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Assessing Multi-Annual Phytoplankton Community Compositions and Biomass Across Tidal Creek and Open Water Estuarine Habitats in South Carolina

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by

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ABSTRACT

The urbanization of coastal environments in recent decades has caused an accelerating increase in nutrient-rich runoff from the landscape. The presence of these excess nutrients in the aquatic environment can result in degradation of water quality and harmful algal blooms. In South Carolina (SC), coastal development continues to threaten the resiliency of salt marsh estuaries, especially in combination with other stressors like climate change and sea level rise. Estuaries are important nurseries and habitats for fisheries and ecotourism. The South Carolina Estuarine and Coastal Assessment Program (SCECAP) is an ongoing monitoring program that assesses the habitat condition along the coast of SC across 30 sites (15 tidal creek and 15 open water estuarine environments) each year. Water samples were collected during the SCECAP to assess phytoplankton biomass and composition. High Performance Liquid Chromatography (HPLC) was used to assess phytoplankton communities between tidal creek and open water estuarine habitat types. Phytoplankton composition was determined using both ChemTax and PhytoClass to compare the two analytical approaches. Phytoplankton biomass was compared to total river discharge in the weeks prior to sampling across several years of the study to determine the influence of coastal run-off. Tidal creeks had significantly higher predicted phytoplankton biomass for the sampling period 1999-2022 ($p < 0.001$) and significantly higher biomass for the sampling year 2023 ($p < 0.001$) compared to open water habitats. PhytoClass resulted in similar concentrations of all algal groups

except haptophytes when compared to ChemTax. Phytoplankton biomass was positively correlated with discharge for Edisto and Santee River Basins for both habitat types ($p < 0.05$). Evaluating management-based methodologies in terms of separating estuaries by habitat type, comparing algal class abundances between different pigment-based taxonomy methodologies, and quantifying the influences of coastal run-off on phytoplankton biomass are important topics in understanding the human impacts on coastal ecosystems and how to implement better management strategies.

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LIST OF ABBREVIATIONS

ALS ..... Alternating Least Squares

APS ..... Annual Phytoplankton Succession

CCAP ..... Coastal Change Analysis Program

DLG ..... Hydrographic Digital Line Graphs

GIS ..... Geographic Information Systems

GRTS ..... Generalized Random Tessellation Stratified

HPLC ..... High Performance Liquid Chromatography

HUC ..... Hydrologic Unit Code

NCCA ..... National Coastal Condition Assessment

NCCR ..... National Coastal Condition Reports

NOS ..... National Ocean Service

NWI ..... National Wetland Inventory

SCDHEC ..... South Carolina Department of Health and Environmental Control

SCDNR ..... South Carolina Department of Natural Resources

SCECAP ..... South Carolina Estuarine Coastal Assessment Program

SDA ..... Steepest Descent Algorithm

USEPA ..... U.S. Environmental Protection Agency

USGS ..... United States Geological Survey

WQP ..... Water Quality Portal

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Although there is only 277, 209 mi^2 of coastal shoreline out of 3,809,525 mi^2 of the total land area in the contiguous United States, approximately 40% of the United States population resides in coastal communities (U.S. Census Bureau, 2022). As a result, the population density in coastal counties is five times larger than the national county average (NOAA, 2024). These coastal zones include estuarine habitats, which comprise a much smaller portion of the coastal zone (~22, 889 mi^2 of intertidal and subtidal estuarine habitats) in the U.S. (Dahl, 2005). Despite the relatively small area of estuaries comprise in the U.S., they are extremely productive ecosystems in the South Atlantic Blight (SAB) region. Estuaries in the SAB have a mean primary production as high as 700 gC/m^2/year and the source of this production comes from riverine input and remineralization in estuarine systems (Verity et al., 1998). Phytoplankton are responsible for the high primary productivity in estuaries because they facilitate the energy transfer from terrestrial nutrient inputs and support the base of the food web (Correll, 1978). Phytoplankton are the most abundant primary producers in the water column of these ecosystems (Correll, 1978). The energy produced by phytoplankton supports the highly diverse and large biomass that local industries rely on for shellfish farming, fisheries, and tourism.

The urbanization and increase in tourism of estuarine ecosystems has decreased the overall habitat quality of estuaries (Sanger et al., 2020). The consequences of declining habitat quality in estuarine systems are an increase in shellfish harvesting closures, harmful algal blooms, pathogens, and hypoxia (Elko et al., 2022; Porter et al., 1996; Yuan et al., 2023). Phytoplankton blooms can be defined as a sustained period of growth where rates of growth exceed those of loss (Daniels et al., 2015). Although phytoplankton blooms provide essential primary productivity in the environment, they can be become harmful when the bloom significantly alters the habitat or produces toxins (Anderson, 2009).

Harmful algal blooms are a historically natural phenomenon. From 1985-2018, it was found that harmful algal blooms were increasing or decreasing in certain regions whereas other regions experienced no change in harmful bloom activity (Hallegraeff et al., 2021). The U.S. coastline has been experiencing increases in algal related biotoxins such as diarrhetic shellfish toxins and amnesic shellfish toxins (Hallegraeff et al., 2021). Harmful algal blooms can be a result of eutrophication, which is the presence of excess nutrients in the aquatic environment typically due to nutrient rich run-off (Freeman et al., 2019; W. Vernberg et al., 1996). The water quality of estuarine and coastal habitats has been decreasing in the past several decades as a result of anthropogenic growth and development (Beck et al., 2018). The water quality for estuarine systems within the SAB can be defined by healthy values of dissolved oxygen, pH, nutrients, chl *a*, total suspended solids, and fecal coliform established by state and national management programs.

In order to assess the quality of the nation's coastal ecosystems, the U.S. Environmental Protection Agency's (USEPA) founded the National Coastal Condition Assessment (NCCA). The NCCA aims to score the nation's coast as good, fair, and poor condition based on sediment quality, water quality, and biological condition scores (USEPA, 2021). This type of survey is conducted every five years with published reports beginning in 2010. In the years prior to these nationwide coastal assessments, the EPA released National Coastal Condition Reports (NCCR) that compiled data from multiple different federal, state, tribal, and local programs that assess water quality and coastal ecological conditions. One of the exemplary state programs included in the NCCR reports was the South Carolina Estuarine Coastal Assessment Program (SCECAP) (USEPA, 2001). SCECAP is a collaborative project between two state agencies, the South Carolina Department of Natural Resources (SCDNR) and the South Carolina Department of Health and Environmental Control (SCDHEC) (Sanger et al., 2020). This project is also in collaboration with the following national agencies: The National Oceanic and Atmospheric Administration's National Ocean Service (NOAA/NOS) and U.S. Environmental Protection Agency's (USEPA) National Coastal Condition Assessment (NCCA) (Sanger et al., 2020). SCECAP is an ongoing monitoring program that started in 1999 where they aim to assess the habitat condition along the coast of South Carolina across 30 sites (15 tidal creek and 15 open water estuarine environments) each year (Sanger et al., 2022). From the years 1999-2006, they monitored 50-60 estuarine sites with half of the sites located in tidal creeks and the other half in open water bodies, but due to a decrease in funding in the following years, the number of sites decreased to 30 starting in 2007 (Bergquist et al., 2009; Sanger et al., 2022). The habitat

condition is comprised of three parameters: water quality index, sediment quality index, and biological condition index (Sanger et al., 2020). The SCDNR assessments compile and analyze data from two consecutive sampling summers in the months of July and August. The NCCA is perhaps the most similar federal program to SCECAP in terms of using a holistic approach to assess habitat condition, but their habitat metrics and incorporation of these metrics differ.

1.2 PURPOSE STATEMENT

The goal of this study is to assess potential weaknesses in estuarine survey management practices in terms of water quality using phytoplankton. Second, to assess new methodologies in quantifying algal class abundances present in the water column. Third, to investigate different methodologies in determining the influence of nutrient rich run-off into estuarine systems using phytoplankton. Each of these goals will be addressed and reviewed independently in the subsequent chapters.

CHAPTER 2

HABITAT TYPE

2.1 PHYTOPLANKTON BIOMASS HYPOTHESIS

The SCECAP reports distinguish two separate regions in an estuary: tidal creeks and open water habitats. The NCCA reports do not recognize distinctions in habitat type within their estuarine systems. Tidal creeks can be defined as the narrow inlets in an estuary (<100 m wide) that are affected by the ebb and flow tides and serve as the first point of entry for run-off (Van Dolah, 2002; Sanger et al., 2022). Open water habitats can be defined as the larger bodies of water in an estuary (>100 m wide) that form tidal rivers, bays, and sounds (Van Dolah, 2002). Open water habitats comprise the majority of the South Carolina coastline. There is not a widely adopted definition of tidal creeks and the SCECAP program defines the habitats by width while other studies define the habitats by depth at low tide (Lerberg et al., 2000).

SCECAP reports from the years 1999-2008 compare the water quality, sediment quality, and biological condition measurements between tidal creek and open water habitat types. Sediment quality and biological measures were also compared with varying results for significant differences between habitat types. The water quality measurements include temperature, salinity, dissolved oxygen, pH, total nitrogen (TN; sum of nitrate/nitrite and total Kjeldahl nitrogen (TKN)), total phosphorus, chl *a*, total suspended solids, BOD, fecal coliform, total organic carbon, alkalinity, and turbidity (Van Dolah et al., 2002, 2004; Van Dolah, 2006; Bergquist et al., 2009, 2011). Chl *a* is useful tool in

examining how phytoplankton biomass varies spatially across coastal ecosystems and with time (Carstensen et al., 2015).

Keppler et al. (2015) investigated SCECAP sites and their own sites within the region of the ACE Basin and found that tidal creeks had significantly higher chl *a*, TN, and TP than open water habitats. The differences in chl *a* concentration between tidal creek and open water habitats from 1999-2008 varies across the entire state of South Carolina with tidal creeks trending slightly higher than open water habitats. During first two years the study (1999-2000), it was found that there was a significant difference in mean chl *a* concentrations between tidal creek and open water habitats with an average of 12.8 µg l^{-1} and 9.7 µg l^{-1}, respectively (Van Dolah, 2002). In the years 2001-2002, mean chl *a* concentrations were not significantly different between the two habitat types (Van Dolah et al., 2004). Tidal creeks had an average chl *a* concentration of 10.2 µg l^{-1} and open water habitats had a mean concentration of 10.0 µg l^{-1} (Van Dolah et al., 2004). From 2003 to 2004, mean chl *a* was significantly higher in tidal creeks than in open water habitats with an average of 11.8 µg l^{-1} and 7.6 µg l^{-1}, respectively (Van Dolah, 2006). The mean chl *a* in tidal creeks was significantly higher than open water habitats from the years 1999 to 2008 (Bergquist et al., 2009, 2011). Subsequent reports from 2009 to 2020 exclude the comparisons between different habitat types and focus solely on the habitat quality scores for each site in their respective habitat types (Van Dolah et al., 2013; Sanger et al., 2016, 2018, 2020, 2022).

The basis for separating these two habitat types and the number of sites sampled within each habitat is supported in the early reports from the years 1999-2006. However, there is a lack of evidence to support why these two habitats should stay distinguished

within the estuary in more recent reports. With 24 years of sampling, the differences between these two habitat types for mean chl *a* should be reexamined to determine the relative importance of separating the two habitat types in terms of phytoplankton biomass. An equal sampling number for each habitat type poses a large sampling bias toward tidal creeks because they comprise a much smaller amount of South Carolina's coastline. Tidal creeks comprise 17% of the coastal zone and open water habitats comprise 83% of the coastal zone (Van Dolah., 2002).

The data should be examined to determine if there is a consistent difference between tidal creek and open water habitats for mean chl *a*. If there are no consistent differences between the two habitat types, then the frequency at which tidal creeks are sampled should not be necessary. It is expected that the mean chl *a* in tidal creeks for the year 2023 will be higher than in open water sites. The mean chl *a* will also be higher in tidal creeks than in open water sites from the years 1999-2023.

2.2 PHYTOPLANKTON DIVERSITY HYPOTHESIS

From a biological standpoint, it is also important to consider the pigment ratios within taxonomic groups of the phytoplankton that comprise estuarine habitats. Mean chl *a* only provides semi-quantitative measures of phytoplankton biomass, but composition of the phytoplankton community is important in terms of defining differences in habitat or quality of habitat. A diverse and balanced phytoplankton community composition could comprise a high mean chl *a*. Whereas an unbalanced community, with little phytoplankton diversity, could present as a typical mean chl *a* concentration. Phytoplankton community composition is an important indicator of ecosystem function and change (Pearl et al., 2003). The importance of considering phytoplankton community

composition is demonstrated in published SCECAP reports from 1999-2006 (Bergquist et al., 2009, 2011). A decrease in funding and changes in administration prioritized the collection and publishing of only mean chl *a* starting in 2007.

The most direct method for acquiring phytoplankton community composition is by microscopy, but it is extremely time consuming in larger surveys of phytoplankton (Mackey et al., 1996). It can also be difficult to identify picoplankton during microscopy, because of the lack of defining taxonomic morphological characteristics (Mackey et al., 1999). A common method for obtaining phytoplankton community compositions across large areas and multiple data sets is High Performance Liquid Chromatography (HPLC) (Wetz et al., 2006). The resulting concentrations of the pigments identified in the sample can be used to identify the abundance of algal groups that are present (Mackey et al., 1996). Scientific programs are used to calculate algal class abundances from pigment concentration measurements so community compositions and phytoplankton diversity can be analyzed across a variety of habitats (Mackey et al., 1996).

Phytoplankton diversity can be driven by the dynamic conditions present in tidal creek environments (Branke, 2012). The varying salinity, nutrient, temperature, and tidal conditions could allow for several phytoplankton groups to reside, whereas stable conditions allow for certain groups to dominate phytoplankton composition based on the competitive exclusion principle (Dodds & Whiles, 2010). However, even in stable conditions, phytoplankton maintain a high diversity in stable resource conditions which violates the competitive exclusion principle, a paradigm is known as the “Paradox of the Plankton” (Hutchinson, 1961). Even if the competitive exclusion principle is violated by

the “Paradox of the Plankton”, tidal creeks are expected to have a higher diversity because of the influence of marine tychopelagic phytoplankton.

Tycho-pelagophytes spend a part of their life cycle attached to the benthos or vegetation in the estuary and become planktonic from physical disturbances (Kennish, 2016). Tidal creeks typically have more vegetation and a greater surface area to volume which leads to greater influences of tychopelagic phytoplankton in the water column. It is hypothesized phytoplankton photopigment diversity will be significantly higher in tidal creeks when compared to open water habitats.

2.3 METHODS: SAMPLING SITES

Tidal creek and open water habitats were defined for South Carolina’s estuarine region by the SCDNR using the following data analyzed in Geographic Information Systems (GIS): Hydrographic Digital Line Graphs (DLG), USGS Digital 7.5’ Topographic Quadrangle Maps, Coastal Change Analysis Program (CCAP) 1995 database, and the National Wetland Inventory (NWI) 1994 database (Van Dolah et al., 2002). The sampling sites were within the coastal zone of South Carolina with little river at the North Carolina-South Carolina border as the northern limit and the Savannah River at the South Carolina-Georgia border as the southern limit (Bergquist et al., 2009). The coastal zone also extended out to the mouth of the drainage basin of each estuary from the saltwater-freshwater interface (Bergquist et al., 2009). Tidal creeks were defined as channels that were less than 100 m wide from marsh bank to marsh bank and open water habitats were defined as channels that were greater than 100 m wide from marsh bank to marsh bank (Van Dolah et al., 2004). A Generalized Random Tessellation Stratified (GRTS) design was used to obtain random sampling locations for each year in each of the

two habitat types. The design also included a minimum of one meter depth for accessibility to the site by water transportation. The total number of sampling locations each year were equally divided between each habitat type. Sampling took place during the months of July and August for the years 2007-2023 and included 30 different sampling sites each year. The 1999-2006 sampling years included 50-60 sampling sites and sampling began in mid-June and ended in August. For the water samples collected in 2023 for HPLC, the same sampling sites provided by the SCDNR were used. A maximum of four sites were sampled in a single day for two subsequent days in a week (Appendix A).

2.4 METHODS: SAMPLE COLLECTION

Seawater was collected at the surface samples of 500 mL of seawater were collected in Nalgene high density polyethylene dark bottles at each site around low tide for the summer 2023 SCECAP sampling (Figure 2.1). Samples were taken at the site for a total of 5 samples per site, at random times and areas around the boat to reduce replication and get a representative sample of the site. Samples were placed on ice and transported to the laboratory for processing. 100 mL of seawater was filtered on a 25 mm diameter Whatman GF/F glass fiber with a 0.7 μm pore size filter with gentle vacuum pressure. The filters were stored in 2 mL microcentrifuge tubes at -80 °C.

2.5 METHODS: HPLC SAMPLE PREPARATION & ANALYSIS

The samples were freeze-dried using a FreeZone 2.5 Liter Benchtop Freeze Dryer for a minimum of 12 hours. 750 μl of a 90 % acetone solvent mixture was added to the samples to extract pigments from the filter and 100 μl of carotenal was added as an internal standard. The acetone carotenal solution was used to extract pigment from the

filter in the period of 24 hours in a -20 °C freezer. The acetone carotenal solution was filtered to remove Whatman GF/F glass fiber filter debris from the mixture using a sterile nylon syringe filter. 400 µl of the filtered acetone carotenal solution was combined with 100 µl of 1 M ammonium acetate in a LCGC Certified Amber Glass vial and capped with a Preslit PTFE/Silicone Septum. A Shimadzu 2050 HPLC that contained both monomeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) and polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) reverse phase C18 columns was used for photopigment analysis. The HPLC was prepped with the mobile phase of 80:20 methanol to acetone ratio solution and a nonlinear gradient of 80:20 methanol to 0.5 M ammonium acetate ratio according to the methods proposed by Pinckney et al. (2001). Chromatograms were obtained using Shimadzu SPD-M10av photodiode array detector and the peaks were identified based on their retention times. The pigment areas were used to calculate the concentration of pigments present in the sample in $\mu g\ l^{-1}$. The total chl *a* concentration in $\mu g\ l^{-1}$ was calculated by adding the concentration of divinyl chl *a* and chlorophyllide *a* to the chl *a* concentration of each sample to represent the biomass of phytoplankton.

2.6 METHODS: SUPPLEMENTAL FLUOROMETRIC DATA

A larger time series of phytoplankton biomass from fluorometric measurements for the years 1999-2022 was used to produce a robust data set to compare phytoplankton biomass between habitat types and correlate with river discharge data. These data were collected in the same way that water samples were collected for HPLC analysis. The samples were processed by filtering 50 ml of water through a Whatman GFC filter. The filter was placed in a microcentrifuge tube with 25 ml of acetone. Samples were frozen until processed by SCDHEC or SCDNR with consistent methods across agencies. The

thawed sample was centrifuged, and the supernatant was quantified using a Turner Model 10-AU fluorometer within 48 hours of collection. Time series data were provided by SCDNR SCECAP colleagues Andrew Tweel and Pamela Marcum. The time series comprised the chl *a* values collected by SCDNR during SCECAP sampling (2002, 2004, 2013-2020) and the supplemental samples collected by SCDHEC within 24 hours of SCDNR SCECAP sampling (1999-2001, 2003, 2005-2012, 2021-2022). Replicates were averaged to obtain mean chl *a* for each station. For the years 2011-2012, data were obtained from the National Water Quality Portal (WQP).

2.7 METHODS: STATISTICAL ANALYSES

A Shannon-Weiner diversity index was used to calculate phytoplankton photopigment diversity for each habitat type. The pigments used to calculate the Shannon-Weiner diversity index were peridinin, alloxanthin, fucoxanthin, zeaxanthin, and chlorophyll b. The mean chl *a* and average photopigment diversity ± standard deviation was calculated for all stations for each habitat type. A Kolmogorov-Smirnov (K-S) test was used to test the chl *a* and photopigment diversity data for normality. A Levene's test was used to determine the homogeneity of variances for the chl *a* data and photopigment diversity data. A natural log, inverse, and square root transformations were all applied to non-normal data in order achieve normally distributed data. A single factor ANOVA was used to determine if there was a significant difference between habitat type for phytoplankton biomass and photopigment diversity. A coefficient of variation for mean chl *a* was calculated for historic data for the years 1999-2022 and for the year 2023 to determine if the variation in 2023 was comparable to historic data sets. The coefficient of variation is a ratio of the standard deviation to the mean that allows for comparisons

between data sets, even if the means are very different from each other to determine the consistency of the data.

2.8 RESULTS

The mean chl *a* concentration ± SD in µg l^{-1} for open water and tidal creek habitats for the sampling year 2023 was 13.38 ± 5.49 and 17.25 ± 8.44, respectively (Figure 2.2). The mean chl *a* data were normally distributed for open water habitats (K-S test, df = 15, p = 0.194) and data were approximately normally distributed for tidal creek habitats (K-S test, df =14, p = 0.047). A Levene's test showed homogeneity of variances for the mean chl *a* data (L = 1.897, df = 1, 27, p = 0.180). A single factor ANOVA with habitat type as the factor (2 levels: open water and tidal creek) revealed there was not a significant difference between tidal creek and open water habitat types for mean chl *a* during the sampling year 2023 (Figure 2.2, $F_{1,27}$ = 2.171, df = 1, 27, p = 0.152). The coefficient of variation for tidal creeks and open water habitats in 2023 was 48.9% and 41 % percent, respectively.

The mean chl *a* concentration ± SD in µg l^{-1} for open water and tidal creek habitats for the sampling years 1999-2022 combined was 9.88 ± 6.23 and 12.20 ± 7.53, respectively (Figure 2.3). The data for mean chl *a* for the sampling years 1999-2022 was not normally distributed and exhibited a skewed distribution to the left for open water and tidal creek habitats (K-S test, df = 440, 433 p < 0.001). A natural log transformation normalized the data for open water (K-S test, df = 440 p = 0.200) and tidal creek habitats (K-S test, df =433, p = 0.060). A single factor ANOVA with habitat type as the factor (2 levels: open water and tidal creek) revealed there was a significant difference between tidal creek and open water habitat types for mean chl *a* across the years 1999-2022

(Figure 2.3, $F_{1,871} = 30.321$, df = 1, 871, $p < 0.001$). The historic (1999-2022) coefficient of variation for tidal creeks and open water habitats in 2023 was 62.2 % and 63.7 % percent, respectively.

The mean ± SD for phytoplankton photopigment Shannon-Weiner diversity for open water and tidal creek habitats for the sampling year 2023 was 0.27 ± 0.07 and 1.06 ± 0.12, respectively (Figure 2.4). The Shannon-Weiner photopigment data were normally distributed for open water habitats (K-S test, df = 15, p = 0.139) and tidal creek habitats (K-S test, df =14, p = 0.137). A Levene's test showed homogeneity of variances for the photopigment data (L = 1.897, df = 1, 27, p = 0.180). A single factor ANOVA with habitat type as the factor (2 levels: open water and tidal creek) revealed phytoplankton photopigment diversity was significantly higher in tidal creek than open water habitat types during the sampling year 2023 (Figure 2.4, $F_{1,27} = 440.321$, df = 1, 27, $p < 0.001$).

2.9 DISCUSSION

Tidal creeks trended slightly higher for mean chl *a* for the sampling year of 2023 (Figure 2.2). However, there was no significant difference in mean chl *a* between tidal creek and open water habitats in 2023, thus we fail to reject the null hypothesis (p = 0.152, Figure 2.2). The data showed a wide range of variability, which could be decreased with a larger sample size. The larger data set that included 23 years of fluorometric measurements for chl *a* decreased the influence of the variability between habitats. Mean chl *a* was significantly higher in tidal creek habitats than open water habitats from 1999-2022, thus the null hypothesis is rejected ($p < 0.001$, Figure 2.3). Tidal creeks had a higher mean chl *a* likely reflecting the influence of nutrient run-off supporting the growth of phytoplankton. The coefficient of variation for the year 2023

was less than the coefficient of variation for the historic dataset. This means that the variation in the data for 2023 falls within the variation of the historic data. Based on the variation observed in 2023, it is unlikely there is a significant difference between the two habitat types during individual years. This is important because the habitat scores are only based on the data from one to two years. If the chl *a* is representing a connected environment, rather than a separated environment, then the habitat condition could not be representative of the habitat.

Tidal creeks exhibited higher diversity than open water habitats, thus hypothesis is support by the results ($p < 0.001$, Figure 2.4). Tidal creeks have a higher surface area to volume ratio which increases the nutrient influence from watersheds (Mallin & Lewitus, 2004). Phytoplankton respond positively to higher concentrations of nutrients in the water column, especially limiting nutrients such as nitrogen in estuarine environments (Zhang et al., 2022). Bazin et al. (2014) studied the phytoplankton diversity and taxonomic abundance gradient along a transect in an estuarine system in Normandy and found that the bay had a lower taxonomic abundance of phytoplankton when compared to upper/inland regions of the estuary. The brackish water that was heavily influenced by river influence, had the highest diversity across the sampling transect (Brazin et al., 2014). The resuspension of many different tycho-pelagic phytoplankton compared to the open water habitats likely led to the increase in diversity observed in tidal creeks. The tidal creeks sampled were typically areas that were higher up along a transect of the estuary compared to the open water sites that were lower along the transect, thus exhibiting the same pattern of decrease in phytoplankton diversity observed in this study.

Tidal creeks provide an important connection between the upland environment and the coastal ocean (Buzzelli, 2008). The biogeochemistry in tidal creeks changes hourly, daily, seasonally, and annually due to fluctuations in tides, material deposits, and allochthonous inputs (Buzzelli, 2008). Defining tidal creek habitats is challenging due to the variability alone between different tidal creeks along the same coastal region (Wetz et al., 2006). Even so, researchers and coastal survey studies still attempt to separate estuaries into two habitat types. The main reason for researchers to define and attempt to understand the link between the terrestrial environment and the coastal ocean is due to the mass flux of energy that is introduced into salt marsh ecosystems from this exchange (Novakowski et al., 2004). This energy exchange supports the high primary productivity observed in salt marsh ecosystem and provides economically valuable fish species with a critical habitat for early and later stages of life (Novakowski et al., 2004). Phytoplankton biomass can serve as an indicator of water quality for tidal creek habitats in estuaries because their growth and abundance are a direct result of nutrients, salinity, temperature, and light availability. However, this may not have been reflected during this study and many other surveys due to the way tidal creek and open water habitats are defined.

Defining tidal creeks and open water habitats by width of the channel (< or > 100 m) is perhaps too broad in that it does not include other important factors. The term “tidal creek” refers to areas that are heavily influenced by tide and become exposed at low tide, which happens to be many of the smaller channels in an estuary (Wetz et al., 2006). The sampling map for the year of 2023 reflects the challenges with drawing a width metric for habitat type. For example, sampling sites RT-23033 and RO-23316 were defined as two separate habitats but sampled within meters of each other. RO-23332 is perhaps one of

the most inland sites, surrounded by terrestrial inputs, but defined as an open water site due to width. This metric could also explain why drawing differences between these two habitat types was challenging for phytoplankton biomass for the year 2023. One study used the depth of < 10 cm at low tide and depth of 2-3 meters at high tide in tidal creeks as a metric for consistency across tidal creeks to assess macrobenthic communities' response to watershed development (Lerberg et al., 2000). The challenge of this concept for the SCECAP program is that their sampling is done at low tide and a depth of at least one meter is required for water transportation and trawls. Another study by Sanger et al. (1999) divides tidal creeks into an upper and lower boundary when comparing salt marsh sediments in underdeveloped and overdeveloped watersheds. A high tide of ~1 m was defined as the upper tidal creek whereas a high tide of ~3 m or where the tidal creek converges with another body of water was defined as a lower boundary (Sanger et al., 1999). Based on these parameters, the SCECAP program is heavily biased toward sampling the lower boundaries of tidal creeks whereas upper boundaries that could reveal important differences between open water and tidal creek habitats. However, it is still important to draw a metric between areas that are directly affected by lower tides and terrestrial inputs. Green & Coco et al. (2007) define tidal creeks as an area that fill with water to significant depth at higher tide but diminishes to a shallow channel that consists of mostly freshwater at low tide.

The concept of tidal creeks lacks a widely accepted definition across many different studies and perhaps needs to be reevaluated. Many studies published in the literature over the past few decades include their own unique definition of tidal creeks that range in detail. The SCECAP program could incorporate habitat metrics to include

sampling in upper tidal creek environments. The current reports could be misleading to the public because habitat condition is scored on the lower boundary of the tidal creek, which could be in better condition than the upper boundary due to the influence other water bodies that converge with the lower boundaries. However, from a management perspective, it is important to define and sample areas that are part of the main exchange between terrestrial inputs and the coastal ocean (Buzzelli, 2008). The main goal of SCECAP is to sample the estuaries to identify areas that may need improvement of coastal management practices and has helped improve our knowledge of the condition of South Carolina's estuaries. Now that most South Carolina's estuaries have been surveyed, there is an opportunity to expand and evolve these coastal assessments.

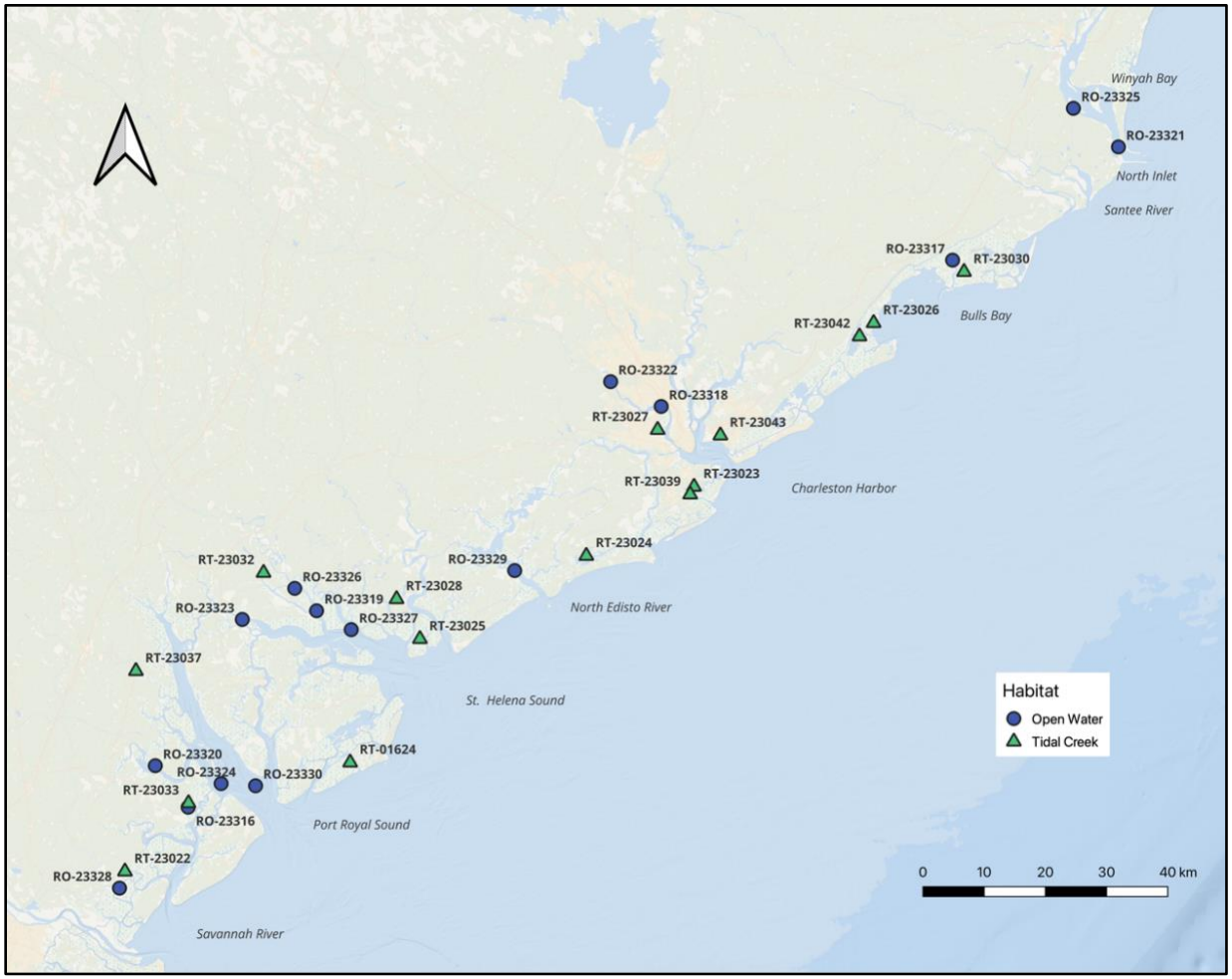

Figure 2.1 Map of the 15 tidal creek and 15 open water estuarine site locations on the coast of South Carolina for the year 2023 that is provided by a random sampling algorithm performed by the SCDNR. Each site location is labeled as either tidal creek (RT-green) or open water (RO-blue) (Appendix A).

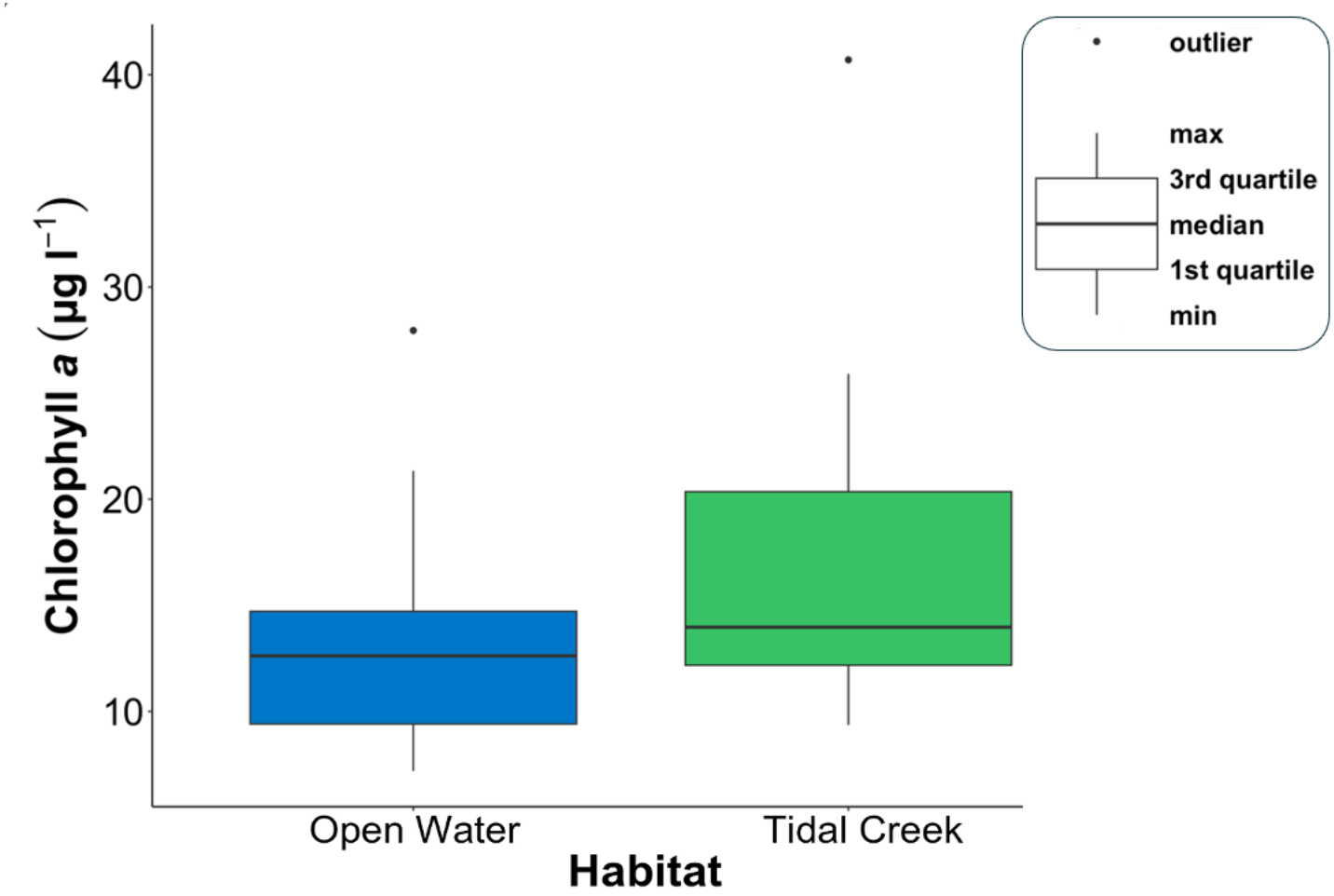

Figure 2.2 Boxplots of phytoplankton biomass (chl *a*) in tidal creek and open water habitats for the year sampling year 2023 ($F_{1,27} = 2.171$, $p = 0.152$).

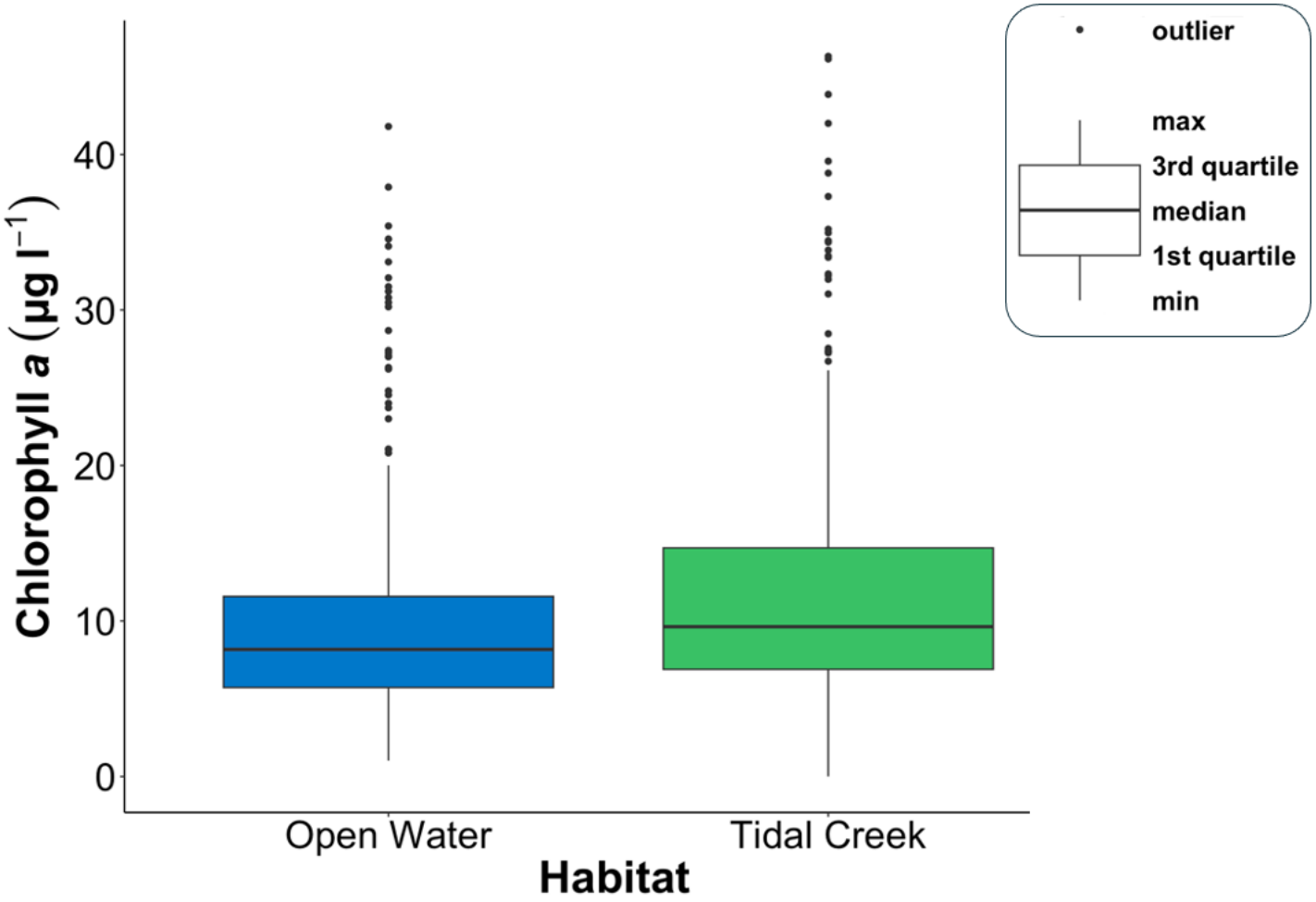

Figure 2.3 Boxplots of phytoplankton biomass (chl *a*) in tidal creek and open water habitats for the sampling years 1999-2022 ($F_{1,871} = 30.321$, $p < 0.001$).

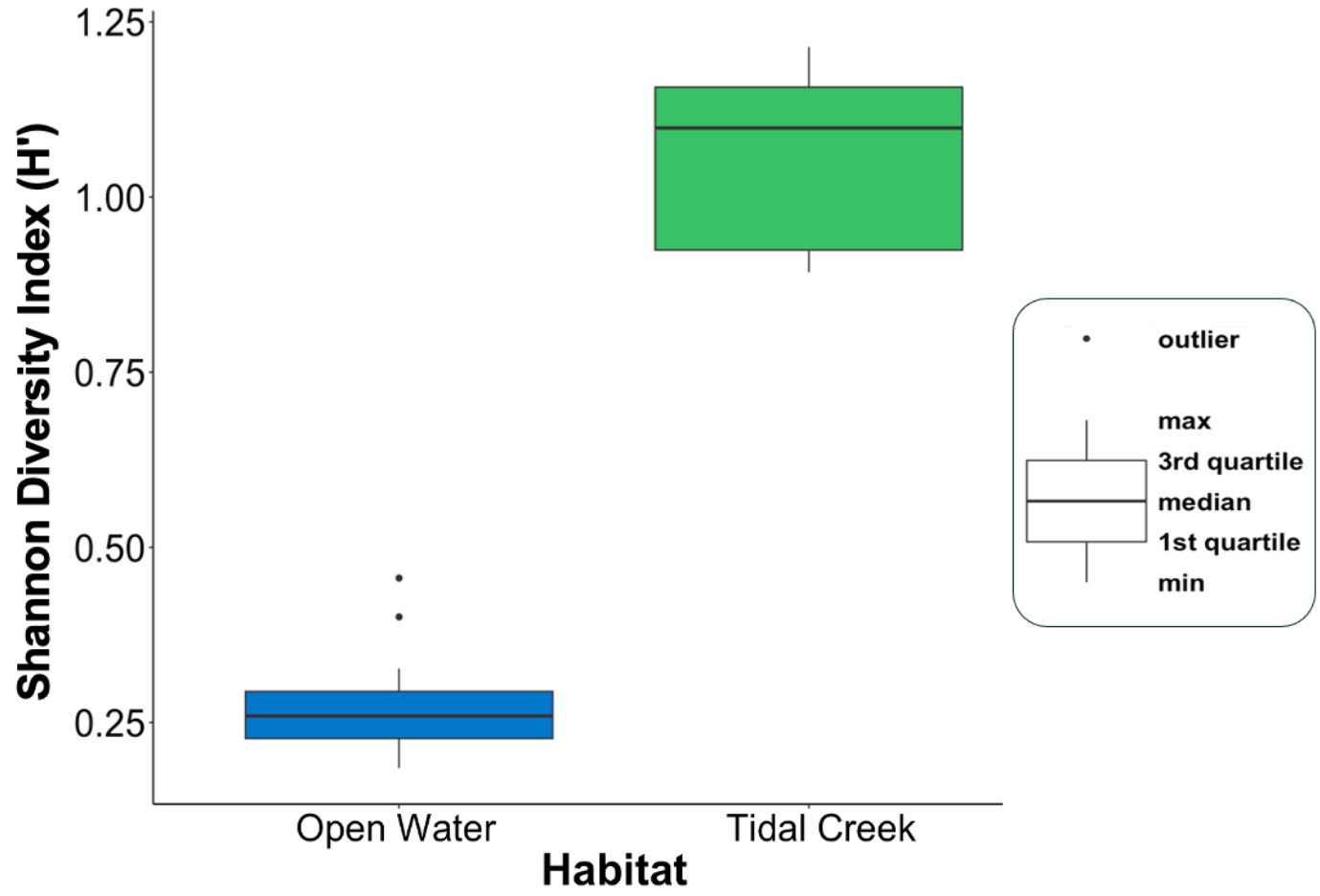

Figure 2.4 Boxplots of phytoplankton photopigment diversity in tidal creek and open water habitats for the sampling year 2023 ($F_{1,27} = 440.321$, $p < 0.001$).

CHAPTER 3

PHYTOPLANKTON TAXONOMY

3.1 TAXONOMIC ALGAL GROUPS

Phytoplankton can be separated into taxonomic groups based on the pigments they contain, rather than classifying to the species level. This methodology works for phytoplankton because they contain unique pigments or biomarker pigments. Biomarker pigments are pigments that overlap between different groups but are found at different concentrations in certain groups (Kramer et al., 2020). In estuarine environments the most common taxonomic phytoplankton groups contributing to the phytoplankton biomass in estuarine environments are diatoms, dinoflagellates, cyanobacteria, green algae, cryptophytes, and haptophytes (Wetz et al., 2006). The biomarker pigments for diatoms are fucoxanthin and violaxanthin (Kramer et al., 2020). Cryptophytes contain alloxanthin, dinoflagellates contain peridinin, and cyanobacteria contain zeaxanthin (Kramer et al., 2020). Haptophytes contain the unique and biomarker pigments fucoxanthin, hexfuco, butfuco, and chl c3 (Kramer et al., 2020). Green algae can be associated with the pigment's lutein, chl b, neoxanthin, and violaxanthin (Kramer et al., 2020).

3.2 DESCRIPTION OF CHEMTAX v1.95

The most widely used method for obtaining algal class abundances is ChemTax in the programming software MatLab (Mackey et al., 1996). The script in ChemTax v1 in MatLab established by Mackey et al. (1996) continues to be the basic algorithm for

almost all advancements for ChemTax. ChemTax v1.95 in Microsoft excel is an equivalent software to the MatLab version and was used in this study (Wright, 2017). ChemTax uses nonnegative matrix factorization, weight of errors, and pigment distribution knowledge (Saggiomo et al., 2023). Nonnegative matrix factorization (NMF) decomposes the sample data matrix (S), where S = pigments x samples, into two lower ranking matrices, F and C, where F = pigments x groups and C = groups x samples (Gillis, 2020). The product of the two lower matrices, F and C, approximately equal matrix S, so that $S \approx F \times C$ (Gillis, 2020). Matrix C contains the resulting algal class abundance for each sample, and matrix F contains the ratios of the unique pigments for each algal class. This factor problem is very complex to solve for a meaningful factorization of the sample matrix (S) unless an initial F matrix, $F_{0,}$ is derived from literature values (Mackey et al., 1996). F_0 is comprised pigment ratios for each algal class where chl *a* is the sum of all pigments equal to 1 (Mackey et al., 1996). Matrices C and F are calculated such that F closely reflects literature values in F_0. The initial matrix of class abundances, C_0, is calculated using a least squares equation (Mackey et al., 1996). The residual error is calculated using matrices S, $C_{0,}$ and F_0 (Mackey et al., 1996). The steepest descent algorithm is iterated to find a residual error below a certain limit or until an iteration limit is reached (Mackey et al., 1996). The steepest descent algorithm (SDA) produces new C and F matrices with each iteration. A single element within the F matrix that results in a lower residual error is kept for the next iteration to produce a matrix with the lowest possible residual error that reflects the most accurate factorization of the sample matrix (Mackey et al., 1996). The algorithm runs for 500 iterations to create 60 separate F matrices and the six matrices with the lowest residual error are averaged to

produce final algal abundances and pigment ratios (Hayward et al., 2023; Wright, 2017). The final matrices are denoted as C_n and F_n, where C_n represents the estimated algal class abundances and F_n represents the estimated pigment ratios.

3.3 COMPETING TAXONOMIC SOFTWARES

The greatest challenge of the ChemTax method of analysis is the pre-existing knowledge of the F_0 matrix pigment ratios. ChemTax accuracy relies heavily on how accurate the literature derived values are. PhytoClass is an emerging method proposed by Hayward et al. (2023) to remove the challenges surrounding ChemTax analysis. Hayward et al. (2023) uses a proxy for ChemTax v1.95 in R studio to compare results with PhytoClass. This study differs from Hayward et al. (2023) in that the latest release, ChemTax v1.95 from 2017, is used to compare algal abundance results with phytoclass instead of R studio ChemTax. Using ChemTax in R studio differs slightly from ChemTax v1.95 in that there are two versions: ChemTax-1 and ChemTax-2. ChemTax-1 focuses on the sensitivity to the starting F matrix values by having a much larger iteration limit of 5,000 compared to 500 iterations in ChemTax v1.95. ChemTax-2 more like ChemTax v1.95 because the starting F matrix values are randomized to the same specified factor of 0.7, but then it is set to an iteration limit of 200 instead of 500.

3.4 DESCRIPTION OF PHYTOCLASS

The PhytoClass software in R studio uses a simulated annealing program that incorporates Alternating Least Squares (ALS) and the Steepest Descent Algorithm (SDA) similarly to ChemTax (Hayward et al., 2023). Simulated annealing is a technique that is used to find the global minima of a mathematical function that has many local minima (Hayward et al., 2023). The terminology in simulated annealing originates from the

physical process of heating a material to a certain temperature and then cooling it down slowly to decrease the likelihood of a defect (Kirkpatrick, 1983). The methodology uses temperature to represent the amount of space the random points cover across the function (Kirkpatrick, 1983). The local minima are found by jumping around randomly at first between maxima and minima without concern for accuracy to explore the entire data set in higher temperatures (Laarhoven & Aarts, 1987). As the process continues and the temperature cools to medium, the focus of the mathematical function is to explore different minima and the paths of constant descent in terms of lowest error (Laarhoven & Aarts, 1987). When the temperature cools to low, the search for a solution is only for local improvements in error to find the absolute global minima (Laarhoven & Aarts, 1987). While PhytoClass does not require a pre-established literature derived F matrix, it does require minimum and maximum values of pigment concentrations for each algal class. The advantage of this method is that the accuracy of the results does not rely as heavily on exact values derived from literature. This new technique claims to be a potential equivalent or improved version of ChemTax. This method of estimating phytoplankton taxonomy using simulated annealing and pigment minimum and maximum values has been made available for scrutiny and use in the package PhytoClass in the programming language R by Hayward et al. (2023).

3.5 HYPOTHESIS & JUSTIFICATION

It is hypothesized that PhytoClass will result in similar concentrations of algal groups when compared to ChemTax 1.95 pigment-based taxonomy methodology. The reason for this is the evidence provided by Hayward et al. (2023) shows that ChemTax in

R and PhytoClass resulted in comparable algal class abundance to the true class abundances.

3.6 METHODS: DATA & ANALYSIS

Historic HPLC data for the years of 1999-2007 were provided by the SCDNR SCECAP program coordinator, Denise Sanger. The historic data were used to compare community composition results of phytoplankton for ChemTax 1.95 and PhytoClass programs. A least-squares linear regression was used to determine the linear equation of the dependent variable PhytoClass and the independent variable ChemTax and determine if the slope for all algal groups was different from zero. Additionally, a linear model with a fixed slope of 1 was compared with the linear model for each algal class using a single factor ANOVA. This was used to test if the slope was significantly different from 1, which represents the equivalence of the two software's. Calculated standardized residuals were tested for normality using a K-S test. The resulting R^2 value and slope of the line was used to determine the similarity between ChemTax and PhytoClass programs for the algal group's dinoflagellates, cryptophytes, diatoms, haptophytes, cyanobacteria, and green algae. The distance of the slope from one was used to infer the percentage in which the concentrations of each algal taxa are underpredicted or overpredicted relative to each to each program.

3.7 RESULTS

The least-squares linear regression analysis between the dependent variable PhytoClass and the independent variable ChemTax 1.95 indicated a significant linear relationship for the algal class dinoflagellates (Figure 3.1A, $n = 510$, adj $r^2 = 0.99$, $p < 0.001$). The equation of this relationship was PhytoClass = (1.2 * ChemTax 1.95) + 0.014

(Figure 3.1A). There was a significant linear relationship for cryptophytes (Figure 3.1B, n = 510, adj $r^2 = 0.98$, $p < 0.001$). The equation of the relationship for cryptophytes was PhytoClass = (1.3 * ChemTax 1.95) + 0.003 (Figure 3.1B). Diatoms had a significant linear relationship (Figure 3.1C, n = 510, adj $r^2 = 0.95$, $p < 0.001$). The equation of the relationship for diatoms was PhytoClass = (0.85 * ChemTax 1.95) + 0.34 (Figure 3.1C). There was a significant weak linear relationship for haptophytes (Figure 3.1D, n = 510, adj $r^2 = 0.51$, $p < 0.001$). The equation for this relationship for the algal class haptophytes was PhytoClass = (2.5 * ChemTax 1.95) + 0.35 (Figure 3.1D). There was a significant linear relationship for cyanobacteria (Figure 3.1E, n = 510, adj $r^2 = 0.82$, $p < 0.001$). The equation for the relationship between ChemTax 1.95 and PhytoClass for cyanobacteria was PhytoClass = (1.3 * ChemTax 1.95) + 0.13 (Figure 3.1E). The regression analysis of green algae indicated a significant linear relationship (Figure 3.1F, n = 510, adj $r^2 = 0.98$, $p < 0.001$). The equation for this relationship was PhytoClass = (0.71 * ChemTax 1.95) - 0.042 (Figure 3.1F).

The slope of the regression line for green algae was significantly different from 1 for all algal groups (Table 3.1, ANOVA, $p < 0.01$). The distance from one can be used to infer the percentage in which the concentrations of each algal taxa are underpredicted or overpredicted relative to each other. PhytoClass predicted 20% more dinoflagellates in the samples compared to ChemTax 1.95 (Figure 3.1A). PhytoClass also predicted 30% more cyanobacteria and cryptophytes than ChemTax 1.95 (Figure 3.1B, E). There were 15% more diatoms using ChemTax 1.95 when compared to PhytoClass (Figure 3.1C). There were also 29% lower green algae concentrations in each sample when using

PhytoClass compared to ChemTax 1.95 (Figure 3.1F). PhytoClass predicted 150% more haptophytes than ChemTax 1.95 (Figure 3.1D).

3.8 DISCUSSION

The two programs exhibited a linear relationship, but they were not similar in their results for the algal groups of diatoms, cryptophytes, cyanobacteria, green algae, and dinoflagellates (ANOVA, $p < 0.01$). The PhytoClass results for haptophytes exhibited a poor linear relationship and a much higher slope than the other algal groups ($n = 510$, adj $r^2 = 0.51$, $p < 0.001$). Due to the nature of each of these programs, the exact taxonomic concentrations of phytoplankton vary each time the program is used. It is also important to note that the concentrations that result from these programs are an estimate of taxa present and not as accurate as methods such as microscopy. However, the slope of the line between these programs should approximately equal one. The results of the two programs are not equivalent based on the results of the ANOVA (Table 3.1). The slope of the haptophyte regression line shows that PhytoClass and ChemTax 1.95 have some major differences when it comes to correctly predicting haptophytes. There is an extensive long-term knowledge of the algal communities that represent South Carolina's estuarine regions with microscopy checks of ChemTax 1.95 F matrix parameters.

Hayward et al. (2023) uses different min and max values of each pigment per each algal group which is to be expected based on the different study locations. However, for haptophytes, Hayward et al. (2023) uses two additional pigment types of Chlorophyll c2-monogalactosyldiacylglyceride ester 18:4 and 14:0 that were not used in this study. These two pigments are biosynthetically related to chlorophyll c2 occur dominantly in haptophytes and they co-occur with fucoxanthin, hex-fuco, and hex-kfuco (Roy et al.,

2011). Diatoms and haptophytes both share fucoxanthin as a common pigment, which makes it difficult to distinguish between the two algal groups. Using these additional pigments could have improved the accuracy of PhytoClass predicting the correct number of haptophytes. Additionally, Hayward et al. (2023) distinguishes between two types of diatoms (A, B) that have the same minimum and maximum values of fucoxanthin, but two separate chlorophyll c pigments of different concentrations. Diatoms A had chlorophyll c1 with a lower min and max concentration and diatoms B had chlorophyll c3 with a higher concentration. This study used concentrations of fucoxanthin and violaxanthin to distinguish diatoms from other algal groups. These differences could explain why haptophytes were overestimated and diatoms were underestimated when using PhytoClass compared to ChemTax 1.95. Another major difference between this study and Hayward et al. (2023) is that chlorophytes, euglenoids, and prasinophytes were grouped together as green algae due to the difficulties of ChemTax 1.95 distinguishing correct concentrations between these groups due to their similarities.

The methods of PhytoClass could be a useful tool in the future for phytoplankton taxonomy. However, the minimum and maximum pigment concentrations will need to be optimized and the use of more pigments may pose a challenge over the widely used ChemTax 1.95 software. The goal of PhytoClass was to reduce the reliance on exact literature derived pigment values, but the minimum and maximum values and the selection of different pigments poses an equivalent challenge for new users. Advancements in eliminating the challenges in estimating taxonomic algal group abundances are important for the scientific community and in the understanding of algal communities across a variety of habitats.

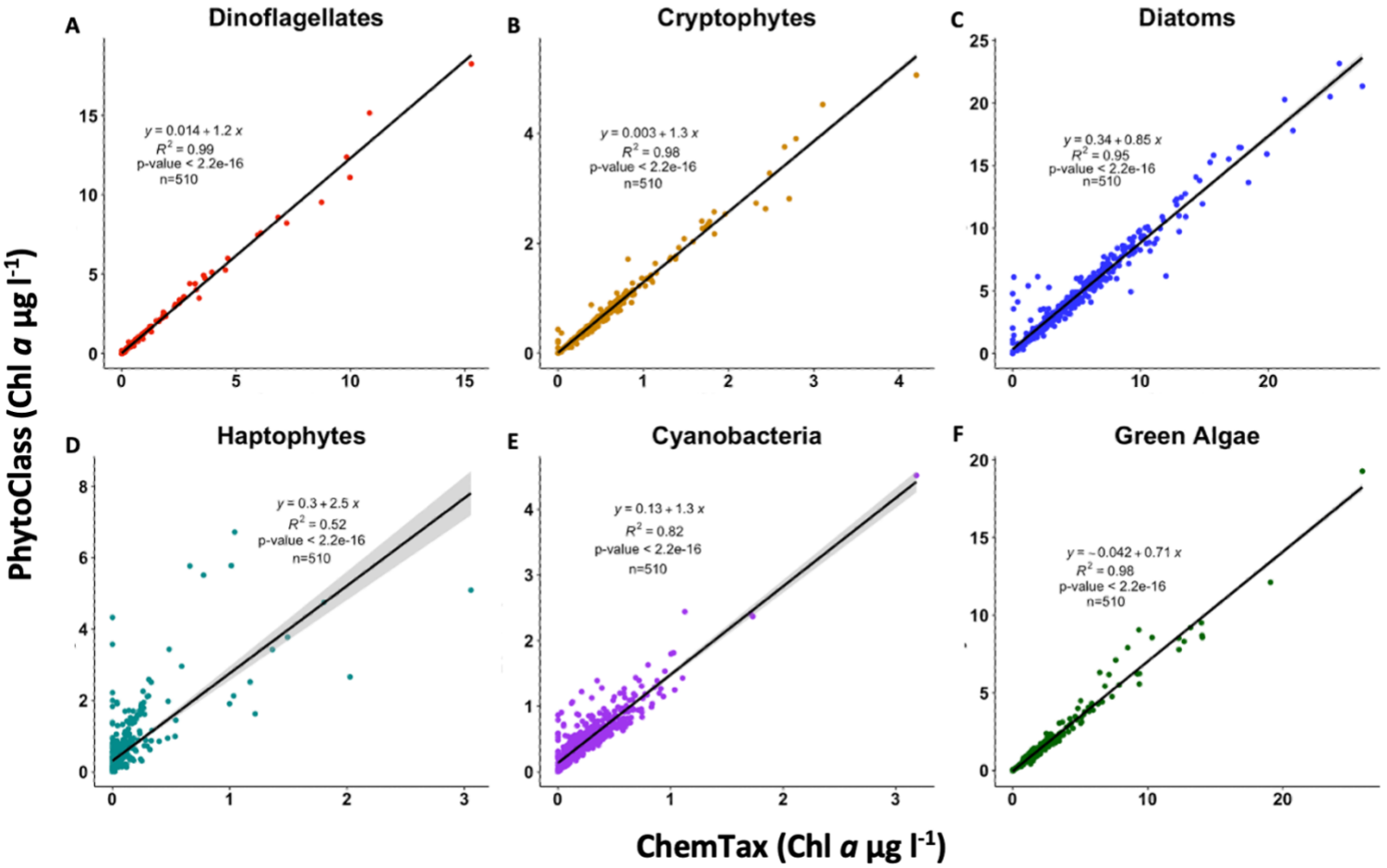

Figure 3.1 Scatter plot comparison of ChemTax 1.95 and PhytoClass pigment-based taxonomy concentrations in µg l^{-1} for each algal group (A. Dinoflagellates, B. Cryptophytes, C. Diatoms, D, Haptophytes, E. Cyanobacteria, and F. Green Algae) with a linear regression line and the corresponding equation, R^2, p-value, and sample number (n).

Table 3.1 Summary table of the results of the linear regression where n is the sample number, R^2 is the coefficient of determination, and the ANOVA F statistic with the significance level for the test if the slope (m) was equal to zero or one.

Algal Class	n	R^2	F m = 0	Significance m = 0	F m = 1	Significance m = 1	Equation
Dinoflagellates	510	0.99	71,081.35	$p < 0.01$	2469.9	$p < 0.01$	$y = 1.2x + 0.014$
Cryptophytes	510	0.98	24,775.10	$p < 0.01$	1222.6	$p < 0.01$	$y = 1.3x + 0.003$
Diatoms	510	0.95	10,558.19	$p < 0.01$	324.33	$p < 0.01$	$y = 0.85x + 0.34$
Haptophytes	510	0.52	542.07	$p < 0.01$	190.59	$p < 0.01$	$y = 2.5x + 0.35$
Cyanobacteria	510	0.82	2,315.03	$p < 0.01$	154.26	$p < 0.01$	$y = 1.3x + 0.013$
Green Algae	510	0.98	19,884.20	$p < 0.01$	3435.1	$p < 0.01$	$y = 0.711x – 0.042$

CHAPTER 4

RIVER DISCHARGE

4.1 FACTORS INFLUENCING PHYTOPLANKTON BIOMASS

A variety of physical and biogeochemical factors can influence mean chl *a* and phytoplankton community composition (Noble et al., 2003). Estuaries are very dynamic ecosystems with tidal, temperature, salinity, and nutrient fluctuations (Twomey & John, 2001). Phytoplankton are influenced by all these factors, and it is difficult to distinguish and characterize relationships between each individual factor in a dynamic environment (Twomey & John, 2001). Phytoplankton are readily consuming and re-mineralizing the nutrients in the water column (Noble et al., 2003). By understanding the role of coastal runoff, especially in highly developed coastal communities, scientists can characterize phytoplankton blooms (Burford et al., 2012).

Projects that include multiple organizations like SCECAP have the resources, personnel, and funding to obtain sediment assessments, biological surveys, and water quality in large scale habitat sampling projects. Nutrient concentrations and their elemental ratios influence phytoplankton abundances and community composition, making phytoplankton important indicators for eutrophication due to coastal runoff (Han et al., 2023). Phytoplankton abundances are specifically controlled by the limiting nutrients phosphorous and nitrogen (Zhang et al., 2022). Algal bioassays are required to determine the uptake of these nutrients and the response of phytoplankton (Burford et al.,

2012). Observational values of nutrient concentrations provide information on what remains in the water column after biological activity which includes consumption and re-mineralization of nutrients. This makes it difficult to draw conclusions or find relationships between algal abundance and nutrient rich runoff. Observations of phytoplankton and nutrient concentrations are more useful in analyzing trends over longer periods of time.

The term Annual Phytoplankton Succession (APS) can be defined as the pattern of phytoplankton abundance observed annually (Caracciolo et al., 2021). This pattern consists of an annual spring phytoplankton bloom that starts in April and persistently increases until it reaches its peak in the months of July and August (Cloern & Jassby, 2010; Noble et al., 2003). Annual phytoplankton observations in the Chesapeake Bay revealed increases in phytoplankton biomass from 1950-1994 (Harding, 1997). The annual observations in phytoplankton abundance also showed variability and changes in phytoplankton abundance according to wet or dry years in terms of rainfall (Harding, 1997). It was found that phytoplankton productivity increased from 1988 to 1989 in the Neuse River due to increased rainfall (Mallin et al., 1991). Coastal runoff has been proven to be a useful tool in interpreting annual fluctuations in phytoplankton productivity and bloom dynamics (Mallin et al., 1993). Years that have increased rainfall typically have peaks in phytoplankton biomass July and August because of the nutrients associated with coastal runoff from land drainage (Harding, 1997). Total river discharge can be utilized to determine the influence of rainfall in analyses because flow is largely affected by weather and climatic events (Depretis, 2021).

4.2 REASONING & HYPOTHESIS

It has been found that residence time of nutrient fluxes in Okatee Estuary, South Carolina is up to five days with and up to two days of flushing time in a river system that was at a distance from the estuary that was approximately less than half of the distance inland used in this study (Moore et al., 2006). To allow time for water from rainfall events to flow into the estuary and then allow the phytoplankton to respond to the influence of nutrients, river discharge from two weeks prior to sampling was chosen in this study. It is expected that river discharge from the sum of the two weeks prior to sampling will be positively correlated with phytoplankton biomass for each major river basin in South Carolina.

4.3 METHODS: DATA & ANALYSIS

River discharge in ft^3/s data were acquired for the years 1999-2022 from the United States Geological Survey (USGS) portal for the 5 major river basins: PeeDee, Santee, Salkehatchie, Edisto, and Savannah (Figure 2.2). The Hydrologic Unit Codes (HUC's) and survey data ranges that corresponded to the coastal river basins of Santee, Edisto, Savannah, Salkehatchie, and PeeDee were 03050112 (2008-2022), 03050206 (1999-2022), 03060109 (1999-2022), 03050208 (1999-2022), and 03040201 (2008-2022), respectively (Figure 2.2). The northernmost PeeDee river basin sites were excluded from the study due to the absence of a long-term hydrologic unit survey period for this location.

The river discharge data in ft^3/s were averaged across the 15-minute time intervals for every hour and the total discharge per hour was summed across 24 hours to equal the total discharge per day. A rolling sum was calculated for the total daily discharge across

14 days prior to sampling and then paired with the sampling date and value for chl *a*. For example, a sample date of 8/14/15 was paired with the total discharge from across the period 8/1/15-8/14/15 to create a two-week lag. A K-S test was used to determine if the chl *a* and total river discharge data were normally distributed for each major river basin. A nonparametric correlation analysis (Spearman's's rank order correlation) was used to compare phytoplankton biomass with mean river discharge.

4.4 RESULTS

The chl *a* data were not normally distributed across the coastal river basins for tidal creek habitats (K-S test, df =301, $p < 0.001$). The river discharge data were also not normally distributed across the coastal river basins for tidal creek habitats (K-S test, df =301, $p < 0.001$). A Spearman's's rank order correlation revealed mean chl *a* per station had a significant, positive correlation with Edisto River Basin discharge ($r = 0.50$, $n = 33$, $p = 0.003$) and Santee River Basin discharge ($r = 0.39$, $n = 67$, $p = 0.001$) for tidal creek habitats (Figure 3.5). In addition, mean chl *a* was not significantly correlated with river basin discharge for the PeeDee ($r = 0.43$, $n = 9$, $p = 0.25$), Salkehatchie ($r = 0.031$, $n = 157$, $p = 0.70$), or Savannah ($r = 0.21$, $n = 35$, $p = 0.23$) rivers within tidal creek habitats (Figure 3.5).

There was a significant positive correlation between river discharge and mean chl *a* in tidal creek habitats for Edisto ($p = 0.003$) and Santee ($p = 0.001$) River Basins, thus the results support the hypothesis (Figure 3.5). The Spearman's rho for the Edisto River Basin in tidal creeks was 0.50, which can be interpreted as moderately correlated (0.50 – 0.70). The Spearman's rho of 0.39 for the Santee River Basin in tidal creek habitats between chl *a* and discharge can be classified as having a low correlation (0.30 – 0.50).

There was not a significant positive correlation between river discharge and mean chl *a* in tidal creek habitats for PeeDee ($p = 0.250$), Salkehatchie ($p = 0.031$), and Savannah ($p = 0.230$) River Basins, thus the results do not support the hypothesis (Figure 3.5).

chl *a* data were not normally distributed across the coastal river basins for open water habitats (K-S test, $df = 320$, $p < 0.001$). The river discharge data were also not normally distributed across the coastal river basins for open water habitats (K-S test, $df = 320$, $p < 0.001$). A Spearman's's rank order correlation revealed mean chl *a* was significantly correlated with river basin discharge for Edisto ($r = 0.61$, $n = 22$, $p = 0.003$) and Santee ($r = 0.41$, $n = 48$, $p = 0.004$) for open water habitats (Figure 3.6). Mean chl *a* was not significantly correlated with river basin discharge for PeeDee ($r = 0.10$, $n = 16$, $p = 0.71$), Salkehatchie ($r = 0.038$, $n = 199$, $p = 0.59$), and Savannah ($r = 0.13$, $n = 35$, $p = 0.46$) for open water habitats (Figure 3.6).

Similar to tidal creeks, open water habitats had a significant positive correlation between river discharge and mean chl *a* for Edisto ($p = 0.003$) and Santee ($p = 0.004$) River Basins, thus the results support the hypothesis (Figure 3.6). The Spearman's rho for the Edisto River Basin for open water habitats can be classified as moderately correlated ($r = 0.61$). The Spearman's rho of 0.41 for Santee River Basin in open water habitats can be interpreted as a low correlation. There was not a significant positive correlation between river discharge and mean chl *a* in open water habitats for PeeDee ($p = 0.710$), Salkehatchie ($p = 0.590$), and Savannah ($p = 0.460$) river basins, the results do not support the hypothesis (Figure 3.6).

4.5 DISCUSSION

Despite the Santee River Basin discharge data survey period comprising only the years 2008-2022, a low correlation between discharge and mean chl *a* was observed for both habitat types. This correlation could strengthen or weaken with an increase in survey years, so it is important to explore more years of data, especially at river basins with low sample sizes. The Edisto River Basin showed the strongest correlation between chl *a* and river discharge across all river basins for both habitat types. This could be due to number of reasons including the geography, stream distribution, and proximity of the monitoring site to the coastal ocean. The characteristic narrowing and convergence of streams in the Edisto River Basin where the monitoring site is located could have isolated the influence of weather events on discharge. The geography and stream distribution of the Edisto River Basin allows for all major streams to meet at a single point and the monitoring site is just below where all the major streams converge. The more rivers and streams that the monitor station encompasses, the better the representation of the water discharge into the surrounding estuaries. The significant correlations between river discharge and mean chl *a* in Edisto and Santee are important because increases in rainfall from more frequent storm events could result increases in annual phytoplankton blooms.

The main challenge for the PeeDee River Basin for both habitat types is that there is large number of streams and rivers that flow to the southern region of the basin, but not the northern region. This challenge was mitigated by removing the northernmost sites because there was not a nearby monitoring station with a long-term data set to analyze this region. The PeeDee River Basin also had the lowest number or sites because of the lack of estuarine habitats in this region. The Salkehatchie River Basin monitoring

location exhibited either very low discharge values or very large outliers while the mean chl *a* values were highly variable. This monitoring site is likely a poor representation for the effect of weather on chl *a* in estuarine habitats because the monitoring station that had long term data did not encompass most of the discharge of rivers and streams flowing into the estuary. The Savannah River Basin is a challenging river basin to measure the effect of discharge on chl *a* because the monitoring sites with long-term data are south of the sampling sites. If chl *a* samples were also collected below the mouth of the Savannah River Basin in the state of Georgia, there may have been a stronger correlation with discharge. However, different intervals could be explored and examined separately for each basin due to the different flow rates and proximity to sampling sites for each river basin. The changes in chl *a* may have not been reflected over the time interval two weeks for every river basin. Exploring nutrient loading in comparison to mean chl *a*, may be a more useful tool in examining the influence of coastal run-off on phytoplankton biomass.

Another study that took place in the Mississippi River Basin found strong positive correlations between streamflow and fluorometric measurements of chl *a* across three years (Lane et al., 2007). They also observed influences in mean chl *a* reflected from rainfall from at least three months prior to sampling (Lane et al., 2007). The influence of river discharge is also contingent upon the nutrients associated with the discharge itself (Jordan et al., 1991). There may have been a significant amount of nutrients associated with river basins that showed significant correlations between river discharge and chl *a*. River discharge can be a useful metric for interpreting influence of nutrient run-off, but it can be even more powerful when paired with the movement of sediment, nitrate, and organic matter (Waite et al., 2023). Based on the results of this study, river discharge for

each river basin was not the main factor influencing the interannual phytoplankton biomass observed.

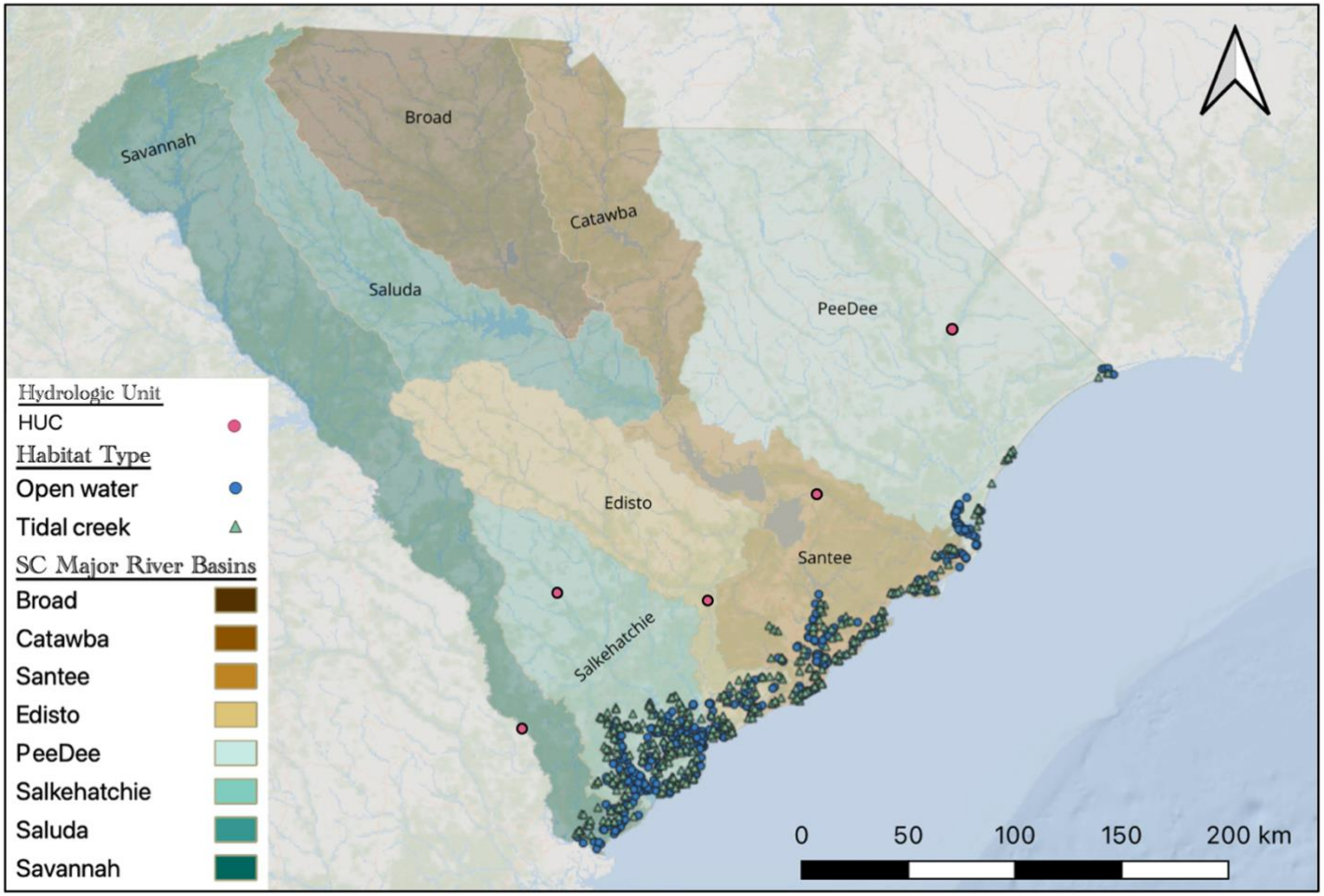

Figure 4.1 Map of South Carolina's major river basins with the 1999-2022 SCECAP site locations of tidal creek (green) or open water (blue) habitats and the hydrologic survey unit locations (pink).

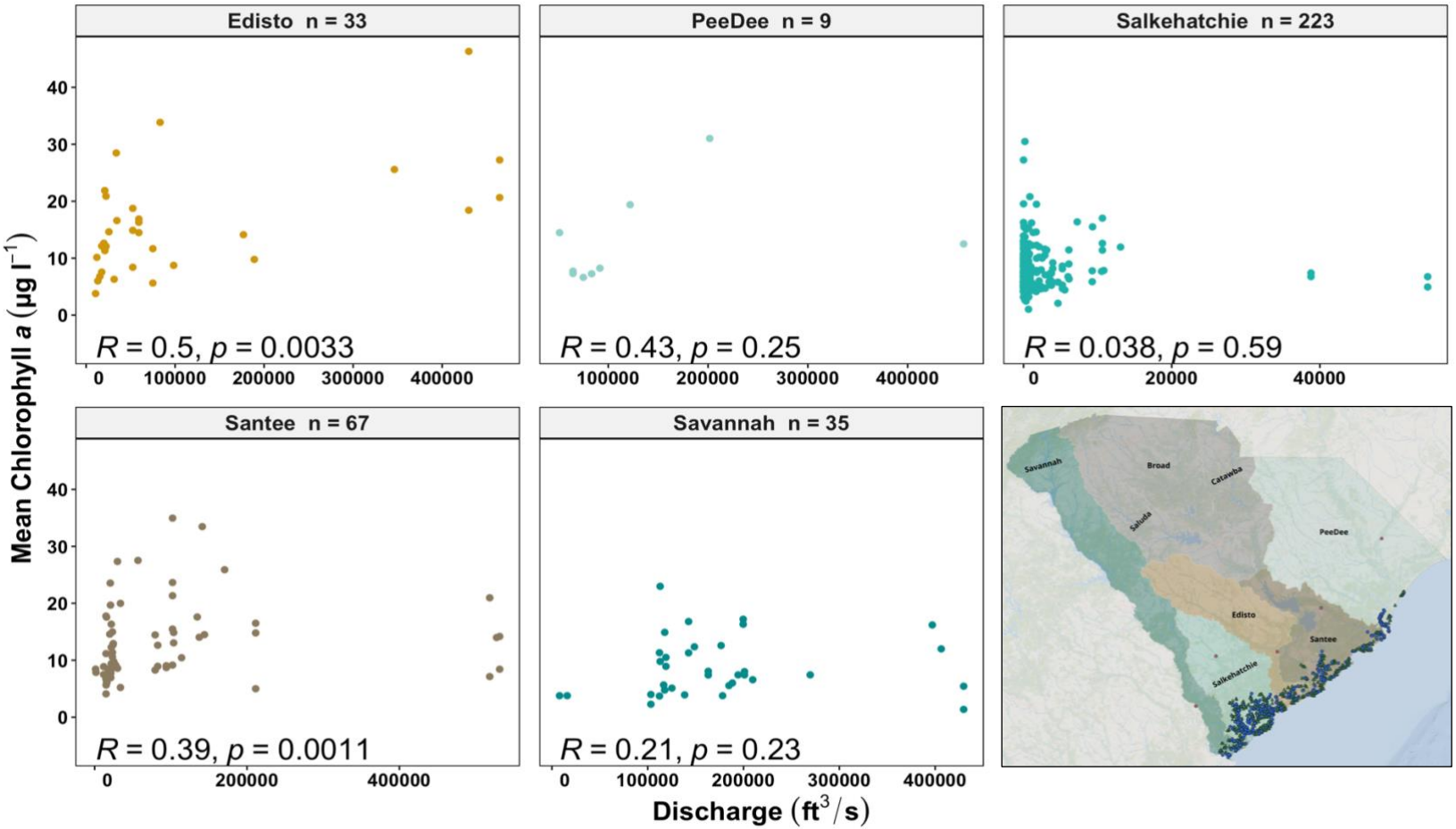

Figure 4.2 Correlation scatter plots of the major river basins Edisto, PeeDee, Salkehatchie, Santee, and Savannah between mean chl *a* in µg l^{-1} and the total discharge in ft^3/s for the two weeks prior to sampling for tidal creek habitats.

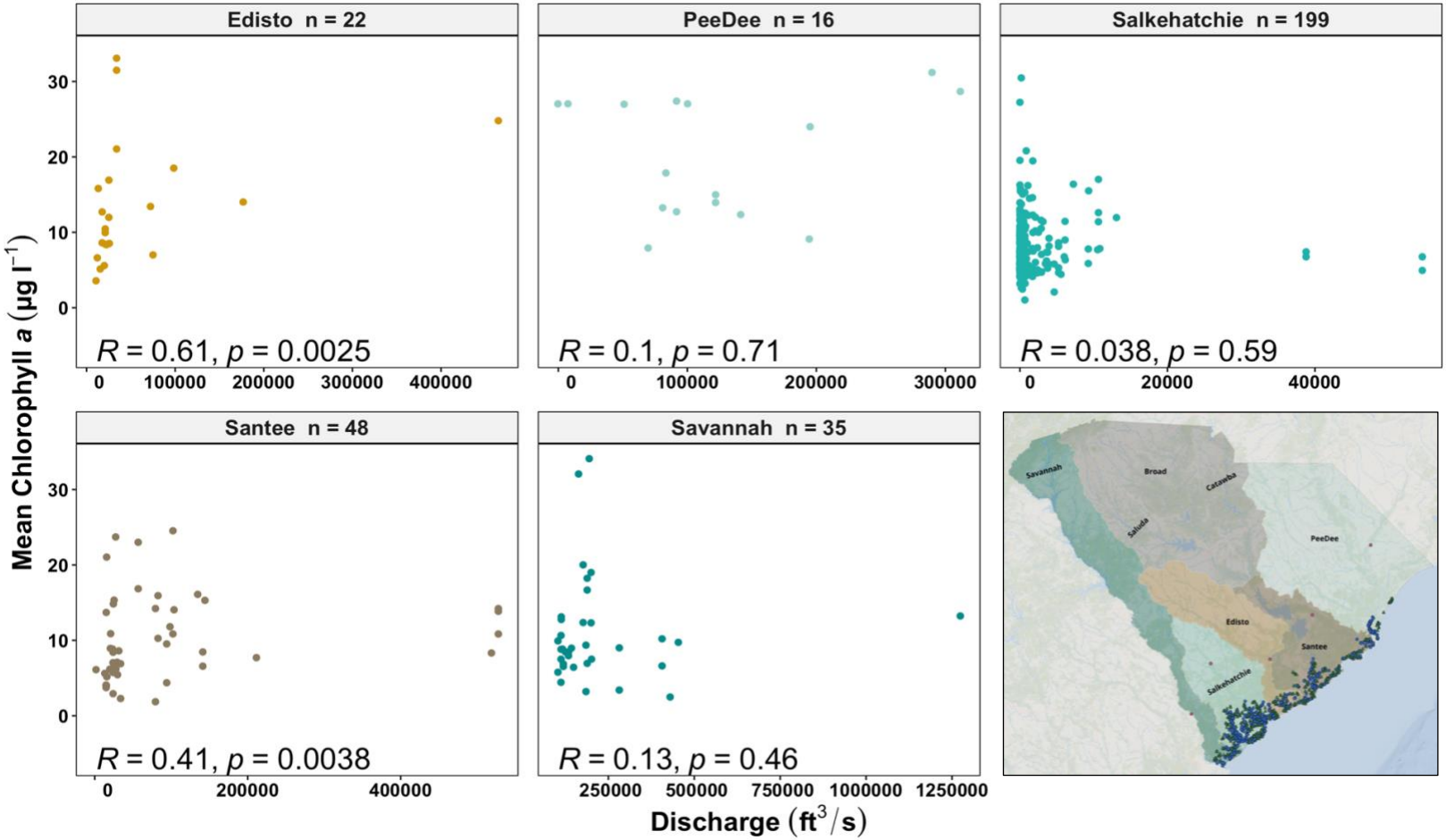

Figure 4.3 Correlation scatter plots of the major river basins Edisto, PeeDee, Salkehatchie, Santee, and Savannah between mean chl *a* in µg l^{-1} and the total discharge in ft^3/s for the two weeks prior to sampling for open water habitats.

CHAPTER 5

CONCLUSION

The goal of this study was to assess potential weaknesses in estuarine survey management practices in terms of water quality using phytoplankton. From a management perspective, phytoplankton biomass is highly variable and in terms of water quality it could be significantly altering habitat condition scores when used as a separate metric, and thus the need for coastal management strategies could be underestimated. The second goal of this study was to assess new methodologies in quantifying concentrations of phytoplankton groups present in the water column. PhytoClass did not result in equivalent or similar algal class concentrations as ChemTax. The final goal of this study was to investigate different methodologies in determining the influence of nutrient rich run-off into estuarine systems using phytoplankton. The resulting significant correlations between river discharge and chl *a* were weak; thus, river discharge is not the main factor influencing phytoplankton biomass and it fails to quantify the influence nutrient rich run-off on mean chl *a* in SC estuarine systems.

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APPENDIX A

2023 SCECAP SITES

Table A.1 Tidal creek sampling site locations with paired physical and chemical data at each site.

Date	Site ID	Time On	Lat	Long	Tide (Ebb/Flood)	Total Depth	pH (S.U.)	DO (mg/L)	Temp (°C)	Salinity (ppt)	Conductivity (µmhos/cm)	Secchi (m)
7/11/23	RT-23042	1048	32.938	-79.6388	slack	3	7.43	4.57	29.39	27.35	44611.29	0.65
7/11/23	RT-23026	1123	32.9574	-79.6145	flood	4.2	7.59	5.57	29.42	25.99	40020.01	0.4
7/11/23	RT-23030	1243	33.0313	-79.4577	flood	3.1	7.49	3.88	29.28	32.61	49019.93	0.45
7/18/23	RT-23037	1148	32.4512	-80.89	ebb	2.4	7.29	4.14	32.19	27.22	41684.61	1
7/19/23	RT-23022	950	32.1581	-80.9092	slack	2	7.28	4.01	30.67	25.54	36248.48	1
7/19/23	RT-23033	1028	32.2579	-80.7991	ebb	2.5	7.59	4.68	30.81	32.67	45186.83	1.25
7/25/23	RT-23032	1226	32.5942	-80.6699	flood	5.1	6.96	3.38	30.48	10.82	18027.48	0.4
7/26/23	RT-23025	1037	32.4977	-80.3993	flood	3	7.39	3.76	31.94	31.7	47762.21	0.7
7/26/23	RT-23028	1221	32.5559	-80.4393	flood	9.7	7.41	5.39	31.01	14.04	22883.29	0.6
8/1/23	RT-23034	943	32.317	-80.5197	flood	6.2	7.76	4.65	29.41	33.37	49985.71	0.5
8/2/23	RT-23024	1120	32.6189	-80.1113	ebb	3.2	7.5	4.17	29.61	32.83	49303.24	0.7
8/29/23	RT-23027	1045	32.8016	-79.988	ebb	1.3	7.08	1.83	30.1	23.44	36457.81	0.25
9/6/23	RT-23023	1128	32.7193	-79.925	flood	2.3	7.69	5.32	28.93	33.06	49623.61	0.65
9/6/23	RT-23039	1156	32.708	-79.9315	flood	2.4	7.67	4.7	28.83	35.27	52561.46	0.5

Table A.2 Open water sampling site locations with paired physical and chemical data at each site.

Date	Site ID	Time On	Lat	Long	Tide (Ebb/Flood)	Total Depth	pH (S.U.)	DO (mg/L)	Temp (°C)	Salinity (ppt)	Conductivity (µmhos/cm)	Secchi (m)
7/11/23	RO-23317	1305	33.0487	-79.4774	flood	5.6	7.62	4.18	29.24	34.39	51394.8	0.6
7/12/23	RO-23321	1044	33.2122	-79.1907	ebb	9.1	7.59	5.46	28.74	13.59	22066.81	0.4
7/13/23	RO-23325	1119	33.2682	-79.2687	ebb	4.1	6.98	4.67	29.45	4.67	8284.68	0.4
7/18/23	RO-23330	930	32.2844	-80.6832	slack	13	7.93	6.02	30.61	33.42	50067.46	1.75
7/18/23	RO-23324	1006	32.286	-80.7437	slack	12	7.84	5.66	30.66	32.27	48531.12	1.5
7/18/23	RO-23320	1048	32.3127	-80.856	ebb	4	7.57	5.26	31.08	22.58	35238.62	1.5
7/19/23	RO-23328	928	32.1331	-80.9188	flood	4.5	7.31	4.57	30.72	23.08	33098.85	1
7/19/23	RO-23316	1049	32.2518	-80.7995	ebb	3.5	7.64	4.81	30.86	32.74	45270.11	1.25
7/25/23	RO-23323	1051	32.5263	-80.7064	slack	3.5	7.38	4.55	30.43	24.05	37308.01	0.6
7/25/23	RO-23319	1134	32.5389	-80.5775	flood	3.8	7.38	5.25	31.08	20.32	32040.7	0.7
7/25/23	RO-23326	1155	32.5716	-80.6155	flood	2.5	7.17	4.29	30.89	14.51	23580.48	0.5
7/26/23	RO-23327	1138	32.5113	-80.5179	flood	2.3	7.63	5.37	30.74	25.67	39547.81	0.45
8/1/23	RO-23329	1237	32.597	-80.2351	ebb	2.6	7.48	3.81	29.99	29.08	44244.94	0.2
8/29/23	RO-23318	1121	32.8361	-79.9814	ebb	3.4	7.2	3.27	31.06	21.3	33433.41	0.15
8/29/23	RO-23322	1203	32.8721	-80.069	ebb	3.4	6.97	3.41	30.37	4.7	8347.22	0.3