these stressors. Our goal was to focus on the May River because the Marine Sensory and Neurobiology Lab at the University of South Carolina Beaufort (under the supervision of Dr. Eric Montie) has been working in this estuary since 2011. This focused approach allowed us to formalize our methodology that could be included in the Town of Bluffton's health assessment of the May River and translated to other watersheds in Beaufort County. The five-part plan focused on:

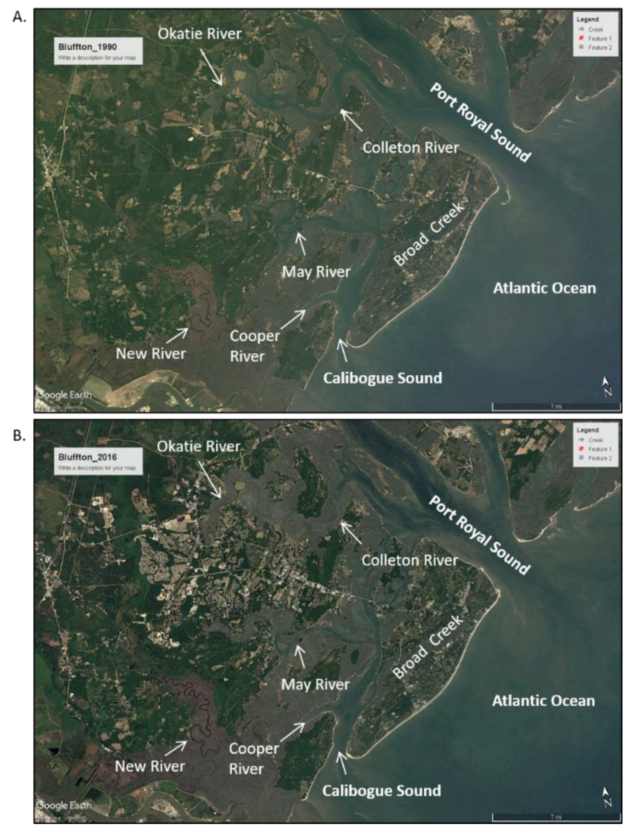

Fig. 2. Google Earth images illustrating the change in urbanization that occurred in the May and Okatie River watersheds from A) 1990 to B) 2016.

A. **Historical analysis of South Carolina Department of Health and Environmental Control (SCDHEC) Shellfish Monitoring Data.** For each SCDHEC Shellfish Monitoring station in the May River, we performed a historical evaluation of salinity and fecal coliform levels from 1999 to 2017. This type of data analysis provides a gauge to determine how effective best management practices (BMPs) have been in managing storm-water runoff and non-point source pollution. Understanding historical trends of salinity and fecal coliform can serve as a proxy for other chemical pollutants (e.g. herbicides, pesticides, petroleum derivatives, pharmaceuticals, and personal care products) and biological pathogens that may be increasing in the May River.

B. **Understanding Factors that Influence Salinity and Fecal Coliform Levels.** We statistically tested factors that influenced salinity and fecal coliform levels in the May River. Factors that we tested included temporal parameters (year, season, lunar phase, tidal phase), geographical parameters (sampling station), and environmental data. We also explored how changes in human activities have affected salinity and fecal coliform levels in the May River by incorporating population level in our models.

C. **Mining of Other Historical Chemical, Physical, and Biological Data.** We performed a search of other long-term datasets collected in the May River and nearby estuaries. These data are important because the health of an estuary depends upon other chemical, physical, and biological parameters beyond fecal coliform measurements.

D. **Comparing Historical Data of the May River to Other Watersheds.** We understand the importance of performing this work for all estuaries in Beaufort County. However, we discovered that this detailed analysis was outside the scope of the 2018-2019 grant award. Using the statistical approach we have designed by working with the May River dataset, future work would focus on analysis of salinity and fecal coliform from all SCDHEC stations from Port Royal Sound to Calibogue Sound. This approach would allow us to identify locations that are resistant or more susceptible to deterioration in water quality and potentially identify underlying factors (e.g. ratio of impervious surface to forested land) that may explain these differences.

E. **Novel Techniques to Monitor Our Natural Resources in the May River.** We understand that the health of the May River estuary is not dependent solely on fecal coliform levels, but it also depends upon other water quality parameters as well as the diversity and abundance of its natural resources. Thus, in this technical report, we summarize the results of our long-term programs that are monitoring water temperature, salinity, pH, and dissolved oxygen (since 2015); fish spawning through the deployment of passive acoustic recorders (since 2013); the diversity and abundance of invertebrates and fish in intertidal creeks (since 2016); and the abundance and distribution of bottlenose dolphins (since 2015). Bottlenose dolphins *Tursiops truncatus* are apex predators (i.e. top predators of the food chain) in estuaries of the Southeast US and are central to ecosystem function (Fig. 3). In the May River,

Fig. 3. Bottlenose dolphin in the May River. (NMFS Permit No. 20060, 2/6/18, Photographer A. Monczak)

bottlenose dolphins are a keystone species (i.e. species that play a critical role in maintaining the structure of an ecological community) and their declines would represent a massive deterioration in the May River ecosystem. Thus, monitoring these predators is a critical component in understanding the health of the May River estuary.

2. METHODS

2.1 Study area

The May River (32°12’49.46’’N; 80°52’23.14”W), SC, is a large subtidal river estuary that is approximately 22 km long and 0.01 km wide near the source and 1 km wide at the mouth. The water depth near the source ranges from ~3 to 7 m while near the mouth ranges from ~4 to 18 m. Bordering the river and creeks are intermittent oyster rubble and live oyster reefs (i.e. eastern oyster *Crassostrea virginica* and vast areas of salt marsh composed of smooth cord grass

Spartina alterniflora. This estuary is strongly influenced by ~2.5 to 3 m semi-diurnal tides.

2.2 Statistical Analysis of SCDHEC Shellfish Monitoring Data

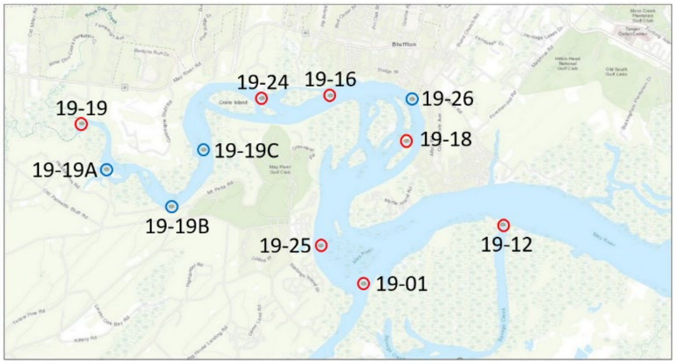

Fig. 4. Locations of SCDHEC long-term shellfish monitoring stations along the May River, SC. Red circles (1999-2017); blue circles (2009-2017).

SCDHEC recorded salinity and fecal coliform data as part of their shellfish-monitoring program. We reported monthly salinity and fecal coliform measurements from 1999 until 2017 at seven stations along the May River, SC (i.e. 19-19, 19-24, 19-16, 19-18, 19-25, 19-01, and 19-12) with four additional stations (i.e. 19-19A, 19-19B, 19-19C, and 19-26) added in 2009 (Fig. 4). In addition to salinity and fecal coliform, SCDHEC measured tidal phase and water temperature at each station. SCDHEC employees divided the tidal cycle into eight categories: early rising tide, mid rising tide, late rising tide, high tide, early falling tide, mid falling tide, late falling tide, and low tide.

Data included in analysis, but not provided by SCDHEC, included Oceanic Nino Index (ONI), rainfall, population, lunar cycle, and season. The Oceanic Nino Index, a monthly temperature anomaly in the equatorial Pacific, was used as an indicator of El Nino Southern Oscillation (ENSO). When the anomaly is positive, El Nino conditions dominate; when the anomaly is negative, La Nina dominates. The source of these data originated from the National Oceanic and Atmospheric Administration (NOAA) database (www.cpc.ncep.noaa.gov/products/precip/CWlink/MJO/enso.shtml). We used daily rainfall data provided by NOAA rain gauges located within the May River watershed (www.ncdc.noaa.gov/cdo-web/search). To create a monthly value, we totaled the rainfall between SCDHEC sampling days. For the annual population levels of Bluffton, SC, we used data obtained from the US Census Bureau (https://www.census.gov/programs-surveys/popest/data/data-sets.html). Season was divided into four categories (i.e. winter, spring, summer and fall) using the solstice and equinox dates for each year. The lunar cycle was divided into four categories: first quarter (lunar days 5-11), full moon (lunar days 12-19), last quarter (lunar days 20-26), and new moon (lunar days 27-4).

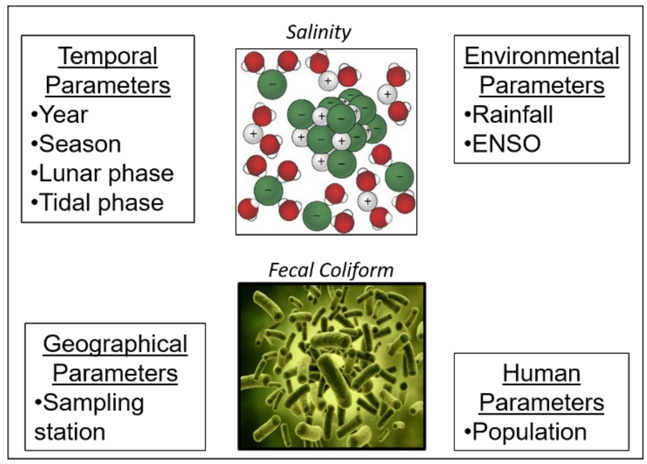

Fig. 5. Temporal, environmental, geographical, and human factors that may influence salinity and fecal coliform levels in the May River, SC.

To understand historical trends, we investigated how temporal, geographical, environmental, and human parameters influenced salinity and fecal coliform levels (Fig. 5). Statistical analyses were conducted using SPSS Statistics 24 software (IBM Corporation, Armonk, NY, USA). We used general linear models (GLM) and correlation tests to understand the effects of these variables on salinity and fecal coliform. Normality was determined through examining the histograms, skewness, and kurtosis of the datasets. Post hoc tests determined significant differences between group means for each categorical variable. Tukey's honest significant difference (HSD) test was used for data with equality of variances, while a Dunnett's C test was used for data that violated this assumption.

Salinity

We separated the salinity data into two different datasets for analysis. The first dataset ranged from 1999 to 2017 and included the seven original stations and but no rainfall data. The second dataset ranged from 2009 to 2017 and included all eleven stations and rainfall data. We performed separate GLMs for each dataset to determine the factors that influenced salinity. Both datasets had an absolute skewness value of <2 and kurtosis of <7 indicating that they were both normally distributed. For the first dataset, we performed a GLM that tested the effect of station, year, season, lunar phase, tidal phase, and ONI on salinity. For the second dataset, we conducted a GLM that tested the effect of station, year, season, lunar phase, tidal phase, ONI, and rainfall on salinity. In both datasets, the variances were equal; thus, Tukey's HSD post hoc tests were conducted. Water temperature was not included in the GLMs, as SCDHEC measurements were not available at each station. We did not include population in the GLMs, since population data were assessed yearly and salinity was sampled monthly.

In addition to general linear modeling, we performed correlation analysis to determine the relationships between salinity (or salinity variability) and ONI, rainfall, population, or year for each SCDHEC station. When data were normally distributed, we used Pearson's correlation tests; when data were not normally distributed, we performed Kendall tau-b correlation tests. To understand historical trends in salinity at each station, we performed a Kendall's tau-b correlation using the 2-year centered salinity moving average. The moving average removed the salinity signal associated with ONI. We conducted a Pearson's correlation test to understand the relationship between the annual population of Bluffton and the annual salinity averages for each station.

Fecal Coliform

We separated the fecal coliform data into three different datasets for analysis. The first dataset ranged from 1999 to 2017 and included the seven original stations but no rainfall data. The second and third datasets ranged from 2009 to 2017 and included all eleven stations. We performed separate GLMs for each dataset to determine what factors (i.e. station, year, season, lunar phase, tidal phase, ONI, rainfall, and salinity) influenced fecal coliform levels. Salinity and rainfall exhibited collinearity, and thus, we included these factors in separate models. The second GLM included salinity levels, while the third dataset included rainfall levels. The data for all three datasets were log transformed since the data were highly variable and were not normally distributed (i.e. absolute skewness value of <2 and kurtosis of <7). We performed Dunnett's C post hoc tests because datasets violated the assumption of equal variance. Similar to the salinity models, water temperature and population were not included.

In addition to general linear modeling, we performed correlation analysis to determine the relationships between fecal coliform levels and salinity, population, or year for each SCDHEC station. When data were normally distributed, we used Pearson's correlation tests; when data were not normally distributed, we performed Kendall tau-b correlation tests. To understand historical trends in fecal coliform levels at each station, we performed a Kendall's tau-b correlation using the 2-year centered fecal coliform moving average. The moving average removed the signal associated with ONI. We conducted a Kendall's tau-b correlation test to understand the relationship between the annual population of Bluffton and the annual fecal coliform averages for each station.

2.3 USCB Environmental Data Monitoring Program

Since 2013 to present, we have deployed water level and temperature loggers (HOBO 100-Foot Depth Water Level Data Logger U20-001-02-Ti and HOBO Water Temperature Pro v2 U22-001, Onset Computer Corporation, Bourne, MA, USA) at three locations in the May River (i.e. 9M, 14M, and 37M) (Fig. 6). Loggers record water depth every hour. These measurements are determined from bottom depth pressure and atmospheric pressure readings

(HOBO 100-Foot Barometric Pressure Level Data Logger U20-001-02-Ti, Onset Computer

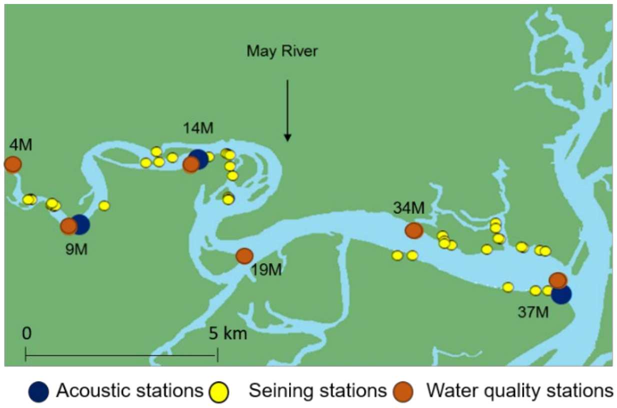

Fig. 6. Map of the May River depicting locations of acoustic, seining, and water quality stations monitored by the USCB Marine Sensory and Neurobiology Lab.

Corporation, Bourne, MA, USA) using formulas provided by Onset Computer Corporation. The factory, calibrated range for the HOBO depth water level logger is between 69 and 400 kPa, which is within our bottom pressure ranges of 100 to 180 kPa. The maximum error for the absolute pressure sensor is ± 1.2 kPa. Temperature loggers record water temperature every hour. These HOBO loggers can measure temperatures between -40°C and 50°C in water with ± 0.21°C accuracy. We place the HOBO loggers in PVC housing and attach to the inside of instrument frames with zip ties. HOBO logger data is downloaded using HOBOware®Pro software (Onset Computer Corporation, Bourne, MA, USA). In this technical report, we present continuous water temperature data from 2013 to 2019 as well as temperature means, minimums, and maximums from 2016 to 2018 for stations 9M, 14M, and 37M.

Since October 2015 to present, we have collected additional environmental data (i.e. salinity, temperature, dissolved oxygen, and pH) once or twice a month at six locations (i.e. 4M, 9M, 14,.19M, 34M, and 37M) with a YSI 556 Handheld Multiparameter Instrument (YSI Inc. / Xylem Inc., Yellow Springs, OH, USA) (Fig. 6). In this technical report, we present data collected from October 2015 to November 2019.

2.4 USCB Passive Acoustic Monitoring Program

Since 2013 to present, we have deployed autonomous, acoustic recorders (DSG-Oceans, Loggerhead Instruments, Sarasota, FL, USA) to monitor the estuarine soundscape (i.e. underwater biological, physical, and human-made sounds) at three locations in the May River (i.e. 9M, 14M, and 37M) based upon previous work (Fig. 6; Montie et al. 2015). Listening and recording sounds of the estuary allows us to detect fish courtship calls, which provide an estimate of spawning of ecologically and economically important fish species including oyster toadfish, silver perch, black drum, spotted seatrout, and red drum (Monczak et al. 2017; 2019) (Fig. 7). These species of fish are also important prey for bottlenose dolphins, an apex

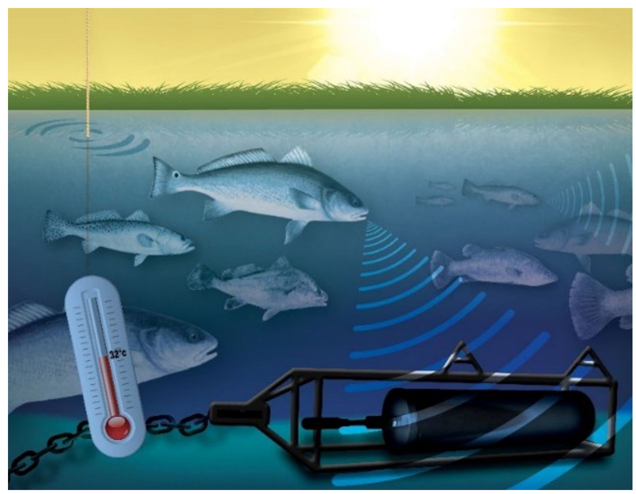

Fig. 7. Long-term acoustic recorders (black instrument in figure) estimate spawning timelines by detecting fish calls associated with courtship. (Monczak et al. 2017. Marine Ecology Progress Series 581:1-19. Feature Article)

predator and keystone species in the May River. Furthermore, listening to the underwater soundscape allows us to detect foraging bouts of dolphins (i.e. echolocation) and communication with conspecifics (i.e. whistles and burst pulses).

To accomplish these tasks, we mount DSG-Ocean recorders in custom-built instrument frames (Mooring Systems, Inc., Cataumet, MA, USA). The instrument frames and DSG-Oceans are painted with antifouling paint (Trilux 33, West Marine, Hilton Head Island, SC, USA). We then deploy the

Fig.8. DSG-Ocean recorder.

instrument frames on the bottom approximately 10 m from the shoreline (Fig. 8). This deployment method is accomplished by attaching a 7 m galvanized chain to the instrument frame. The chain is then attached to a line, which stretches along the river bottom to an auger that is inserted into the sediment along the side of the marsh. This method allows deployment and retrieval of instruments without the need of scuba diving. In addition, this setup minimizes moving parts and noise artifacts and protects the recorder and loggers.

The DSG-Ocean recorder is equipped with a High Tech Inc. hydrophone (i.e. sensitivity of -185 dBV µPa-1) with a flat frequency response between ~0.1 and 30 kHz. The system is calibrated by the manufacturer with a 0.1 V (peak) frequency sweep from 2 – 100 kHz and it is powered by 24 D-cell alkaline batteries housed in a cylindrical PVC tube (i.e. 0.65 cm length, 11.5 cm diameter). Acoustic recordings are saved as DSG files on a 128 GB SD card. DSG files are downloaded and batch converted into wav files using DSG2wav© software (Loggerhead Instruments, Sarasota, FL, USA). We schedule the DSG-Oceans to record the soundscape for 2 minutes every 20 minutes at a sampling rate of 80 kHz.

We manually review each 2 min wav file using Adobe Audition CS5.5 software (Adobe Systems Incorporated, San Jose, CA, USA). Spectrograms are visualized using a spectral resolution of 2048 (i.e., the number of vertical bands used to draw frequencies in the Adobe Audition spectrogram) and a 10 second time window (i.e., zooming in the Adobe Audition spectrogram to show 10 sec at a time). We focus analysis on black drum, oyster toadfish, silver perch, spotted seatrout, and red drum (Fig. 9). For each 2 min wav file, an observer scores the file based upon the intensity of calling for each fish species. The calling intensity score is based on four categories (i.e. 0 = no calls; 1 = one call; 2 = multiple calls; 3 = overlapping calls or chorus). An observer also records other sounds and noises originating from boats, rain, bottlenose

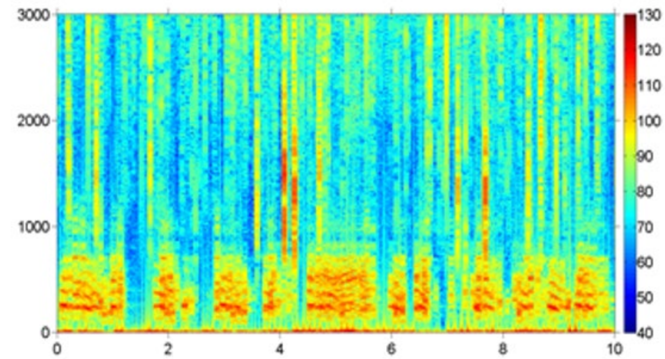

Fig. 9. Spotted seatrout calling in the May River. Males produce calls to attract females to a spawning location.

dolphins (i.e. echolocation, whistles, and burst pulse sounds), and unknown sounds. We enter these data into a standardized spreadsheet in Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). From these data, we sum calling intensity scores per night (12:00 to 11:40 the next day) for black drum, silver perch, oyster toadfish, spotted seatrout, and red drum and plot the sums against date with corresponding water temperature, day length, and lunar cycle. In this technical report, we present fish calling timelines (i.e. a proxy for spawning) from 2013 to 2018.

2.5 USCB Invertebrate and Fish Monitoring Program

Since 2016 to present, we have performed invertebrate and fish sampling one to two times per month in the May River using a haul seine (i.e. seine width = 9.1 m, height = 1.2 m, and mesh diameter = 3 mm) and block nets (i.e. additional stationary seine nets to stop fish from escaping). Near each passive acoustic station (i.e. 9M, 14M, and 37M), we seine two to four sites per month (Fig. 6). This sampling equates to six to twelve seines per month selected randomly from a list of sites. Seine sites include tidal pools (i.e. shallow pools of water created on the low tide), intertidal creeks (i.e. small secondary or tertiary creeks feeding from the main river accessible on the low tide), and shoreline habitats (i.e. sites located along the bank of the primary river) (Fig. 10). For each haul seine, we measure the length and width of the

Fig. 10. USCB students Bradshaw McKinney and Shaneel Bivek seine an intertidal creek in the May River at low tide.

pool or creek sampled. Once the seine is completed, we transfer the catch into a live well, quantify the abundance of each species, determine lengths, and then release the live organisms at the original sampling location. For each seine, we calculate the species richness (i.e. the number of different species) and species abundances, both standardized by the seine area. This work is conducted under SCDNR permit numbers 5135 and 5136 as well as IACUC protocol 2233-101181-022217. In this technical report, we provide species richness and abundance data from 2016 to 2018.

2.6 USCB Bottlenose Dolphin Monitoring Program

Since October 2015 to present, we have conducted vessel-based dolphin surveys once or twice a month of the entire May River, SC (i.e. station 4M to 37M; Fig. 6). Each survey includes a boat operator and two or three visual observers. Observers visually scan a 180° area around the bow of the boat in search of dolphins. When a dolphin group is sighted, we record the GPS location along with the time, total number of individuals and mother/calf pairs, environmental conditions, water quality, and behavior. If possible, we capture high quality photographs of each animal's dorsal fin. Each sighting ends after a group is lost, a picture of each dorsal fin is captured, or when 15 min elapses. Our NMFS permit #20066 authorizes these marine mammal surveys.

We use photographs of dorsal fins to identify individual dolphins from a catalog of known animals using computer Darwin software (Fig. 11; Eckerd College Dolphin Research Group, Eckerd College, Florida) (Stanley 1995) . Through years of

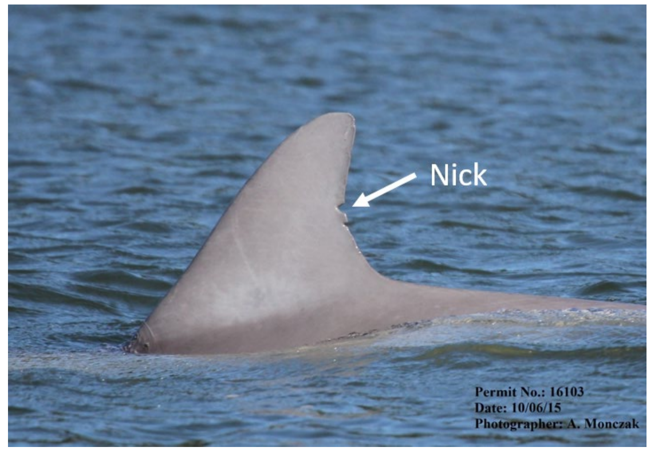

Fig. 11. Dorsal fin of Warny (USCB ID_0148), a bottlenose dolphin photographed in the May River on 10/16/15.

dolphin surveys (since 2011), we created a dorsal fin catalog by adding photos of fins that contained distinguishing features (i.e. notches, nicks, and unique marks). For each survey, we use only high quality photographs that contain the entire trailing edge of an animal's dorsal fin for identification of individuals. From these data, we calculate site fidelities for each individual dolphin:

$$\left(\frac{Number\ of\ surveys\ a\ dolphin\ was\ sighted}{Number\ of\ surveys\ conducted}\right) \times 100\%.$$

These data provide a way to distinguish a resident from a migrant. In this technical report, we present site fidelity calculations from 2016 to 2018. In addition, we report the total number of dolphins and the number of mother/calf pairs for each survey.

3. RESULTS AND DISCUSSION

3.1 Historical Analysis of SCDHEC Shellfish Monitoring Data and Understanding Factors that Influence Salinity and Fecal Coliform

Salinity Levels in the May River

We evaluated long-term salinity trends using two different datasets. From 1999 to 2017, we found that station, year, season, lunar cycle, tidal cycle, and ONI significantly influenced salinity levels (see Appendix 1, Table 1). Year influenced salinity the most with a η^2 of 0.296, while lunar cycle had the smallest influence on salinity with a η^2 of 0.006. From 2009 to 2017, we determined that station, year, season, tidal cycle, rainfall, and ONI significantly influenced salinity (Table 2). Year had the largest influence on salinity with a η^2 of 0.306, while tidal cycle had the smallest influence on salinity with a η^2 of 0.027. For both datasets, these factors helped explain 41% and 61% of the salinity variability, respectively, in the May River, SC.

Station (i.e. the location of where sampling occurred along the river) significantly influenced salinity. For both datasets, we found that average salinity increased from the headwaters to the mouth (Fig. 12). Locations closer to the headwaters are more sensitive to factors that influence salinity (i.e. rainfall, runoff, tidal cycles, and lunar cycles), while areas located closer to the mouth are buffered by larger volumes of water (Van Dolah et al. 2007). Station 19-19, located closest to the headwaters, had the lowest average salinity and largest range for the 1999-2017 dataset (i.e. average salinity was 27.23‰ and the range was 31‰) and the 2009-2017 dataset (i.e. average salinity was 26.16‰ and the range was 30‰). Station 19-12, located closest to the mouth, had the highest average salinity and the smallest range for the 1999-2017 dataset (i.e. average salinity was 30.36‰ and the range was 13‰) and the 2009-2017 dataset (i.e. average salinity was 30.39‰ and the range was 12‰).

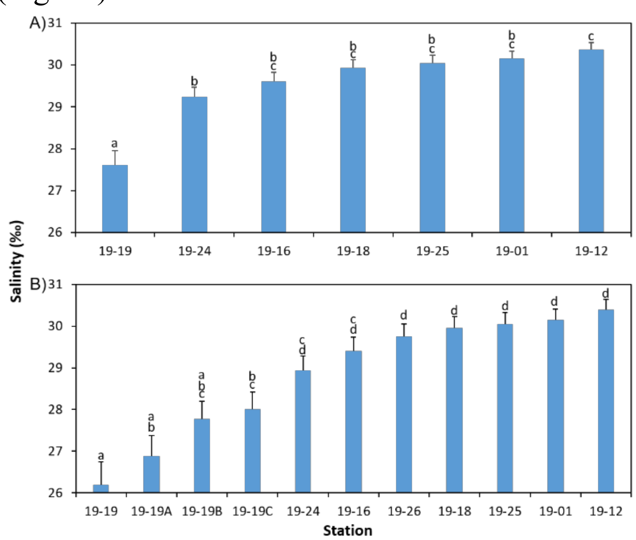

Fig. 12. Average salinity (‰) at each SCDHEC shellfish monitoring station from (A) 1999 to 2017 and (B) 2009 to 2017. Stations that share a letter are not significantly different from each other.

We found that year had the greatest influence on salinity levels in the May River (Tables 1-2). Average salinity for each year fluctuated in a cyclical pattern from 1999 to 2017 due to the El Nino Southern Oscillation (ENSO) (Fig. 13). We determined that years with positive ONIs decreased salinity, while years with negative ONIs increased salinity (Fig. 14). Salinity levels correlated negatively with ONI, and this pattern was most dramatic at stations near the mouth of the May River (Table 3; Fig. 14). In addition to affecting the equatorial Pacific, ENSO affects the southeastern United States. During El Nino episodes, the temperatures are colder, and there is an increase in precipitation. The opposite is true during La Nina. ENSO exhibits inter-annual variability meaning that El Nino and La Nina episodes occur every 2-7 years. We detected this cyclical pattern in the long-term salinity dataset of the May River and did note a recent increase in El Nino events, which may be associated with climate change (Fig. 13; Meehl et al. 2000). The periods of dry weather and warm temperatures associated with La Nina caused increased salinity levels; the periods of wet weather and cool temperatures associated with El Nino caused decreased salinity levels. Previous research of seven estuaries along the Texas coast showed similar findings, where increased salinity levels occurred during La Nina and decreased levels during El Nino (Tolan, 2007).

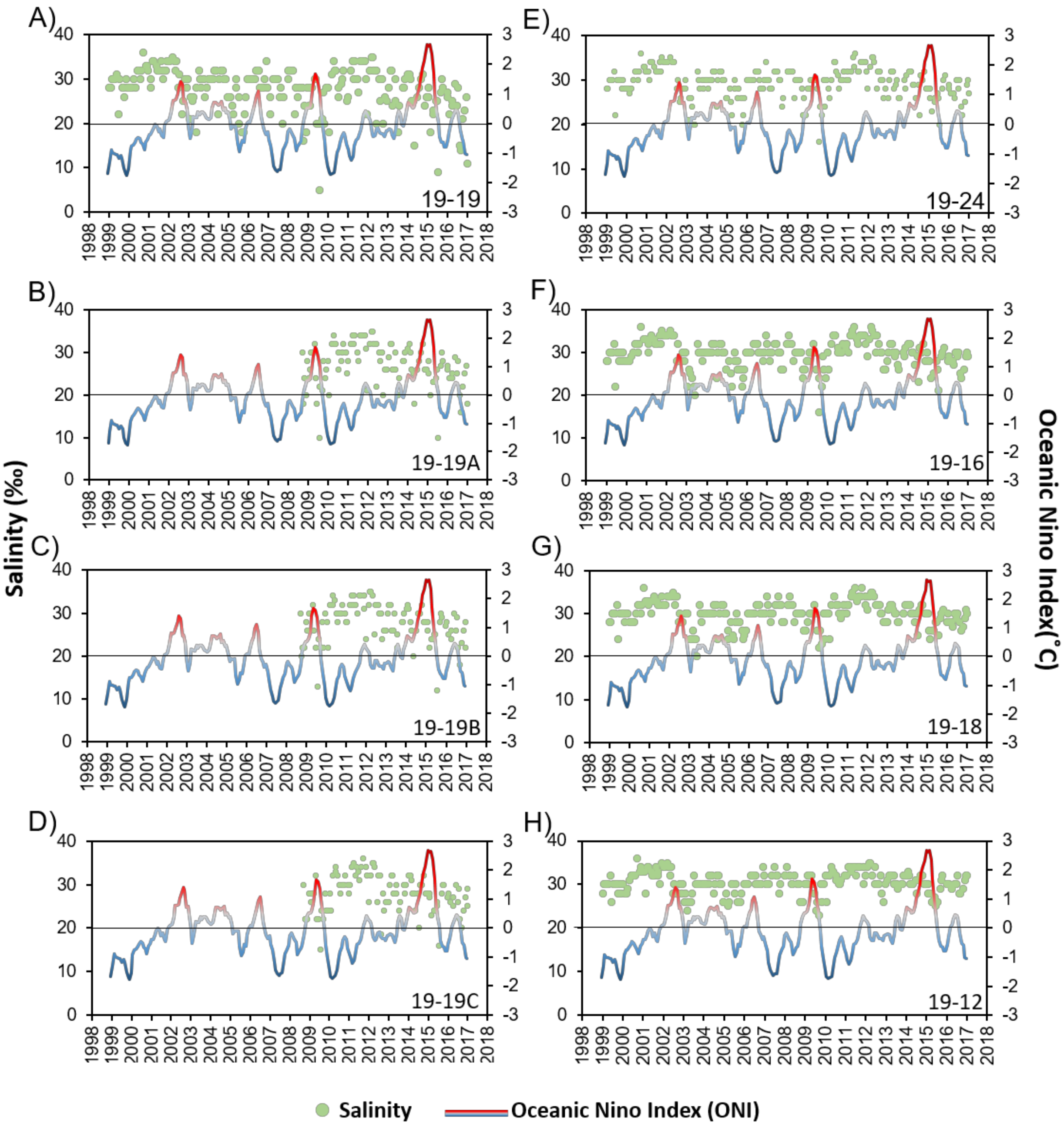

Fig. 13. SCDHEC monthly salinity (‰) measurements at eight of the eleven SCDHEC stations along the May River, SC from 1999 to 2017. Oceanic Nino Index (˚C) is plotted on the secondary y-axis. The blue sections of the line indicate La Nina phases, while the red sections of the line denote El Nino phases. Stations 19-19, 19-19A, 19-19B, and 19-19C were located closer to the source, stations 19-24 and 19-16 were located along the middle of the May River, and stations 19-18 and 19-12 were located closer to the mouth of the May River.

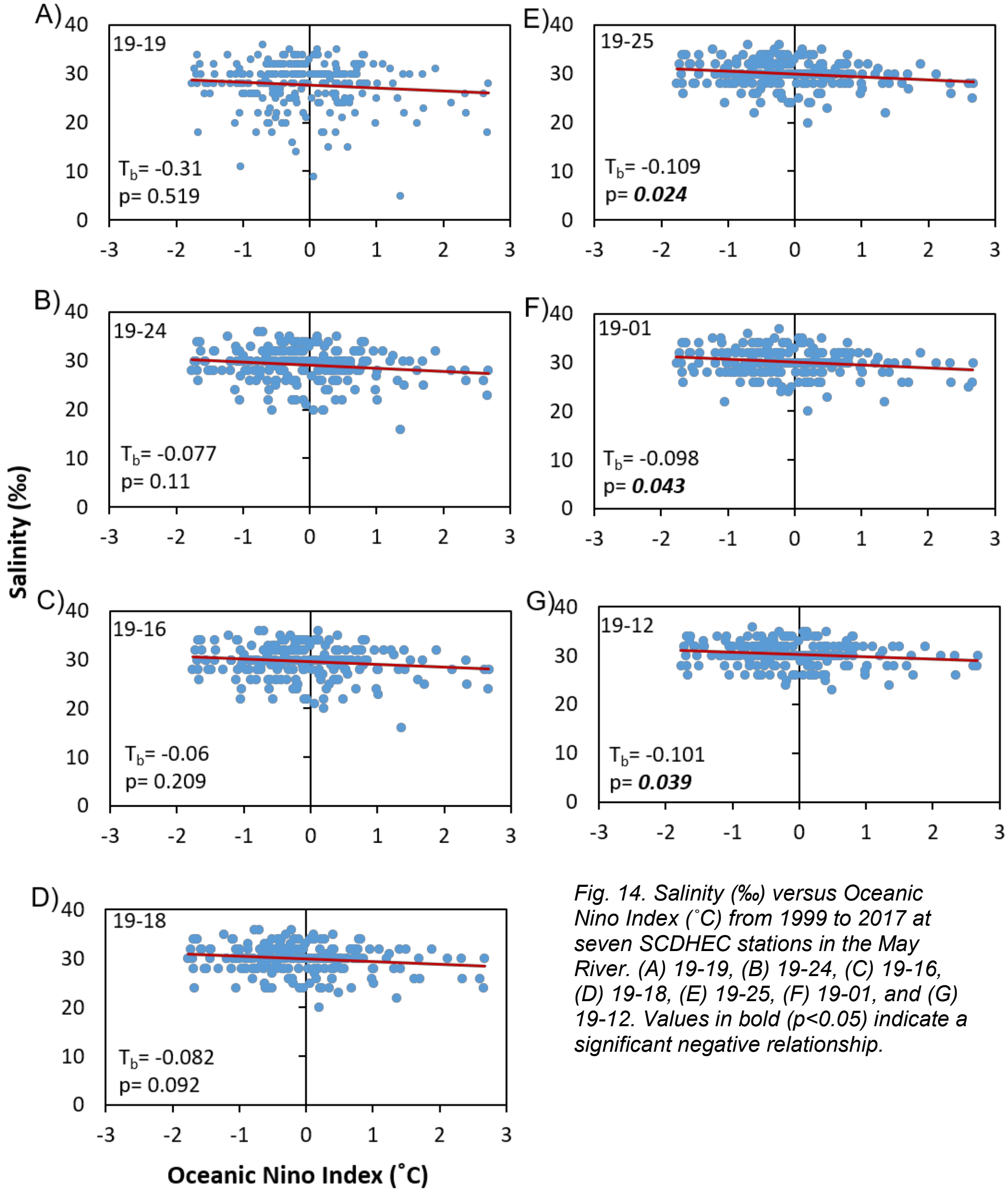

Fig. 14. Salinity (‰) versus Oceanic Nino Index (˚C) from 1999 to 2017 at seven SCDHEC stations in the May River. (A) 19-19, (B) 19-24, (C) 19-16, (D) 19-18, (E) 19-25, (F) 19-01, and (G) 19-12. Values in bold (p<0.05) indicate a significant negative relationship.

We observed that other temporal factors influenced salinity including season, lunar cycle, and tidal cycle. Interactions of evaporation and rainfall amounts affected seasonal salinity trends in the May River (Hollins & Ridd 1997; Seager et al. 2009). Fall had significantly higher salinity levels compared to the other seasons (i.e. spring, summer, and winter) due to the lowest rainfall (Fig. 15A). Although summer had the highest amount of rainfall, it did not have the lowest salinity because freshwater input was buffered by high levels of evaporation associated with higher summer temperatures. Salinity was the highest during the new and full moons, due to larger volumes of oceanic water entering the estuary during high tide (Fig. 15B). We observed increases in salinity during the higher tidal phases (i.e. early rising, mid rising, late rising and high tide) and decreases during the lower tidal phases (i.e. early falling, mid falling, late falling, and low tide) (Fig. 15C). Ocean water floods the river during the rising tide, increasing the salinity, while on the low tide, a combination of

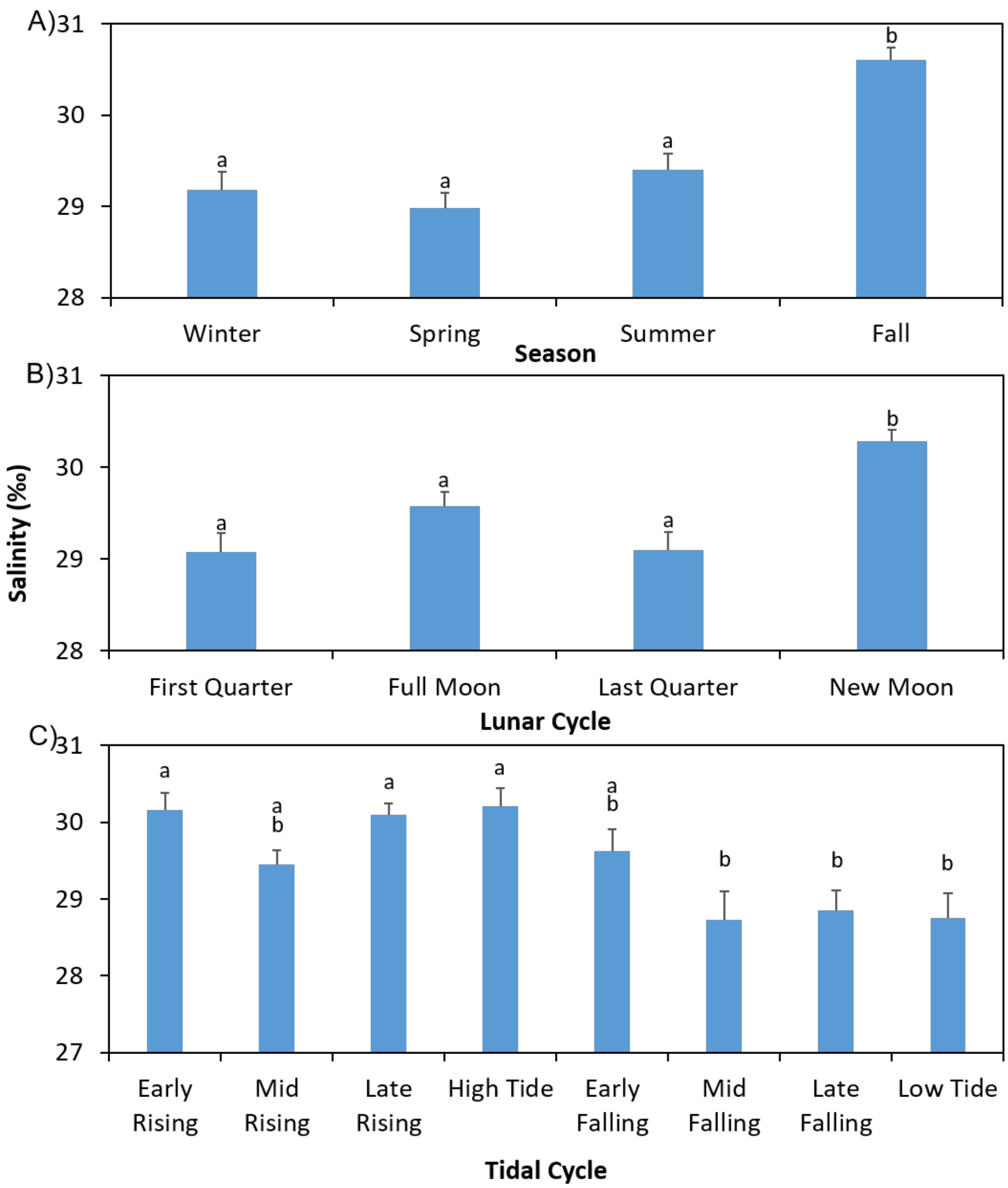

Fig. 15. Mean salinity (‰) for each (A) season, (B) lunar phase, and (C) tidal phase for all SCDHEC stations sampled from1999 to 2017. Means that share a letter are not significantly different from each other.

receding oceanic water and fresh, groundwater intrusion lowers the salinity.

We detected an increase in salinity variability in the headwaters of the May River from 1999 to 2017 (Table 4; Fig. 16). Previous research reported that salinity variance increases in response to increased impervious surface around the watershed, and headwaters of tidal rivers can serve as an early warning sign of potential degradation of the estuary (Holland et al. 2004). Population, which can be an indicator of impervious surface, has rapidly increased in the town of Bluffton from 1999 to 2017 and was negatively correlated with salinity in the headwaters (i.e. station 19-19) (Table 5; Fig. 17). We showed that salinity negatively correlated with rainfall at all eleven stations in the 2009 to 2017 dataset with more negative correlations in the headwaters (Table 6; Fig. 18). If we reduced the salinity signal from ENSO (which causes more rainfall in the Southeast) by applying a 2-year centered moving average for each station, there is evidence that many of the sampling locations along that May River have undergone a significant decrease in salinity from 1999 to 2017 (Table 7; Fig. 19).

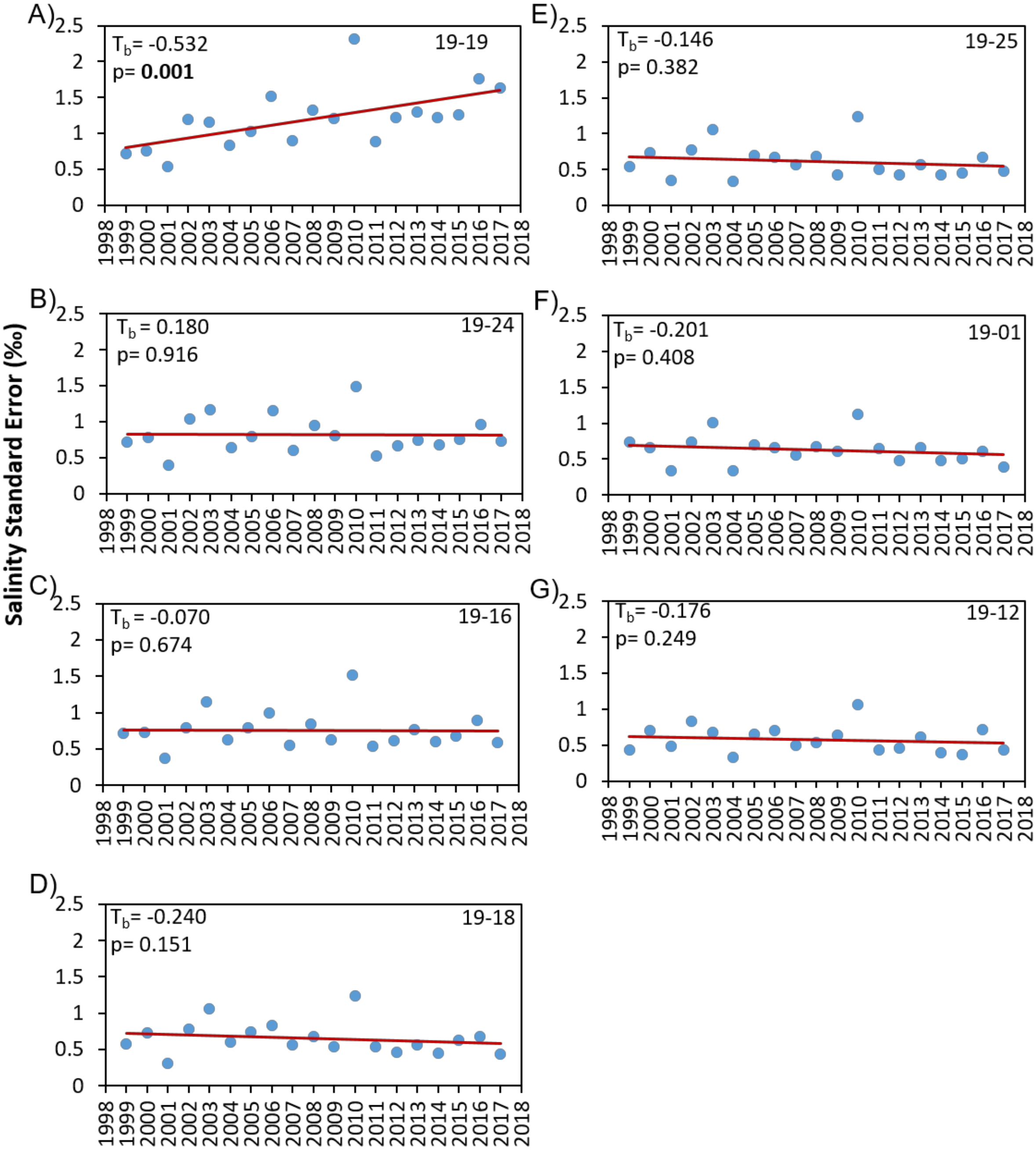

Fig. 16. Salinity standard error for each year sampled from 1999 to 2017 at each of the seven SCDHEC stations: (A) 19-19, (B) 19-24, (C) 19-16, (D) 19-18, (E) 19-25, (F) 19-01, and (G) 19-12. Values in bold (p<0.05) indicate a significant positive relationship.

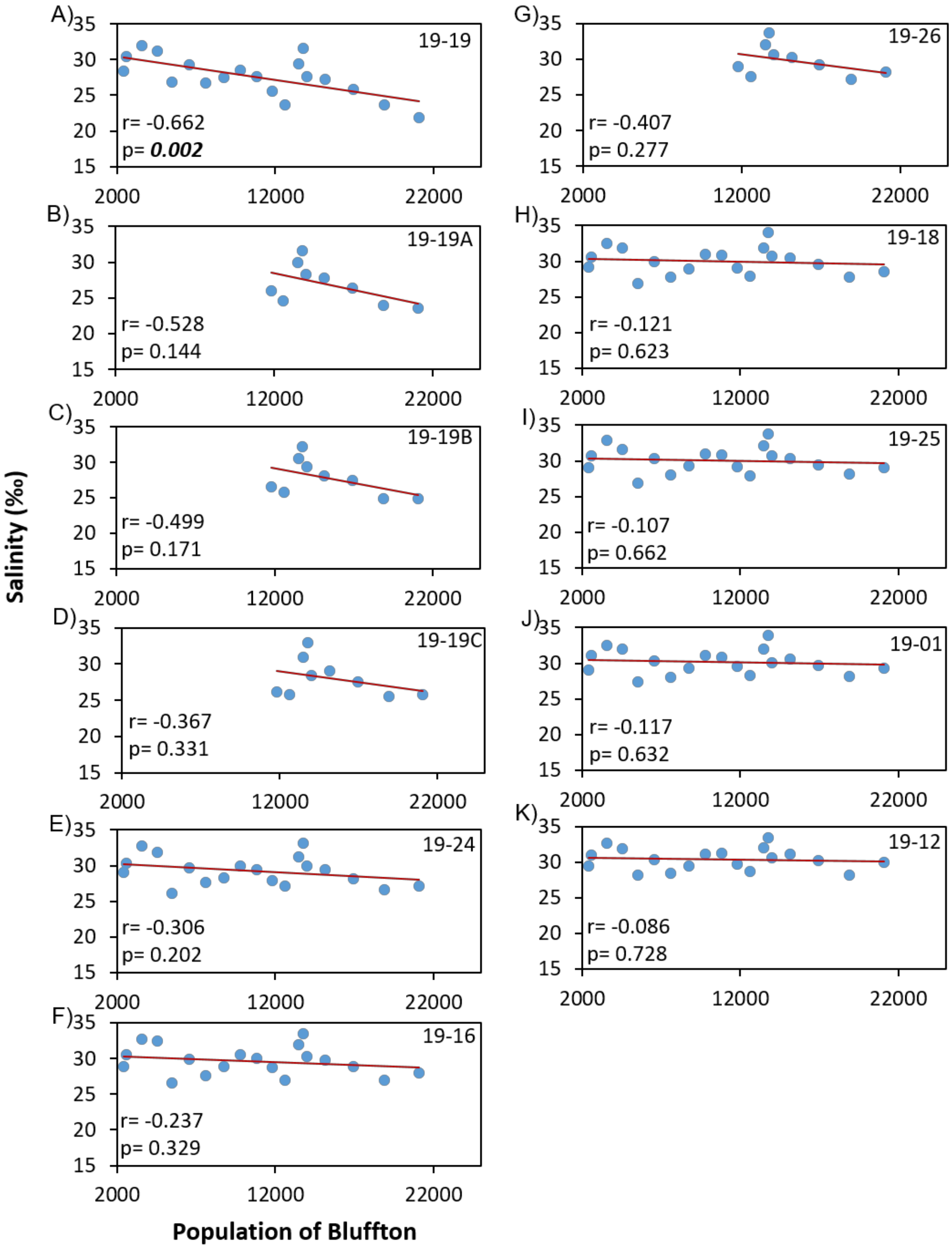

Fig. 17. Annual salinity average (‰) plotted versus annual population from 1999 to 2017 at (A) 19-19, (E) 19-24, (F) 19-16, (H) 19-18, (I) 19-25, (J) 19-01, and (K) 19-12 and from 2009 to 2017 at (B) 19-19A, (C) 19-19B, (D) 19-19C, and (G) 19-19-26. Values in bold (p<0.05) indicate a significant negative relationship.

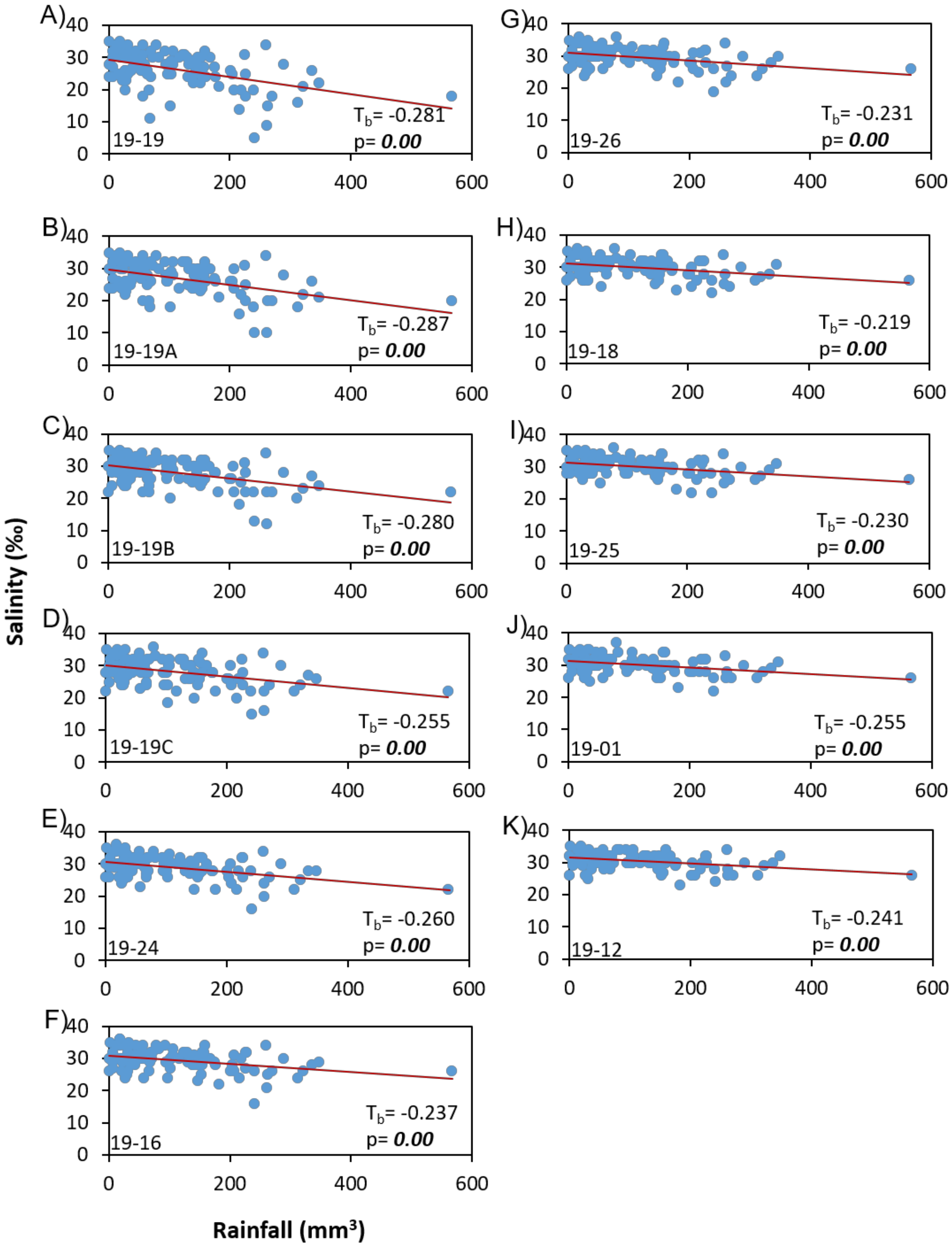

Fig. 18. Salinity (‰) versus summed rainfall sampled from 2009 to 2017 at all eleven SCDHEC stations: (A) 19-19, (B) 19-19A, (C) 19-19B, (D) 19-19C, (E) 19-24, (F) 19-16, (G) 19-26, (H) 19-18, (I) 19-25, (J) 19-01, and (K) 19-12. Values in bold ($p<0.05$) indicate a significant negative relationship.

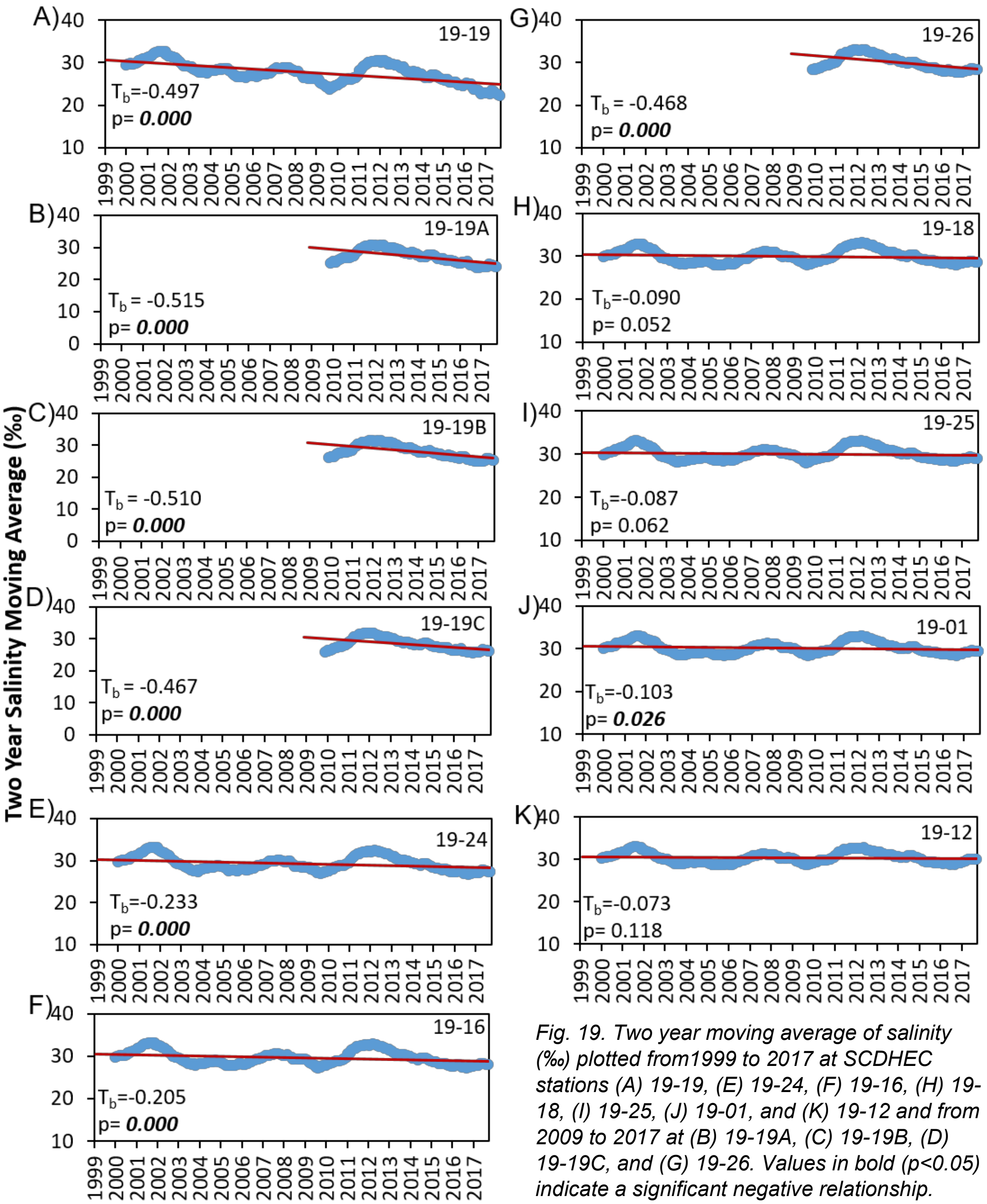

Fig. 19. Two year moving average of salinity (‰) plotted from1999 to 2017 at SCDHEC stations (A) 19-19, (E) 19-24, (F) 19-16, (H) 19-18, (I) 19-25, (J) 19-01, and (K) 19-12 and from 2009 to 2017 at (B) 19-19A, (C) 19-19B, (D) 19-19C, and (G) 19-26. Values in bold (p<0.05) indicate a significant negative relationship.

A combination of increased development and climate change may have led to decreased salinity levels (and increased variability) observed in the headwaters of the May River. Developed and deforested lands have higher levels of freshwater input into estuaries, which leads to decreased salinity levels and increased salinity variability (Holland et al. 2004). Additionally, scientific evidence suggests that climate change is resulting in more frequent and intense El Nino events, which have been shown to reduce salinity levels in estuaries (Timmerman et al. 1999, Tolan 2007, Lee & McPhaden, 2010, Wang et al. 2017). Our analysis may indicate that, as both watershed development increases and climate change progresses, there is potential for further decreases in salinity in the headwaters of the May River, SC. It is possible that sea level rise could impede these changes.

Fecal Coliform Levels in the May River

We investigated long-term, fecal coliform levels over two decades using three different datasets as described in the methods. For the first dataset (i.e. 1999 – 2017), we found that station, year, salinity, tidal cycle, ONI, and season significantly influenced fecal coliform levels (Table 8; Fig. 20). Station

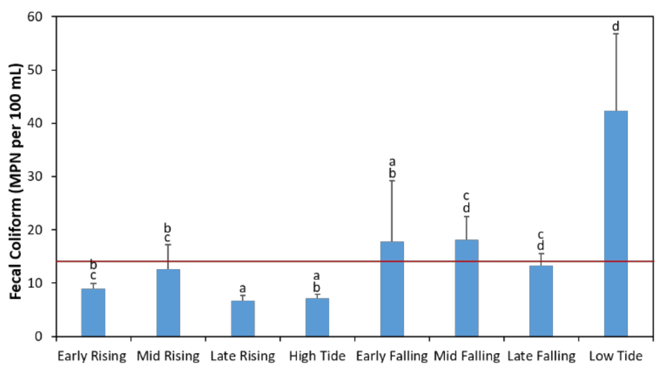

Fig. 20. Average fecal coliform (MPN per 100 mL) during each phase of the tidal cycle for all SCDHEC stations (i.e. 19-19, 19-24, 19-16, 19-18, 19-25, 19-01, and 19-12) sampled from 1999 to 2017. Tidal phases that share a letter are not significantly different from each other. Below the red line indicates SCDHEC's approved fecal coliform levels at 14 MPN/100 mL

influenced fecal coliform the most with a η^2 of 0.170, while season had the smallest influence on fecal coliform with a η^2 of 0.006. For the second dataset (i.e. 2009 – 2017; salinity included in GLM), we determined that station, tidal cycle, year, salinity, lunar cycle, and ONI significantly influenced fecal coliform levels (Table 9). Station influenced fecal coliform the most with a η^2 of 0.375, while ONI had the smallest influence on fecal coliform with a η^2 of 0.008. For the third dataset (i.e. 2009 – 2017; rainfall included in GLM), we found that station, tidal cycle, year, lunar cycle, and rainfall significantly influenced fecal coliform levels (Table 10). Station influenced fecal coliform the most with a η^2 of 0.482, while rainfall had the smallest influence on fecal coliform with a η^2 of 0.008. For all three datasets, these factors helped explain 40%, 56%, and 54% of the fecal coliform variability, respectively, in the May River, SC.

We observed that the sampling location (i.e. station) had the greatest influence on fecal coliform levels for all three datasets (Tables 8-10; Fig. 21). Average fecal coliform levels were the highest in the headwaters and decreased moving towards the mouth (Fig. 21). The fecal coliform levels at locations closest to the headwaters were well above the approved SCDHEC fecal coliform maximum of 14 MPN per 100 mL. From 1999 to 2017, station 19-19 (i.e. closest to the headwaters) had the highest average fecal coliform level (63.66 MPN/100 mL) and the largest range (1698.10 MPN/100 mL). During this time period, we observed that stations closer to the mouth had lower fecal coliform averages and smaller ranges; station 19-01 had the lowest average (4.52 MPN/100 mL) and station 19-25 had the smallest range (31.3 MPN/100 mL).

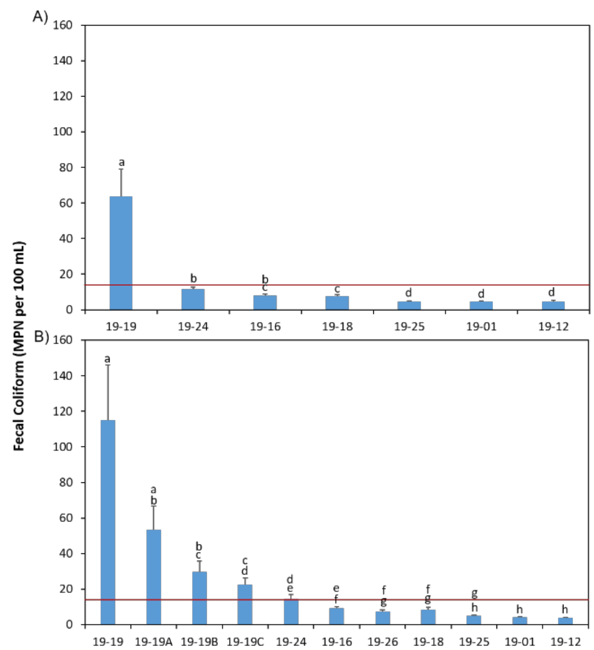

Fig. 21. Average fecal coliform (MPN/100 mL) at each SCDHEC shellfish monitoring station from (A) 1999 until to 2017 and (B) 2009 to 2017. Below the red line indicates SCDHEC's approved fecal coliform levels at 14 MPN/100 mL. Stations that share a letter are not significantly different from each other.

We determined that year was a significant factor that influenced fecal coliform levels in the May River (Tables 8-10). Since 1999, the mean fecal coliform levels near the headwater stations have increased dramatically, as high as 3150% at station 19-19 (Table 11). At stations 19-19, 19-19A, 19-19B, 19-19C, and 19-24 (i.e. locations closest to the headwaters), fecal coliform levels have been above SCDHEC's approved limit since 2009 (Fig. 22).

Since ONI influenced fecal coliform levels (Tables 8-10), we removed the signal from ENSO (i.e. by using a 2-year centered moving average) to better understand the strength of the change (i.e. as measured by the magnitude of the Kendall's tau-b correlation coefficient). Since 1999, fecal coliform levels have significantly increased at all stations except for the station closest to the mouth (i.e. 19-12) (Table 12; Fig. 23).

We observed that fecal coliform levels were higher when salinity levels were lower, and that this negative relationship was strongest at sampling stations closest to the headwaters (Table 13; Figs. 24-25). Additionally, we found that fecal coliform levels in the headwaters increased as population levels grew in the Town of Bluffton, and that this positive relationship was strongest at sampling locations closest to the headwaters (Table 14; Fig. 26). As other studies in different estuaries and watersheds have indicated, we suggest that the rising levels of fecal coliform in the May River are associated with the loss of forested land and the increase of impervious surfaces within the watershed (Holland et al. 2004). The headwaters can serve as an early warning signal of potential degradation of the entire watershed as they experience a greater sensitivity to factors that influence fecal coliform (i.e. rainfall, salinity, and population growth), while the areas closer to the mouth are buffered by larger, more stable bodies of water (Van Dolah et al. 2007; Holland et al. 2004). In other watersheds, studies have reported that larger amounts of freshwater input and storm-water runoff decrease salinity levels and increase fecal coliform; in fact, these studies have shown that lower salinity levels increase the survival rate of fecal coliform bacteria (Chigbu et al. 2004; Lipp et al. 2001; Šolić & Krstulović, 1992).

In addition to septic leakage, we suggest that rising fecal coliform levels in the May River are also associated with the loss of forested land, increased impervious surface, and perhaps climate change. Transformation of forested land to urbanized areas increases the amount of impervious surface surrounding the watershed, resulting in larger amounts of storm-water runoff entering estuaries (Holland et al. 2004). The loss of wetlands and forests also decreases natural sinks for storm-water runoff. The result being a decrease in salinity of volume-sensitive waters, which is more favorable for the survival of fecal coliform bacteria. In addition, stronger and more frequent El Nino events, associated with climate change, provide more rainfall to the Southeast, decreasing salinity levels in volume-sensitive waters (Timmerman et al. 1999; Wang et al. 2017; Lee & McPhaden, 2010). Our analyses indicate that the synergistic nature of urbanization and climate change may lead to further increases in fecal coliform levels in the May River.

3.2 Mining of Other Historical Chemical, Physical, and Biological Data

In addition to the long-term, monthly salinity and fecal coliform monitoring conducted by SCDHEC, we have collected water depth, temperature, salinity, pH, and dissolved oxygen levels at various locations in the May River since 2013 / 2015 (see Section 3.4). Other agencies and organizations have monitoring programs beyond fecal coliform and salinity in SC, but these programs do not consistently measure chemical, physical, and biological parameters in the May River. The South Carolina Estuarine and Coastal Assessment Program (SCECAP) was established in 1999 to begin evaluating the overall health of the state's estuarine habitats on a periodic basis using a combination of water quality, sediment quality, and biotic condition measures, but sampling occurs only at the mouth of the May River and infrequently. We could review these data for the Town of Bluffton (http://www.dnr.sc.gov/marine/scecap/index.html).

NOAA's National Estuarine Research Reserves (NERRs) program have stations within the ACE basin (located within Beaufort, Colleton, and Charleston Counties) and in North Inlet-Winyah Bay (located within Georgetown County). Continuous monitoring of various biological, chemical, and physical parameters has occurred since 1992 at both of these reserves. Biological parameters include chlorophyll and nutrients (i.e.

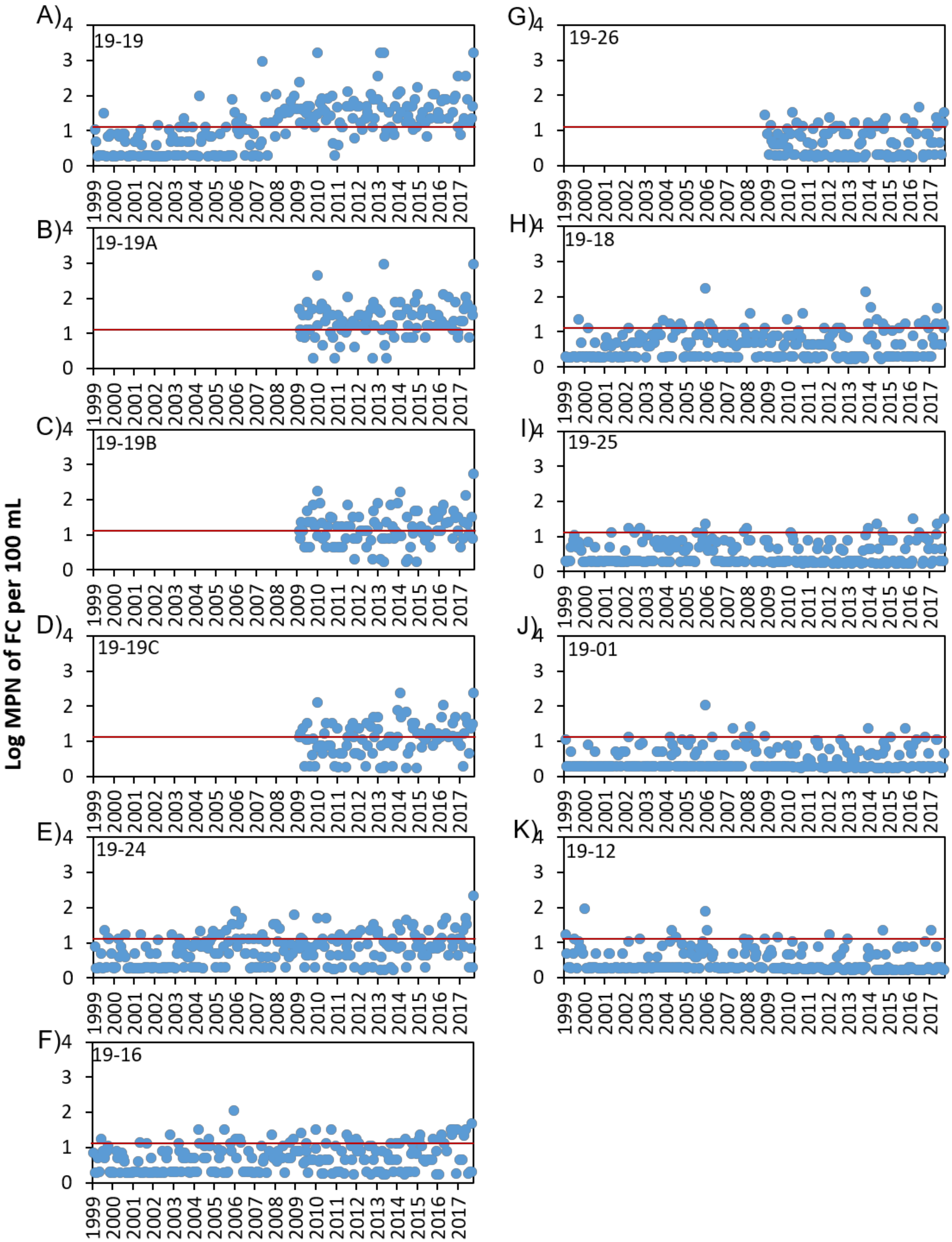

Fig. 22. Fecal coliform levels (log FCMPN/100mL) at each SCDHEC station along the May River, SC from 1999 or 2009. Below the red line indicates SCDHEC's approved fecal coliform levels at log 14 MPN/100 mL.

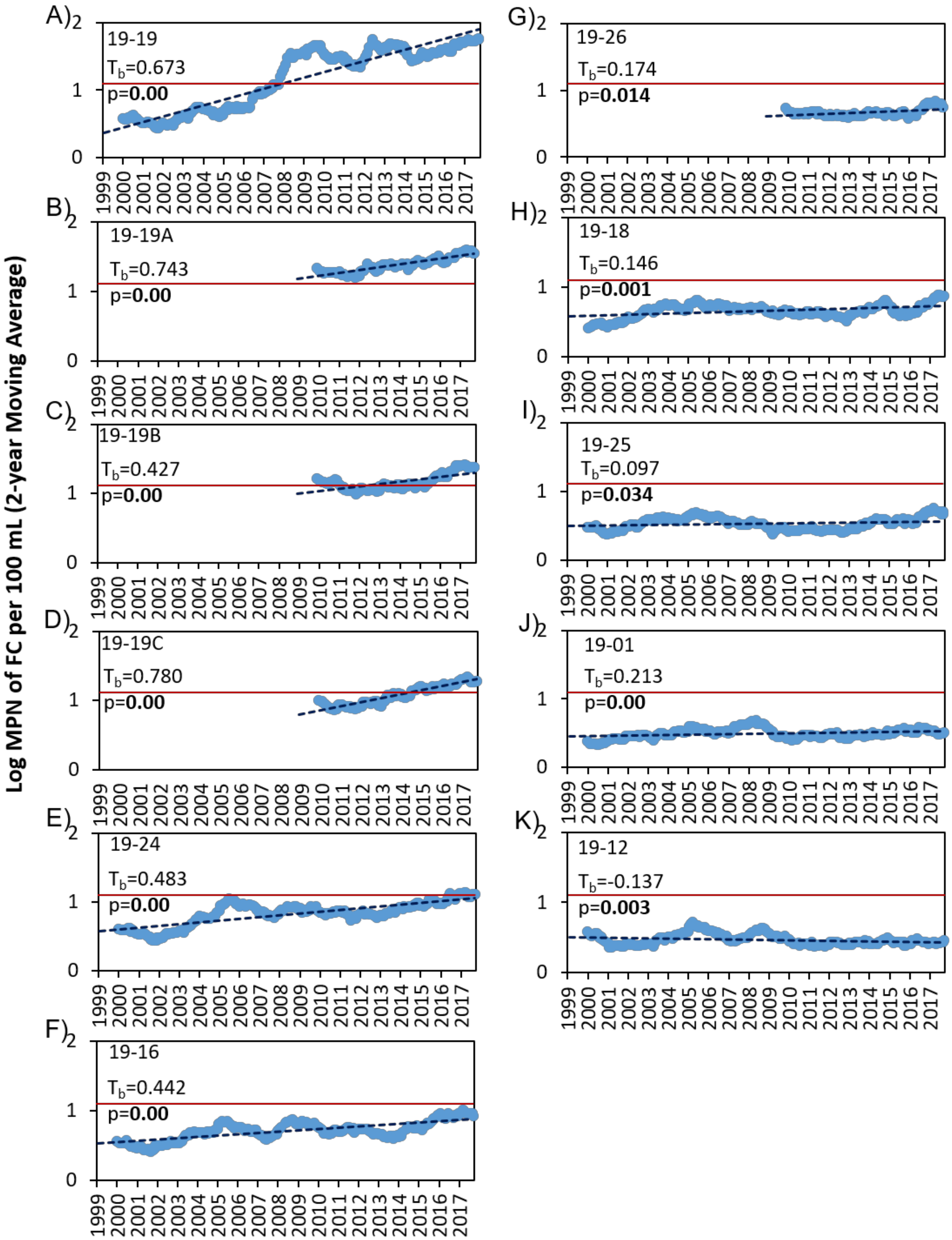

Fig. 23. Two year centered moving average of fecal coliform (log FCMPN/100mL) at all SCDHEC stations in the May River, SC. Below the red line indicates SCDHEC's approved fecal coliform levels at log 14 FCMPN/100 mL.

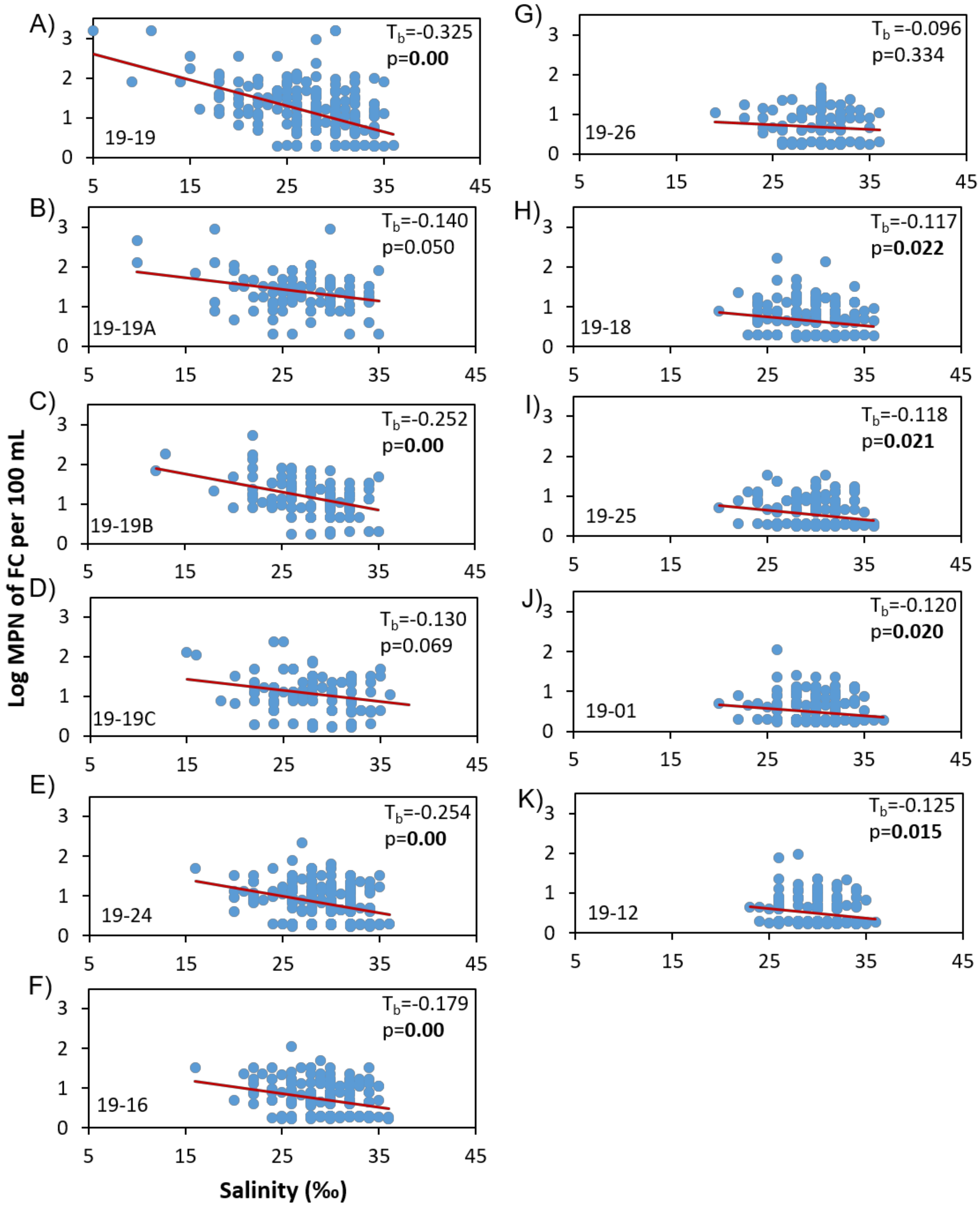

Fig. 24. Fecal coliform (log FCMPN/100mL) versus salinity (‰) at all SCDHEC stations in the May River, SC. Stations sampled from 1999 to 2017 are (A)19-19, (E) 19-24, (F) 19-16, (H) 19-18, (I) 19-25, (J) 19-01, and (K) 19-12. Stations sampled from 2009 to 2017 are (B) 19-19A, (C) 19-19B, (D) 19-19C, and (G) 19-26. Values in bold (p<0.05) indicate a significant negative relationship.

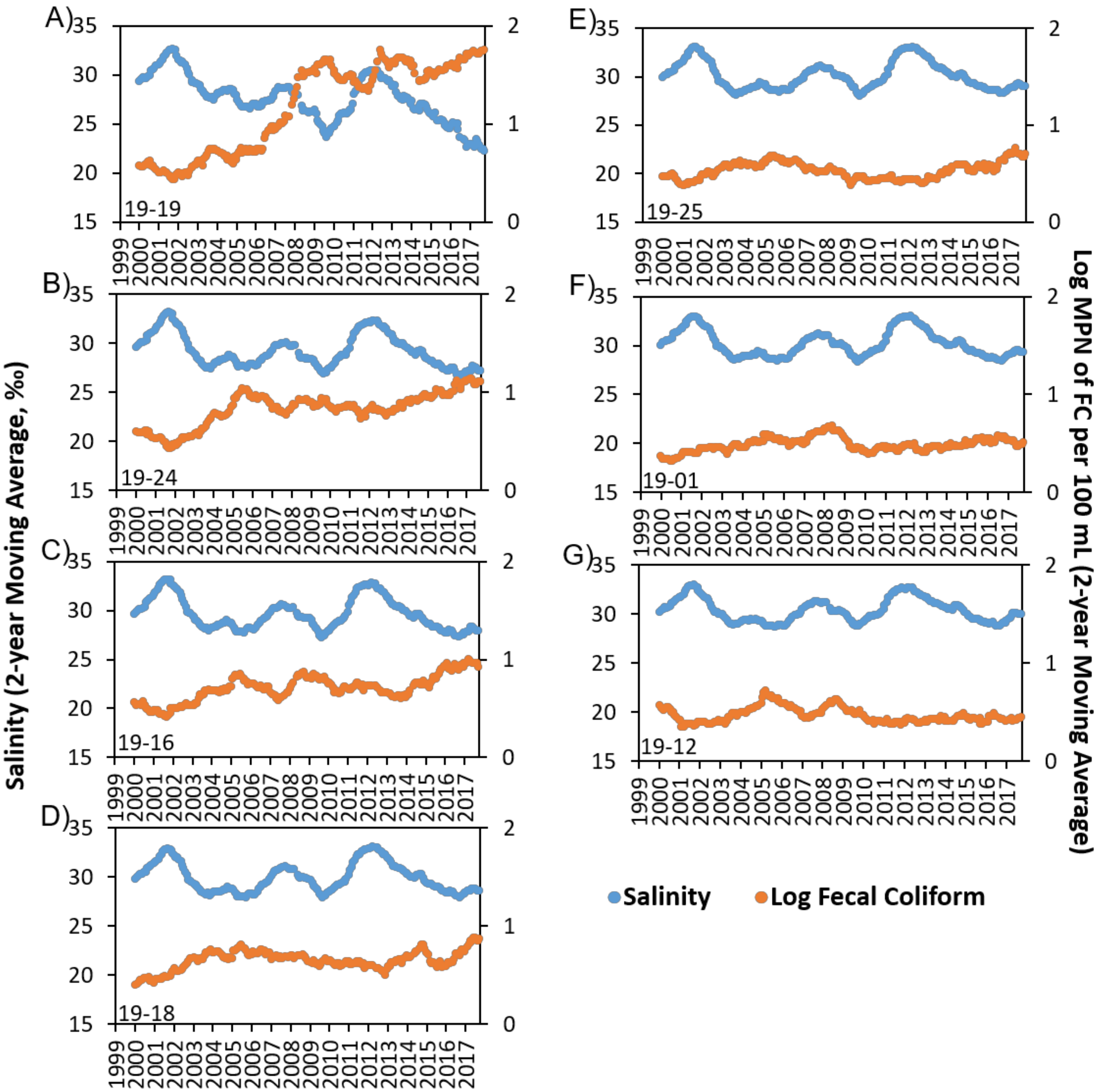

Fig. 25. Two year centered moving averages of fecal coliform (log FCMPN/100mL) and salinity (‰) plotted in orange and blue respectively from 1999 to 2017 at seven SCDHEC stations: (A) 19-19, (B) 19-24, (C) 19-16, (D) 19-18, (E) 19-25, (F) 19-01, and (G) 19-12.

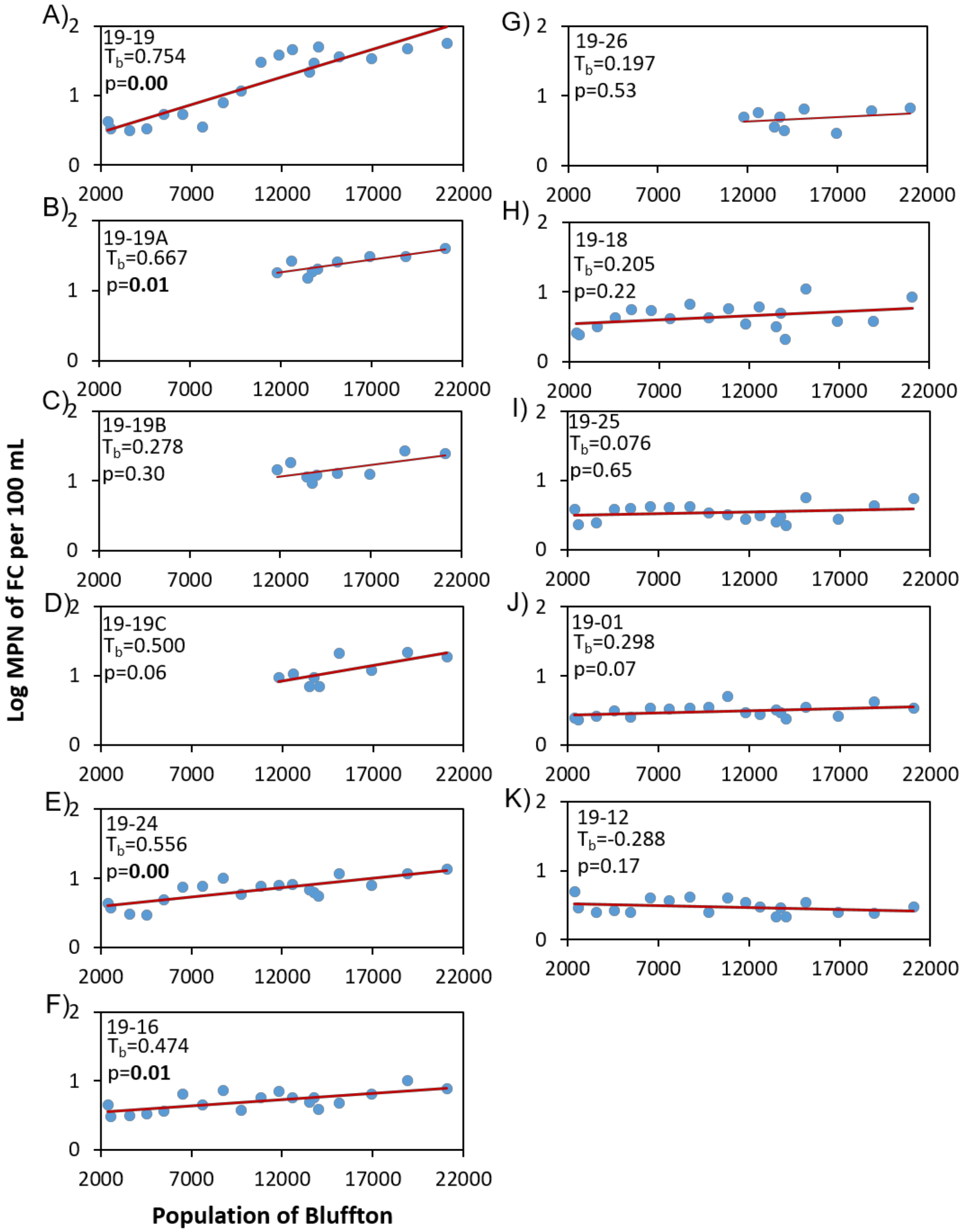

Fig. 26. Annual average fecal coliform (log FCMPN/100mL) versus annual population from 1999 to 2017 at SCDHEC stations (A) 19-19, (E) 19-24, (F) 19-16, (H) 19-18, (I) 19-25, (J) 19-01, and (K) 19-12 and from 2009 to 2017 at SCDHEC stations (B) 19-19A, (C) 19-19B, (D) 19-19C, and (G) 19-26. Values in bold (p<0.05) indicate a significant positive relationship.

phosphorus and nitrogen); chemical parameters include pH, salinity, and dissolved oxygen; and physical parameters include water turbidity, depth, temperature, and rainfall. NOAA also oversees the National Data Buoy Center, which collects various climatic parameters including water temperature, water depth, and salinity. Buoys along the South Carolina coastline have been recording measurements since as early as 1978. These datasets could be analyzed to determine long-term trends, which would provide insight into processes and patterns occurring in the May River.

3.3 Comparing Historical Data of the May River to Other Watersheds

SCDHEC monitors salinity and fecal coliform levels at shellfish stations located in other watersheds within Beaufort County, SC. SCDHEC Areas 17, 18, 19, and 20 are all located within Beaufort County. Through the work outlined in this technical report, we could perform a comparative approach to all shellfish stations extending from Port Royal Sound to Calibogue Sound. This analysis would identify estuaries that are resistant or more susceptible to deterioration in water quality and potentially identify underlying factors that may explain these differences. In addition to determining the impact of population growth on these long-term datasets, we could include land-use data (e.g. impervious surface, forested land, number of building permits) to generate a more powerful model.

3.4 USCB Environmental Data Monitoring Program – The Importance of Measuring Temperature, Salinity, pH, and Dissolved Oxygen

From 2013 to March 2015, we recorded water temperature and water depth continuously every hour in the spring, summer, and fall at three locations in the May River (i.e. 9M, 14M, and 37M); from March 2015 to present, we began recording these parameters year round (depth data not shown; Fig. 27). Between 2016 and 2018, we found that the lowest mean water temperature occurred in 2018 and the highest in 2017 for all stations (Fig. 28A). In addition, we recorded the lowest maximum and lowest minimum water temperatures in 2018 (Fig. 28B-C).

Since October 2015, we have monitored salinity, pH, dissolved oxygen (DO), and water temperature at six locations (i.e. 4M, 9M, 14M, 19M, 34M, and 37M) in the May River (Fig. 29). These environmental parameters exhibited strong seasonal and spatial patterns. We detected the lowest pH and DO in the summer and the highest in the spring and winter months at all stations (Fig. 29B-C). The headwaters (i.e. station 4M) exhibited the lowest mean levels in salinity, pH, DO, and temperature for all years (Table 15). In addition, we detected the highest variability in salinity, pH, DO and temperature ranges in the headwaters (i.e. station 4M) and the lowest variability closest to the mouth (i.e. stations 19M, 34M, and 37M).

Our long-term monitoring program of environmental data is important because these parameters affect the health of marine life such as mollusks (e.g. oysters, clams) and fish. Variability in salinity levels can be challenging for fish because the energetic cost of ion regulation increases with salinity variability, which is experienced in the headwaters of the May River and has increased since 1999 (Fig. 16; Boeuf 2001). Furthermore, captive studies with juvenile eastern oysters have shown that low salinity exposure (i.e. 15 compared to 30) for prolonged periods of time increases mortality (e.g. Dickinson et al. 2012).

Despite the natural variability of pH in estuaries compared to less variability in open ocean waters, monitoring the pH of estuaries is becoming increasingly important because of rising CO_2 levels and ocean acidification. Prolonged decreases in pH levels have the potential to affect marine calcifying organism (i.e. like oysters and clams that form hard shells) (Ringwood & Keppler 2002, Miller et al. 2009, Dickinson et al. 2012). In fact, recent wild and captive studies have shown that increased CO_2 levels (which lowers pH) decreased the survival and/or growth of juvenile clams *Mercenaria mercenaria* and eastern oysters (Ringwood & Keppler 2002, Dickinson et al. 2012). Results indicated that when average pH levels fell below 7.5, growth rates of clams were < 50% than the rates when deployed under higher pH conditions (Ringwood & Keppler 2002). Recent studies have shown that the combined exposure of elevated CO_2 levels and low salinity jeopardizes the survival of eastern oysters because of the weakening of their shells (Dickinson et al. 2012). Interestingly and potentially worrisome, we have shown that salinity levels in the headwaters of the May River have decreased since 1999 (which correlated with

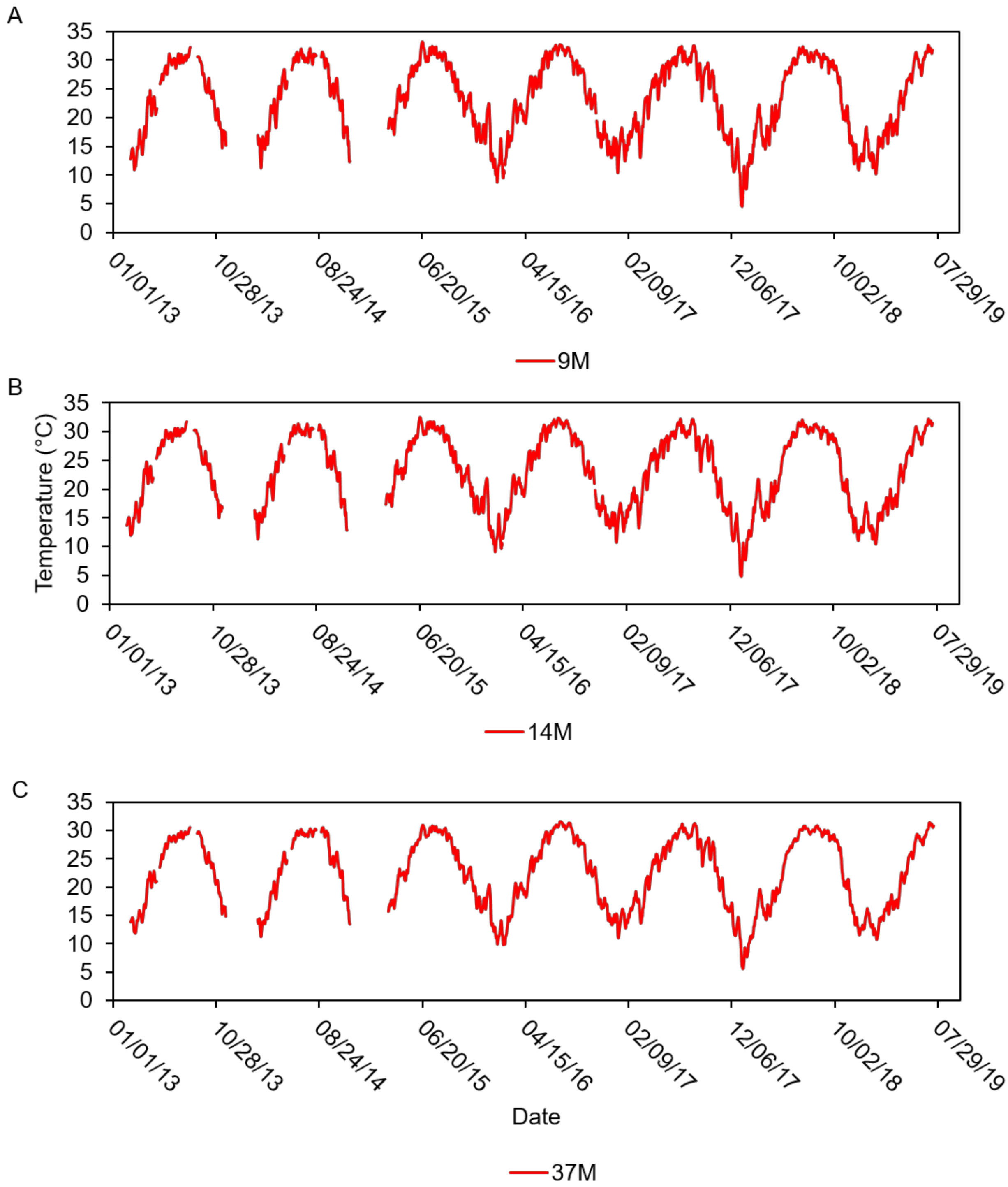

Fig. 27. Long-term, continuous water temperature recorded in the May River at stations A) 9M, B) 14M, and C) 37M from 2013 to 2019. These data are useful to help us understand how climate variability affects natural resources. Additionally, we use these data to understand the behavior of other water quality parameters, such as dissolved oxygen (DO) levels. In the summer, water temperature reaches its maximum and this can decrease the DO to hypoxic conditions in the May River (see Fig. 28). This could decrease the survival and growth of invertebrates and fish in the headwaters. Thus, over longer time scales, monitoring water temperature allows us to understand the consequences of global warming and the interactive effects of climate change on DO levels.

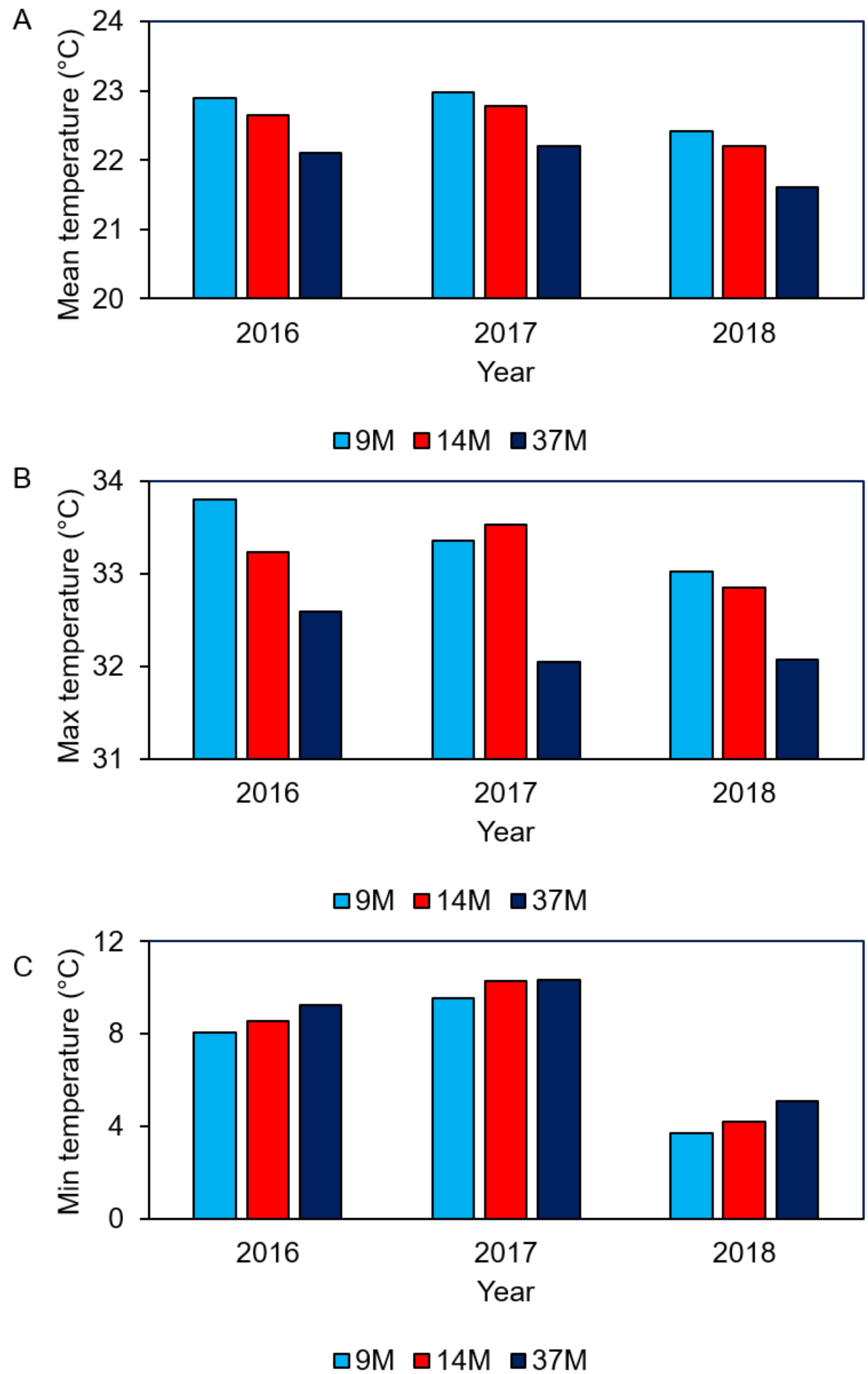

Fig. 28. Comparisons of A) mean, B) maximum, and C) minimum water temperature between 2016 and 2018 at stations 9M, 14M, and 37M in the May River. These endpoints can be monitored to understand the impact of climate change on our estuary.

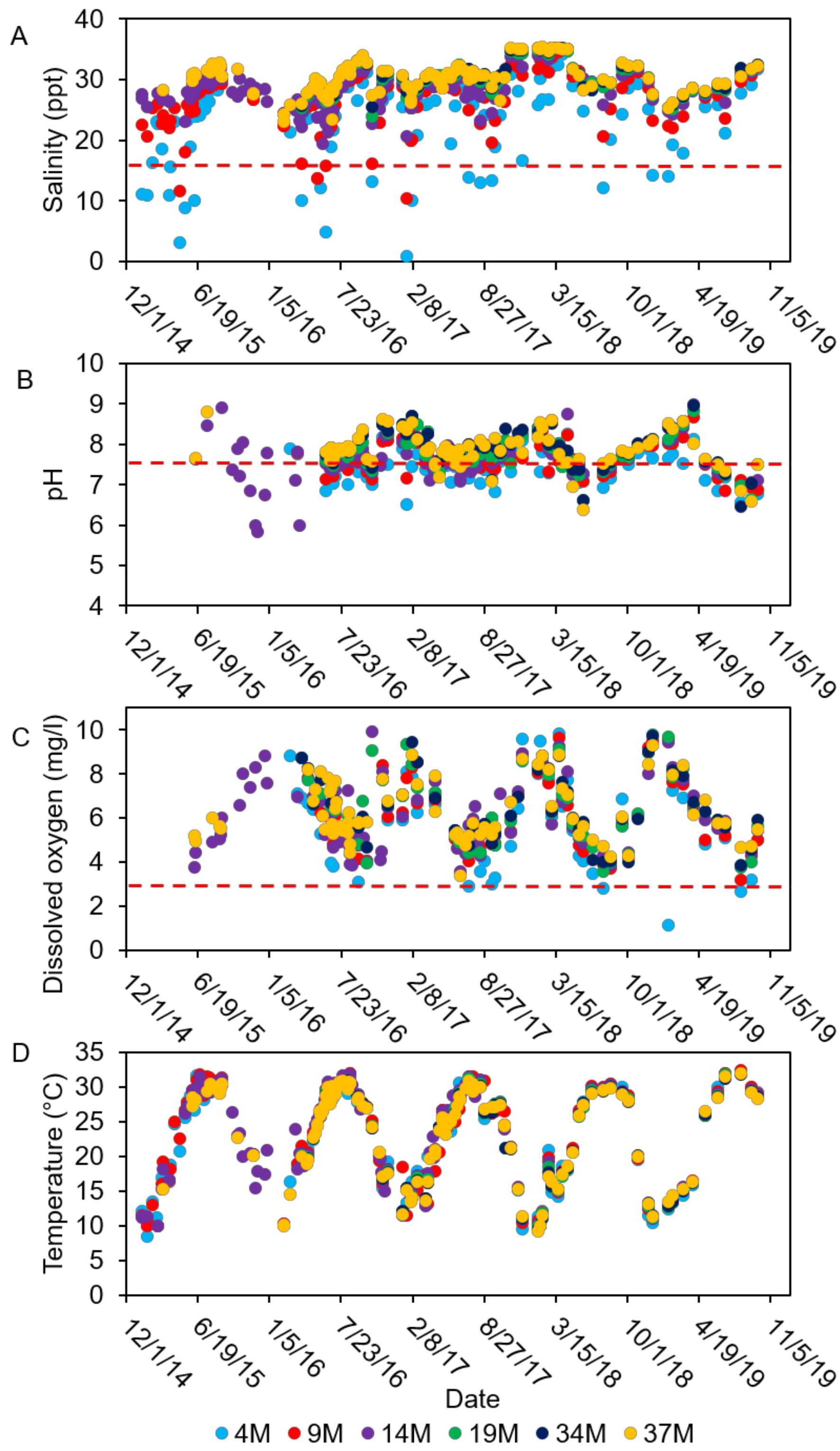

Fig. 29. Comparisons of A) salinity, B) pH, C) dissolved oxygen, and D) water temperature between 2015 and 2019 at stations 4M, 9M, 14M, 19M, 34M, and 37M in the May River. Red dash line indicates A) salinity of 15 ppt; B) pH of 7.5; and C) dissolved oxygen of 3 mg/L. It has been shown that prolonged exposure of juvenile eastern oysters to salinity levels of 15 ppt increases mortality rate by ~ 20% as compared to salinity of 30. A pH below 7.5, can decrease the growth of juvenile clams by 50%. Hypoxic waters can occur when DO falls below 3 mg/L.

increased population levels). Additionally, we routinely observe pH levels below 7.5 in the May River, albeit more consistently in the headwaters (Fig. 29B).

Over the past half century, human activity and coastal development has greatly accelerated nutrient flow to estuaries, increasing primary production and causing widespread eutrophication (e.g. Verity et al. 2006, Howarth et al. 2011). Simply speaking, eutrophication occurs when excessive nutrients in a body of water, usually from runoff, causes a bloom of phytoplankton; the organic material then sinks, gets decomposed, then this depletes DO levels in bottom water, which causes hypoxic conditions. Hypoxic waters occur when DO falls below 2-3 mg/L, and many organisms like shrimp, crabs, and fish avoid or become stressed in waters with DO at or below this level (Diaz and Rosenberg 1995). For non-motile organisms like oysters, DO levels that fall below their threshold can be lethal or impair recovery from other stresses like harvesting (Verity et al. 2006). In the past, researchers thought that estuaries in the South Atlantic Bight were relatively safe from hypoxia because high amplitude tides increase mixing (Verity et al. 1993). However, recent evidence suggests oxygen saturation has steadily declined from 1986 to 2006 in the headwaters of rivers and estuaries of Georgia (Verity et al. 2006). In the headwaters of the May River, recently, we have recorded DO levels below 3 mg/L in the summer, when oxygen levels reach their lowest (Fig. 29C).

Studies have revealed that climate related threats to saltmarshes include sea level rise, rising temperatures and CO_2 levels, and increasing storm frequency (Easterling et al. 2000, Brierley & Kingsford 2009). Rising temperatures could accelerate low DO levels in eutrophic waters, which could increase hypoxic conditions. Rising CO_2 levels may decrease the pH of estuaries, which could affect the growth and survival of oysters and other bivalves. In addition to climate driven stressors, as we have discussed, Bluffton is the fourth fastest growing town in South Carolina with a population growth of 40.7% between 2010 and 2016 (https://www.census.gov/programs-surveys/popest/data/data-sets.html). In this technical report, we found that salinity levels significantly decreased from 1999 to 2017 in the headwaters of the May River. This pattern correlated with increasing population (and presumably impervious surface). Thus, it is crucial to continue monitoring salinity, water temperature, DO, and pH from the headwaters to the mouth of the May River. Currently, our research lab is the only organization to monitor water temperature, pH, and DO along the May River on a routine basis.

3.5 USCB Passive Acoustic Monitoring Program – The May River Provides Critical Habitats for Fish Spawning Aggregations

By analyzing underwater sound data from 2013 to 2018 and quantifying fish courtship sounds, we have determined the spawning timelines for a community of fishes (Montie et al. 2015, Monczak et al. 2017, 2019) (Figs. 30-32). The calling season of black drum occurs between February and March, silver perch between February and June, oyster toadfish between February and November, spotted seatrout between May and September, and red drum between September and October (Figs. 30-32). Our results indicate that spawning behavior at the locations monitored (i.e. 9M, 14M, and 37M) continues from one year to the next (Figs. 30-32). We have detected only one red drum spawning aggregation, and this aggregation congregates each year at the mouth of the May River.

Due to the precise sampling of our acoustic recorders, we can determine the first and last day of the spawning season. Thus, these data may be especially helpful in comparing the timing and length of reproductive seasons from one year to the next, which may fluctuate according to the climatic patterns present during that year. It is possible that differences in these calling parameters (i.e. timing, length, and frequency) may influence year class strength of recreationally important fish species like spotted seatrout and red drum, and that acoustic monitoring may provide a powerful analysis tool (Monczak et al. 2017).

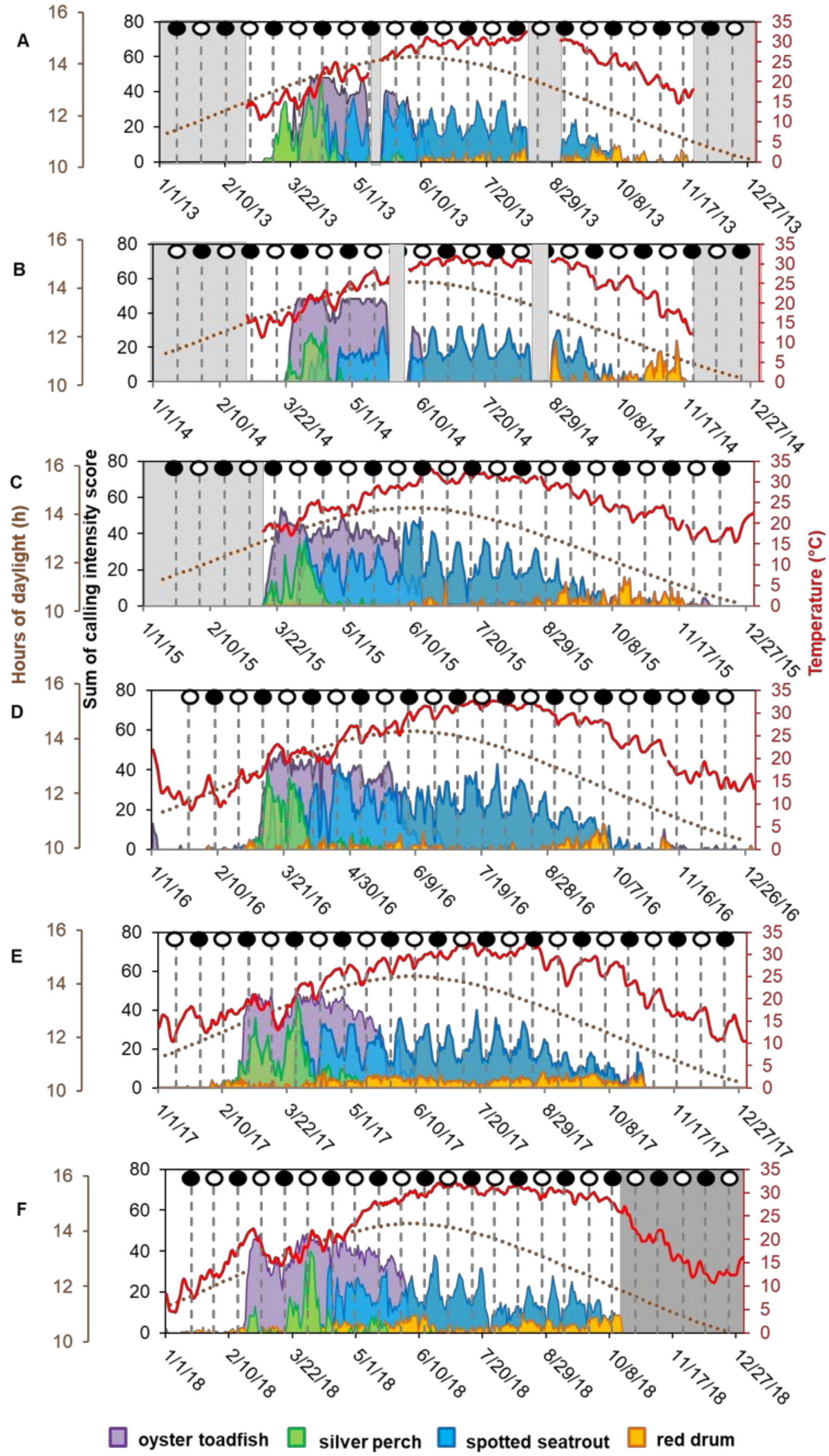

Fig. 30. Seasonal patterns of fish sound production in the May River at station 9M. Sum of calling intensity scores in A) 2013, B) 2014, C) 2015, D) 2016, E) 2017, and F) 2018. Also shown are water temperature (red line), hours of daylight (brown dotted line), new (dark circles), full (white circles) moon phase, and no data = gray box.

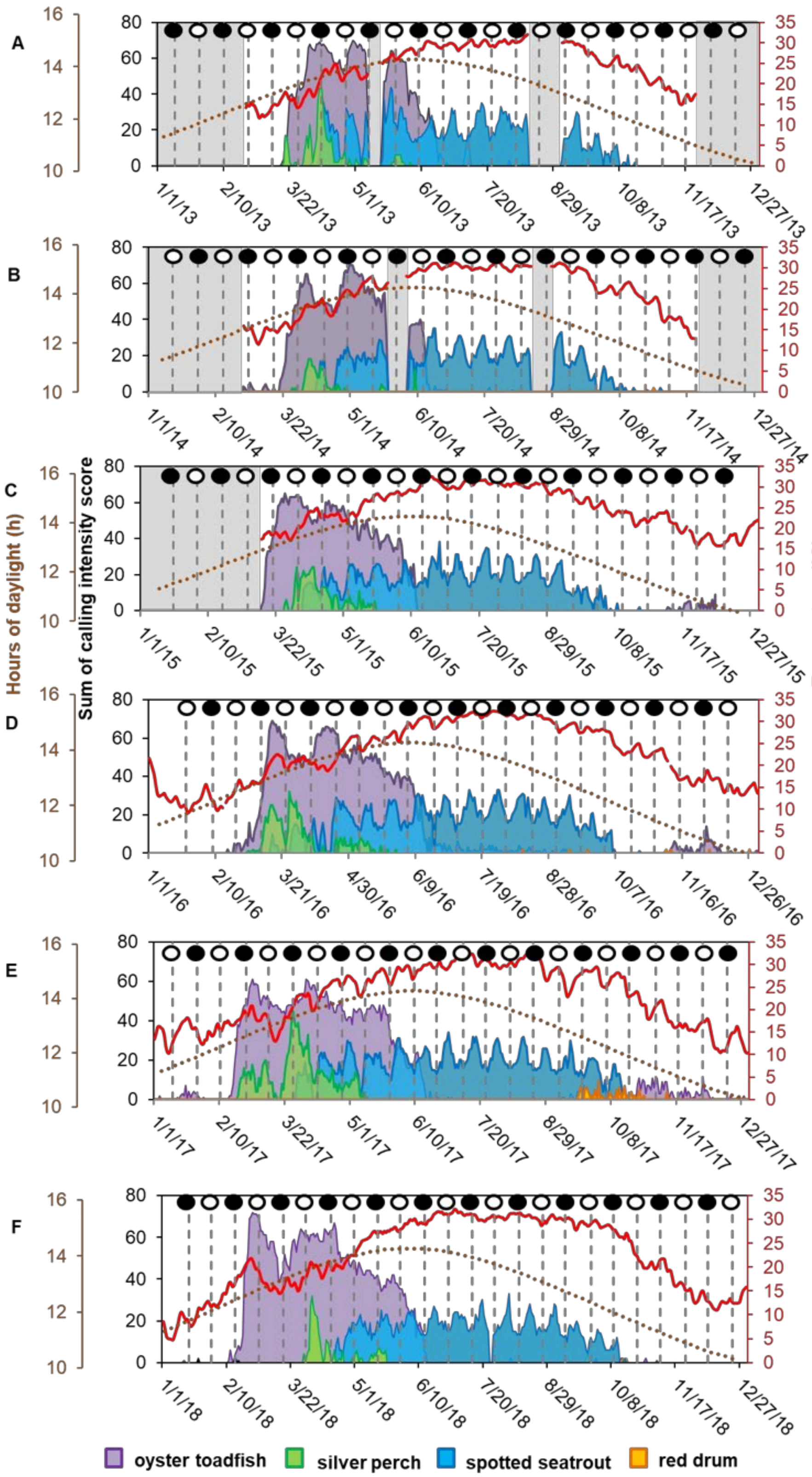

Fig. 31. Seasonal patterns of fish sound production in the May River at station 14M. Sum of calling intensity scores in A) 2013, B) 2014, C) 2015, D) 2016, E) 2017, and F) 2018. Also shown are water temperature (red line), hours of daylight (brown dotted line), new (dark circles), full (white circles) moon phase, and no data = gray box.

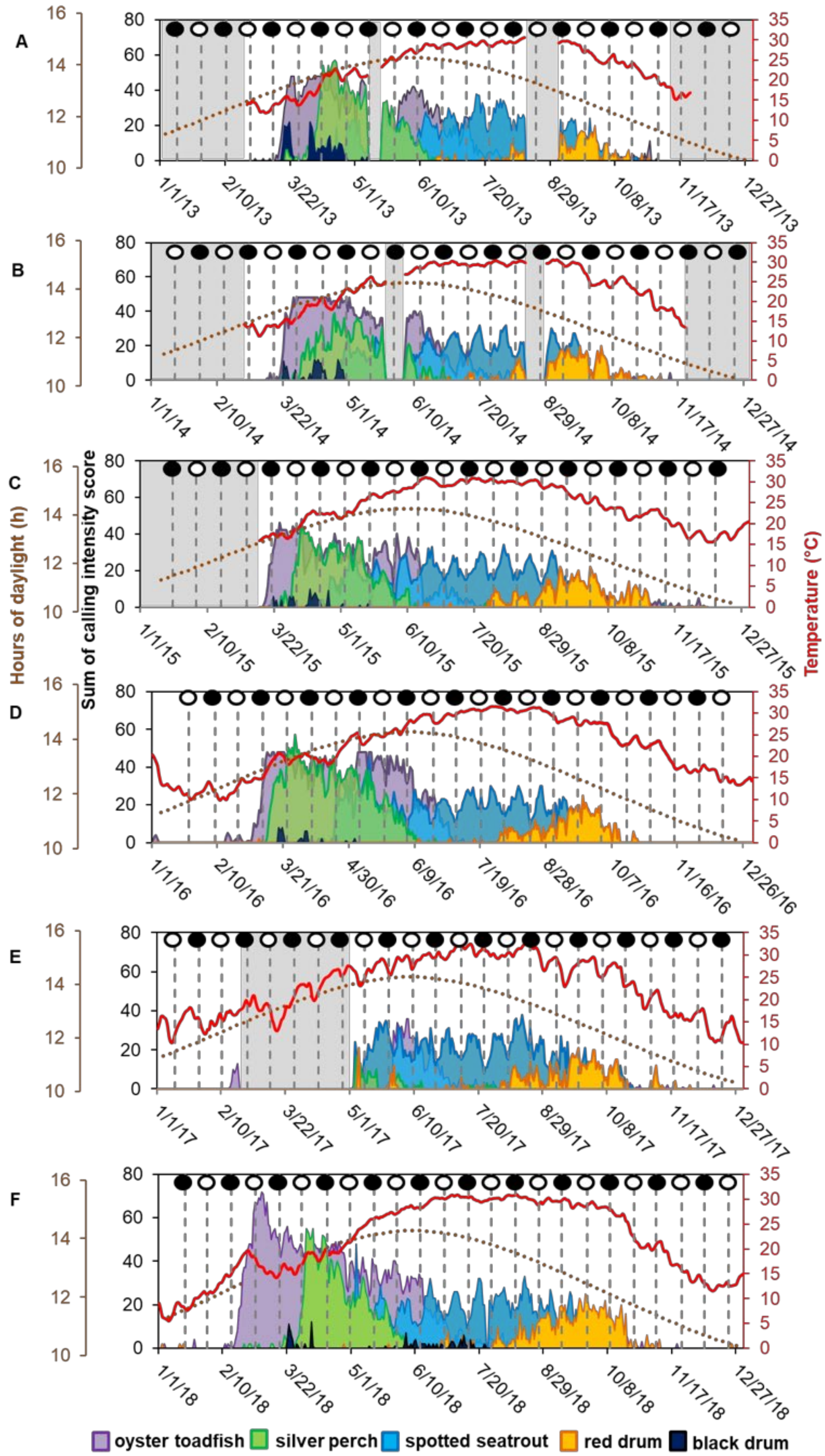

Fig. 32. Seasonal patterns of fish sound production in the May River at station 37M. Sum of calling intensity scores in A) 2013, B) 2014, C) 2015, D) 2016, E) 2017, and F) 2018. Also shown are water temperature (red line), hours of daylight (brown dotted line), new (dark circles), full (white circles) moon phase, and no data = gray box.

3.6 USCB Seining Program – The May River Provides Critical Nursery Habitats for Juvenile Fish

For this technical report, we report species richness and abundance in the May River from 2016 to 2018. In total, we caught five species of invertebrates and 54 species of fish while seining (Tables 16-17). We detected temporal patterns in species richness and total abundance (i.e. summed for all species) with a peak during springtime and minimum during wintertime (Fig. 33). This seasonal pattern followed the warming and cooling patterns of the estuary. We found a significant positive regression between water temperature and species richness and abundance. Spatially, overall all years, the highest species richness and species abundance occurred at station 14M.

Many of the fish species caught in intertidal pools and creeks represent young-of-the-year and are sensitive to low salinity and DO levels as well as pollutants associated with storm-water runoff. Through long-term monitoring efforts, we can track changes in species richness, abundances, and growths with shifts in environmental data (water temperature, salinity, pH, and DO). We can monitor how these endpoints respond to increased development of the May River watershed, changes in water quality, and other human stressors such as global warming, ocean acidification, micro-plastics, and noise pollution.

3.7 USCB Dolphin Monitoring Program – the May River Supports a Resident and Migrant Population of Dolphins

Since October of 2015 to October 2019, we performed 75 dolphin surveys in the May River; three of these surveys were conducted in 2015; 20 in 2016; 23 in 2017; 19 in 2018; and to date, 10 in 2019 (Table 18). From photo identification of dorsal fins, we have identified 185 individuals that inhabit the May River; most of these individuals we added to our catalog in 2016, which was the first full year of surveying (Table 19). The numbers of new individuals added to the catalog have decreased over time, indicating that we have identified a large portion of the dolphin population in the May River (Fig. 34).

Nonetheless, the number of unique dolphins in the May River catalog continues to increase slowly, which could be due to the influx of migratory animals. Of the 185 dolphins in the catalog, we have sighted approximately 61 of them only once. Thus, it is very possible that a large percentage (i.e. at least 32%) of the dolphins identified are migrants. For example, from 2016 to 2018, we conducted 62 dolphin surveys, and we identified 165 unique individuals. Site fidelity for these dolphins ranged from 1.61% to 46.77% (Table 20). Those with a site fidelity of 1.61 were sighted only once, while those with high site fidelity were seen in almost half of the surveys conducted and likely represent year round residents of the May River estuary (Fig. 35).

Dolphin abundance varied throughout the year ranging from 2 to 55 dolphins sighted per survey (Fig. 36A). The abundance of mo/ca pairs also varied, ranging from 0 to 9 per survey (Fig. 36B). We found that the average number of dolphins sighted per survey ranged from 16 to 23 and the average number of mother/calf (mo/ca) pairs sighted per survey ranged from 2 to 4 (Table. 18). We observed that the number of dolphins sighted varied depending upon the month (Fig. 37). We observed the greatest abundance of dolphins during late spring and summer – a time of high productivity in the May River estuary (Fig. 37). We observe more sightings of dolphins towards the mouth of the May River with less sightings in the headwaters (Fig. 38). It is possible that deterioration of water quality in the headwaters has decreased prey abundance, and therefore, dolphins spend less time in this area. More research is needed to determine if this pattern is due to water quality deterioration or natural distribution patterns in volume-sensitive waters.

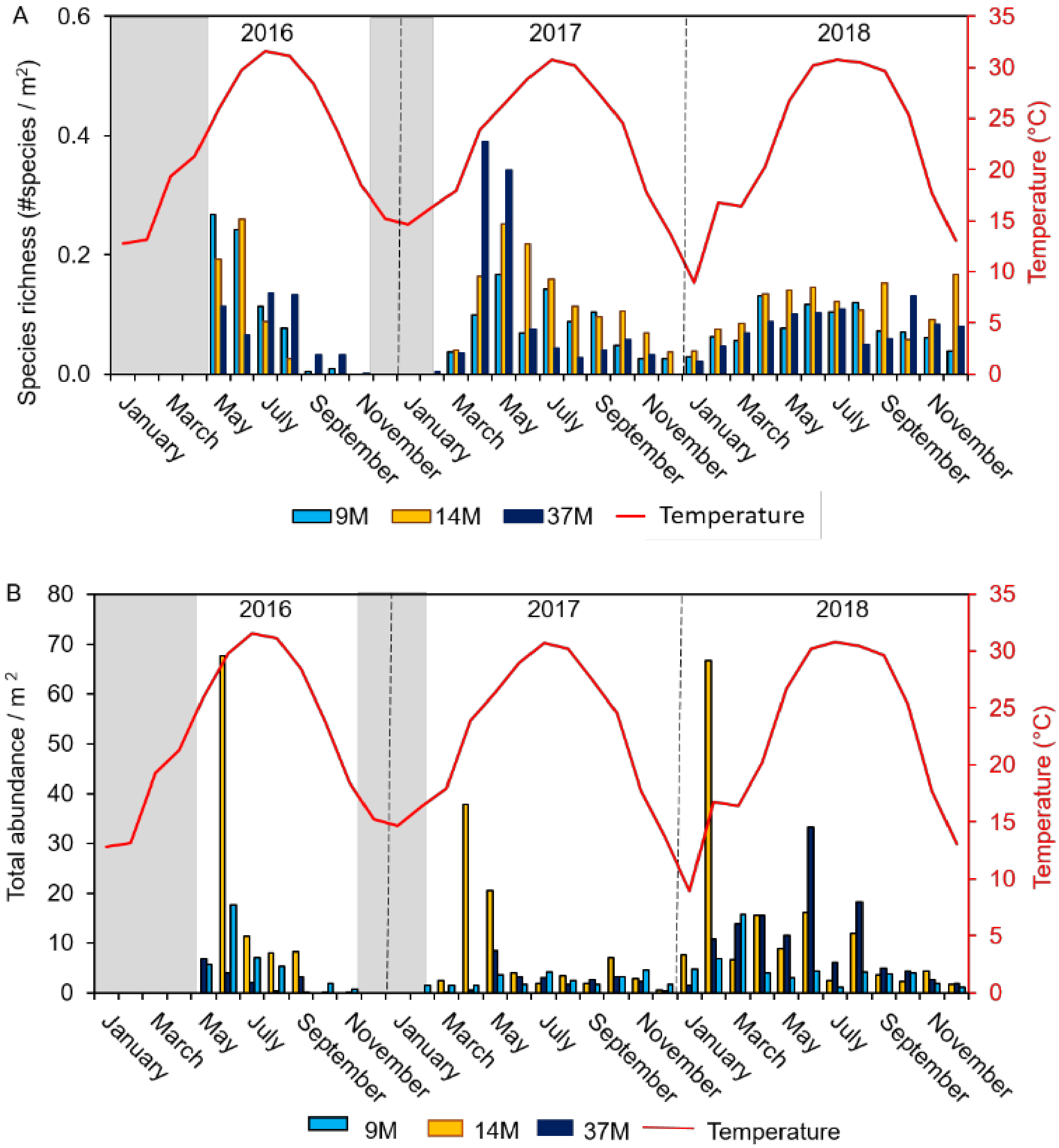

Fig. 33. A) Species richness and B) total abundance from haul seines between 2016 and 2018 at stations 9M, 14M, and 37M in the May River. Total abundance includes all fish counted. Also shown is monthly average temperature (red). Gray box = no data.

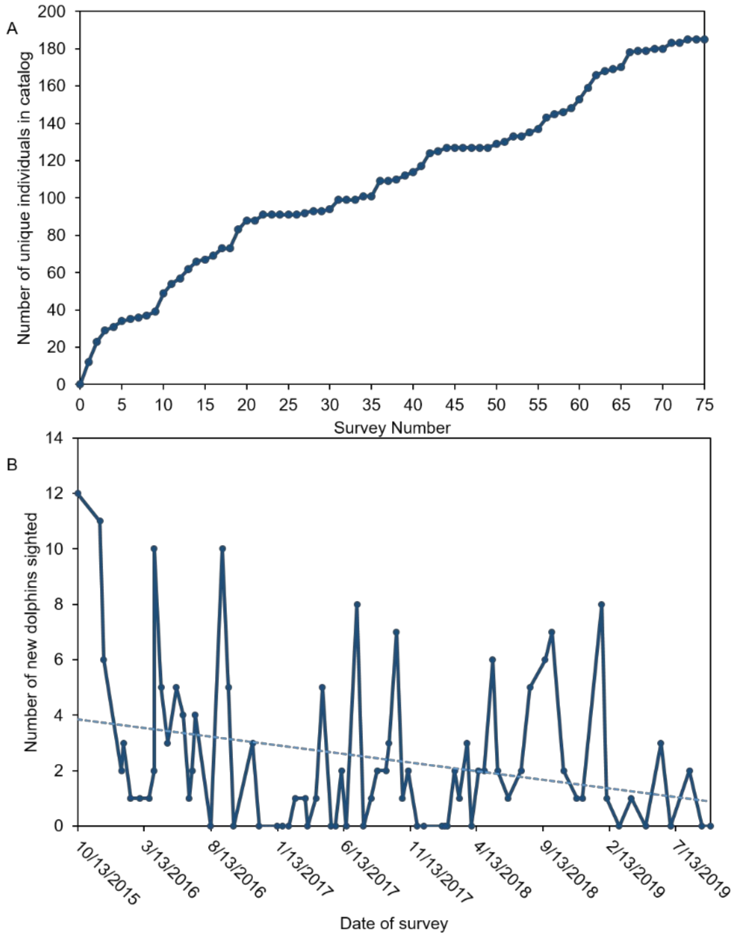

Fig. 34. Discovery of dolphins from October 2015 to October 2019. A) The number of unique dolphins in the May River catalog after each survey. B) The number of new dolphins sighted on each survey.

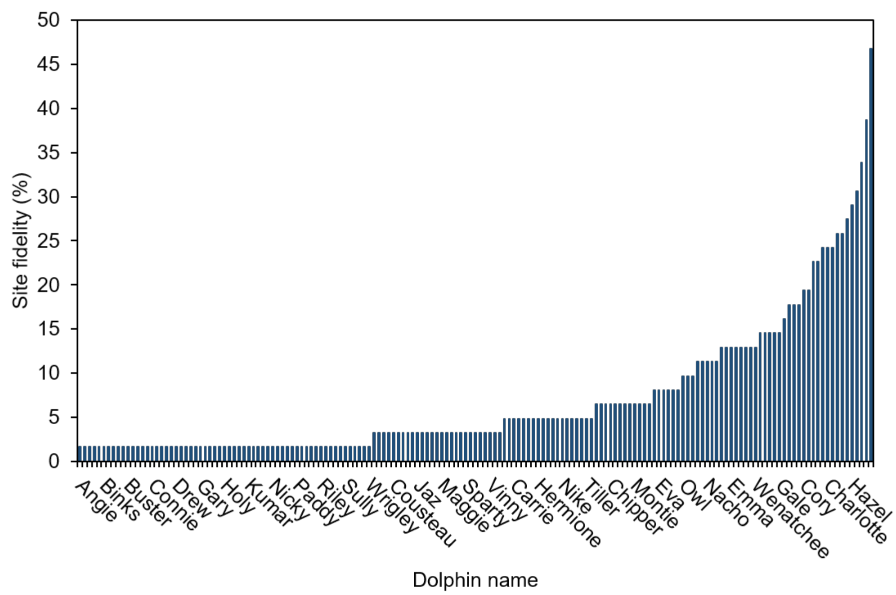

Fig. 35. Site fidelity calculated for all identified dolphins in the May River catalog from 2016 to 2018. High site fidelity indicates that dolphins are sighted more frequently in the May River.

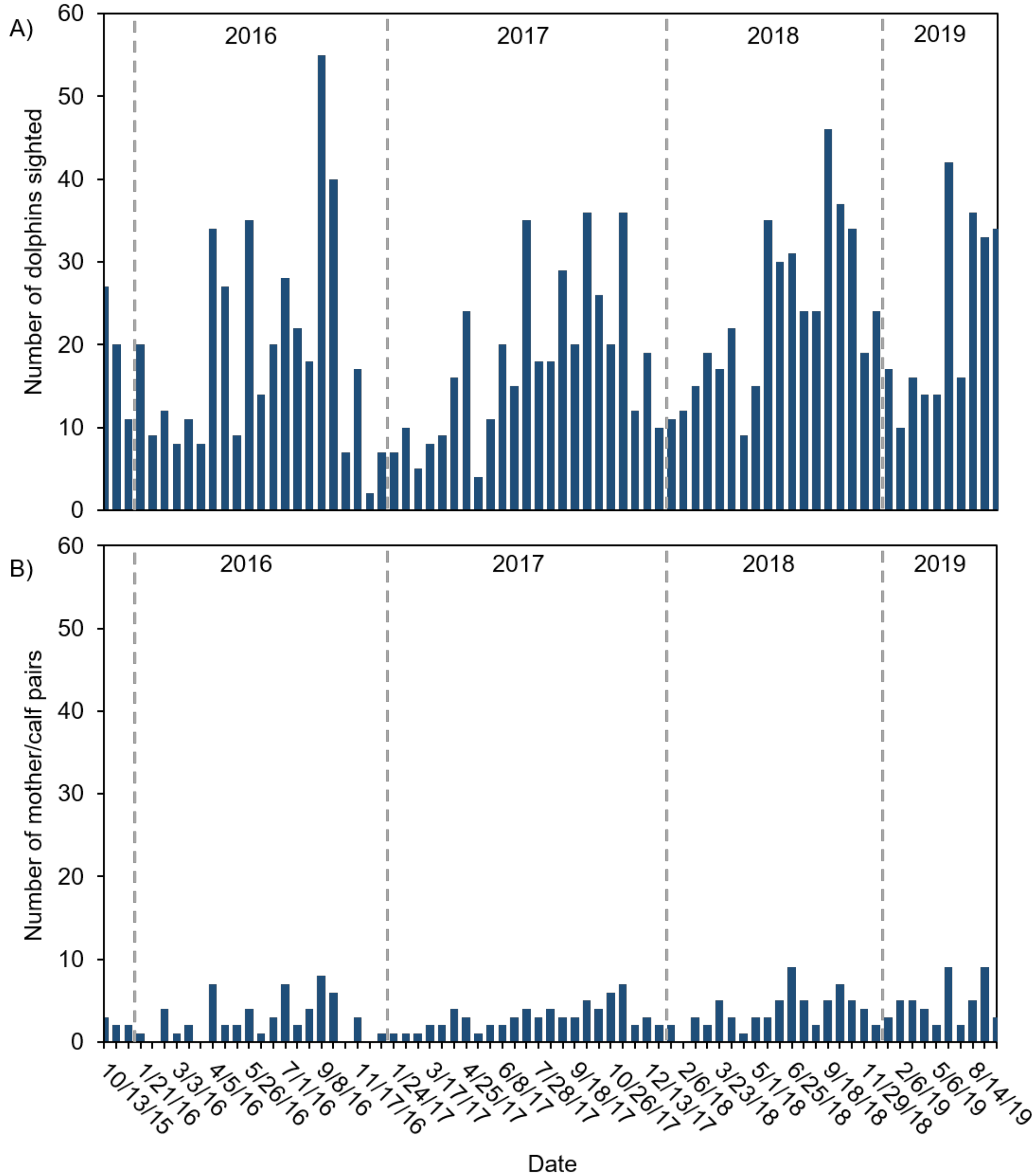

Fig. 36. Data collected from dolphin surveys conducted from October 2015 to October 2019. A) Total number of dolphins sighted each survey. B) Total number of mother/calf pairs sighted each survey. The dashed grey line indicates the end of each year.

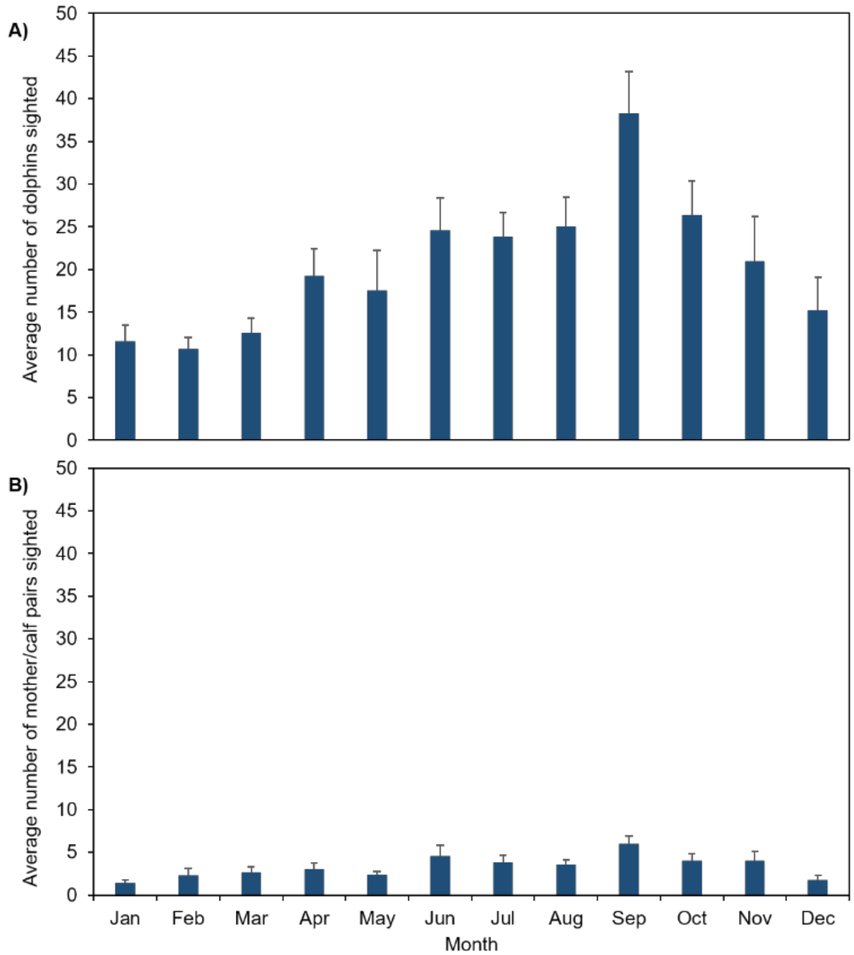

Fig. 37. A) Average number of dolphins sighted each month. B) Average number of mother/calf pairs sighted each month.

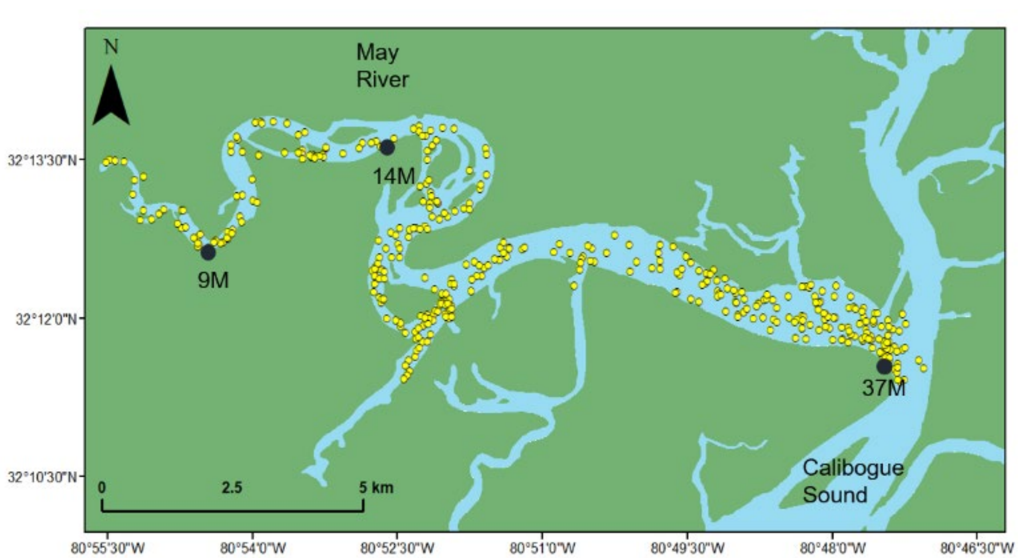

Fig. 38. Map of the May River showing locations of all dolphin sightings (yellow) from 2016 to 2018. There was 438 sightings with each sighting consisting of one or more dolphins. Locations of acoustic stations 9M, 14M, and 37M (blue).

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6. APPENDIX

6.1 Tables

Table 1. Results of the general linear model that tested the significance of specific factors on long-term salinity levels from 1999 to 2017 in the May River, SC. Values in **bold** are significant at p<0.05.

	df	F	Partial η^2	p
Year	18	35.483	0.296	**0.00**
Station	6	27.831	0.099	**0.00**
Season	3	29.050	0.054	**0.00**
Tidal Cycle	7	5.202	0.023	**0.00**
Oceanic Nino Index	1	27.246	0.018	**0.00**
Lunar Cycle	3	2.940	0.006	**0.03**
R Squared	0.41			

Table 2. Results of the general linear model that tested the significance of specific factors on long-term salinity levels from 2009 to 2017 in the May River, SC. Values in **bold** are significant at p<0.05.

	df	F	Partial η^2	p
Year	8	61.859	0.306	**0.00**
Station	10	33.748	0.231	**0.00**
Rainfall	1	205.997	0.155	**0.00**
Season	3	43.735	0.105	**0.00**
Oceanic Nino Index	1	98.430	0.081	**0.00**
Tidal Cycle	7	4.487	0.027	**0.00**
Lunar Cycle	3	1.113	0.003	0.34
R Squared	0.61			

Table 3. Kendall's tau-b correlation that tested the relationship between Oceanic Nino Index (ONI) and salinity from 2009 to 2017 at each station in the May River, SC. Values in **bold** are significant at p<0.05.

Station	T_b	p
19-19	-0.31	0.52
19-24	-0.08	0.11
19-16	-0.06	0.21
19-18	-0.08	0.09
19-25	-0.11	**0.02**
19-01	-0.10	**0.04**
19-12	-0.10	**0.04**

Table 4. Kendall's tau-b correlation that tested the relationship between year and salinity standard error from 1999 to 2017 at each station in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	0.532	**0.001**
19-24	0.180	0.916
19-16	-0.070	0.674
19-18	-0.24	0.15
19-25	-0.15	0.38
19-01	-0.20	0.41
19-12	-0.18	0.29

Table 5. Pearson's correlation that tested the relationship between population and salinity levels from 1999 to 2017 at stations 19-19, 19-24, 19-16, 19-18, 1-25, 19-01, and 19-12 and from 2009 to 2017 at stations 19-19A, 19-19B, 19-19C, and 19-26 in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	r	p
19-19	-0.662	**0.00**
19-19A	-0.528	0.14
19-19B	-0.499	0.17
19-19C	-0.367	0.33
19-24	-0.306	0.20
19-16	-0.237	0.33
19-26	-0.407	0.28
19-18	-0.121	0.62
19-25	-0.107	0.66
19-01	-0.117	0.63
19-12	-0.086	0.73

Table 6. Kendall's tau-b correlation that tested the relationship between rainfall and salinity from 2009 to 2017 at each station in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	-0.281	**0.00**
19-19A	-0.287	**0.00**
19-19B	-0.280	**0.00**
19-19C	-0.255	**0.00**
19-24	-0.260	**0.00**
19-16	-0.237	**0.00**
19-26	-0.231	**0.00**
19-18	-0.219	**0.00**
19-25	-0.230	**0.00**
19-01	-0.255	**0.00**
19-12	-0.241	**0.00**

Table 7. Kendall's tau-b correlation that tested the relationship between year and the two year salinity moving average from 1999 to 2017 at stations 19-19, 19-24, 19-16, 19-18, 1-25, 19-01, and 19-12 and from 2009 to 2017 at stations 19-19A, 19-19B, 19-19C, and 19-26 in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	-0.490	**0.00**
19-19A	-0.515	**0.00**
19-19B	-0.510	**0.00**
19-19C	-0.467	**0.00**
19-24	-0.233	**0.00**
19-16	-0.205	**0.00**
19-26	-0.468	**0.00**
19-18	-0.090	0.052
19-25	-0.087	0.062
19-01	-0.103	**0.026**
19-12	-0.076	0.118

Table 8. Results of the general linear model that tested the significance of specific factors on long-term fecal coliform levels from 1999 to 2017 in the May River, SC. Values in **bold** are significant at p<0.05.

	df	F	Partial η^2	p
Station	6	51.057	0.170	**0.00**
Year	18	9.438	0.102	**0.00**
Salinity	1	157.701	0.095	**0.00**
Tidal Cycle	7	8.005	0.036	**0.00**
Oceanic Nino Index	1	25.814	0.017	**0.00**
Season	3	3.000	0.006	**0.03**
Lunar Cycle	3	2.395	0.005	0.07
R Squared	0.40			

Table 9. Results of the general linear model that tested the significance of specific factors on long-term fecal coliform monitoring from 2009 to 2017 in the May River, SC. Salinity was used as a factor in this model but not rainfall. Values in **bold** are significant at p<0.05.

	df	F	Partial η^2	p
Station	10	65.825	0.375	**0.00**
Tidal Cycle	7	19.587	0.111	**0.00**
Year	8	7.985	0.055	**0.00**
Salinity	1	60.620	0.052	**0.00**
Lunar Cycle	3	4.762	0.013	**0.00**
Oceanic Nino Index	1	8.435	0.008	**0.00**
Season	3	1.383	0.004	0.25
R Squared	0.56			

Table 10. Results of the general linear models that tested the significance of specific factors on long-term fecal coliform monitoring from 2009 to 2017 in the May River, SC. Rainfall was used as a factor in this model but not salinity. Values in **bold** are significant at p<0.05.

	df	F	Partial η^2	p
Station	10	101.998	0.482	**0.00**
Tidal Cycle	7	21.622	0.121	**0.00**
Year	8	7.891	0.054	**0.00**
Lunar Cycle	3	4.688	0.013	**0.00**
Rainfall	1	9.159	0.008	**0.00**
Season	3	1.871	0.005	0.13
Oceanic Nino Index	1	0.889	0.001	0.35
R Squared	0.54			

Table 11. Fecal coliform (MPN/100 mL) means ± standard error in 1999, 2009, and 2017 at each station along the May River, SC. % increase was calculated using the formula:
((*2017 measurement – 1999 measurement)/1999 measurement*)*100%

	1999	2009	2017	% Increase
19-19	6.73 ± 2.38	52.69 ± 16.68	218.57 ± 130.59	3150.06
19-19A	-	27.25 ± 7.19	115.22 ±73.72	322.74
19-19B	-	21.41 ± 6.26	71.26 ± 43.70	232.84
19-19C	-	12.82 ± 2.78	39.88 ± 18.77	211.15
19-24	6.64 ± 1.95	11.80 ± 4.45	33.43 ± 17.45	403.26
19-16	5.89 ± 1.35	8.79 ± 1.82	15.92 ± 4.91	170.30
19-26	-	6.90 ± 1.91	10.09 ± 2.82	46.26
19-18	3.96 ± 1.75	4.49 ± 0.97	12.61 ± 3.57	218.53
19-25	4.57 ± 0.84	3.28 ± 0.65	8.86 ± 2.81	93.98
19-01	2.97 ± 0.77	3.71 ± 0.96	4.74 ± 1.25	59.83
19-12	6.48 ± 1.41	4.65 ± 1.17	4.87 ± 1.86	-24.94

Table 12. Kendall's tau-b correlation that tested the relationship between year and the 2-year fecal coliform moving average (log FCMPN/100 mL) from 1999 to 2017 for stations 19-19, 19-24, 19-16, 19-18, 19-25, 19-01, and 19-12, and from 2009 to 2017 at stations 19-19A, 19-19B, 19-19C, and 19-26 in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	0.673	**0.00**
19-19A	0.743	**0.00**
19-19B	0.427	**0.00**
19-19C	0.780	**0.00**
19-24	0.483	**0.00**
19-16	0.442	**0.00**
19-26	0.174	**0.01**
19-18	0.146	**0.00**
19-25	0.097	**0.03**
19-01	0.213	**0.00**
19-12	-0.137	**0.00**

Table 13. Kendall's tau-b correlation that tested the relationship between salinity and fecal coliform levels (log FCMPN/100 mL) from 1999 to 2017 for stations 19-19, 19-24, 19-16, 19-18, 19-25, 19-01, and 19-12, and from 2009 to 2017 at stations 19-19A, 19-19B, 19-19C, and 19-26 in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	-0.325	**0.00**
19-19A	-0.140	**0.05**
19-19B	-0.252	**0.00**
19-19C	-0.130	0.07
19-24	-0.254	**0.00**
19-16	-0.179	**0.00**
19-26	-0.053	0.46
19-18	-0.117	**0.02**
19-25	-0.118	**0.02**
19-01	-0.120	**0.02**
19-12	-0.125	**0.02**

Table 14. Kendall's tau-b correlation that tested the relationship between population and fecal coliform levels (log FCMPN/100 mL) from 1999 to 2017 at stations 19-19, 19-24, 19-16, 19-18, 1-25, 19-01, and 19-12 and from 2009 to 2017 at stations 19-19A, 19-19B, 19-19C, and 19-26 in the May River, SC. Values in **bold** are significant at $p<0.05$.

Station	T_b	p
19-19	0.754	**0.00**
19-19A	0.667	**0.01**
19-19B	0.278	0.30
19-19C	0.500	0.06
19-24	0.556	**0.00**
19-16	0.474	**0.01**
19-26	0.197	0.53
19-18	0.205	0.22
19-25	0.076	0.65
19-01	0.298	0.07
19-12	-0.228	0.17

Table 15. Mean salinity, pH, dissolved oxygen, and water temperature recorded during monthly monitoring at six stations in the May River.

Salinity

Year	4M	9M	14M	19M	34M	37M
2016	21.81	24.03	27.22	28.91	29.42	29.19
2017	21.77	26.47	28.91	30.25	30.66	30.47
2018	27.55	30.31	31.70	32.21	32.31	32.36

pH

Year	4M	9M	14M	19M	34M	37M
2016	7.34	7.53	7.59	7.81	7.91	7.93
2017	7.40	7.66	7.66	7.85	7.96	7.84
2018	7.59	7.79	7.90	7.77	7.85	7.80

Dissolved oxygen (mg/L)

Year	4M	9M	14M	19M	34M	37M
2016	5.58	5.94	5.94	6.84	6.64	6.37
2017	5.51	6.07	5.77	6.23	6.18	6.07
2018	6.03	6.42	6.33	6.53	6.63	6.71

Water temperature (°C)

Year	4M	9M	14M	19M	34M	37M
2016	24.16	24.24	25.02	25.21	25.12	25.42
2017	22.11	22.46	23.81	22.53	22.40	22.82
2018	20.84	20.35	20.17	19.98	19.99	19.93

Table 16. List of species caught and quantified during seining conducted one or two times per month in close proximity to passive acoustic stations in the May River, SC.

Family	Scientific name	Common name
Invertebrates		
Alpheidae	*Alpheus heterochaelis*	Big clawed snapping shrimp
Loliginidae	*Lolliguncula brevis*	Brief squid
Portunidae	*Callinectes sapidus*	Blue crab
Squillidae	*Squilla mantis*	Mantis shrimp
Palaemonidae	*Palaemonetes vulgaris*	Grass shrimp
Fish		
Atherinopsidae	*Menidia menidia*	Atlantic silverside
Belonidae	*Strongylura marina*	Atlantic needlefish
Blenniidae	*Chasmodes bosquianus*	Striped blenny
Carangidae	*Oligoplites saurus*	Leatherjack
Clupeidae	*Alosa pseudoharengus*	Blueback herring
Clupeidae	*Dorosoma petenense*	Threadfin shad
Cynoglossidae	*Symphurus plagiusa*	Blackcheek tonguefish
Diodontidae	*Chilomycterus schoepfii*	Striped burrfish
Elopidae	*Elops saurus*	Ladyfish
Engraulidae	*Anchoa mitchilli*	Bay anchovy
Ephippidae	*Chaetodipterus faber*	Atlantic spadefish
Fundulidae	*Fundulus heteroclitus*	Mummichog
Fundulidae	*Fundulus majalis*	Striped killifish
Gerreidae	*Eucinostimus harengulus*	Tidewater mojarra
Gerreidae	*Diapterus auratus*	Irish mojarra
Gobidae	*Ctenogobius boleosoma*	Darter goby
Gobidae	*Ctenogobius smaragdus*	Emerald goby
Gobidae	*Gobiosoma bosc*	Naked goby
Gobidae	*Microgobius thalassinus*	Green goby
Gobiidae	*Evorthodus lyricus*	Lyre Goby
Haemulidae	*Orthopristis chrysoptera*	Pigfish
Lutjanidae	*Lutjanus griseus*	Gray snapper
Lutjanidae	*Lutjanus synagris*	Lane snapper
Monacanthidae	*Stephanolepis hispidus*	Planehead filefish
Mugilidae	*Mugil cephalus*	Striped Mullet
Mugilidae	*Mugil curema*	White mullet
Paralichthyidae	*Ancilopsetta omata*	Ocellated flounder
Paralichthyidae	*Citharichthys spilopterus*	Bay whiff
Paralichthyidae	*Etropus crossotus*	Fringed flounder
Paralichthyidae	*Paralichthys albiguttata*	Gulf flounder
Paralichthyidae	*Paralichthys dentatus*	Summer flounder
Paralichthyidae	*Paralichthys lethostigma*	Southern flounder
Phycidae	*Urophycis regia*	Spotted hake
Sciaenidae	*Bairdiella chrysoura*	Silver perch
Sciaenidae	*Cynoscion nebulosus*	Spotted seatrout
Sciaenidae	Cynoscion regalis	Weakfish
Sciaenidae	*Sciaenops ocellatus*	Red drum
Sciaenidae	*Leiostomus xanthurus*	Spot
Sciaenidae	*Pogonias cromis*	Black drum
Sciaenidae	*Menticirrhus americanus*	Southern kingfish
Sciaenidae	*Micropogonias undulatus*	Atlantic croaker
Scombridae	*Scomberomorus maculatus*	Spanish mackerel
Serranidae	*Centropristis philadelphica*	Rock sea bass
Sparidae	*Archosargus probatocephalus*	Sheepshead
Sparidae	*Lagodon rhomboides*	Pinfish
Sphyraenidae	*Sphyraena barracuda*	Great barracuda
Stromateidae	*Peprilus Triacanthus*	American butterfish
Syngnathidae	*Syngnathus fuscus*	Northern pipefish
Syngnathidae	*Syngnathus louisianae*	Chain pipefish
Synodontidae	*Synodus foetens*	Inshore lizardfish
Tetraodontidae	*Sphoeroides maculatus*	Northern puffer
Trichiuridae	*Trichiurus lepturus*	Atlantic cutlassfish
Triglidae	*Prionotus tribulus*	Bighead searobin
Uranoscopidae	*Astroscopus y-graecum*	Southern stargazer

Table 17. Abundance of fish species caught in haul seines from 2016 to 2018 in the May River, SC.

Common Name	Total Catch			Average Catch	CPUE			Average CPUE
	2016	2017	2018	2016-2018	2016	2017	2018	2016-2018
Mummichog	15938	11687	15869	14498.00	146.22	89.90	125.94	120.69
Spot	32	2553	39587	14057.33	0.29	19.64	314.18	111.37
Bay anchovy	3108	12393	11222	8907.67	28.51	95.33	89.06	70.97
Atlantic silverside	2482	4464	12519	6488.33	22.77	34.34	99.36	52.16
Silver perch	4184	5887	8055	6042.00	38.39	45.28	63.93	49.20
Striped mullet	204	4	8046	2751.33	1.87	0.03	63.86	21.92
Pinfish	44	322	6740	2368.67	0.40	2.48	53.49	18.79
Tidewater mojarra	579	1895	1568	1347.33	5.31	14.58	12.44	10.78
White mullet	0	318	56	124.67	0.00	2.45	0.44	0.96
Spotted seatrout	82	123	66	90.33	0.75	0.95	0.52	0.74
Chain pipefish	11	114	99	74.67	0.10	0.88	0.79	0.59
Red drum	48	91	76	71.67	0.44	0.70	0.60	0.58
Southern flounder	1	1	145	49.00	0.01	0.01	1.15	0.39
Leatherjack	21	57	62	46.67	0.19	0.44	0.49	0.37
Pigfish	3	42	77	40.67	0.03	0.32	0.61	0.32
Blackcheek tonguefish	12	35	72	39.67	0.11	0.27	0.57	0.32
Blueback herring	10	91	2	34.33	0.09	0.70	0.02	0.27
Bay whiff	23	30	32	28.33	0.21	0.23	0.25	0.23
Naked goby	16	22	28	22.00	0.15	0.17	0.22	0.18
Striped burrfish	4	9	44	19.00	0.04	0.07	0.35	0.15
Bighead searobin	2	16	32	16.67	0.02	0.12	0.25	0.13
Inshore lizardfish	17	10	22	16.33	0.16	0.08	0.17	0.14
Atlantic spadefish	0	14	27	13.67	0.00	0.11	0.21	0.11
Southern kingfish	2	8	18	9.33	0.02	0.06	0.14	0.07
Striped killifish	2	6	16	8.00	0.02	0.05	0.13	0.06
Planehead filefish	1	14	8	7.67	0.01	0.11	0.06	0.06
Green goby	2	12	5	6.33	0.02	0.09	0.04	0.05
Ladyfish	0	14	4	6.00	0.00	0.11	0.03	0.05
Northern pipefish	17	0	0	5.67	0.16	0.00	0.00	0.05
Gray snapper	0	14	3	5.67	0.00	0.11	0.02	0.04
Darter goby	2	13	0	5.00	0.02	0.10	0.00	0.04
Lyre goby	0	15	0	5.00	0.00	0.12	0.00	0.04
Atlantic needlefish	2	1	10	4.33	0.02	0.01	0.08	0.04
Threadfin shad	0	13	0	4.33	0.00	0.10	0.00	0.03
Ocellated flounder	0	1	10	3.67	0.00	0.01	0.08	0.03
Emerald goby	0	7	1	2.67	0.00	0.05	0.01	0.02
Atlantic menhaden	0	0	8	2.67	0.00	0.00	0.06	0.02
Weakfish	0	5	2	2.33	0.00	0.04	0.02	0.02
Striped blenny	2	3	2	2.33	0.02	0.02	0.02	0.02
Northern puffer	4	1	2	2.33	0.04	0.01	0.02	0.02
Irish mojarra	0	0	7	2.33	0.00	0.00	0.06	0.02
Atlantic croaker	0	0	7	2.33	0.00	0.00	0.06	0.02
American butterfish	0	4	2	2.00	0.00	0.03	0.02	0.02
Lane snapper	0	1	3	1.33	0.00	0.01	0.02	0.01
Black drum	3	0	1	1.33	0.03	0.00	0.01	0.01
Sheepshead	0	4	0	1.33	0.00	0.03	0.00	0.01
Great barracuda	0	0	3	1.00	0.00	0.00	0.02	0.01
Rock sea bass	1	1	0	0.67	0.01	0.01	0.00	0.01
Southern stargazer	0	2	0	0.67	0.00	0.02	0.00	0.01
Fringed flounder	1	0	0	0.33	0.01	0.00	0.00	0.00
Feather Blenny	1	0	0	0.33	0.01	0.00	0.00	0.00
Gulf flounder	0	1	0	0.33	0.00	0.01	0.00	0.00
Summer flounder	1	0	0	0.33	0.01	0.00	0.00	0.00
Spotted hake	0	0	1	0.33	0.00	0.00	0.01	0.00
Spanish mackerel	1	0	0	0.33	0.01	0.00	0.00	0.00
Atlantic cutlassfish	0	1	0	0.33	0.00	0.01	0.00	0.00

Table 18. Data collected for dolphin surveys conducted from October 2015 to October 2019.

Year	# of surveys	Total dolphins sighted	Total mo/ca pairs sighted	Avg. dolphins sighted per survey	Avg. mo/ca pairs sighted per survey
2015	3	54	7	18.00	2.33
2016	20	396	58	19.80	2.90
2017	23	375	63	16.30	2.74
2018	19	429	70	22.58	3.68
2019	10	231	46	23.10	4.60

Table 19. Number of new dolphins added to the May River dolphin catalog each year.

Year	Number of surveys	Number of dolphins added to catalog
2015	3	29
2016	20	62
2017	23	36
2018	19	43
2019	10	15

Table 20. Site fidelity (SF) information for dolphins sighted from three full survey years.

Year	Number of surveys conducted	Number of individuals sighted	SF range	SF mean	SF standard error
2016	20	88	5-50	11.65	1.01
2017	23	97	4-61	9.13	0.93
2018	19	110	5-58	13.06	1.00
2016-2018	62	165	2-47	6.96	0.62