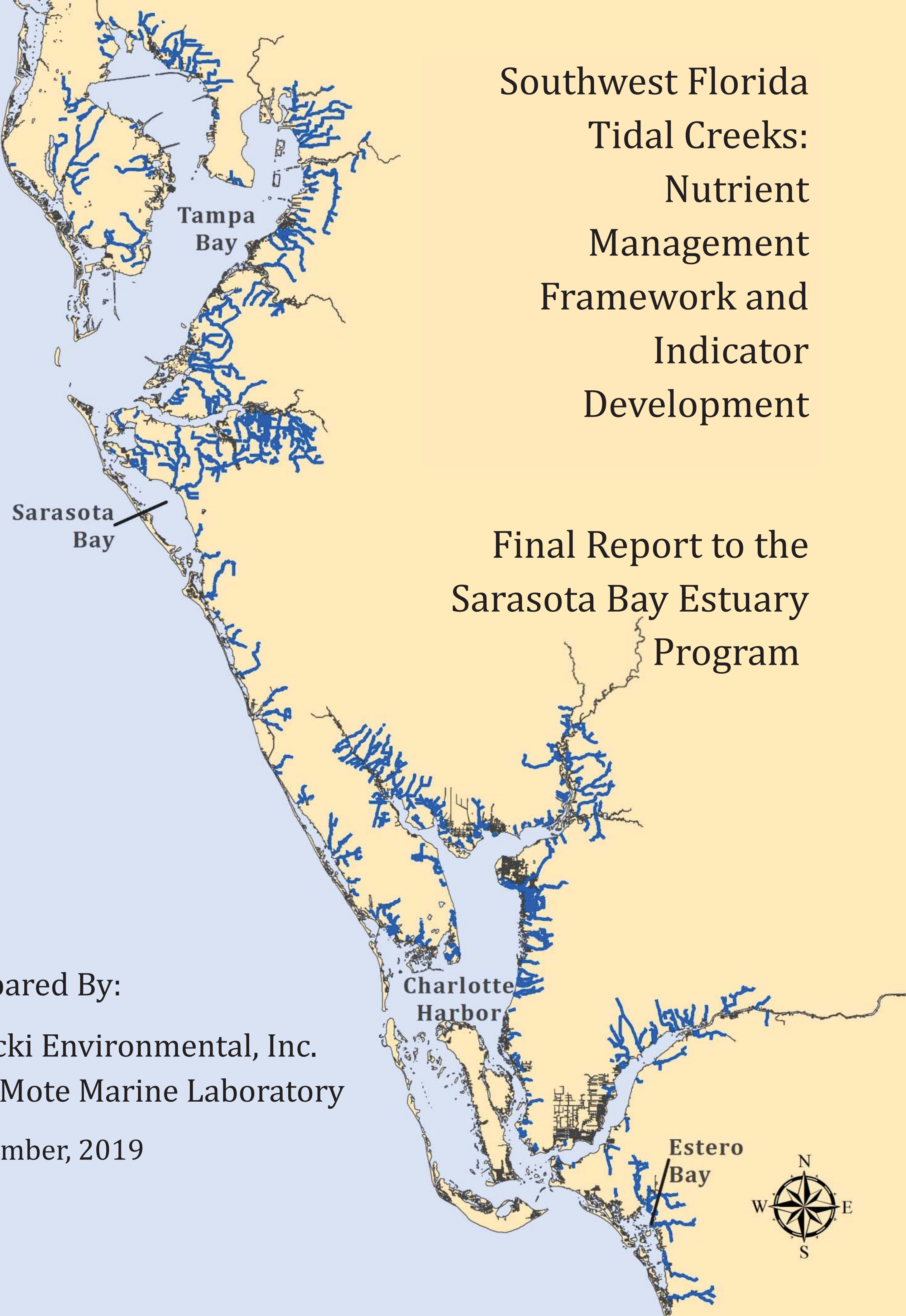


Southwest Florida Tidal Creeks: Nutrient Management Framework and Indicator Development

Final Report to the Sarasota Bay Estuary Program

Prepared By:

Janicki Environmental, Inc.
and Mote Marine Laboratory

December, 2019

Forward

The Sarasota Bay Estuary Program (SBEP) was awarded a Wetlands Program Development Grant by the United States Environmental Protection Agency (US EPA Region 4) to conduct a multidisciplinary study of southwest Florida tidal creeks to forward the science relating nutrient inputs, instream processes and biological responses in southwest Florida tidal creeks. Project and grant management was provided by Dr. Jay Leverone, Sarasota Bay Estuary Program with EPA project oversight by Felicia Burks (Technical Project Officer). The study was a collaborative effort by the three southwest Florida Estuary Programs including SBEP, Tampa Bay Estuary Program (TBEP), and Coastal & Heartland National Estuary Partnership (CHNEP, formerly Charlotte Harbor National Estuary Program) with in-kind services provided by six counties to assist in the sampling effort. The six counties in southwest Florida include Pinellas, Hillsborough, Manatee, Sarasota, Charlotte, and Lee counties. This report was principally authored by Mike Wessel of Janicki Environmental with contributions to both the analysis and reporting of the nutrient isotope analysis by Dr. Emily Hall of Mote Marine Laboratory and edits to content and format by Dr. Jay Leverone and Ed Sherwood (Tampa Bay Estuary Program).

The report was written as a mid-level synopsis of the analytical efforts conducted to develop the proposed nutrient management framework in such a way that a variety of readers with varying levels of expertise could conveniently assimilate the information. There is a wealth of information provided in the appendices that provide detailed descriptive plots, summary statistics of the raw data, and detailed results from the analytical phase of the project. This report is a complimentary study to a previous tidal creeks nutrient study completed in 2016 (Janicki Environmental and Mote 2016). Readers interested in more detailed background information on these creeks and the nutrient management framework proposed to manage these creeks that may not be conveyed in the body of this report should refer to that report. Of course, any questions regarding this work can also be directed to Mike Wessel, Janicki Environmental, Inc. at mwessel@janickienvironmental.com or Dr. Jay Leverone at jay@sarasotabay.org.

The previous report (Janicki Environmental, Inc. and Mote, 2016) referred a sampled creek in Charlotte County as “East Spring” when in fact it is more commonly referred to as “West Spring”. This creek is referred to in this report as “West Spring”. In addition, the Estero River is referred to as simply “Estero” as the distinction between the terms “river” and “creek” for these tributaries is mostly semantic (for example, a much longer and more voluminous “Phillippi Creek” is traditionally referred to as a creek while the shorter “Estero River”” is traditionally referred to as a river). The term “tributary” has also been historically used to describe systems within this population. Since most of the tidal creeks are first order in that they drain directly to the larger estuary rather than a tidal river, the term creek was adopted for this report.

Acknowledgements

We would like to thank all the members of the Joint Technical Advisory Committee of the three contiguous National Estuary Programs of southwest Florida (including other individuals too numerous to mention) for their contributions and commitment to the successful completion of this project. Kris Kaufman (SWFWMD) and Amber Whittle (FWC) served as TAC co-chairs for the joint meetings and are warmly acknowledged. The following participants were directly involved in implementing this study and are also gratefully acknowledged for their commitment to the protection of southwest Florida tidal creeks:

United States Environmental Protection Agency: Felicia Burks

Sarasota Bay Estuary Program: Dr. Jay Leverone and Mark Alderson

Tampa Bay Estuary Program: Ed Sherwood and Gary Raulerson

Coastal & Heartland National Estuary Partnership: Jennifer Hecker and Nicole Iadevaia

Pinellas County: Mark Flock, Sarah Malone, Rob Burnes and Robert McWilliams

Hillsborough County: Tom Ash, Chris Pratt, Brett Ursin and Julie Christian

Manatee County: Rob Brown, Greg Blanchard and Kerry Harkinson

Sarasota County: John Ryan, Nicole Iadevaia and Sheila Scolaro

Charlotte County: Roger Debruler, Phillip Brouse and Judith Ott (Volunteer)

Lee County: Brett O'Brien and Susan Fite

Mote Marine Laboratory: Dr. Emily Hall, Camia Charniga and Dr. Ari Nissanka

Mote Marine Laboratory (Retired): Dr. L. Kellie Dixon

Janicki Environmental: Tony Janicki, Jon Perry and Steve West

Executive Summary

The Sarasota Bay Estuary Program (SBEP) was awarded two Wetlands Program Development Grants by the United States Environmental Protection Agency (USEPA) Region 4 to conduct multidisciplinary studies of southwest Florida tidal creeks. The overarching goal of these grants was to coordinate and accelerate research relating to the causes, effects, extent, prevention, reduction, and elimination of water pollution in tidal creek wetlands.

The first study included one year of bimonthly sampling of 16 creeks and was intended to provide information that may support the development of numeric interpretations of the Florida Department of Environmental Protection's (FDEP) narrative water quality standard for nutrients in southwest Florida tidal creeks. The first study focused on identifying stressor-response relationships between water column nutrient concentrations and water quality and biological response endpoints. Response endpoints included dissolved oxygen, chlorophyll (water column and benthic) and fish community structure. Additionally, the study looked at landscape-level effects at varying spatial scales for analysis of both near and far-field effects of watershed stressors on tidal creek biological integrity. The results of that study confirmed that these creeks are critical habitats for estuarine-dependent fish species of ecological, recreational and commercial value, but also highlighted the complexity of defining stressor-response relationships in such dynamic aquatic ecosystems.

The follow-up study described by this report concentrated on monthly Spring and Fall sampling over a two year period to describe nutrient and chlorophyll dynamics when the creeks were most susceptible to nutrient loading. This study did not include fish sampling; instead it focused on total and dissolved pools of water column and sediment nutrients, presence of macrophytes, and stable isotope analysis from both suspended particulate organic matter and macroalgae. A special focus of this stud was to describe nutrient dynamics along the salinity gradient of these creeks relative to conservative mixing principles.

These two studies have provided a wealth of data derived directly from sampling within southwest Florida tidal creeks to better understand the role of source water nutrient inputs, instream processes, habitat interactions, and biological responses in these critically

important tidal wetland habitats but to date, tidal tributaries in Florida remain governed under Florida Administrative Code (FAC) by the Narrative Criteria, with impairment evaluations guided by chlorophyll and dissolved oxygen crtieria that were not developed based on data collected strictly within tidal creeks. Tidal creeks possess water quality characteristics that differ from freshwater systems and from the open estuary. As a result of their direct connection and close proximity to watershed sources of nutrient inputs and their smaller volumes and shallower depths relative to the open estuary, tidal creeks have relatively high nutrient and chlorophyll concentrations and low dissolved oxygen (DO) compared to downstream waterbodies. The NEPs remain interested in developing a management framework based specifically on data collected within tidal creeks to provide complimentary, additional information on tidal creek condition that can be used to support management actions based on commonly collected water quality data. The concept of a management strategy for southwest Florida tidal creeks was first promoted by the Tampa Bay Regional Planning Council in 1987 (Clark 1987). Since that time a number of very important but disparate studies have been conducted on different aspects of tidal creek integrity, but there is still need for a unified management framework to proactively identify creeks with elevated nutrient concentrations for further research and potential management actions prior to the creeks exceeding their assimilative capacity or becoming "Impaired" according to State regulations. This study proposes a nutrient management framework based on an objective assessment of nutrient concentrations for creeks with sufficient data within the populaton of over 300 southwest Florida tidal creeks. The term "**Framework**" is defined to provide a unified, objective, method to evaluate nutrient data for the entire population of creeks and includes a **Report Card** and a host of **Indicators** developed to provide evidence as to the potential underlying causes of the observed outcomes. The Framework also provides a mechanism by which to display the reults in a manner that can be readily understood by the regional NEPs, local resource managers, and the general public to help inform science-based decision making.

It is important to note that this study and its findings, including the Framework outcomes, do not carry any regulatory authority and are not intended to supercede any State or Federal regulatory standards currently in place, including aquatic life criteria used to evalaute waterbody impairments or pollutant load reduction goals associated with existing or forthcoming total maximum daily loads (TMDLs). The nutrient management framework was developed to serve as a screening tool to identify creeks with elevated nutrient concentrations for further evaluation to protect their in-stream ecology, but is not designed to explicitly identify regulatory impairments (or lack thereof) or the needs of downstream waterbodies such as larger estuarine waters that may be in need of nutrient load reductions.

Application of the Report Card to all southwest Florida creeks with appropriate data is provided in Figure E1 below and has resulted in 29 creeks within the study area being identified by one of the three management categories: “Caution”, “Investigate” or “Act”. The teal color represents the tidal creek boundary (Class 2 and 3M), while the grey area represents the freshwater segments (Class 1 and 3F) which have numeric nutrients standards and are independently assessed by FDEP.

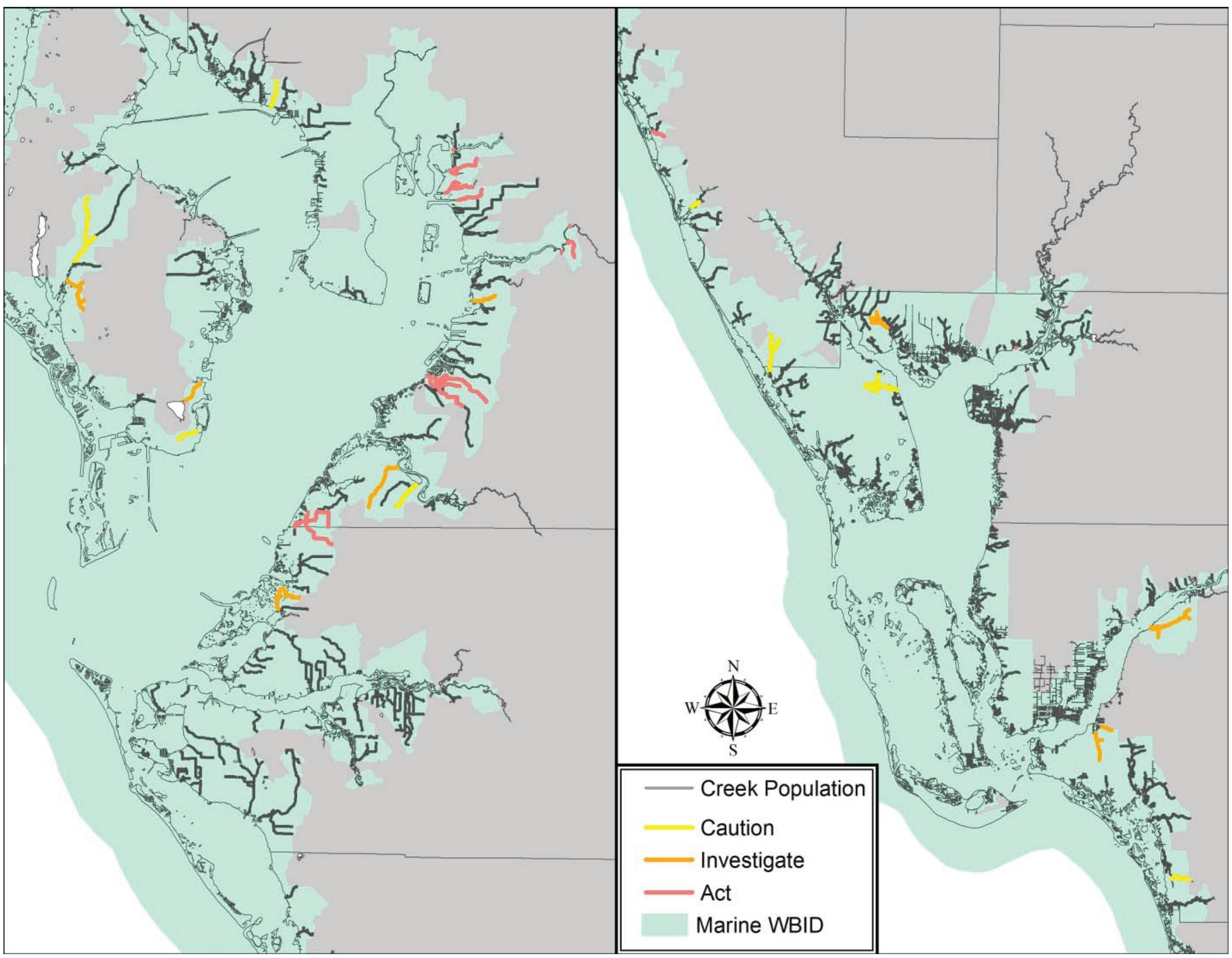


Figure E 1. Application of revised nitrogen management framework to the population of southwest Florida tidal creeks.

Creeks colored in either coral, orange, or yellow provide an actionable list of tidal creeks for which the identification, investigation and prioritization of potential local management responses can be pursued. The list is based on an objective assessment of water quality conditions based on nutrient concentrations that can be used to identify appropriate responses.

In addition to the Report Card, this study has produced a number of water quality indicators to characterize tidal creek condition. The following indicators are proposed to provide supporting evidence regarding tidal creek condition and identify potential contributing factors to observed outcomes.

- A median chlorophyll to nitrogen threshold ratio no greater than 15. A higher ratio indicates that a creek may be highly physically altered or have severed or restricted hydrologic connections to the estuary.
- A trophic state index score less than 60, which limits a creek to the "fair" category for estuarine waters.
- A macrophyte index based on frequency-of-occurrence with less than 50% of samples with macrophytes present, similar to FDEP's rapid periphyton index.
- A total nitrogen Annual Geometric Mean (AGM) concentration no more than 1.1 mg/L, which was built on the weight-of-evidence that higher concentrations are associated with an increased chlorophyll exceedence frequencies.
- A nitrate concentration ratio between the source water and estuary greater than 1, with lower index values suggesting nitrate inputs within the tidal portion of the creek.

These indicators are not individually deterministic of tidal creek condition; however, they do provide evidence to evaluate potential causes of over-enrichment in these creeks and their susceptibility to adverse effects. An aggregated index was also constructed as a possible mechanism to provide additional support for the management framework. The individual indicators, as well as the aggregated index scores, allow resource managers to quickly evaluate a host of individual metrics that relate to actual nutrient concentrations and respond in a proactive manner that improves stewardship of these critical wetland habitats. Individual and aggregated indices will require consistent monitoring and evaluation over time to confirm their utility in adequately describing tidal creek condition but can be applied to any creek with available and sufficient water quality data making the

Framework a useful surveillance tool that can be refined as more data and scientific findings become available to inform stewardship of these systems.

In addition to the Framework Report Card and Indicator development, a wealth of scientific information was generated from this study to accelerate research into causes, extent and potential remediation of nutrient pollution to tidal creek wetlands. One consistent observation was that nutrient addition was common within the estuarine portion of the creeks relative to that expected by conservative mixing equations. Frequently, the source water and tidal nutrient concentrations were nearly equivalent, and in some cases the tidal nutrient concentration was higher than the source water concentration. These outcomes depended largely on the magnitude of the source water nitrogen concentration, wetland habitat availability, and antecedent rainfall. For example, when source water nitrogen concentrations were low, source and tidal nitrogen concentrations were similar despite large changes in salinity along the length of the creek. This was particularly true for organic nitrogen and colored dissolved organic matter lending support to the hypothesis that addition may not necessarily be due to anthropogenic sources. Conversely, pulse rainfall events tended to result in elevated nutrient concentrations in the freshwater delivered to the tidal portion of the creek and a more typical dilution curve as a function of salinity. This report proposes that nutrient dynamics in these creeks can be generally characterized by three expected phases:

- Dry Phase: In this phase a low volume of freshwater is delivered to the tidal portion of the creek under “base flow” conditions. Source water nutrient concentrations tend to be lower than average and nutrient concentrations in the tidal portion of the creeks are similar to source water concentrations. The tidal concentrations represent an integration of source water inputs, assimilation, organic decomposition of both allochthonous and autochthonous material, outwelling from adjacent mangroves, and potentially sediment flux.

- Pulse Wet Phase: Characterized by conditions that generate storm water runoff and direct storm water infrastructure inputs. Freshwater pulses containing higher nutrient concentrations are delivered to the tidal portion of the creek and attenuated by the differential between source and tidal water concentrations. Salinity is a reasonable surrogate for estimation of the dilution of nutrients within the tidal portion of the creek under this phase.

- Saturated Wet Phase: Under these conditions the entire creek is essentially freshwater. Nutrient concentrations tend to be (but are not necessarily) higher and there is little to no gradient in nutrient concentrations along the length of the creek.

These are expectations developed based on our study, but we observed that the empirical dynamics may take many shapes based on site specific conditions. For example, to validate outcomes of our study with independent data, the State's master water quality database was queried for creeks within our study area that had multiple stations routinely sampled on the same date, with at least a single station in both the freshwater and tidal sections. The objective of this exercise was to identify the distribution of date-specific nutrient salinity slopes within creeks across the range of creeks in the population with available data. We found a wide range of outcomes ranging from Delaney Creek which had only 14% negative slopes (indicating that nitrogen concentrations were higher in the tidal portion) to (Phillippi Creek which had 88% negative slopes). Overall the average creek had 52% negative slopes indicating nutrient pulses from the freshwater source for the average creek. The end members of this distribution represent valuable indicators of tidal creek nutrient dynamics and efforts should be made to distribute future sampling effort across this range of conditions to characterize creek condition in future studies. The data requirements for this assessment require that at least one freshwater station and one tidal station are sampled on the same date, as close in time as possible. Unfortunately, many routine water quality monitoring programs don't currently meet this requirement and this design is a recommendation for future sampling efforts.

The nutrient management framework, including the report card and the indicator results, provides a locally developed approach to evaluate tidal creek condition and promote, prioritize and accelerate natural resource management actions where they are most likely to result in nutrient load reductions that provide benefits to water quality in these critical habitats. The framework complements the regulatory assessment process by identifying waterbodies with elevated nutrient concentrations instead of the narrative criteria for DO and chlorophyll concentrations which were not developed directly from tidal creek data. The recommendations also include a pathway to forward the development of additional biological and landscape metrics to refine further the assessments of tidal creek condition as well as sampling strategies and protocols to evaluate tidal creek condition. Recommendations are provided for minimum sampling requirements (i.e. quarterly sampling with at least a single station in the fresh and tidal portion of the creek sampled on the same date, as close in time as possible). This revised management framework, and the development of additional indicators of tidal creek condition, is intended to assist resource managers in prioritizing investigative actions and management responses, thereby improving the stewardship, management and protection of these critical coastal wetland habitats.

Table of Contents

List of Figures

List of Tables

Acronyms and Abbreviations

ANOSIM	Multivariate Analysis of Similarity
CDOM	Colored dissolved organic matter
CHLA	Water Column Chlorophyll a Corrected for Pheophytin (µg/l)
CHNEP	Coastal & Heartland National Estuary Partnership
CWA	Clean Water Act
DO	Dissolved Oxygen (mg/l)
DO%	Percent Dissolved Oxygen
EPA	United States Environmental Protection Agency
F.A.C.	Florida Administrative Code
FDEP	Florida Department of Environmental Protection
FIM	Fisheries Independent Monitoring Program
FWRI	Florida Wildlife Research Institute
H2O_UAL	Hydrologic Unit Area Load
IWR	Impaired Waters Rule
mg/l	Milligrams per Liter
NH3	Dissolved Ammonia (mg/l)
NNC	Numeric Nutrient Criteria
NO23	Nitrite-nitrate as N (mg/l)
OPO4	Orthophosphorus (mg/l)
OrgP	Organic Phosphorus (mg/l)
PC	Particulate Carbon
PCA	Principal Components Analysis
PN	Particulate Nitrogen
PP	Particulate Phosphorus
PSU	Practical Salinity Units
SBEP	Sarasota Bay Estuary Program
TBEP	Tampa Bay Estuary Program
TKN	Total Kjeldahl Nitrogen (mg/l)
TMDL	Total Maximum Daily Load
TN	Total Nitrogen (mg/l)
TP	Total Phosphorus (mg/l)
TTHI	Tidal Tributaries Habitat Initiative
µg/l	Micrograms per Liter
uS/*cm*	microsiemens
WBID	Water Body Identifier
%sat	Percent Saturation

** A “D” preceding any nutrient in the text indicates that the constituent is reported as the dissolved fraction.

1 Background

The Sarasota Bay Estuary Program, in partnership with the Tampa Bay Estuary Program, Coastal & Heartland National Estuary Partnership, and the 6 contiguous counties within the 3 National Estuary Program watersheds recently developed a local nutrient management framework ("Framework") for southwest Florida tidal creeks (Janicki Environmental, Inc. and Mote 2016). The Framework included both a nutrient evaluation "Report Card" and a management strategy to help identify and prioritize creeks for further evaluation based on annual geometric average nutrient concentrations. In addition to establishing management framework, the study identified several areas of further research to inform more site specific indicators of tidal creek condition. The study focused on identifying stressor response relationships between water column nutrient concentrations and water quality and biological response endpoints. Response endpoints included dissolved oxygen (DO), chlorophyll (water column and benthic), and fish community structure to test paradigms associated with current regulatory standards applied to tidal creeks and identify additional indicators of creek condition. Additionally, the study looked at landscape level effects at varying spatial scales for analysis of both near and far-field effects of watershed stressors on tidal creek biological integrity. The results of that study confirmed that these tidal creeks are critical habitats for estuarine dependent fish species of ecological, recreational and commercial value and also highlighted the complexity of defining stressor-response relationships in such dynamic aquatic ecosystems. A bulleted summary of those findings is provided is section 1.1 below.

1.1 Findings of the 2016 Tidal Creeks Study

The principal findings of the Janicki Environmental, Inc. and Mote 2016 study (subsequently referred to as "Previous Study") are listed in the bullet points below.

- The studied creeks well represented the range of expected nutrient conditions in the larger population of 306 southwest Florida tidal creeks.

- Tidal creeks were characterized by water quality conditions typical of wetland environments with the potential for episodic low dissolved oxygen and elevated chlorophyll concentrations.

- The annual geometric chlorophyll averages for all creeks were below 20 ug/l though in some individual samples chlorophyll a concentrations measured over 100 ug/l. indicating sporadic, site-specific and transient bloom conditions.

- There was not a clear, uniform relationship between water column chlorophyll and dissolved oxygen concentrations among creeks that could be used to establish a chlorophyll threshold concentration indicative of adverse effects to biological integrity based on data collected in tidal creeks.

- Creeks exhibiting chlorophyll bloom conditions (i.e. West Spring, Phillippi, Spring, and Sugarhouse) were not necessarily the creeks with the highest geometric average total nutrient concentrations, indicating mediating factors influence the probability of bloom conditions in these creeks including shading, residence times, and estuarine mixing.

- Nutrient concentrations did not follow conservative mixing principles along the estuarine salinity gradient suggesting that a linear dilution curve approach may not be appropriate for establishing tidal creek targets and thresholds.

- Southwest Florida tidal creeks are critical wetland habitats for estuarine dependent fish of high economic and ecological value including some of the most natural creeks in the study that failed the water quality standards being applied to tidal creeks.

While these creeks are critical habitats for estuarine dependent fishes, and a healthy fish community represents a principal ideal of what constitutes a “healthy” creek, fish communities as a biological response endpoint were not acutely sensitive to the nutrient concentrations we observed, including creeks that approached the numeric nutrient criteria for freshwater streams. Therefore, this follow up study did not include fish as a biological response endpoint and instead was conducted to validate outcomes of the previous water quality findings and collect additional information on instream nutrient dynamics that relate nutrient delivery from the watershed, instream processing, and water quality and biological responses (other than fish) indicative of tidal creek condition. The goals and objectives of this study are presented in the next section.

1.2 Goals and Objectives

Based on outcomes of the previous study, the goals of this study were to collect additional data and conduct additional analyses to refine the existing tidal creeks nutrient management framework to help local resource managers promote, prioritize and accelerate management actions to improve tidal creek nutrient conditions in these critical habitats. The objectives were to describe empirial nutrient dynamics along the salinity gradient withn the creek, evaluate sediment nutrient concentrations in relationship to water column nutrients, conduct water column and macroalgal stable isotope assessments, and develop additional water quality indicators that can be applied to the entire population of creeks to provide a weight of evidence to complement the existing management framework. The sampling scheme was designed to evaluate seasonal nutrient dynamics along the salinity gradients. Stable isotope evaluations were used to characterize and help partition the nutrients based on findings from the previous study suggesting that nutrient addition resulted from a combination of landscape level anthropogenic stressors, nutrient inputs from emergent wetland vegetation and riparian buffer habitats, and instream processes related to the decomposition of organic materials. Sediment nutrient concentrations and stoichiometric ratios were used to compare the water column and sediment nutrient characteristics and identify relative differences in ratios among creeks. A rapid macroalgal survey was developed to evaluate contributions of algal to observed water column nutrient concentrations and better understand potential sinks and sources of nutrients in the tidal portion of these systems. Together, these objectives were identified to complement previously collected data and promote the coordination and acceleration of research relating to the causes, effects, extent, prevention, reduction, and elimination of water pollution in southwest Florida tidal creek wetlands. **We recognize that regulatory aquatic life criteria must focus on the most sensitive components of the ecosystem under study and it is important to note that this study, its findings, and the management framework, including the report card results do not carry any regulatory authority and are not intended to supercede any State or Federal regulatory standards currently in place, including aquatic life criteria used to evaluate waterbody impairments or pollutant load reduction goals associated with existing or forthcoming total maximum daily loads (TMDLs).**

2 Methods

The following sections describe the methods used for data collection and assessment of nutrient dynamics in southwest Florida tidal creeks.

2.1 Study Design

This study design maintained many aspects of the first tidal creeks study; however, there were several important differences. As described by the bullet points below, this study:

- Focused on fewer creeks and a seasonal (Spring and Fall) monthly sampling frequency while extending the sampling over two years to capture inter-annual variation in water quality across the same temporal window.
- Used a fixed station design for samples in the tidal portion of the creek.
- Used analytical methods to explicitly quantify the dissolved fraction of total nutrient constituents that are most readily available for biological uptake.
- Analyzed water column samples for particulate carbon, nitrogen, and phosphorus to evaluate stoichiometric ratios within the water column.
- Collected sediment nutrient concentrations during 4 sampling events at all stations.
- Included a rapid macroalgal survey.
- Included stable isotope assessment of water column and algal samples.

Importantly, despite the focus on the dry season in this study, both studies meet the temporal requirements established in F.A.C 62-302.531 for evaluation of annual geometric averages for assessment against water quality standards described as follows in Florida administrative code [*.... To calculate an annual geometric mean for TN, TP, or chlorophyll a, there shall be at least four temporally independent samples per year with at least one sample taken between May 1 and September 30 and at least one sample taken during the other months of the calendar year. To be treated as temporally independent, samples must be taken at least one week apart*].

The creeks selected for sampling are shown in Figure 2-1 along with an illustration of the fixed station sampling scheme. The fixed station design was chosen to eliminate the potential spatial variability within strata established during the first study while assessing temporal changes in water quality and to locate the most downstream stations nearest the downstream mangrove fringe that tends to dominate the mouth of these creeks. A downstream station (Station 1) was located near the downstream end of the first sampling stratum created for the previous study while Station 3 was located near the upstream boundary of the upper stratum. The fixed station location for the middle stratum was located in the center of that stratum (Figure 2-1).

Figure 2-1. Location of six creeks identified for sampling with example of fixed station sampling for South Creek inset.

Details of the sampling methods are provided in the Quality Assurance Project Plan (QAPP) provided in Appendix A.

2.2 Dissolved and Total Nutrients

Abbreviations for water quality constituents measured during this study are provided in Table 2-1.

Table 2-1. Abbreviations for measured water quality constituents.

CDOM	Colored dissolved organic matter	PC	Particulate Carbon
CHLA	Water Column Chlorophyll a Corrected for Pheophytin (µg/l)	PN	Particulate Nitrogen
DIN	Dissolved Inorganic Nitrogen	PP	Particulate Phosphorus
DO	Dissolved Oxygen (mg/l)	Psu	Practical Salinity Units
DO%	Dissolved Oxygen (% Saturation)	TDN	Total Dissolved Nitrogen
mg/l	Milligrams per Liter	TKN	Total Kjeldahl Nitrogen (mg/l)
NH3	Ammonia (mg/l)	TN	Total Nitrogen (mg/l)
NO23	Nitrite-nitrate as N (mg/l)	TP	Total Phosphorus (mg/l)
OPO4	Orthophosphorus (mg/l)	µg/l	Micrograms per Liter
OrgP	Organic Phosphorus (mg/l)	uS/*cm*	microsiemens

**** A "D" preceding any nutrient in the text indicates that the constituent is reported as the dissolved fraction.**

The analytical methods for nutrients are listed in Table 2-2. Ammonia (DNH3), nitrite-nitrate (DNO23), and orthophosphorus (DOPO4) were preserved via freezing instead of acidifying. This avoided additional potential for contamination from acid addition and enabled improved detection limits and analytical range (Bran+Luebbe/Seal, 2005a, 2005b, 2010). Absorption of chromophoric dissolved organic matter (CDOM) was determined with a spectrophotometric scan of a filtered (0.45 µ) sample, at multiple wavelengths. CDOM analyses are analogous to that for True Color and absorption can also be reported in PCU by scanning platinum-cobalt standards. Multiple wavelengths for color permitted the calculation of humification indices of CDOM and allowed evaluation of color as a proxy for dissolved organic carbon. CDOM samples were frozen according to Conmy (2008). Total dissolved N, Total dissolved P, and suspended particulate P were determined via methods of Solorzano and Sharp (1980a, 1980b) with modifications for segmented flow analysis. Inorganic and total N and P were analyzed according to Dixon et al., 2014a; Dixon et al., 2014b; and Dixon and Hounshell, 2013 based on EPA methods (Murphy and Hick, 1986). Particulate C and N were determined via high temperature combustion and chromatographic separation of combustion products on an industry standard CHN analytical instrument. Methods are in accordance with EPA 440.0 (Zimmerman et al., 1997). Sediments were analyzed for total kjeldahl nitrogen (TKN) and total phosphorus (TP) via EPA methods. Though sediment NO23 was not analyzed, total kjeldahl nitrogen was considered equivalent to sediment total nitrogen for the purpose of computing sediment stoichiometric ratios. Previous experience indicated that NO23 is typically on the order of 10^{-5} of sediment TKN. Sediments were also analyzed for percent moisture (to allow sediment data to be reported on a dry weight basis) and percent organics. Percent organics were used to compute a proxy for sediment carbon based on literature values. Sediment grain size was determined via laser particle size analyzer (Coulter, 1994), and provide estimates of sediment porosity, relevant to measures of sediment flux. Grain size distribution statistics of standard deviation, skewness and kurtosis were calculated using geometric statistics (Coulter, 1994).

Table 2-2. Analytical methods for nutrient analyses. * - NELAP certification of method.

Analyte	**Reference**	**Method**
WATER		
Turbidity*	Standard Methods 2130B (2001, 2011)	Nephelometric
Chlorophyll *a* (free of pheophytin)*	EPA 445.0 (1997)	Acetone extraction, fluorometry
Nitrite-Nitrate-N, Diss.	Bran+Luebbe/Seal (2010)	Cd reduction, diazotization
Ammonia-N, Diss.	Bran+Luebbe/Seal, (2005a)	Salicylate, Berthelot
Phosphorus-ortho P, Diss.	Bran+Luebbe/Seal, (2005b)	Molybdate, ascorbic acid
CDOM Absorption [Color, True (Diss.)]	Mitchell et al., (2000)	Absorption at 465, 365 nm [relative to PCU standards]
Total N, Diss.	Solorzano and Sharp (1980a), modified for segmented flow	Alkaline persulfate digestion, Cd reduction, diazotization
Total P. Diss.	Solorzano and Sharp (1980b), modified for segmented flow	Ash 450-500ºC, molybdate, ascorbic acid
Total N, Particulate	EPA 440.0 (1997), Thermo Electron, 2004	High temp combustion (FlashEA1 1112 Elemental Analyzer)
Total C, Particulate	EPA 440.0 (1997), Thermo Electron, 2004	High temp combustion (FlashEA1 1112 Elemental Analyzer)
Total P. Particulate	Solorzano and Sharp (1980b), modified for segmented flow	Ash 450-500ºC, molybdate, ascorbic acid
SEDIMENT		
Total Kjeldahl Nitrogen	351.2	Kjeldahl digestion, salicylate
Total Phosphorus	365.4	Kjeldahl digestion, molybdate, ascorbic acid
% Moisture, Organics*	Standard Methods 2540G (1997, 2011)	Dry at 103-5ºC, Combustion at 550ºC
Grain size	Coulter (1994)	Particle size analyzer (laser)

2.3 Stable Isotope Analysis

Water samples for suspended particulate organic matter [SPOM] and macroalgae samples (when available) for isotope analyses were collected at each station during four different sampling events, placed on ice, and returned immediately to the lab. Zooplankton and macro-detritus was removed from the sample by pre-filtering water through 150µm mesh. After pre-filtering, the water sample was filtered through an ashed (450°C x 4 h) 47 mm glass fiber filter (GF/F) at low pressure (5 in. Hg vacuum). Samples were treated with 1N HCl to remove carbonates and rinsed thoroughly with distilled water. After filtering and rinsing, samples were stored in a desiccator until analyses. All of the collected macroalgae was dried at 55°C for 48 hours. Dried tissues were powdered (mortar and pestle) and stored in a desiccator prior to isotopic analysis. All samples were sent to the stable isotope laboratory at the University of South Florida for processing. δ15N and δ13C values were measured using a ThermoFisher Scientific Delta V Advantage Isotope Ratio Mass Spectrometer. Carbon and nitrogen isotope values are reported in conventional per mil notation (‰) relative to Pee Dee belemnite limestone and nitrogen gas in air. Carbon and nitrogen isotopic composition of primary producers reflects the assimilated nutrient sources and the carbon metabolic pathway used (Peterson and Fry, 1987).

2.4 Macroalgal Surveys, Stream Morphology, and Canopy Cover

Qualitative macroalgal surveys were conducted during each water quality sampling, using an assessment technique similar to that of Lyons at al. (2009). Assessment was performed at the three water quality locations within the tidal reach and at two intermediate locations roughly equidistant between each water quality station. Macroalgae were not assessed at the freshwater station. Observations were made on both sides of the channel using a four-tined garden rake on both banks of the creek to determine algal presences. Data allowed for the quantitative assessment of the frequency of algal presence and the collection of algal samples for stable isotope analysis.

Stream morphologies for the estimation and comparison of approximate residence times were extracted from ArcGIS products associated with the riparian habitat, bathymetry, and sediment quality evaluations performed during a previous study (Eilers 2013). Bathymetric data was collected using a 12' johnboat equipped with a small outboard motor and a Lowrance HDS 5 Gen2 HD Fish-finder with side scan sonar and GPS mapping system. Data collection began at the stream mouth and continued upstream as far as navigable. The data were post-processed in ArcGIS to create a raster file of depths. These cross sectional vertical profiles were site verified at three locations in association with the fixed station locations to verify the utility of using these data for generating volumetric estimates of the sampling area associated with each creek.

Canopy cover was estimated using a combination of methods including qualitative estimates from field surveys, ITree Software, and site visits using mobile cameras equipped with fisheye lens (Tichy, 2015). These methods were compared to provide a qualitative estimate of the relative difference in light climate among creeks.

2.5 Rainfall Estimates

Rainfall estimates for each of the six creeks were calculated using NEXRAD (next Generation Radar) data clipped to the creek watershed boundary (Figure 3-35 left). NEXRAD data was provided by the Southwest Florida Water Management District (SWFWMD) from data collected by the National Weather Service and summed daily for each pixel to calculate a daily rainfall total for each pixel. The Standardized Precipitation Index (SPI: McKee et al. 1993) was used to characterize rainfall droughts and surpluses over a variety of time scales from 1 to 60 months. The SPI is a probability-based index that considers only precipitation and not evapotranspiration or other seasonal effects, but is useful as a characterization of drought over various time scales. The probabilities are standardized so that an index of zero indicates the average or median precipitation amount. A negative index value indicates lower than normal conditions, and a positive index indicates above normal conditions. This procedure was implemented in R statistical software(R Core development Team, 2014) using the SPEI package (Vincente-Serrano 2010).

2.6 Statistical Methods

Statistical methods included general linear mixed effects models, conditional probability analysis, quantile regression, and nonparametric multivariate analysis.

General linear mixed effects models were used to evaluate differences in water quality constituents among creeks and years with an interaction term to assess changes between studies within a creek while accounting for the potential correlation that existed from taking multiple samples within the same creek on the same date (i.e., correlation among stations within date) (Clark 2007). The model allows for statistical inference to account for correlated errors as specified by the error covariance (R) matrix. Compound symmetry was selected to define the R matrix of the model. Data were natural log transformed prior to analysis. Because the geometric average is a result of exponentiating the natural log transformed mean, the evaluation based on natural log transformed values are essentially equivalent to evaluating the differences in geometric averages as defined by State water quality standards.

The model as specified is:

$$Y_{ijk} = a + B_1 * X_j + B_2 * X_k + B_3 * X_{jk} + e_{ijk} + e_{ij}$$

Where:

a=grand intercept

X_j = Creek Effect

X_k = Year Effect

X_k = Year*Creek Interaction

B_{1-3} = Level specific means relative to a reference

e_{ijk} = Random normal error component

e_{ij} = Error component expressing correlation among observations collected in the same creek on the same date

The model was implemented using the GLIMMIX procedure in SAS 9.4 (SAS Institute 2014).

Decision trees are a class of methods used for data mining and predictive analysis (Rokach and Maimon 2008). Statistically based decision trees have been successfully used to assist in the development of numeric nutrient criteria for lakes in Michigan (Soranno et al., 2008). Conditional inference trees (Hothorn et al., 2006) and ensemble models, such as RandomForest (Brieman 2001), are types of decision trees used to identify a set of classifiers that maximize the difference in the response distribution using binary splits of the data. All of these models fall under a category of models known as conditional probability analysis. We conducted decision tree analysis using the R software language (R Core Development Team 2014) and specifically using the PARTY (Hothorn et al., 2006) and RandomForest (Brieman 2001) packages in R. These packages recursively partition the response variable into binary splits ("nodes") based on statistical relationships among a host of predictors. The partitioning process iteratively searches for a point in the predictor variable that maximizes the difference in the distribution between two groups of response data. The point in the stressor variable at which the p value is minimized, after adjustment for multiple comparisons, is assigned as the breakpoint defining the split of the response variable into 2 groups. Once the first split is made, the process continues the subsequent nodes conditional on the first split. Hence, the term “conditional inference” or “conditional probability analysis” that has been popularized recently by the EPA as a potential approach for establishing numeric nutrient criteria. This class of decision tree is applicable to all kinds of regression problems, including nominal, ordinal, numeric, censored as well as multivariate response variables and arbitrary measurement scales of the covariates (Rokach and Maimon 2008). The random forest implementation of the conditional inference tree uses bootstrap aggregation of 1000’s of model runs based on random subsets of the data. An advantage of this approach is its ability to identify the most influential variables that have increased robustness in generalizing to new data. However, the disadvantage of this approach is that the model results are not directly attributable to specific threshold values of the predictor that maximizes the difference between two groups. We used both techniques as exploratory methods to identify conditional relationships among a host of parameters that might inform more formal statistical analysis on stressor-response relationships based on a certain subset of environmental conditions.

Multivariate analyses were conducted using Primer V6 (Clark and Gorley, 2006). Multidimensional scaling of a square root transformed and normalized Euclidian distance matrix was used to portray differences among samples in multidimensional space and Similarity percentage analysis (SIMPER: Clarke and Gorley 2006) was used identify water quality constituents contributing most to the dissimilarity among creeks.

3 Results

The following sections summarize the data collected and analyses conducted to support the tidal creek nutrient management framework. In an effort to produce a relatively concise synthesis of the results, advanced statistical graphics techniques (e.g. panel plots, heat maps, decision trees, etc.) were used to summarize the results with a focus on interpretation. Detailed data summaries, including tables and plots, are presented as appendices. This chapter includes results of water quality (3.1), statistical analyses used to make inference regarding between and within creek differences (3.2); results of the algal surveys (3.3), results of isotope analysis (3.4), sediments (3.5), stream morphology and canopy cover (3.6), and rainfall (3.7). This Chapter is followed by Chapters on Inference (4), revisions to the management framework (5), and recommendations for future research (6).

3.1 Descriptive Assessment of Water Quality

Two hundred and eighty-eight (288) water quality samples were collected among the six creeks over the course of the study. Twelve samples were collected at each of the four stations within a creek (Station 1 is most downstream and Station 4 is most upstream). An example summary table of the laboratory water quality results for Estero is provided in Table 3. These tables were produced for every creek. Additional descriptive statistics, and a host of spatial and temporal distribution plots for each creek, are provided in Appendix B.

Table 3-1. Descriptive statistics for constituents sampled at the four stations in Estero (n=12 for all Parameters and stations).

	Station											
	1			2			3			4		
Parameter (Unit)	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
CDOM Abs @ 254 nm (m-1)	69.15	50.70	140.00	65.41	46.70	131.00	62.83	45.50	135.00	82.47	55.80	136.00
CDOM Abs @ 275 nm (m-1)	54.17	39.10	112.00	51.29	35.70	106.00	49.20	35.10	109.00	64.03	43.10	109.00
CDOM Abs @ 295 nm (m-1)	38.54	27.20	83.30	36.47	24.80	78.40	34.96	23.90	81.20	44.21	29.10	80.30
CDOM Abs @ 350 nm (m-1)	15.51	10.20	36.50	14.63	9.00	34.30	14.08	9.00	36.00	16.86	10.70	34.70
CDOM Abs @ 355 nm (m-1)	14.21	9.30	33.60	13.46	8.30	31.40	12.92	8.10	33.20	15.40	9.70	31.80
CDOM Abs @ 365 nm (m-1)	11.96	7.70	28.70	11.22	6.80	26.80	10.81	6.60	28.30	12.83	7.80	27.00
CDOM Abs @ 400 nm (m-1)	6.28	3.90	15.60	5.91	3.40	14.40	5.66	3.00	15.40	6.61	3.70	14.40
CDOM Abs @ 440 nm (m-1)	3.11	1.70	8.10	2.88	1.40	7.50	2.79	1.30	8.20	3.20	1.70	7.40
CDOM Abs @ 465 nm (m-1)	2.07	1.00	5.60	1.97	0.90	5.20	1.87	0.80	5.60	2.11	1.10	5.20
Carbon, Particulate (µg/L)	991.75	304.00	4,490.00	684.25	272.00	1,150.00	463.00	228.00	850.00	2,197.67	579.00	4,930.00
Chlorophyll a free of pheophytin (µg/L)	27.32	2.15	228.50	11.78	2.07	48.68	4.52	0.99	19.87	13.35	1.88	53.00
Color, True (365) (Pt-Co)	23.25	16.00	56.00	22.00	14.00	52.00	21.17	12.00	55.00	25.08	14.00	53.00
Ammonia (as N), Diss. (mg/L)	0.12	0.00	0.26	0.12	0.01	0.26	0.11	0.06	0.19	0.07	0.00	0.21
Nitrate-Nitrite (as N), Diss. (mg/L)	0.17	0.00	0.36	0.23	0.03	0.42	0.28	0.09	0.43	0.06	0.00	0.25
Nitrogen, Particulate (µg/L)	157.42	36.00	767.00	103.67	10.00	211.00	67.67	23.00	127.00	255.33	64.00	560.00
Nitrogen, Total, Diss. (mg/L)	0.75	0.51	1.01	0.77	0.51	0.96	0.78	0.56	0.99	0.69	0.49	0.95
Orthophosphate (as P), Diss. (mg/L)	0.03	0.01	0.08	0.03	0.01	0.08	0.03	0.01	0.09	0.01	0.00	0.02
Phosphorus, Particulate (µg/L)	28.09	14.30	99.20	22.10	12.90	42.50	17.56	9.80	38.90	37.97	13.50	72.30
Phosphorus, Total, Diss. (mg/L)	0.05	0.02	0.10	0.05	0.01	0.11	0.04	0.02	0.10	0.03	0.01	0.05
Salinity, Lab (PSU)	9.19	0.30	24.10	6.76	0.20	21.20	4.30	0.20	15.80	0.26	0.20	0.30
Specific Conductance, Lab (mmhos/cm)	15.14	0.65	38.00	11.34	0.47	33.70	7.42	0.49	25.90	0.55	0.31	0.71
Turbidity (NTU)	2.69	1.40	5.30	2.62	1.40	4.10	2.65	1.60	4.20	11.21	4.90	27.00

3.1.1 Common Water Quality Indicators

As expected, surface salinity increased downstream in all creeks (Figure 3-1. Creeks had a similar rate of change in salinity between stations and, at times, the entire creek could be nearly fresh even though sampling occurred predominantly during the “Dry” season. The salinity at most upstream stations was near zero for most creeks, though there were occasions when salinities above 5 PSU were recorded (predominantly West Spring but also occasionally in Frog and South Creeks). Salinity was highly statistically different among stations in all creeks which is an important consideration when evaluating changes in nutrient concentrations along the salinity gradient. The rate of change in surface salinity was not significantly different among creeks indicating the sampling scheme well represented the salinity gradient consistently across creeks. These results are further discussed in Section 4.2 of this report and provided in Appendix E, but are important outcomes for context when evaluating the descriptive results for the other constituents described in this section.

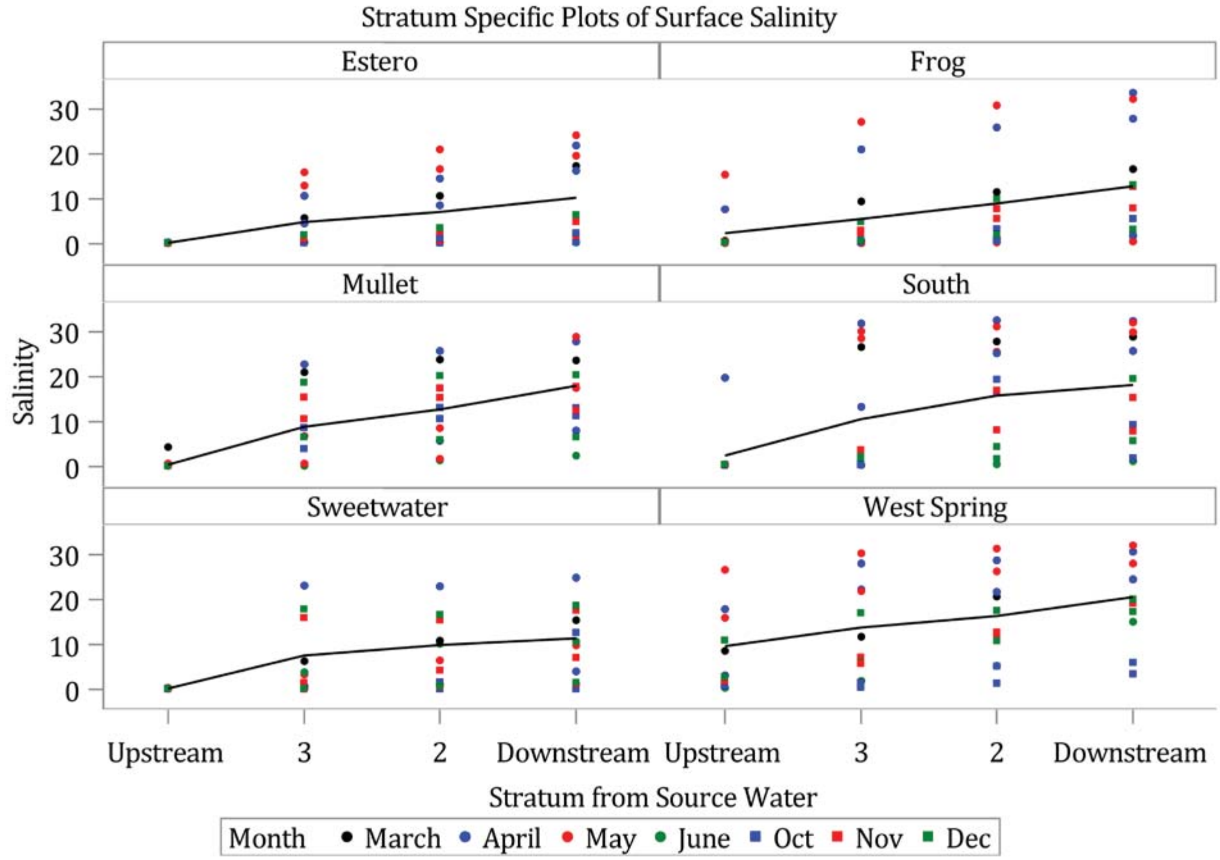


Figure 3-1. Surface salinity distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Bottom salinity distributions displayed more variability in the distribution of salinities in the downstream stations, resulting in differential rates of change in bottom salinity with distance downstream (Figure 3-2). West Spring and South Creek never became totally fresh in the most downstream stations, indicating more stratification potential in these creeks relative to the others.

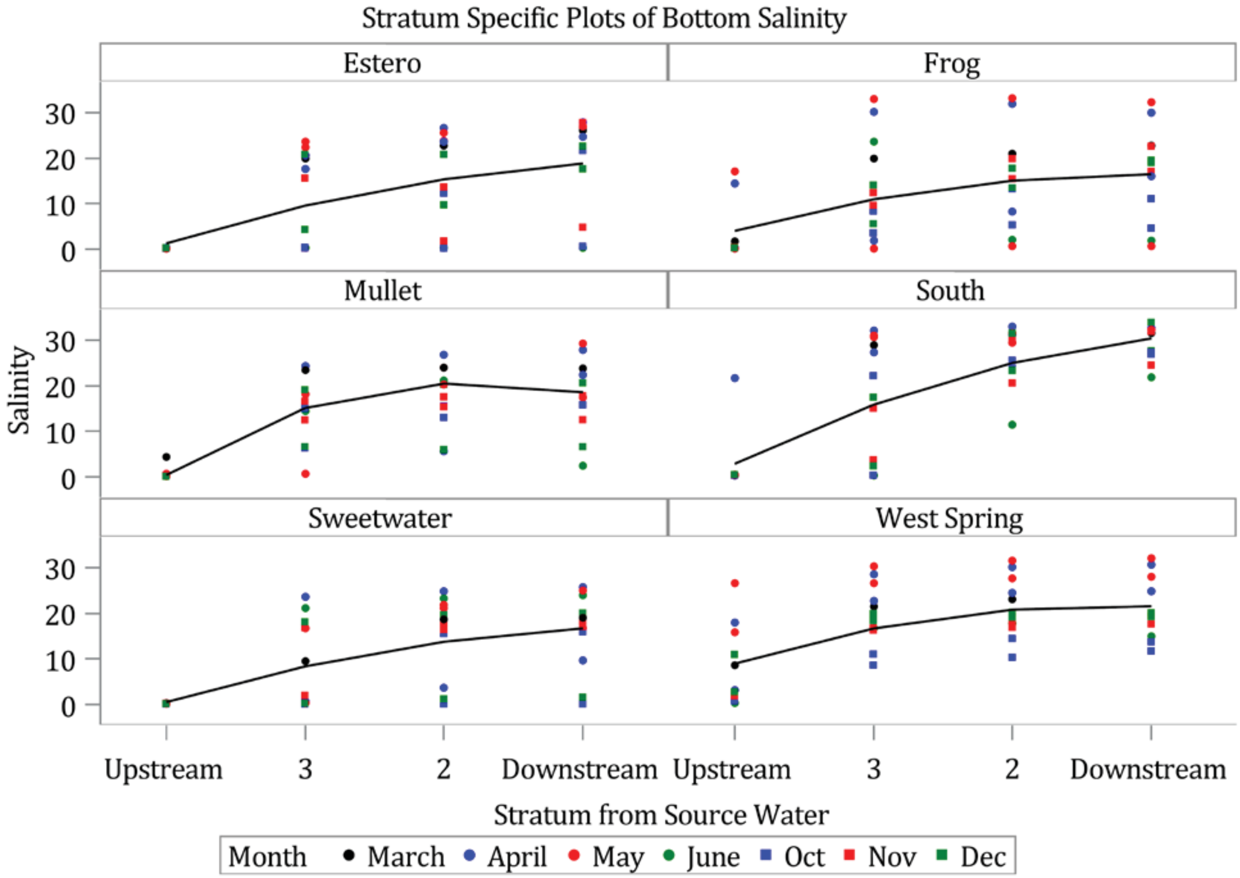


Figure 3-2. Bottom salinity distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Dissolved oxygen (% saturation) distributions were clearly creek-specific and site dependent (Figure 3-3). Upstream station had the highest DO for some creeks and the lowest DO for other creeks. For instance, in West Spring the upstream station DO tended to be lowest while the next site downstream tended to be highest. In Sweetwater, South, and Mullet Creeks, DO tended to be lower in the middle sites while Frog and Estero had similar distributions irrespective of location in the system.

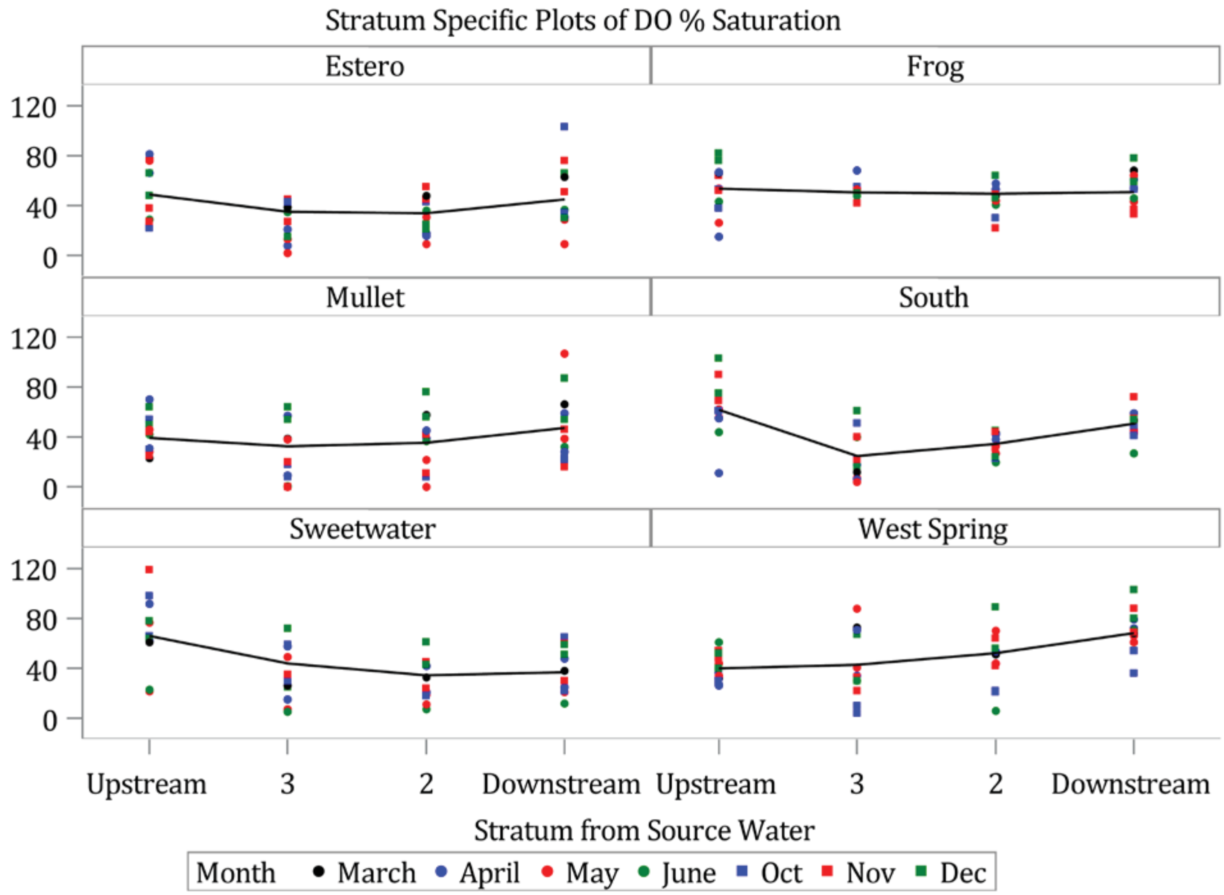


Figure 3-3. Dissolved oxygen (% saturation) distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

True color distributions were similar both within and across creeks (Figure 3-4) with average concentrations between 20-30 PCU. South Creek had the highest episodic concentrations, exceeding 60 PCU during June and October events. Though all creeks were sampled on the same dates, only Estero showed a similar deviation from the rest of the distributions for those two events.

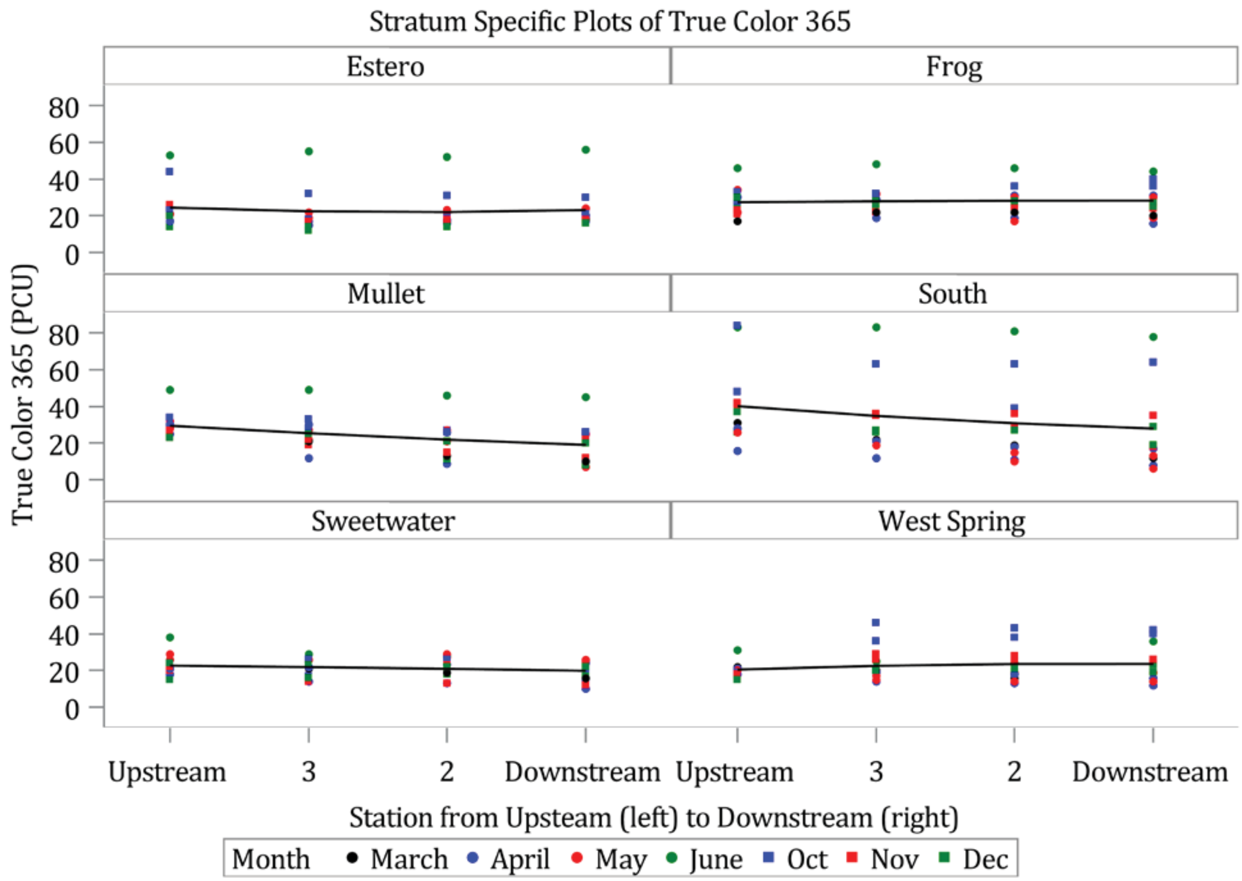


Figure 3-4. Color distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Corrected chlorophyll distributions are plotted in Figure 3-5 using the log (base 2) scale since the distribution is skewed by high chlorophyll concentrations. Chlorophyll was highly variable with no consistent spatial patterns among creeks. Temporal distributions were also variable with episodic high chlorophyll during May, June, and October and lower chlorophyll in November, December and March. The relationship between chlorophyll and nutrients and dissolved oxygen is explored further in the following chapters of this report.

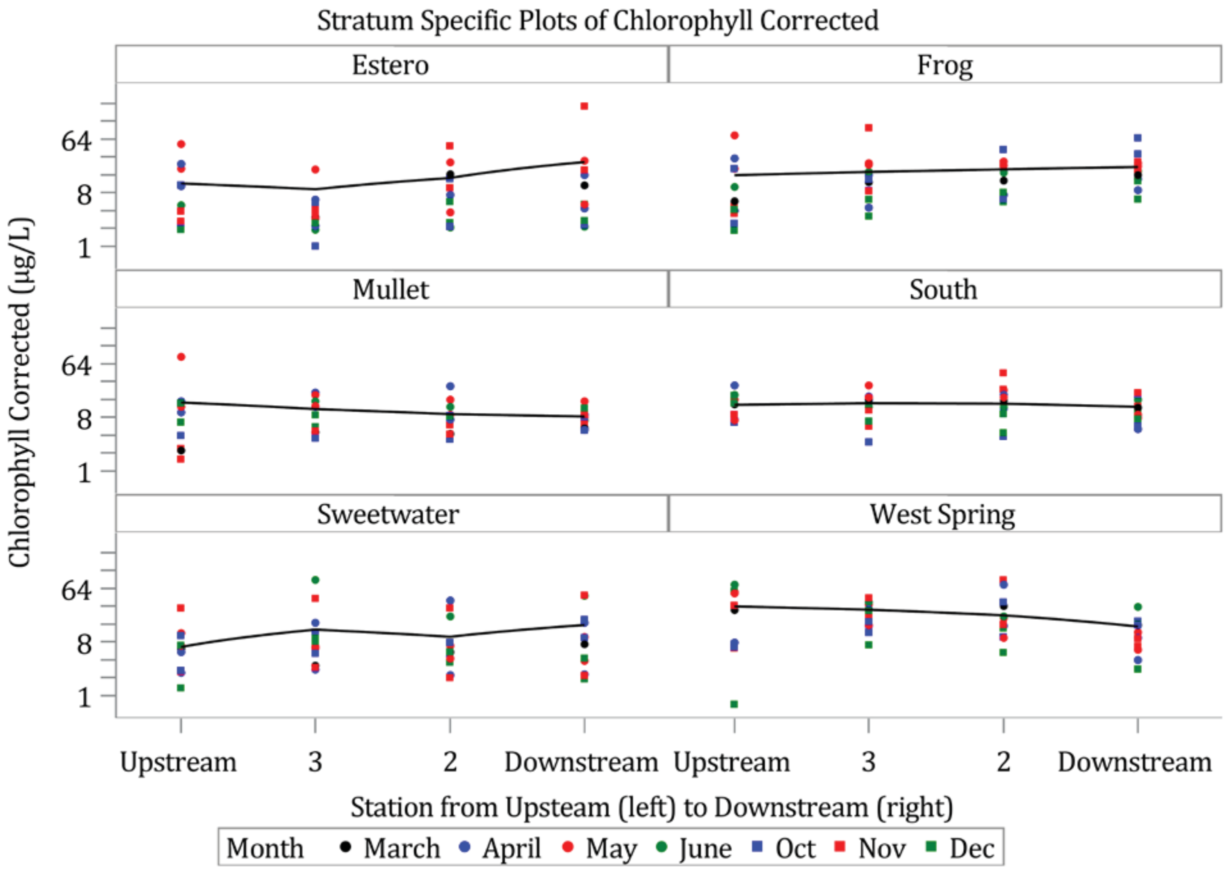


Figure 3-5. Corrected chlorophyll distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration. Note, chlorophyll expressed on natural log base 2 scale.

Total nitrogen distributions tended to decrease with distance downstream in 3 of the creeks while being stable in the other 3 creeks (Figure 3-6). Frog and South Creeks had the highest total nitrogen at the most upstream station. South Creek TN decreased markedly with increasing distance downstream while Frog Creek TN was generally stable the length of the creek. South and Frog Creeks also displayed the highest within station TN variability of all creeks.

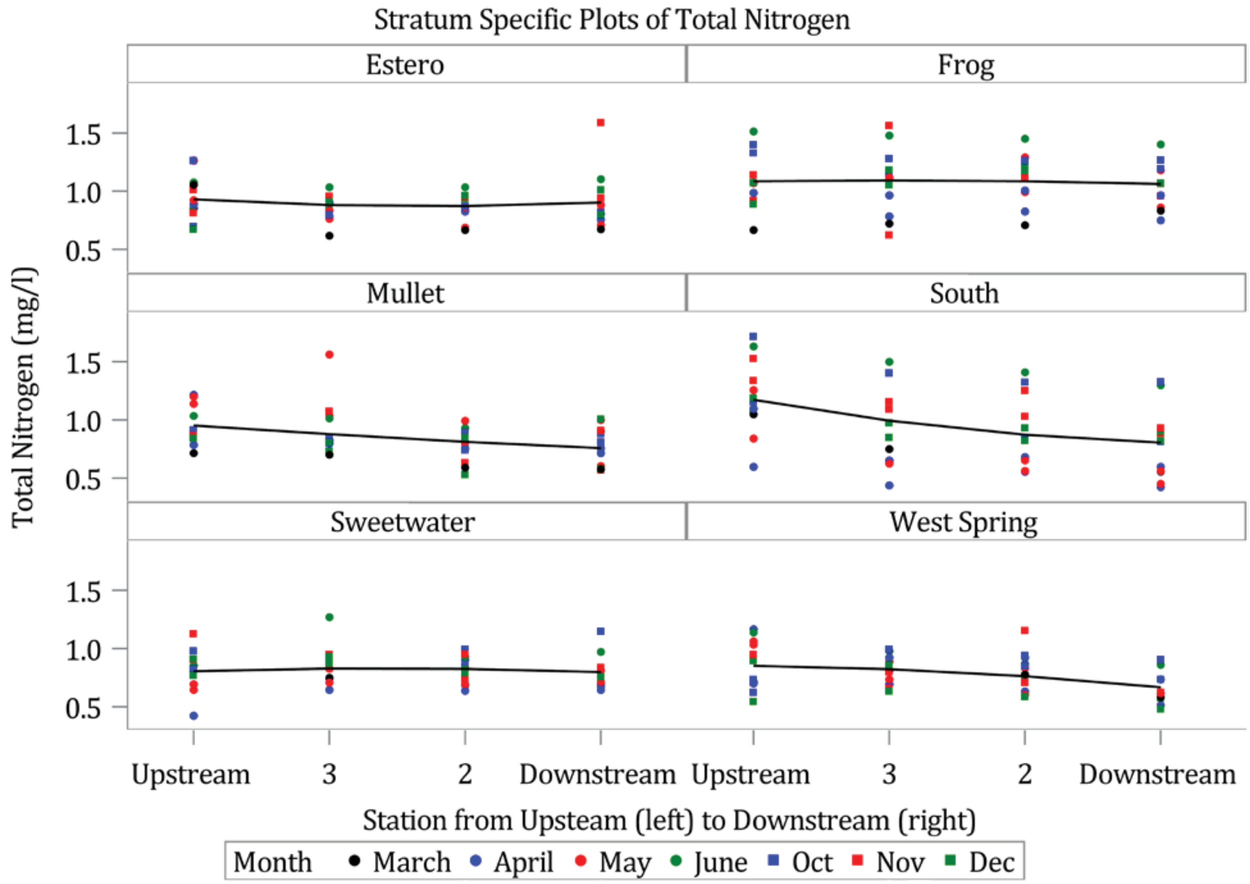


Figure 3-6. Total nitrogen distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Total phosphorus distributions (Figure 3-7) were generally stable and in some cases increased slightly moving downstream (but without the marked variability seen for total nitrogen). The exception was Frog Creek, which exceeded 1 mg/l TP at 3 downstream stations in May 2018. TP plots are also shown with a log (base 2) scale.

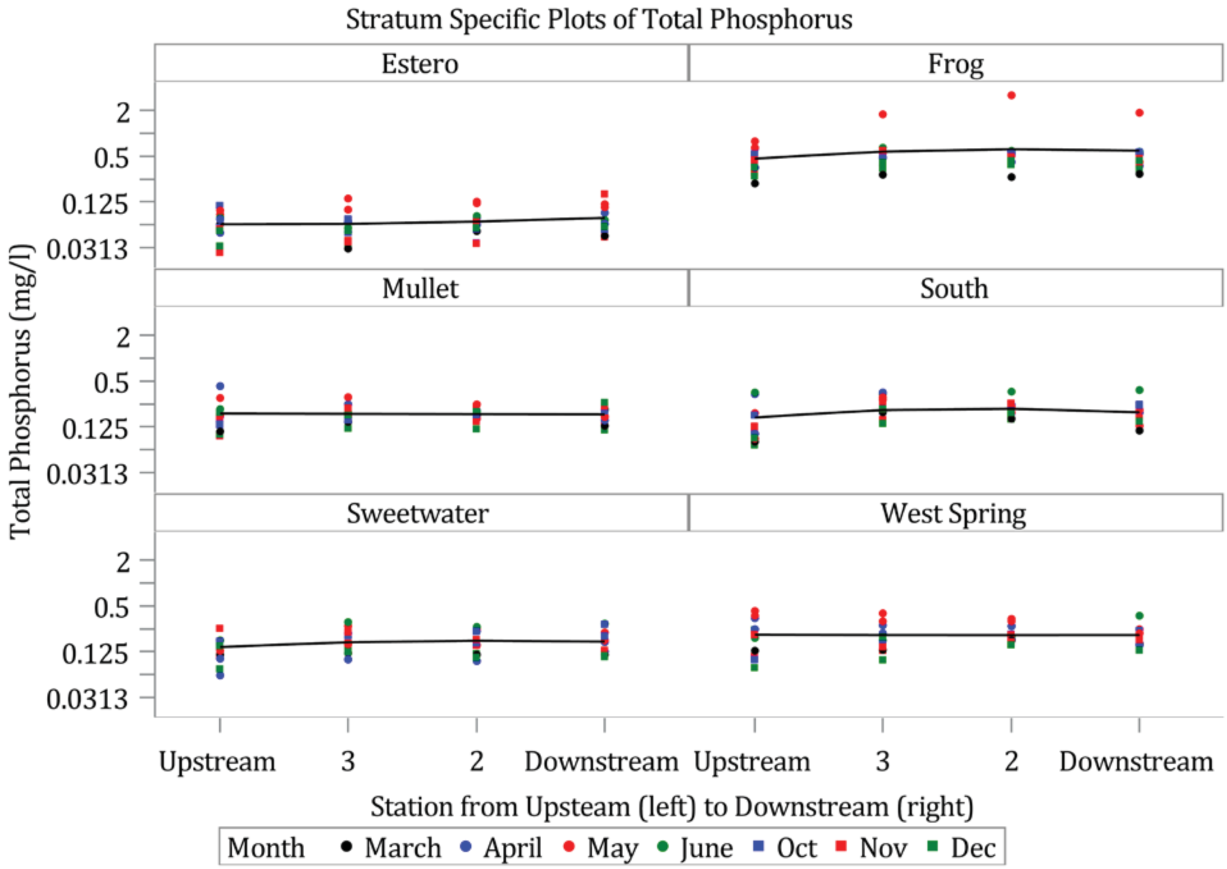


Figure 3-7. Total phosphorus (log base 2) distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

3.1.2 Dissolved Inorganic Nutrients

This study included explicit measurements of the dissolved fraction of nitrogen and phosphorus to help quantify that portion of the total concentrations most readily available for biological uptake. The dissolved inorganic nutrient forms were of particular interest. The spatio-temporal distributions of dissolved inorganic nitrogen and dissolved orthophosphate are described below.

Dissolved inorganic nitrogen (DIN) includes nitrate, nitrite and ammonia in dissolved forms. The distribution of DIN displayed high temporal variability (Figure 3-8). The spatial trends were consistently declining with increased distance downstream with the exception of Estero where the average concentration increased substantially from the headwater station to the next station downstream and then declined (except during December of both 2017 and 2018).

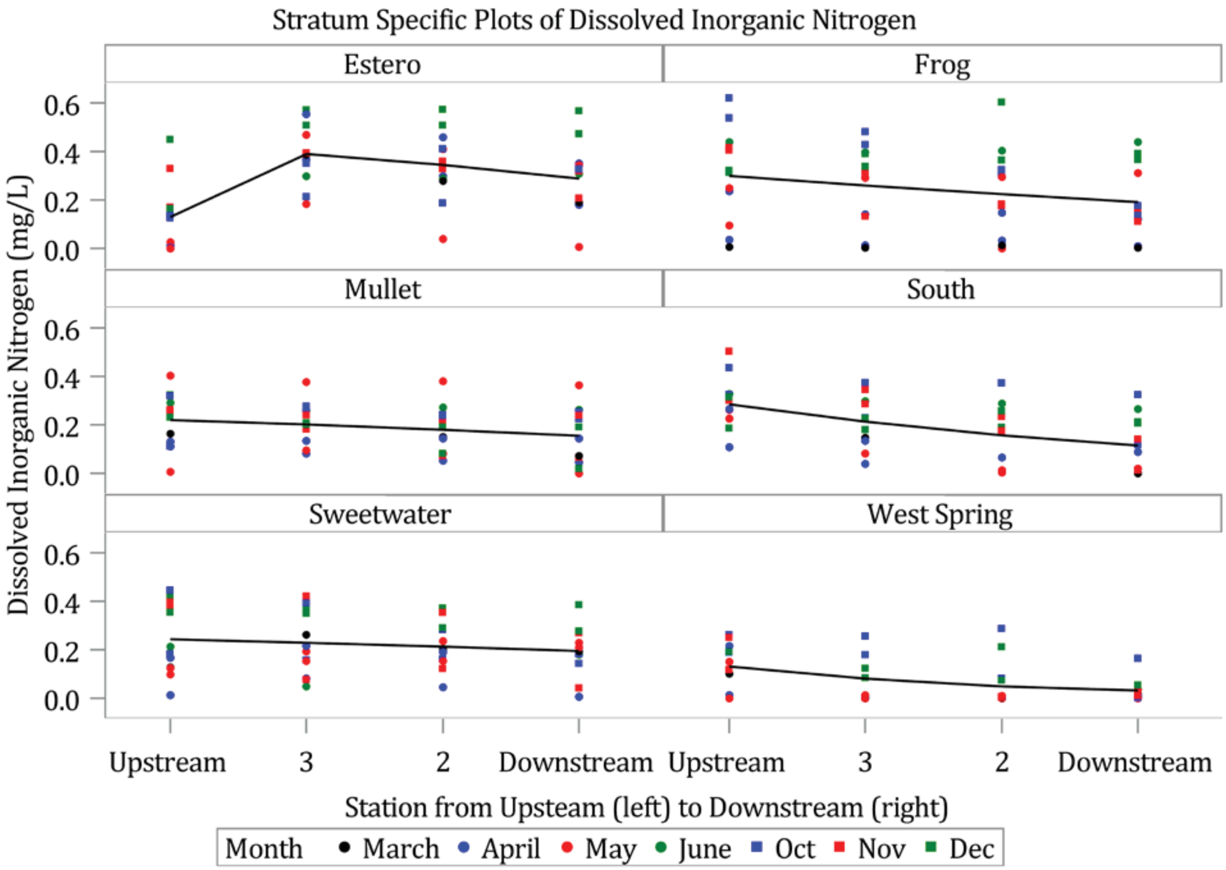


Figure 3-8. Dissolved inorganic nitrogen distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Dissolved orthophosphate distributions tended to be highest and most temporally variable in Frog Creek (Figure 3-9). Three of the 6 creeks had very little temporal variability relative to Frog Creek. There was no spatial trend in orthophosphate for any creek.

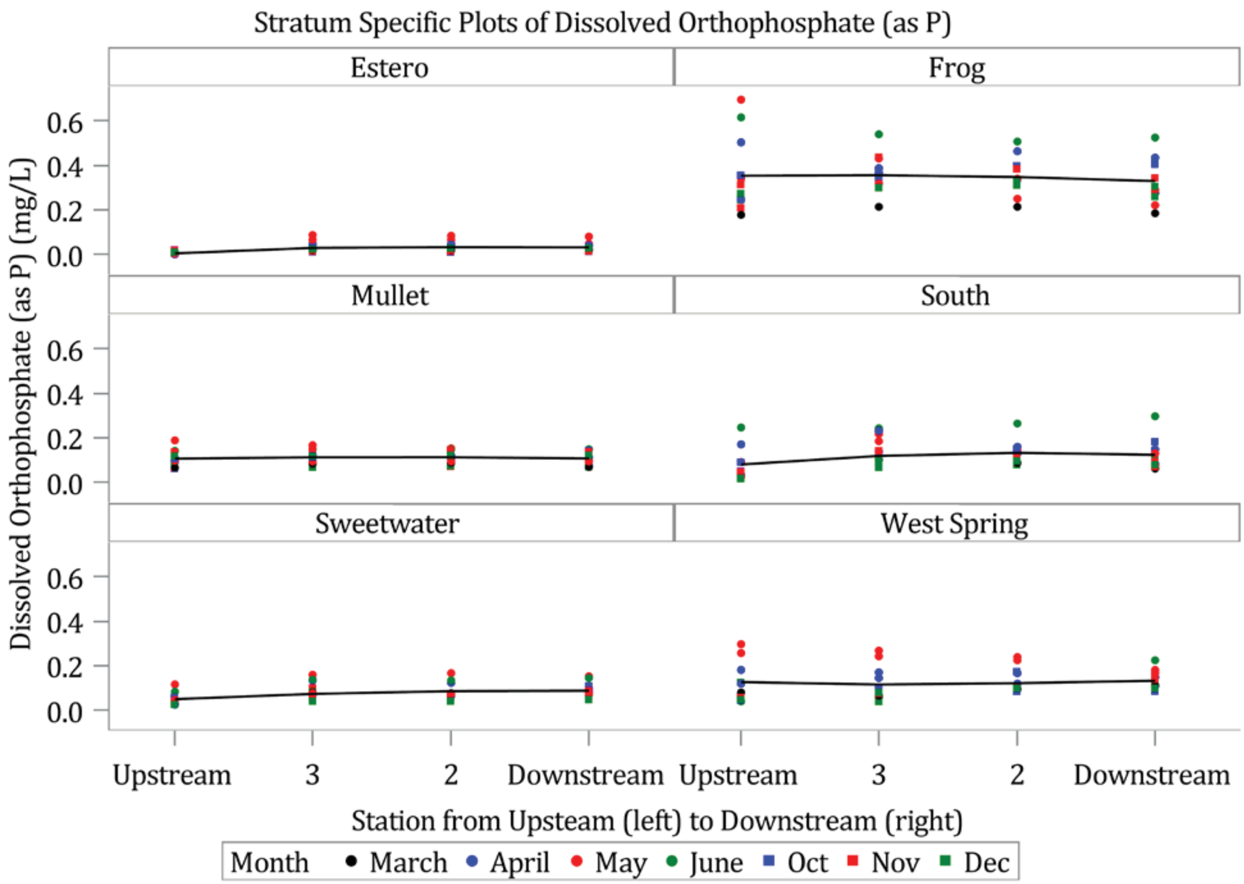


Figure 3-9. Dissolved orthophosphate distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Spatio-temporal distribution plots for all constituents are provided in Appendix C.

3.1.3 Particulate Nutrients and Carbon

Separately analyzing dissolved and total pools of nutrients allowed for the specific quantification of particulate forms of nutrients and carbon. Particulate nitrogen (PN) distributions were spatially variable both across and within creeks (Figure 3-10). Source water concentrations tended to be near 200 ug/l though the two southernmost creeks (West Spring and Estero) had the highest source water PN concentrations. West Spring PN concentrations were variable for all stations while in Estero, much lower concentrations were observed at the downstream stations. In Frog Creek, the average concentration increased with distance downstream while concentrations in the other creeks tended to be stable or declining. Episodic events of high concentrations in the downstream stations in Estero and Sweetwater Creeks increased the averages in the most downstream stations for those creeks.

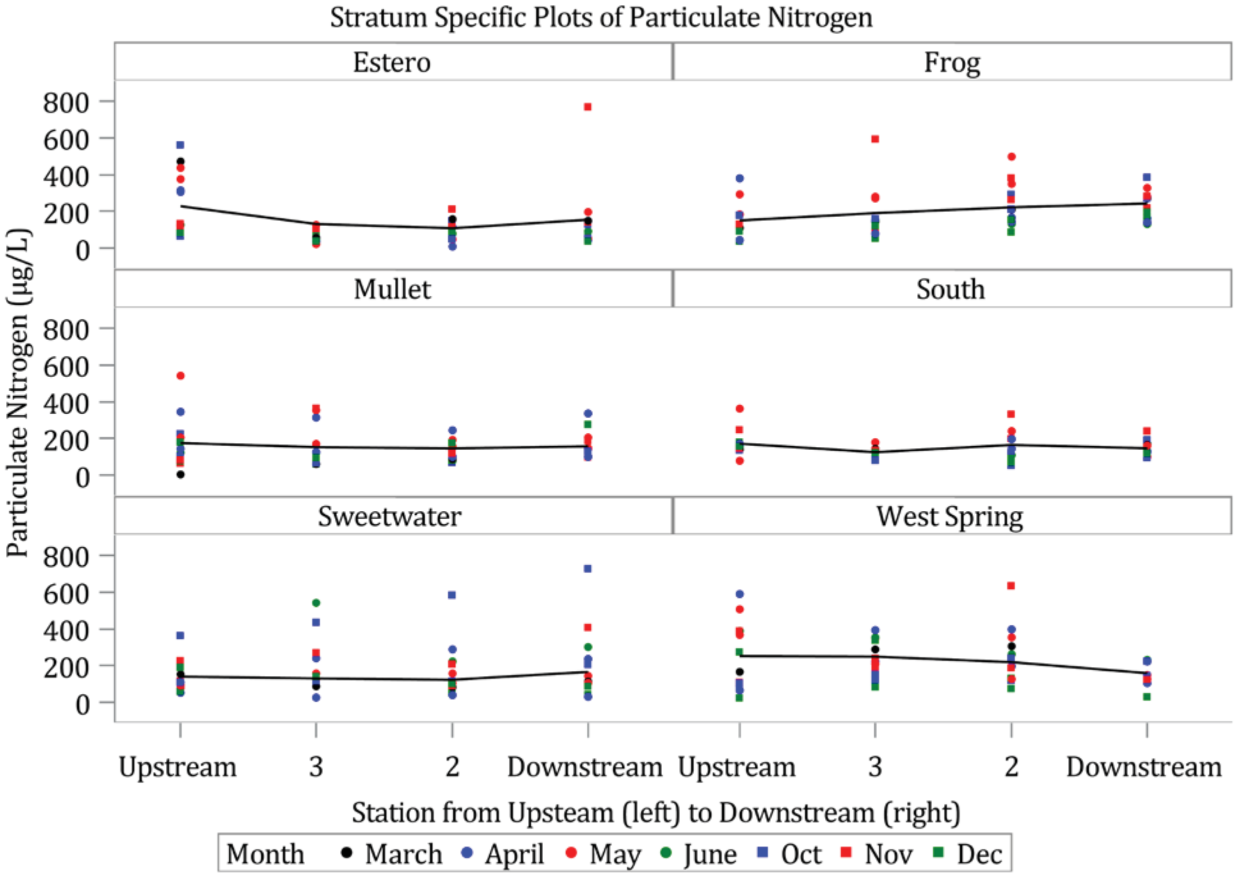


Figure 3-10. Particulate nitrogen distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

In comparison to PN, particulate phosphorus distributions were relatively stable (Figure 3-11) except for the episodic event in Frog Creek in May 2108. These plots are log base 2 scale to account for the extreme Frog Creek values.

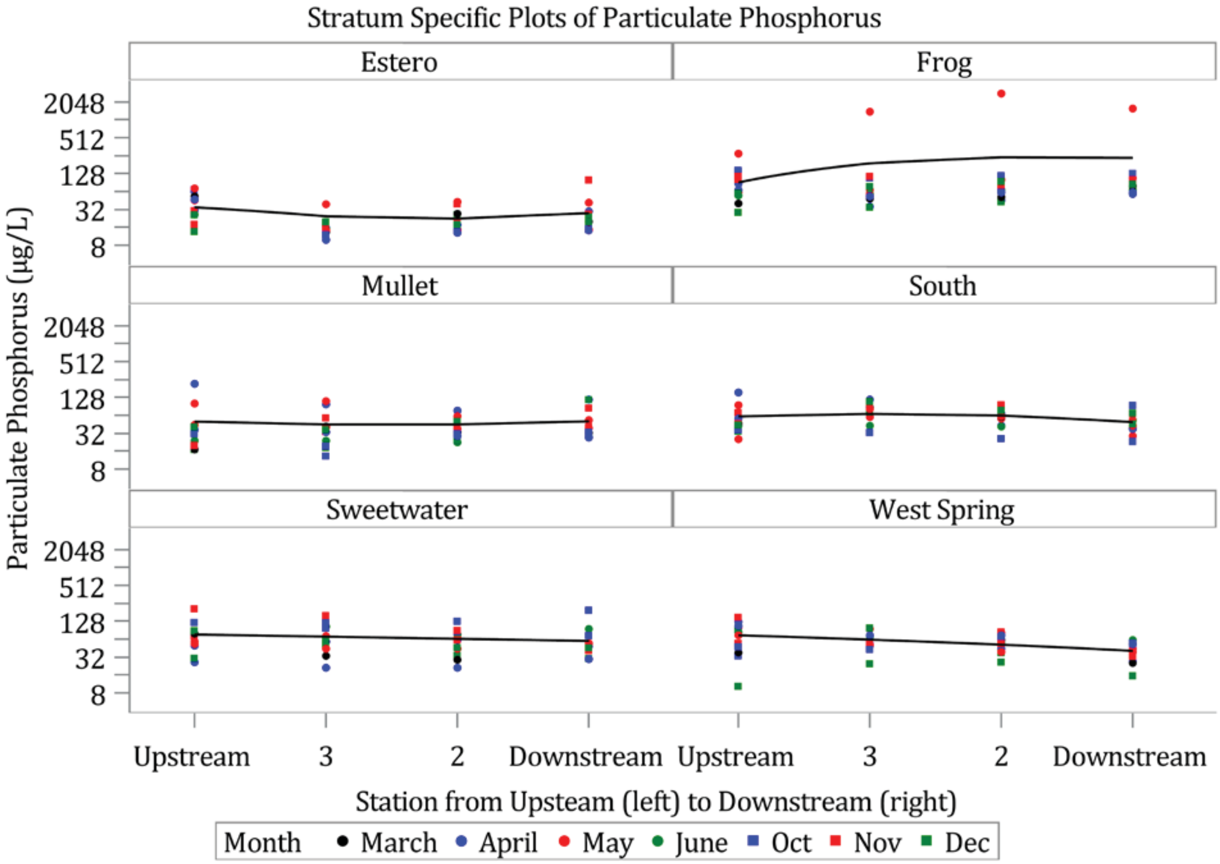


Figure 3-11. Particulate phosphorus distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

Particulate carbon distributions were creek dependent with Estero and West Spring displaying the highest variability in the source water and average concentrations generally stable within the tidal section (Figure 3-12).

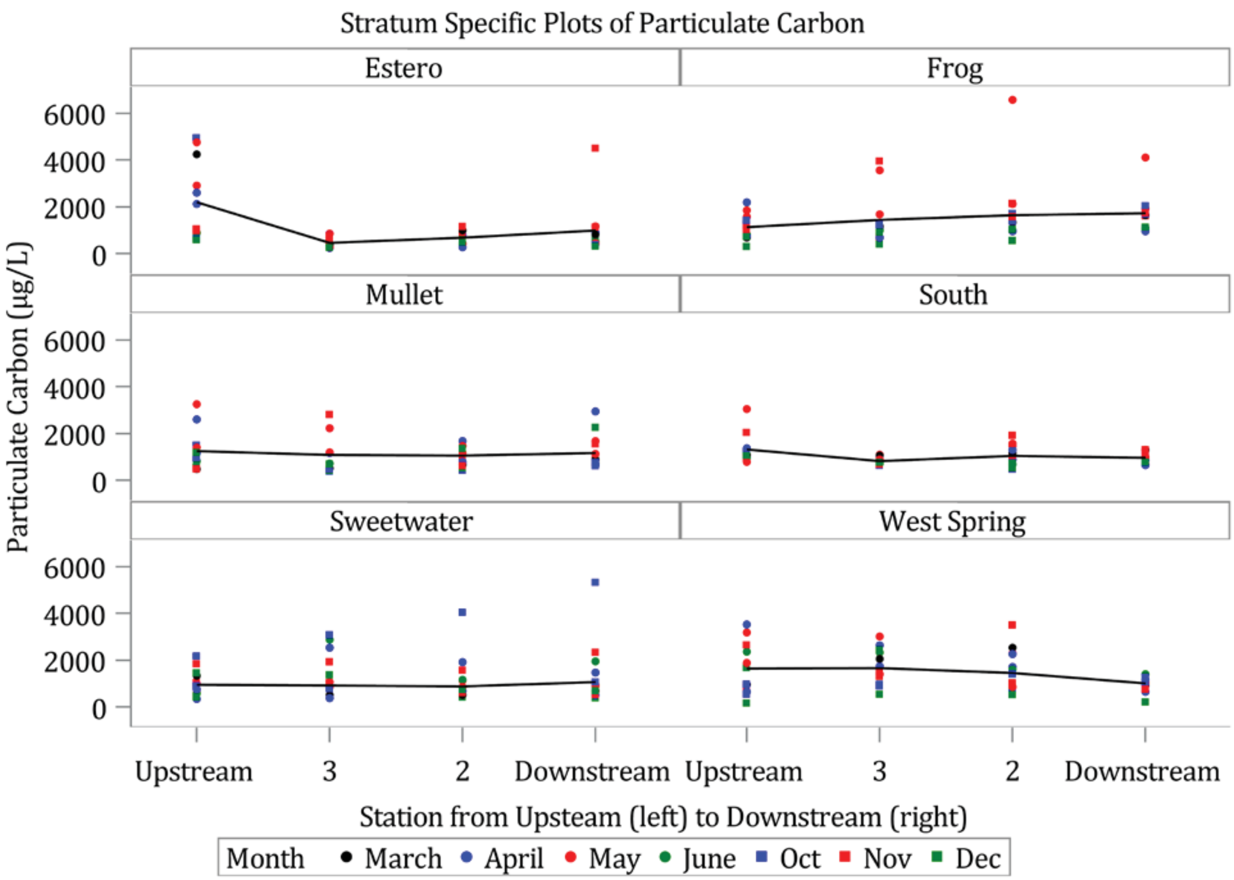


Figure 3-12. Particulate carbon distributions for the 6 creeks sampled between 2017 and 2018. Plots are oriented from upstream to downstream with color coding to define sampling month and shape to define season. Solid line is a locally weighted smoother of the average concentration.

3.1.4 Multivariate Differences among Creeks

One way to summarize the differences in water quality among creeks is the use of the "Heatmap" (Figure 3-13). The values in this heatmap are grand median concentrations across stations, normalized to the highest median value of any creek for that constituent. For example, South Creek had the highest median CDOM_275 value of any creek and therefore is valued at 1 while the other creek values represent the proportion of that highest value. The color ramp (blue to red) in the heatmap represents the deviation from the highest value (red). For example, DIN concentrations in West Spring were only 5% of DIN concentrations in Estero and therefore West Spring is assigned a blue color gradient. Conversely, for CDOM 275, there was less difference relative to the highest creek value (South); therefore all cells are some shade of red. The heatmap results highlight the differences in phosphorus between Frog Creek and all other creeks and also highlight the differences in dissolved nitrogen species in West Spring from all other creeks.

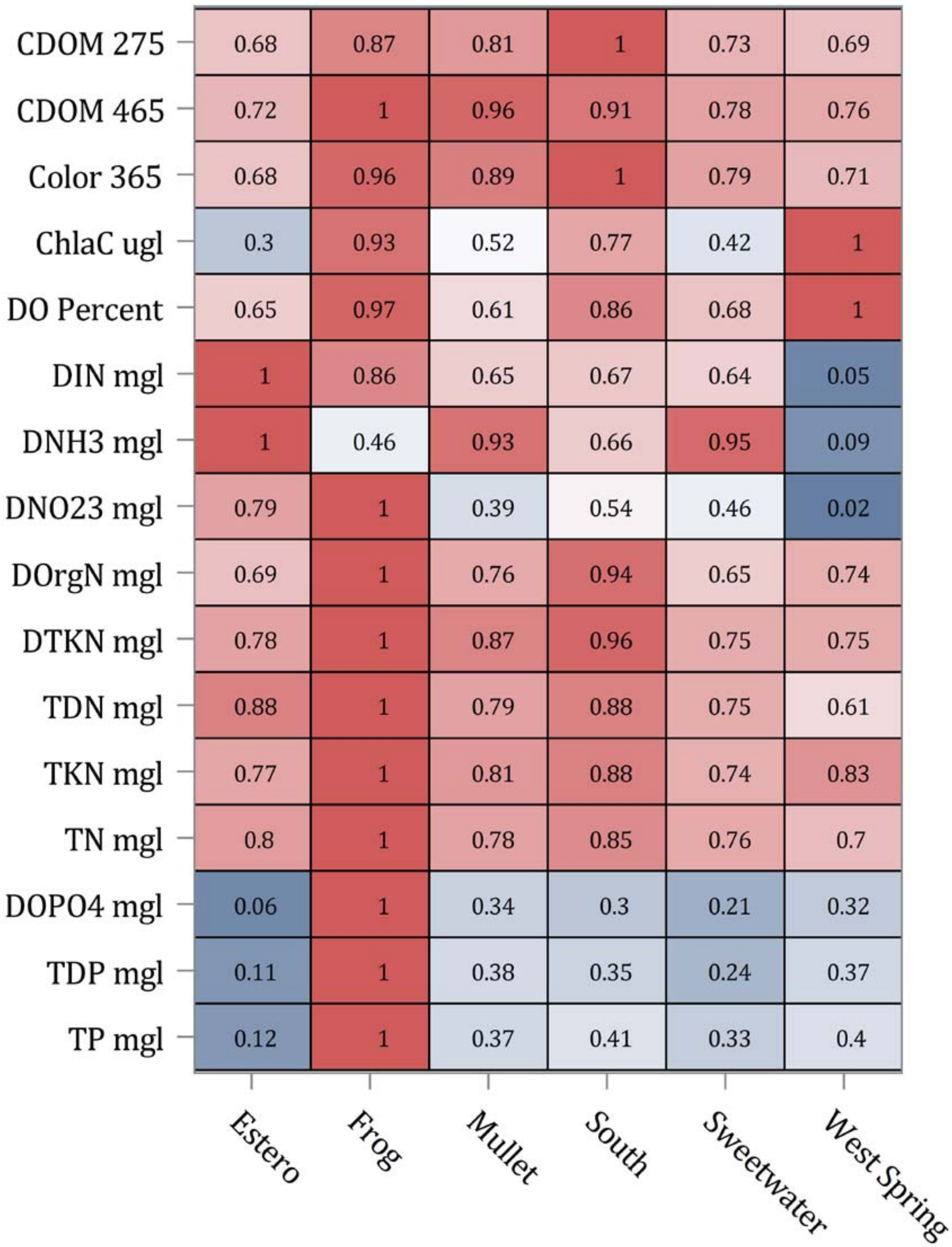


Figure 3-13. Heatmap displaying all water quality constituents examined with values representing the proportion of the highest grand median concentration for each constituent.

Heat map results are displayed separately for source and tidal waters in Figure 3-14. These maps demonstrate the relative differences, particularly for nitrogen species, between source and tidal waters.

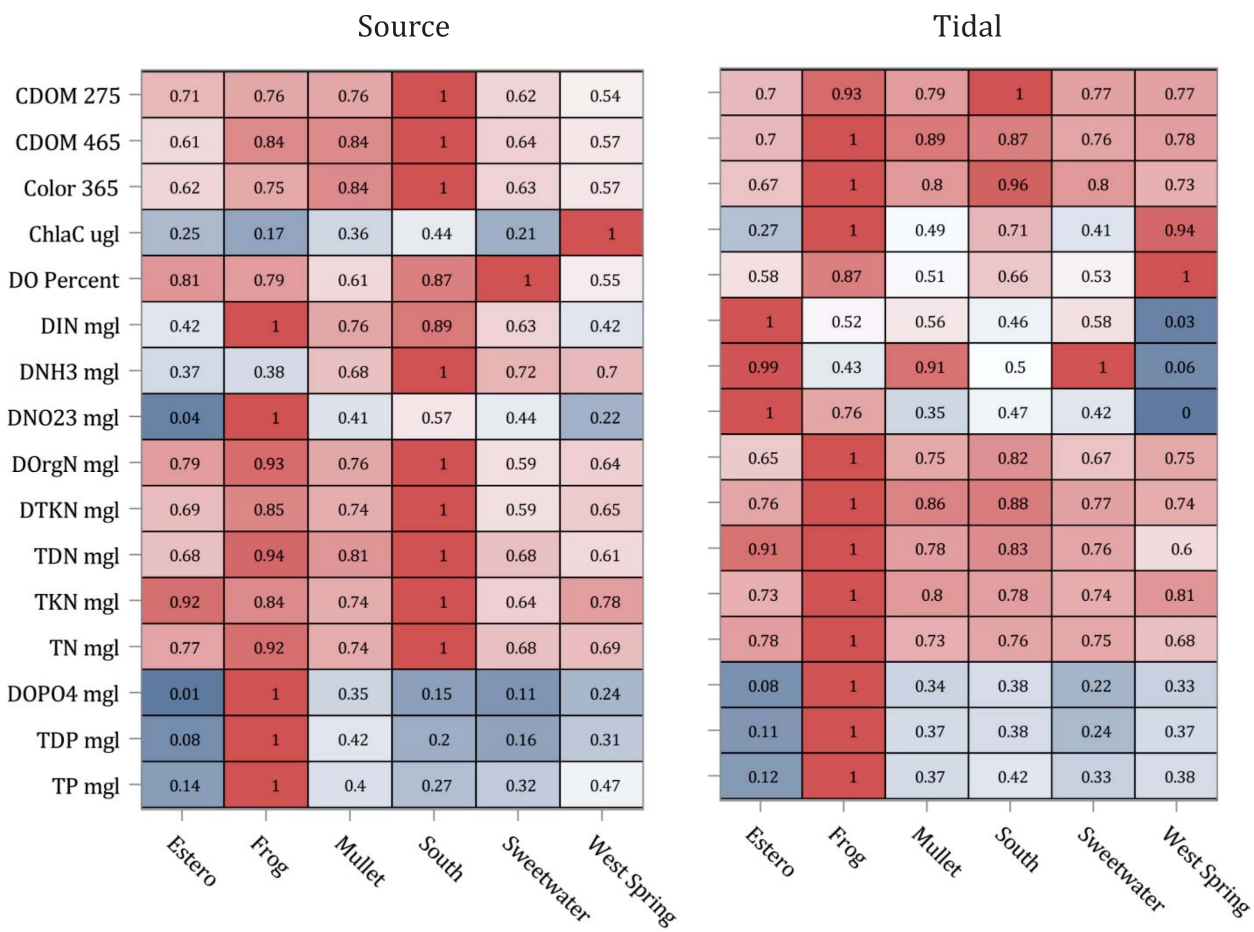


Figure 3-14. Heat map results for source water and tidal water segments within the six creeks examined for this study.

Multivariate analysis was conducted to describe the principal water quality constituents contributing to differences among the creeks. Principal components analysis and similarity percentage analysis (SIMPER) were conducted using Primer (V6: Clarke and Gorley 2006) to identify the principal water quality constituents contributing to differences among the 6 creeks. Seasonal averages for each of the 4 sites within each creek were calculated, natural log transformed, and normalized prior to principal components analysis. A resemblance matrix was generated using euclidean distance to conduct SIMPER to evaluate dissimilarities in water quality among creeks.

The results of PCA suggest three principal component axes with PC1 dominated by the TN to TP ratio (and DIN to DIP ratio), as well as carbon to phosphorus ratio and ammonia which tended to separate Estero and Frog from the remaining creeks. PC2 was dominated by dissolved nitrogen, including organic and inorganic fractions and CDOM, and resulted in more seasonal separation of creeks. PC3 was dominated by all forms of phosphorus including total, dissolved, and fractionations which separated the high phosphorus Frog Creek from the other creeks. A three dimensional graphical representation of the results of the PCA, with symbols representing creeks, is presented in Figure 3-15.

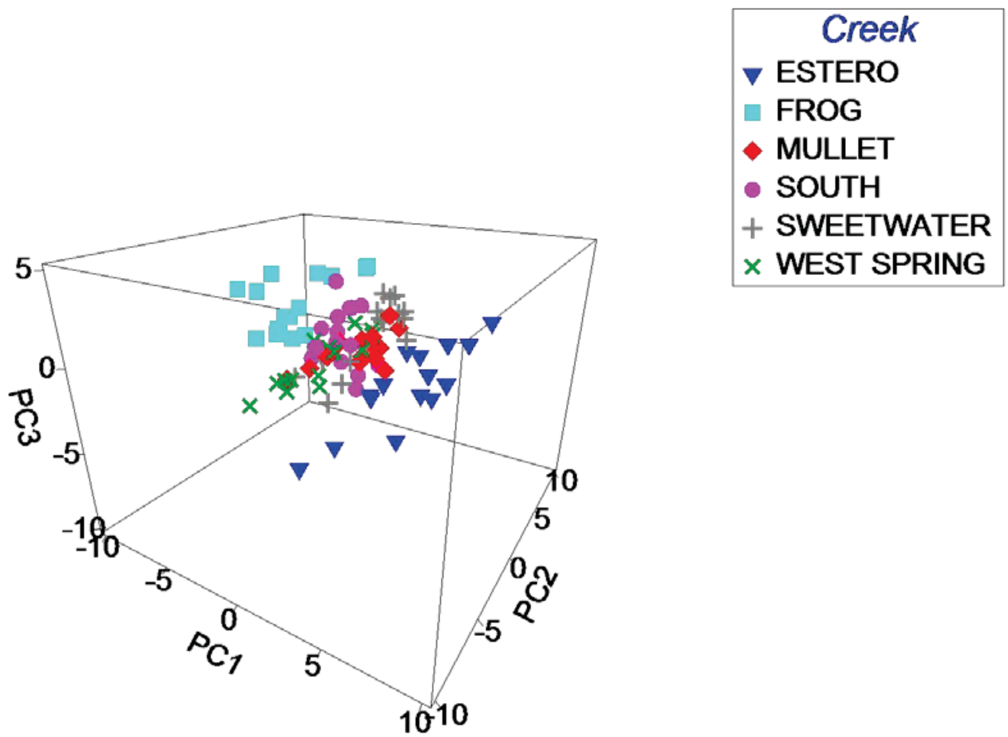


Figure 3-15. Multivariate water quality pca based on normalized euclidean distance.

SIMPER was used to evaluate the pairwise differences in water quality among creeks (Table 3-2). Results suggest dissolved ammonia (DNH3_mgl) was a principal determinant of difference between West Spring and most other creeks along with nitrogen: phosphorus ratios and chlorophyll. High phosphorus concentrations in Frog Creek were again a principal determinant in its dissimilarity from other creeks, while CDOM was the principal determinant in the dissimilarity between South Creek and Mullet, Sweetwater, and West Spring.

Table 3-2. Top 5 water quality constituents contributing to differences between creeks in pairwise comparisons.

	Estero	Frog	Mullet	South	Sweetwater
West Spring	DIN_DIP_ratio	TP_mgl	DNH3_mgl	TDN_mgl	DNH3_mgl
	DIN_mgl	Turb_NTU	PC_PN_Ratio	CDOM_275	ChlaC_ugl
	TN_TP_Ratio	TDN_mgl	TDN_fraction	Color_365	DIN_mgl
	DNH3_mgl	DOPO4_mgl	ChlaC_ugl	CDOM_465	TDN_fraction
	TDN_fraction	TDP_mgl	PN_ugl	DTKN_mgl	TDP_fraction
Estero		TN_TP_Ratio	PC_PN_Ratio	DIN_DIP_ratio	N_P_Ratio
		DOPO4_mgl	DIN_DIP_ratio	PC_PP_Ratio	PN_PP_Ratio
		TDP_mgl	TN_TP_Ratio	CDOM_465	PC_PP_Ratio
		TP_mgl	TDP_fraction	TN_TP_Ratio	TN_TP_Ratio
		PP_ugl	PC_ugl	Color_365	PC_ugl
Frog			TP_mgl	TP_mgl	DOPO4_mgl
			DOPO4_mgl	DOPO4_mgl	TDP_mgl
			Turb_NTU	TDP_mgl	TP_mgl
			TDP_mgl	Turb_NTU	DOrgN_mgl
			PP_ugl	PP_ugl	TDP_fraction
Mullet				CDOM_275	PC_PN_Ratio
				PC_PN_Ratio	TDP_fraction
				Color_365	PN_ugl
				CDOM_465	TDN_fraction
				TDN_mgl	PC_ugl
South					CDOM_275
					Color_365
					DOrgN_mgl
					DTKN_mgl
					CDOM_465

Based on the descriptive assessment provided in Section 3.1 above, the following summary is provided:

- Salinity consistently increased with distance downstream (as expected) and the rate of change between stations was consistent among creeks indicating that the sampling design was well balanced spatially.

- Dissolved oxygen (expressed as percent saturation) was spatially variable both within and between creeks and had expected temporal variation.

- Color concentrations were similar across creek and consistent within a creek without a great deal of spatial variability.

- Total nitrogen decreased with distance downstream in 3 creeks and was spatially consistent in 3 creeks. Total nitrogen was highest in Frog Creek; both Frog and South Creeks exhibited the largest within-site variation.

- Total phosphorus was generally stable temporally and spatially within creeks. Frog Creek had the highest overall total phosphorus while Estero had the lowest.

- Dissolved inorganic nitrogen generally decreased with distance downstream. Estero was the exception, where DIN tended to increase between the most upstream station and the next station downstream.

- Multivariate analysis suggests differences among creeks were due to differences in total phosphorus concentrations, TN to TP (and DIN to DIP) ratios, carbon to phosphorus ratio, ammonia, dissolved nitrogen forms, and CDOM.

The next sections describe statistical data comparisons, comparisons to data collected during the previous study, and evaluations of stable isotope, sediment and algal results.

3.2 Water Quality Comparison to Previous Study

To compare findings of the previous study to the results of this study, data from the two studies were combined and only months common to both studies were selected for comparison. As a result, data from January, July, and September were eliminated from the first study despite both studies meeting the temporal requirements established in F.A.C 62-302.531 for evaluation of annual geometric averages. Geometric averages were then calculated using the three downstream estuarine sites. For example, the geometric average total nitrogen concentrations for the 6 creeks examined in this study are provided in Figure

3-16. For convenience, the previous study is referred to as year 2014 though sampling actually began in November 2013.

An ANOVA for clustered data was conducted using a linear mixed effects model to account for the potential that observations taken on the same date with a creek were correlated. Natural log transformed individual samples were used to test for statistically significant differences in average concentration between years and creeks, with an interaction term to assess changes between studies within a creek. Because the geometric average is a result of exponentiating the natural log transformed mean, the ANOVA on natural log transformed concentrations is essentially equivalent to evaluating the differences in geometric averages. The results of the ANOVA suggest a significant difference in least squares means between Frog Creek and all other creeks (Figure 3-16) and a marginally significant difference ($p<0.07$) between the 2014 and 2017 samples. After adjusting for multiple comparisons, no other creeks were significantly different from one another. This result is illustrated by the diffogram plot of Figure 3-17 where lines in the multiple comparison plot that do not cross the diagonal broken line represent statistically significant differences.

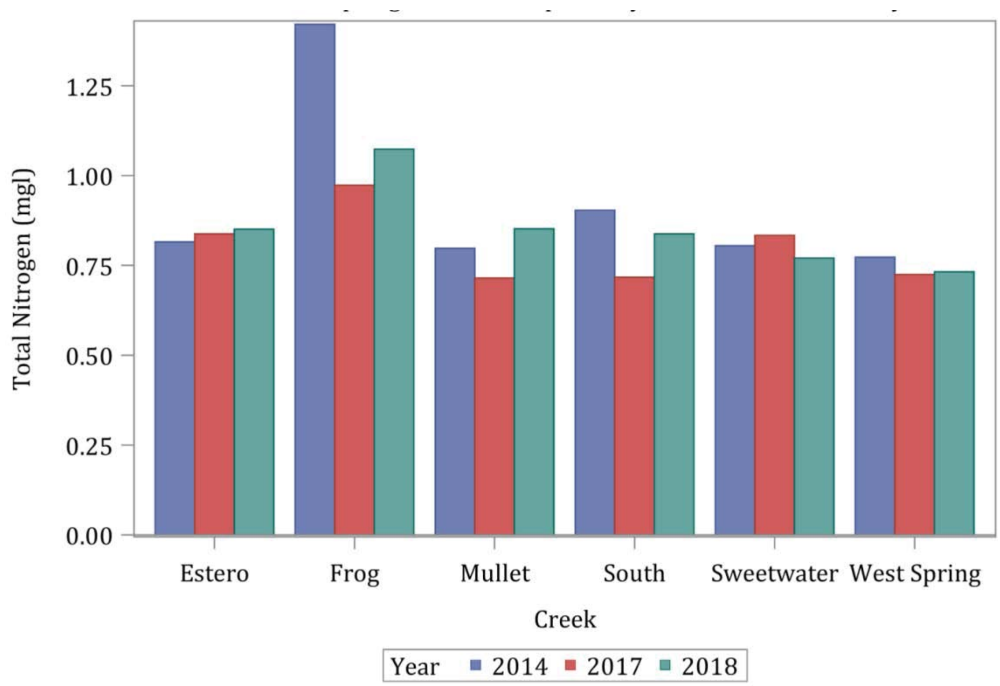


Figure 3-16. Geometric average total nitrogen distributions for the 6 creeks sampled for each year in the study. Note that Sampling for year 2014 began in November of 2013 and that no samples collected in January, July, or September were included in the calculation of the geometric averages for 2014 in order to match the temporal distribution of samples with the more recent study.

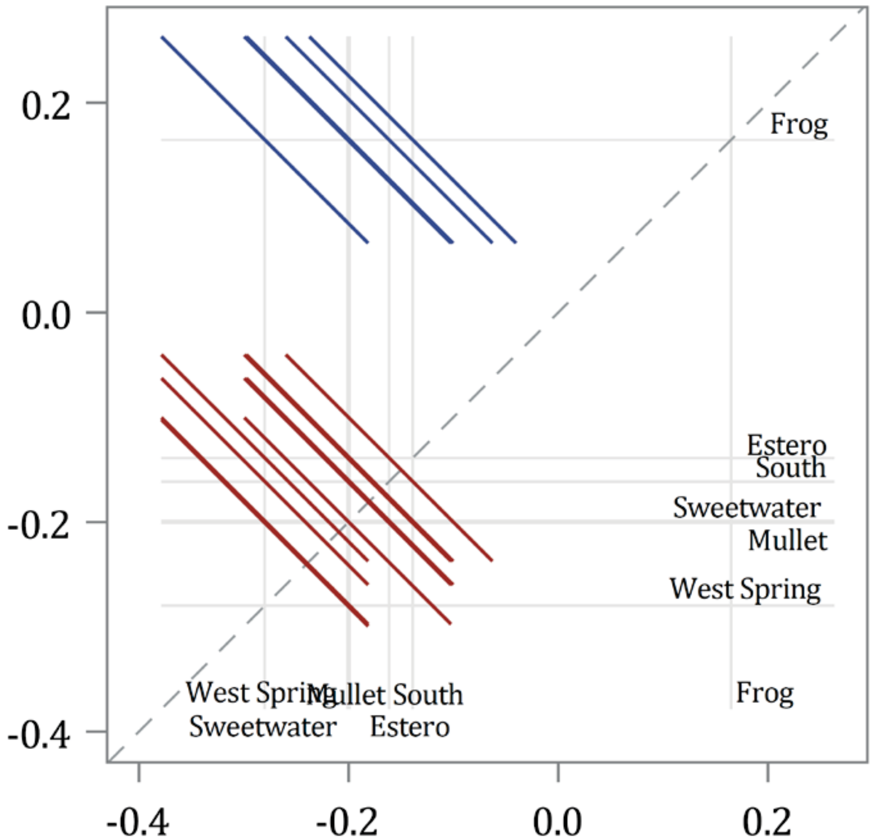


Figure 3-17. Diffogram displaying results of multiple comparison adjusted natural log scale differences between creeks. This plot suggests Frog Creek is statistically different from all other creeks in terms of natural log transformed average nitrogen concentrations and that there is no evidence to suggest that the other creeks differ in total nitrogen concentration.

Geometric average total phosphorus concentrations are shown in Figure 3-18. Estero and Frog deviated from the other creeks as described above and the ANOVA confirmed this (Figure 3-19). The Year effect and the interaction term were not significantly different for TP indicating consistency among studies in the characterization of TP concentrations.

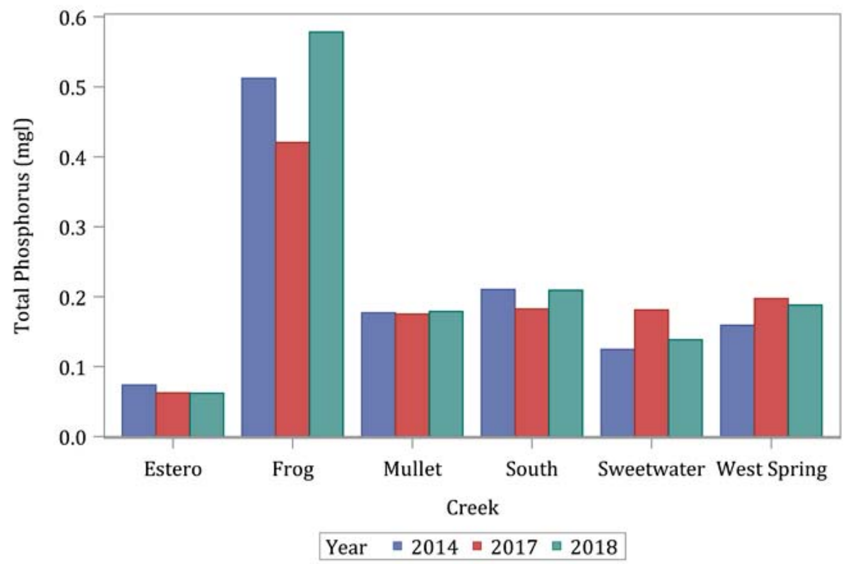


Figure 3-18. Geometric average total phosphorus distributions for the 6 creeks sampled for each year in the study. Note that sampling for year 2014 began in November of 2013 and that no samples collected in January, July, or September were included in the calculation of the geometric averages for 2014 in order to match the temporal distribution of samples with the more recent study.

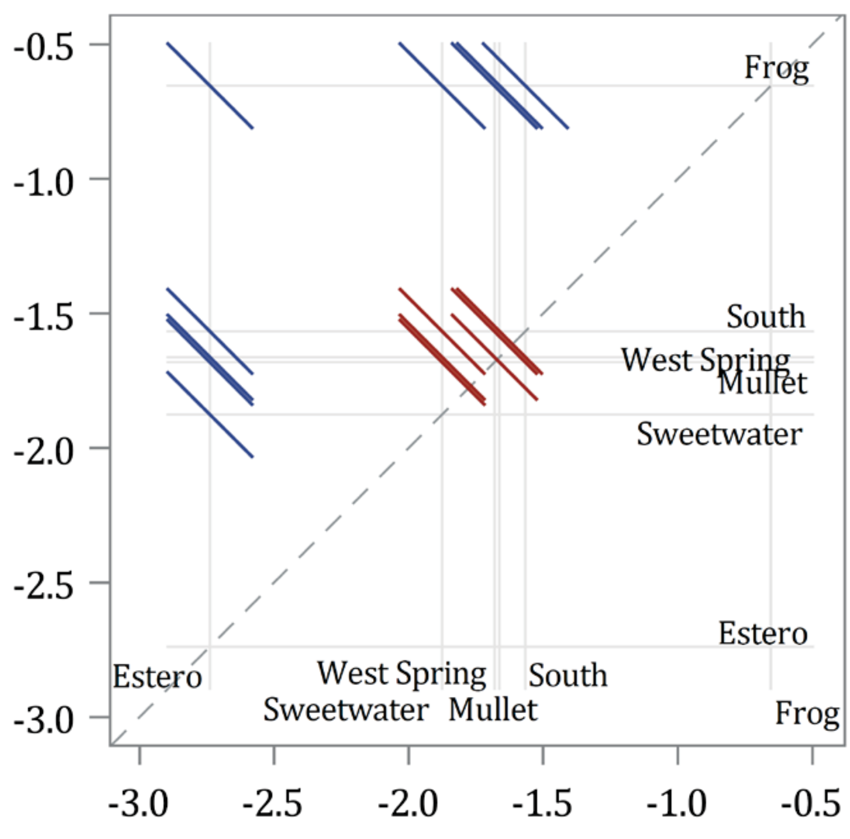


Figure 3-19. Diffogram displaying results of multiple comparison adjusted natural log scale differences in total phosphorus among creeks.

These results suggest several important findings. 1) Most of the creeks appear to be in a stable state regarding total nutrient concentrations over time. 2) Frog Creek experienced a marginally significant reduction in total nitrogen between 2014 and 2017 and 3) the difference in analytical methods between studies did not affect the overall estimates of total nutrient concentrations.

To confirm the findings for Frog Creek, we obtained independently collected data for the Frog Creek fixed station location which is co-located with our most upstream site and collected by Manatee County under its routine monitoring program. The annual geometric average TN for this site is displayed in Figure 3-20 which shows similarities in annual geometric averages in 2014, 2017 and 2018 between the two sampling programs. A decrease in TN between 2014 and 2017 was also observed between the two programs. These results also confirm findings of the previous tidal creeks study suggesting that nutrient concentrations in the tidal portion of the system tend to reflect the source water inputs because the source water and tidal concentrations were similar in Frog Creek. The Frog Creek source water site occasionally experiences salt water intrusion, but is typically below 5 psu salinity. Recent upgrades to Manatee County's northwest wastewater treatment facility may be a potential explanation for the recent reductions in TN concentrations in Frog Creek.

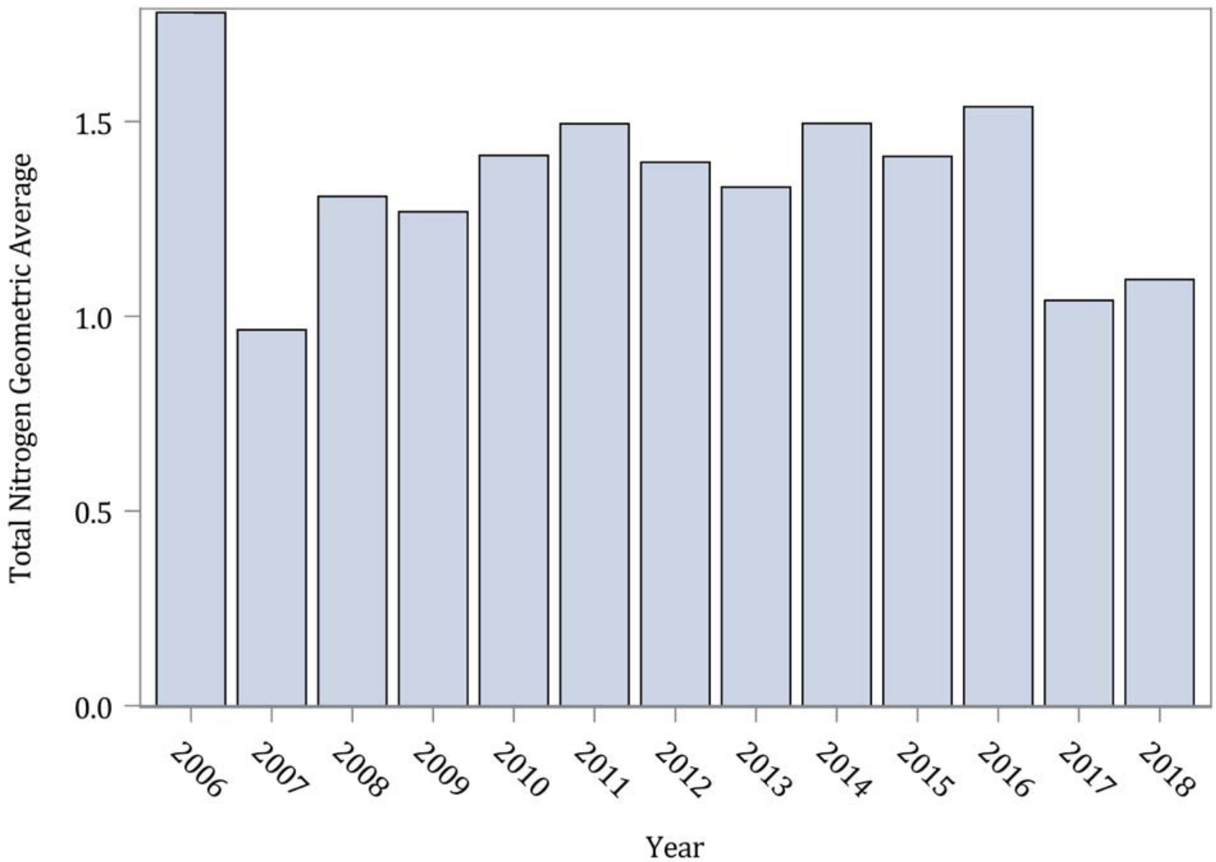


Figure 3-20. Annual geometric averages for Manatee County fixed station in Frog Creek which is also the same location as the most upstream site for this study.

Additional efforts to evaluate the source water distribution of TN across creeks using independent data was evaluated by a timeseries plot of annual geometric total nitrogen for the 5 tributaries with established fixed-station source water monitoring (Figure 3-21: note that no independently sampled fixed station monitoring exists in West Spring). Numbers above the bars represent the number of samples collected that year. A horizontal line at 1.0 mg/l is provided as a reference line. These results using independent data confirm that the our study well represented the source water total nitrogen conditions found using independent data for these creeks with the highest AGM concentrations in Frog and South Creek and lower concentrations in the other creeks.

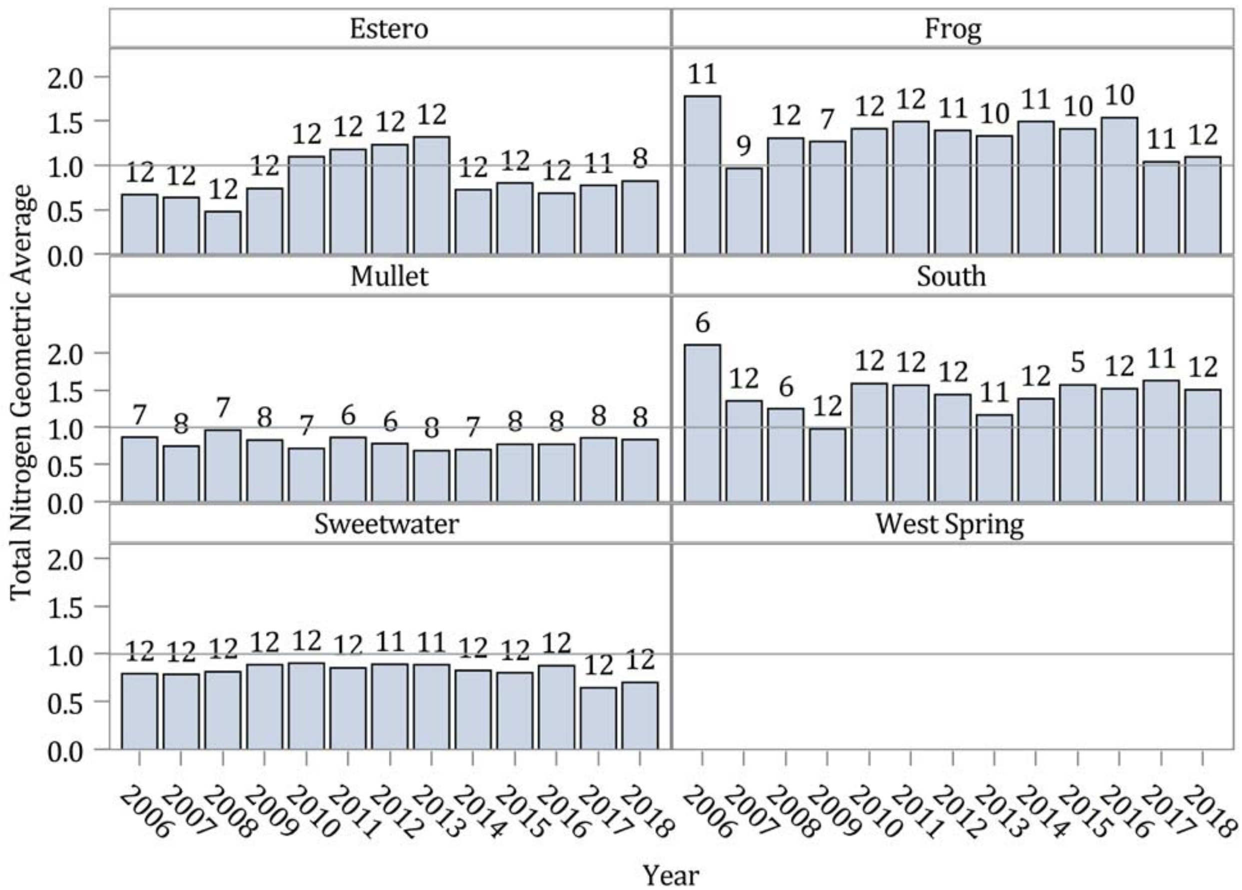


Figure 3-21. Annual geometric average total nitrogen concentration (mg/l) for independent fixed station locations co-located with the source water site for 5 of the 6 creeks sampled for this study. Horizontal line of 1.0 mg/l is provided for reference.

Chlorophyll was also significantly different among creeks (Figure 3-22) and while there were large differences in chlorophyll concentrations between years in Estero, the interaction term was only marginally significant and probably suffers from low power to detect between-year differences within a creek given the variability in chlorophyll concentrations. This variability is an important consideration with regard to regulatory evaluations based on AGM chlorophyll concentrations as uncertainty in the estimate of the AGM are not considered when determining impairments for chlorophyll.

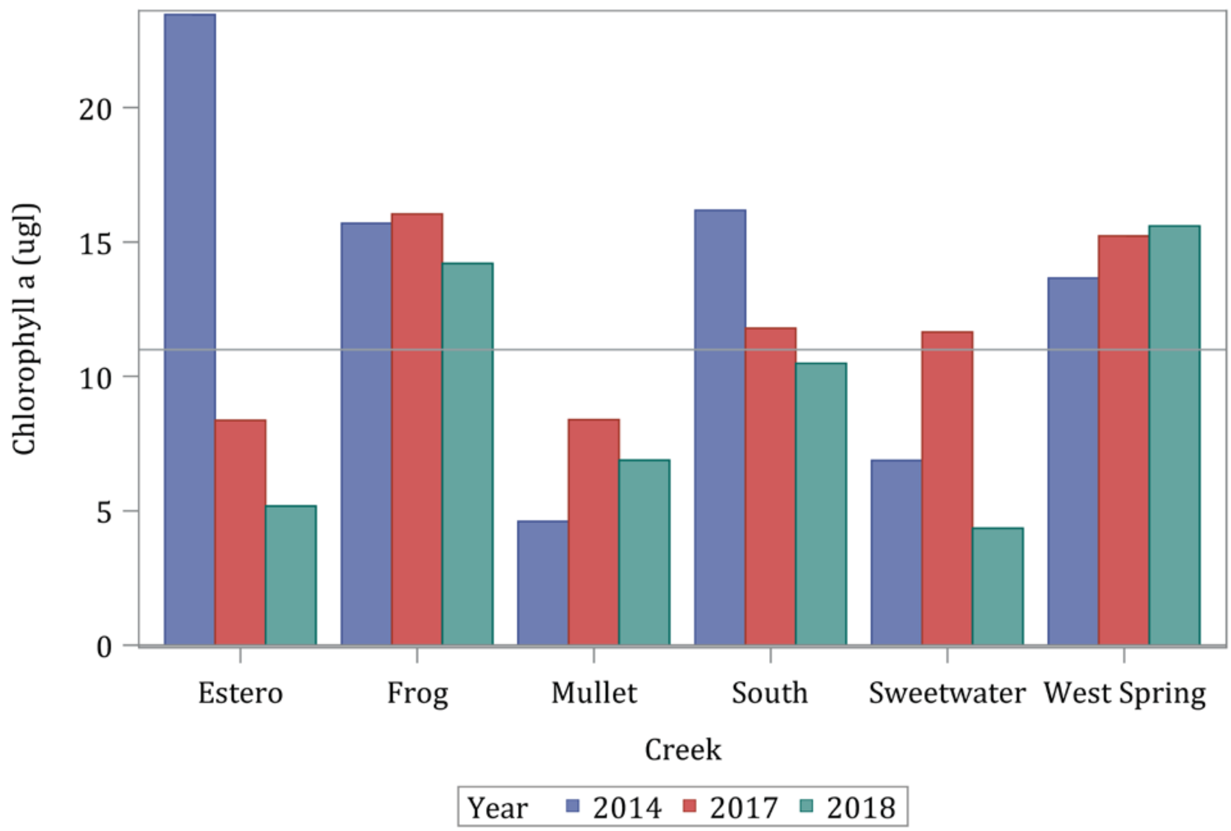


Figure 3-22. Geometric average chlorophyll distributions for the 6 creeks sampled for each year in the study. Note that sampling for year 2014 began in November of 2013 and that no samples collected in January, July, or September were included in the calculation of the geometric averages for 2014 in order to match the temporal distribution of samples with the more recent study.

Plots and output from the linear mixed models for these and other constituents of interest are provided in Appendix D.

3.3 Algal Surveys

Macroalgal surveys were conducted in association with each of the three water quality sampling stations in the estuarine portion of the creek using four-tined garden rake on both banks of the creek to determine algal presence. Data allowed for the quantitative assessment of the frequency of algal presence and the algal stable isotope analysis.

Despite having the highest nutrient concentrations in our study, there were no algae collected in Frog Creek. A single occurrence was reported in South Creek, while Sweetwater Creek, which had lower nutrient concentrations, had the highest occurrence of algae. Sweetwater Creek, the most quiescent creek despite being in an intensely developed watershed, has a fairly broad and natural floodplain in its upstream reaches and our

County partners have reported their observations from implementing FDEPs rapid periphyton survey in freshwater streams that algae occurrence is highly dependent on stream velocity. The only creek with drift algae was West Spring; all other algae collected were filamentous.

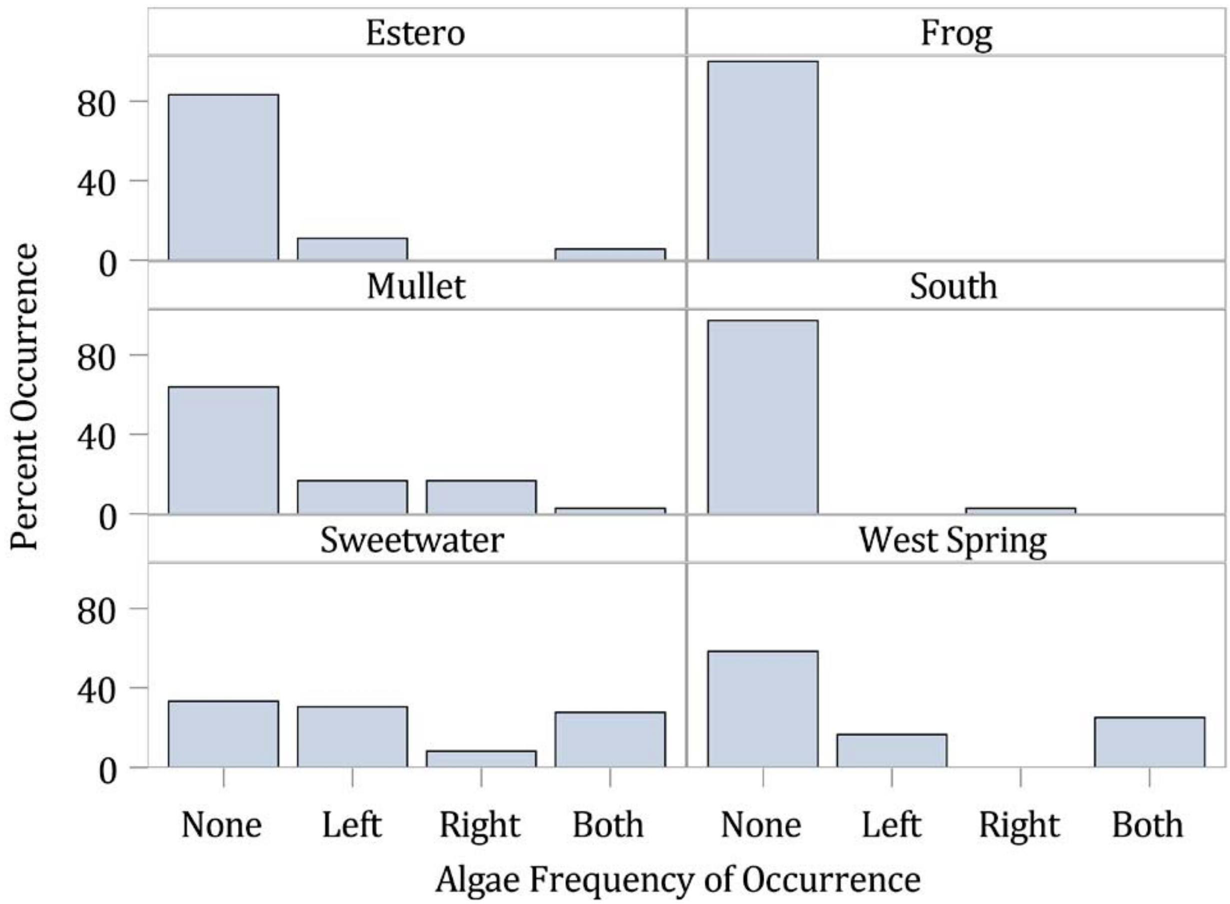


Figure 3-23. Percent occurrence of algae for left and right bank collected in association with the tidal water quality sampling stations

3.4 Stable Isotope Assessment

Stable isotope analysis provides information on nutrient sources to the creeks and dominant nutrient production pathways within the creeks. The objective of this analysis was to assess the dominant sources of nutrients for suspended particulate organic matter (SPOM – a proxy for phytoplankton) and macroalgae. The nutrient pathways that support algal food webs (phytoplankton or macroalgae) are relatively straightforward. Algal production depends on nutrients that originate from either the watershed or from in-situ recycling. Since different land-use types produce distinct isotopic signatures (Kendall, 1998), studies have succeeded in attributing the isotopic composition of various consumers to differential patterns of land-use (Wigand et al., 2007). However, urbanized watersheds have a variety of potential nitrogen sources including reclaimed wastewater, septic systems (Valiela et al., 2000), fertilizer, and atmospheric deposition (Anisfield et al., 2007). Ammonia from artificial fertilizer (inorganic N) carries a characteristic δ15N ratio generally less than 0‰ (Peterson and Fry 1987, Goffman et al 2006). On the other hand, septic systems and wastewater treatment plants produce ammonia, nitrate, and organic nitrogen compounds with relatively heavier ratios, often greater than +10‰ (Valiela et al. 2000). Wastewater treatment plants that employ an alternating aerobic/anaerobic cycle to achieve a higher degree of nutrient reduction generate effluent and residuals with δ15N ratios more enriched than most other naturally occurring signatures, reportedly as high as +26‰ (Savage and Elmgren 2004) and +40‰ (Jordan et al. 1997). SPOM δ15N tends to be slightly lighter than its source (so if wastewater is 10‰, then we expect to see SPOM around 6-8‰). Therefore, the source of nutrients used by algae may be estimated by comparing the isotope ratio of algae with those of the potential nutrient sources for a particular environment. For this study, the isotopic signatures of local nutrient sources (e.g. septics, reclaimed, atmospheric, etc.) were not directly assessed. Instead, results of the isotopic analysis for the SPOM samples were compared to previously reported isotopic signatures in the region (Demers, 2008; Lapointe and Bedford, 2007; Greenwood et al., 2008; Malkin, 2010; Lapointe et al. 2016; Yang and Toor, 2016;Table 3-3).

SPOM δ15N to δ13C for all creeks and all dates are presented in Figure 3-24. δ13C signatures indicate that carbon is mostly terrestrial (vs. marine) while δ15N signatures indicate multiple sources. None of the SPOM δ15N were above 10‰, but according to other regional studies (Demers, 2008; Lapointe and Bedford, 2007; Greenwood et al., 2008; Malkin, 2010; Lapointe et al. 2016; Yang and Toor, 2016), values above 3‰ represent potential wastewater/septic signatures rather than fertilizer or atmospheric. Regarding δ15N, Frog Creek had the most enriched SPOM δ15N signatures compared to other creeks (Figure 3-25) which agreed with previous studies (Malkin, 2010). However, it is important to look at each creek separately and as a time series, which is presented in Figure 3-26 and Figure 3-27**.** SPOM in West Spring and Frog Creeks were the only creeks that showed elevated δ15N, suggesting a wastewater/septic source of nitrogen. However, when looking at sampling period, West, Estero, Frog and Sweetwater Creeks showed at least one time when SPOM δ15N signatures were above 3‰. The most enriched δ15N SPOM signatures were in Frog Creek. October and November appeared to have more enriched signatures than the other months, which may reflect the cumulative effects of wet season nutrient delivery from the surrounding landscape.

Table 3-3. Reference source δ15N from within the sampling region.

Nitrogen Source	δ15N (‰)	Reference
DONAX WRF AFBC*	30.45	Hale et al., 2008
DONAX WRF AFAC*	8.00	Hale et al., 2008
15 0 15 fertilizer	-8.89	Hale et al., 2008
6 6 6 fertilizer	-10.59	Hale et al., 2008
Other Synthetic Fertilizers	-4 to 4	IAEA, 2013
Manure	10 to 20	IAEA, 2013
Other municipal wastewater treatments	10 to 30	IAEA, 2013
Synthetic fertilizer	-2 to 2	Bateman and Kelly, 2007
Atmospheric deposition	-3 to 1	Paerl and Fogel, 1994
Wastewater/septic	>3	Lapointe et al., 2015

*** These samples are from treated wastewater collected from the Donax Water Reclamation Facility (WRF). AF refers to “after filtration” treated wastewater either before or after chlorination, identified as AFBC and AFAC, respectively (only AFAC water is used in “re-use” applications, i.e. irrigation).**

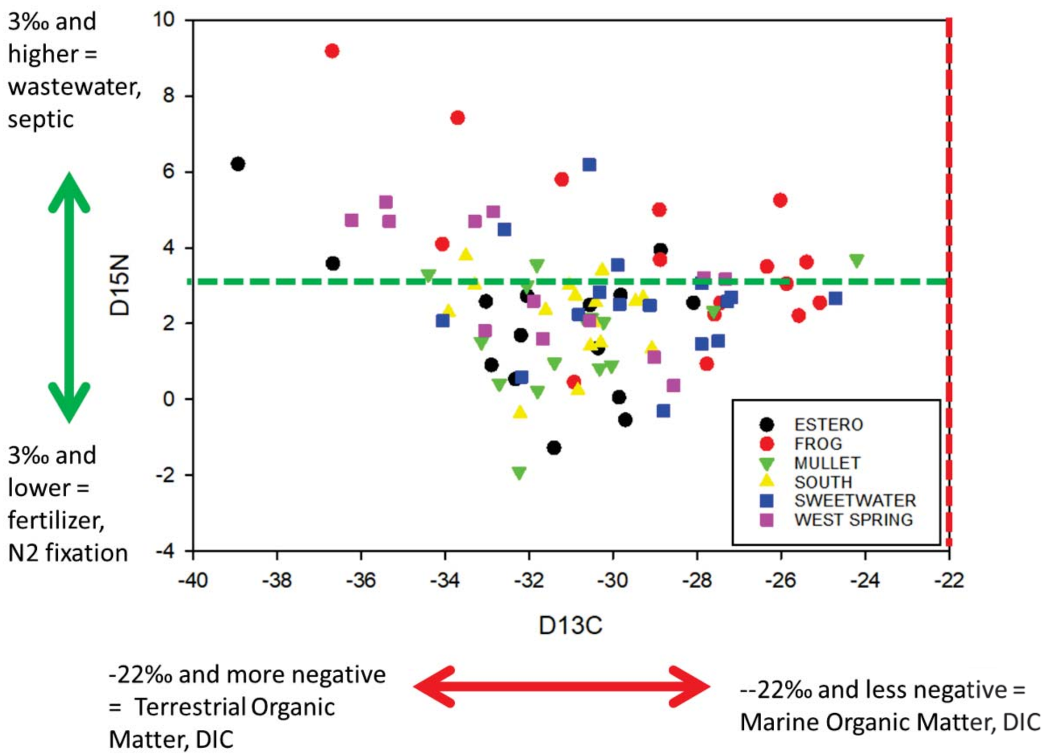


Figure 3-24. D15N vs D13N for SPOM by creek for all sampling events. Axis labels adapted from Peebles et al, 2010.

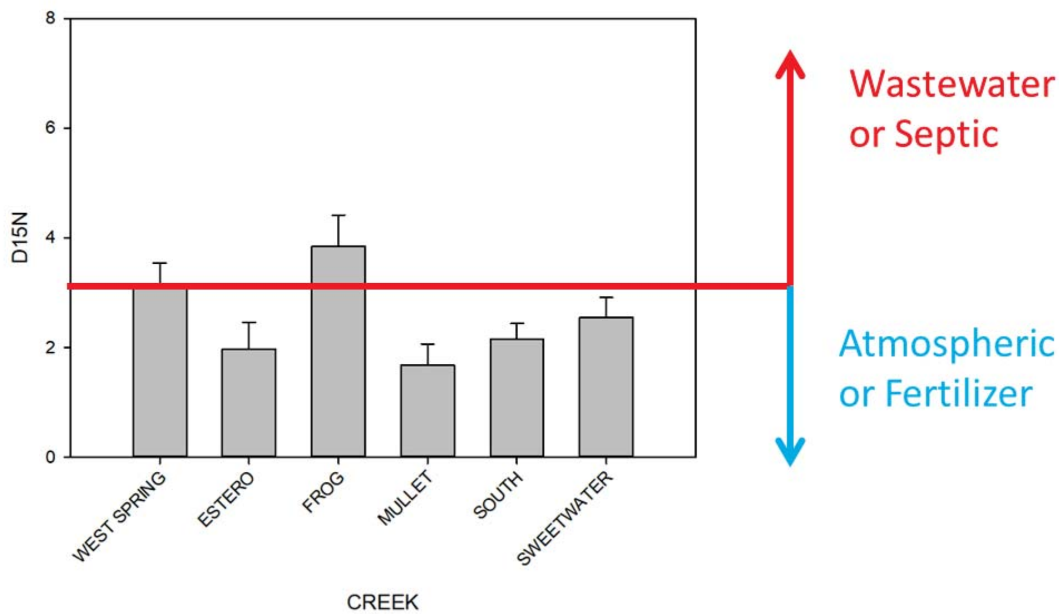


Figure 3-25. Creek specific average (+1SE) δ15N ratios of SPOM for all creeks over all sampling periods.

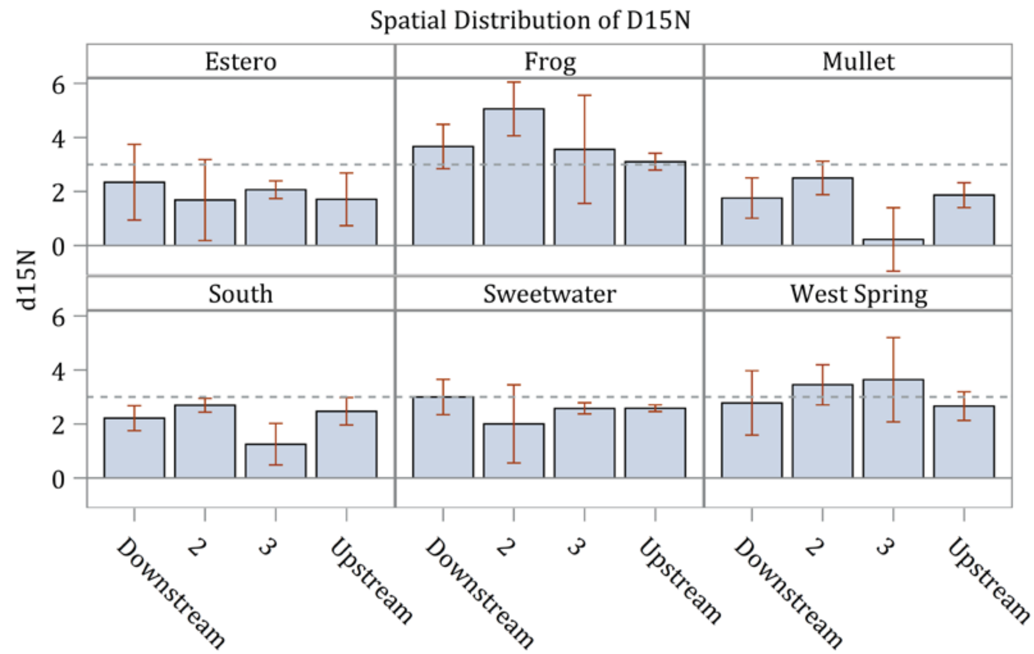


Figure 3-26. Site specific average δ15N ratios of SPOM in each creek with standard error bars.

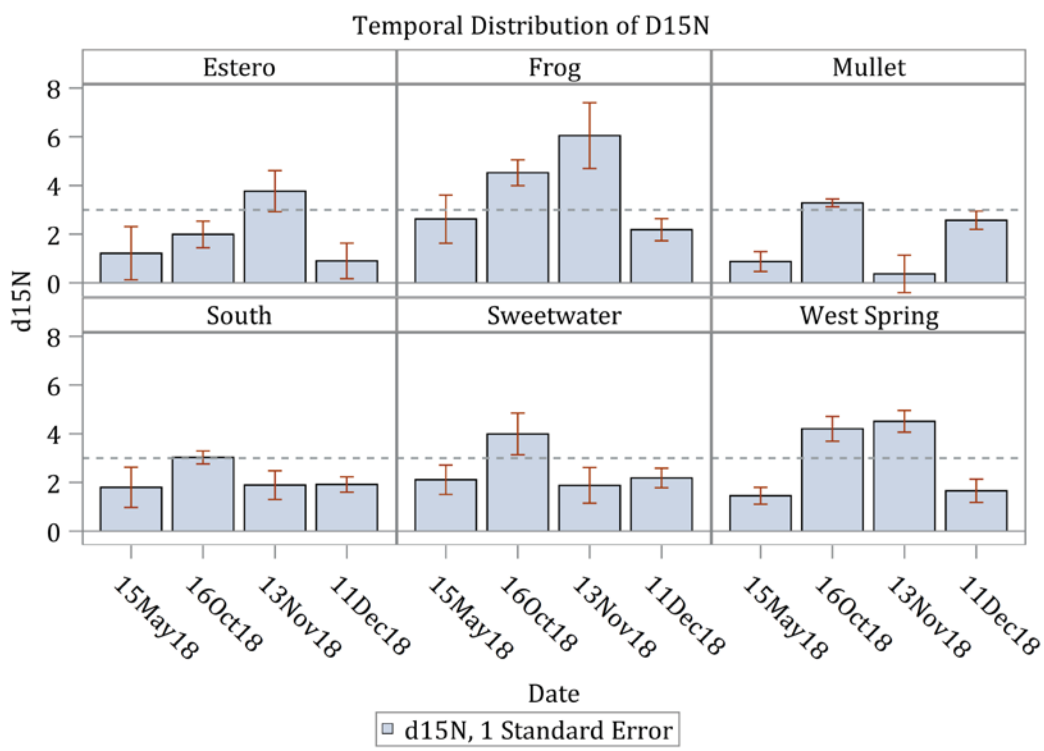


Figure 3-27. Date specific average δ15N ratios of SPOM in each creek with standard error bars.

Roughly two-thirds of the macroalgal δ15N generally revealed a heavier isotopic signal (δ15N>3), especially in West Spring, Sweetwater and Mullet Creeks (Figure 3-28 and Figure 3-29). Several macroalgal δ13C signatures in Sweetwater Creek indicated a marine source of carbon.

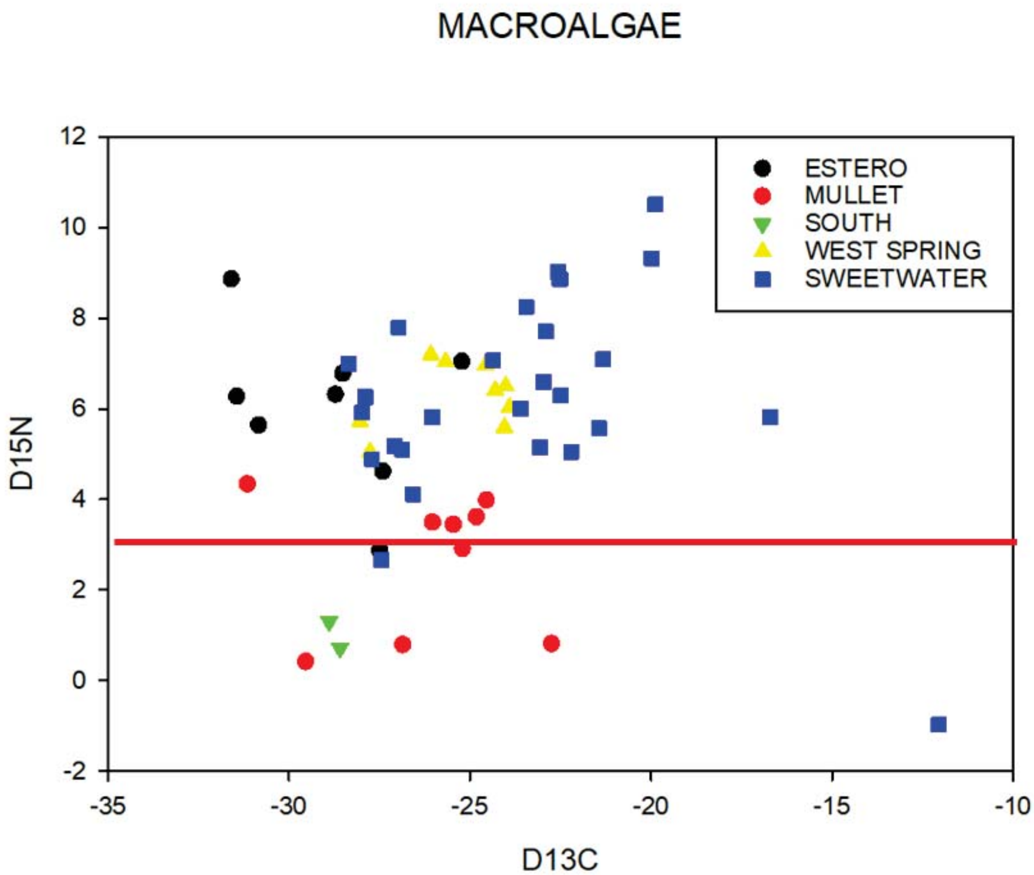


Figure 3-28. δ15N vs δ13C for all available macroalgae samples in all creeks.

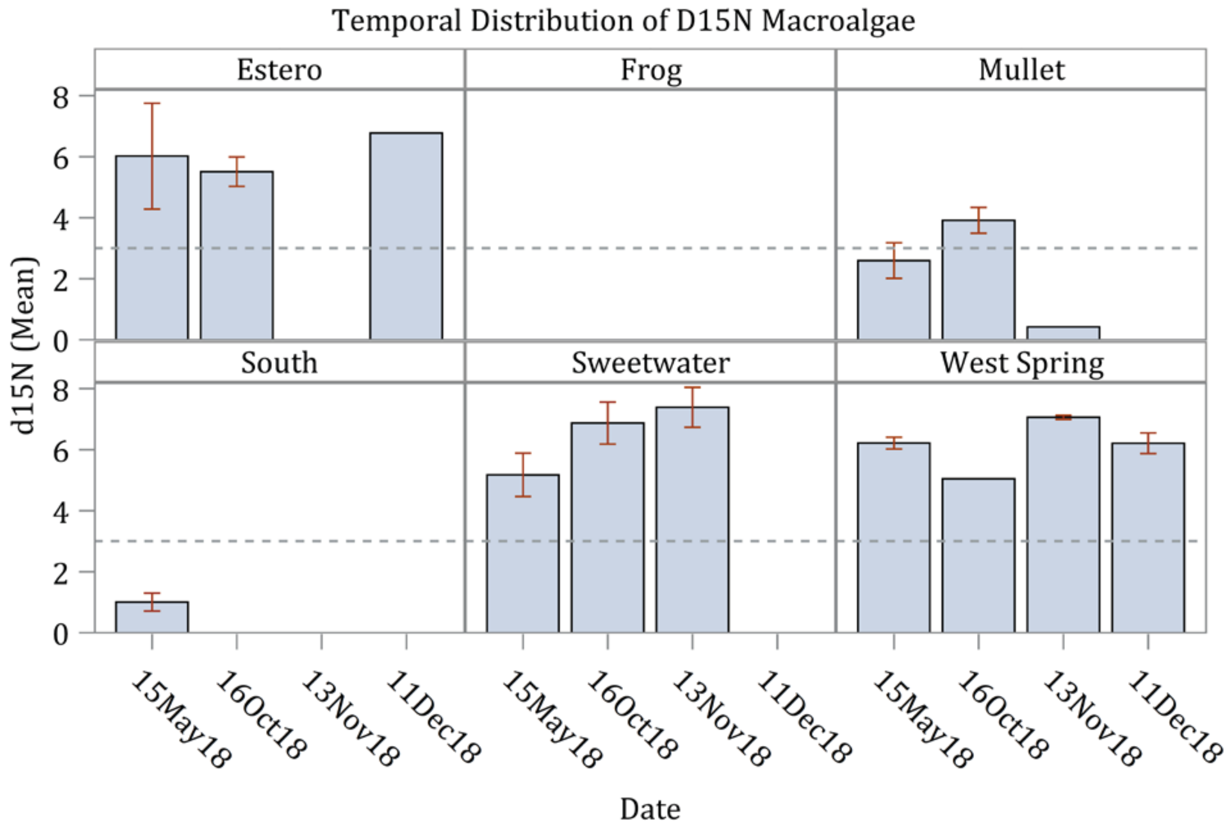


Figure 3-29. Average δ15N for macroalgae in each creek where macroalgae was present (no macroalgae was present in West or Frog Creeks), separated by sampling date.

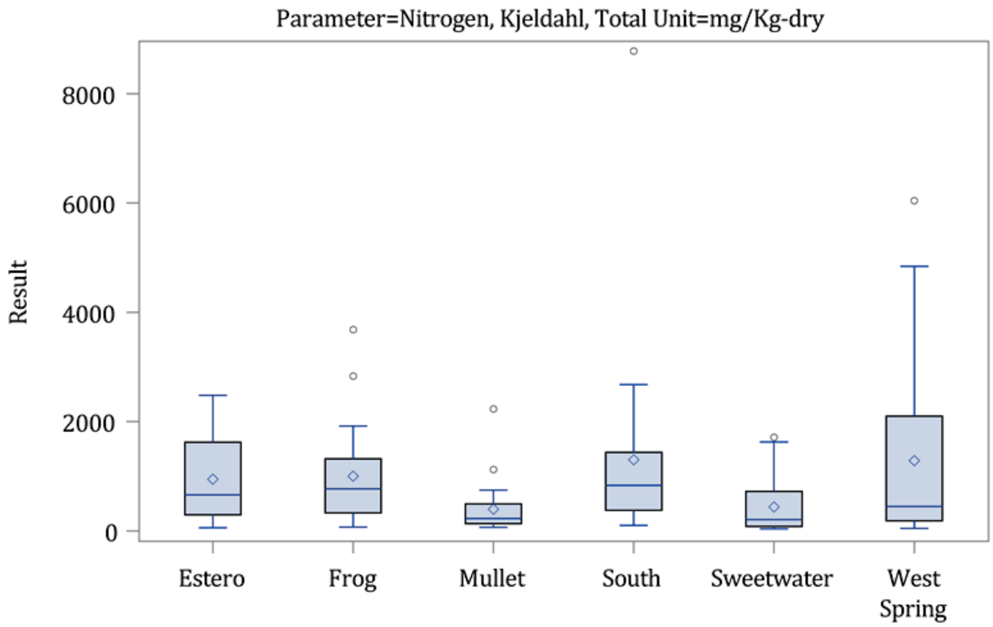
3.5 Sediments

Sediment collections were conducted in all six creeks during three water quality sampling events (May 23, 2017, November 14, 2017, and November 13, 2018) resulting in 144 samples for sediment nutrients and grain size analysis. Results comparing sediment nutrients among creeks were similar to the water column nutrients indicating that sediment nutrient concentrations reflect water column concentrations. There were high positive correlations (Table 3-4) between sediment nutrients, particularly nitrogen, and percent silt (PSilt), percent moisture (pmoist), and total volatile solids (TVSolids) and negative correlation with percent sand (PSand). Frog Creek sediments had much higher average TP than the other creeks, while Mullet Creek had the lowest average (and median) sediment TKN and the second lowest (i.e. slightly higher than Estero) TP (Figure 3-30).

Table 3-4. Spearman rank correlation among sediment metrics across creeks for the 2017-2018 sampling events.

Spearman Correlation Coefficients, N = 143
Prob > |r| under H0: Rho=0

	G2mm	PClay	PSand	PSilt	Pmoist	TKN	TP	TSolids	TVSolids
G2mm	1.00000	-0.16452 0.0496	-0.01206 0.8863	0.03643 0.6658	0.17556 0.0360	0.16719 0.0460	0.22087 0.0080	-0.17556 0.0360	0.27127 0.0010
PClay	-0.16452 0.0496	1.00000	-0.86853 <.0001	0.84593 <.0001	0.59640 <.0001	0.63146 <.0001	0.30243 0.0002	-0.59640 <.0001	0.59473 <.0001
PSand	-0.01206 0.8863	-0.86853 <.0001	1.00000	-0.99847 <.0001	-0.84849 <.0001	-0.86450 <.0001	-0.41284 <.0001	0.84849 <.0001	-0.82749 <.0001
PSilt	0.03643 0.6658	0.84593 <.0001	-0.99847 <.0001	1.00000	0.86221 <.0001	0.87758 <.0001	0.42284 <.0001	-0.86221 <.0001	0.84125 <.0001
Pmoist	0.17556 0.0360	0.59640 <.0001	-0.84849 <.0001	0.86221 <.0001	1.00000	0.96329 <.0001	0.42446 <.0001	-1.00000 <.0001	0.95604 <.0001
TKN	0.16719 0.0460	0.63146 <.0001	-0.86450 <.0001	0.87758 <.0001	0.96329 <.0001	1.00000	0.43201 <.0001	-0.96329 <.0001	0.95915 <.0001
TP	0.22087 0.0080	0.30243 0.0002	-0.41284 <.0001	0.42284 <.0001	0.42446 <.0001	0.43201 <.0001	1.00000	-0.42446 <.0001	0.47950 <.0001
TSolids	-0.17556 0.0360	-0.59640 <.0001	0.84849 <.0001	-0.86221 <.0001	-1.00000 <.0001	-0.96329 <.0001	-0.42446 <.0001	1.00000	-0.95604 <.0001
TVSolids	0.27127 0.0010	0.59473 <.0001	-0.82749 <.0001	0.84125 <.0001	0.95604 <.0001	0.95915 <.0001	0.47950 <.0001	-0.95604 <.0001	1.00000



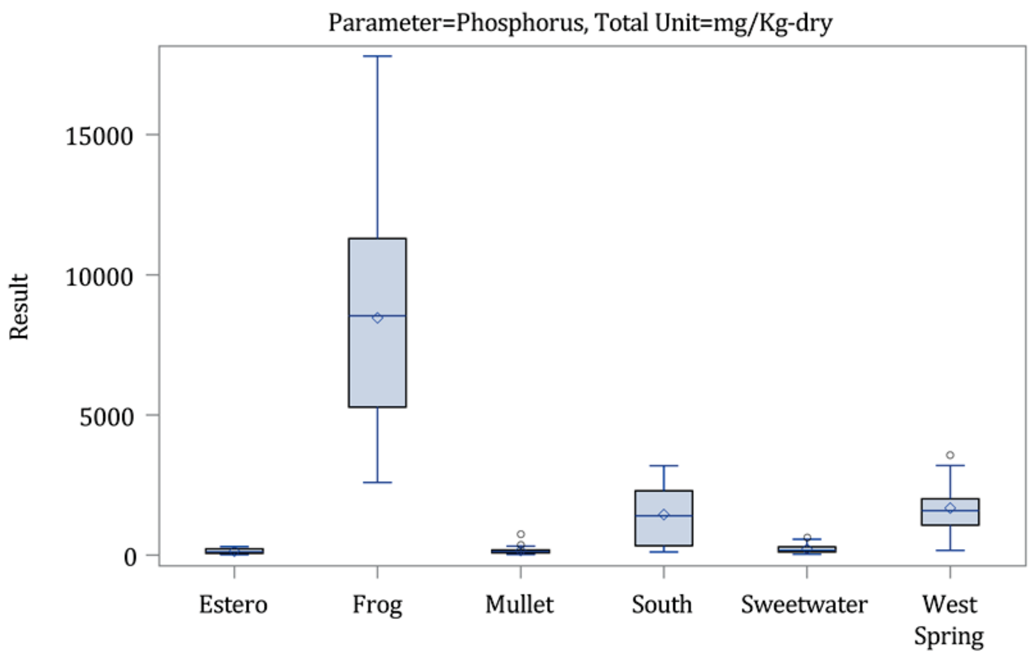


Figure 3-30. Sediment concentrations for total kjeldahl nitrogen (top) and total phosphorus (bottom) based on.

3.6 Stream Morphology and Canopy Cover

The six creeks vary dramatically in size and form. This study design was intended to capture a broad range of creek types with respect to their size and degree of physical alteration. Figure 3-31 displays the creeks on a common scale (1:50,000) to illustrate this point. The bathymetry layers associated with the original creek surveys (such as the one shown for South Creek in Figure 3-32) were used to estimate the relative volumes of the tidal portion for all 16 creeks sampled during the previous study. Those estimates were site-verified at three locations in association with the fixed stations for the six creeks resampled as part of this study. Those volumetric estimates are provided in Table 3-5. Bishop and Mullet Creeks are small shallow creeks in Pinellas County and had the lowest volumetric estimates while West Spring in Charlotte County had the largest tidal volumes.

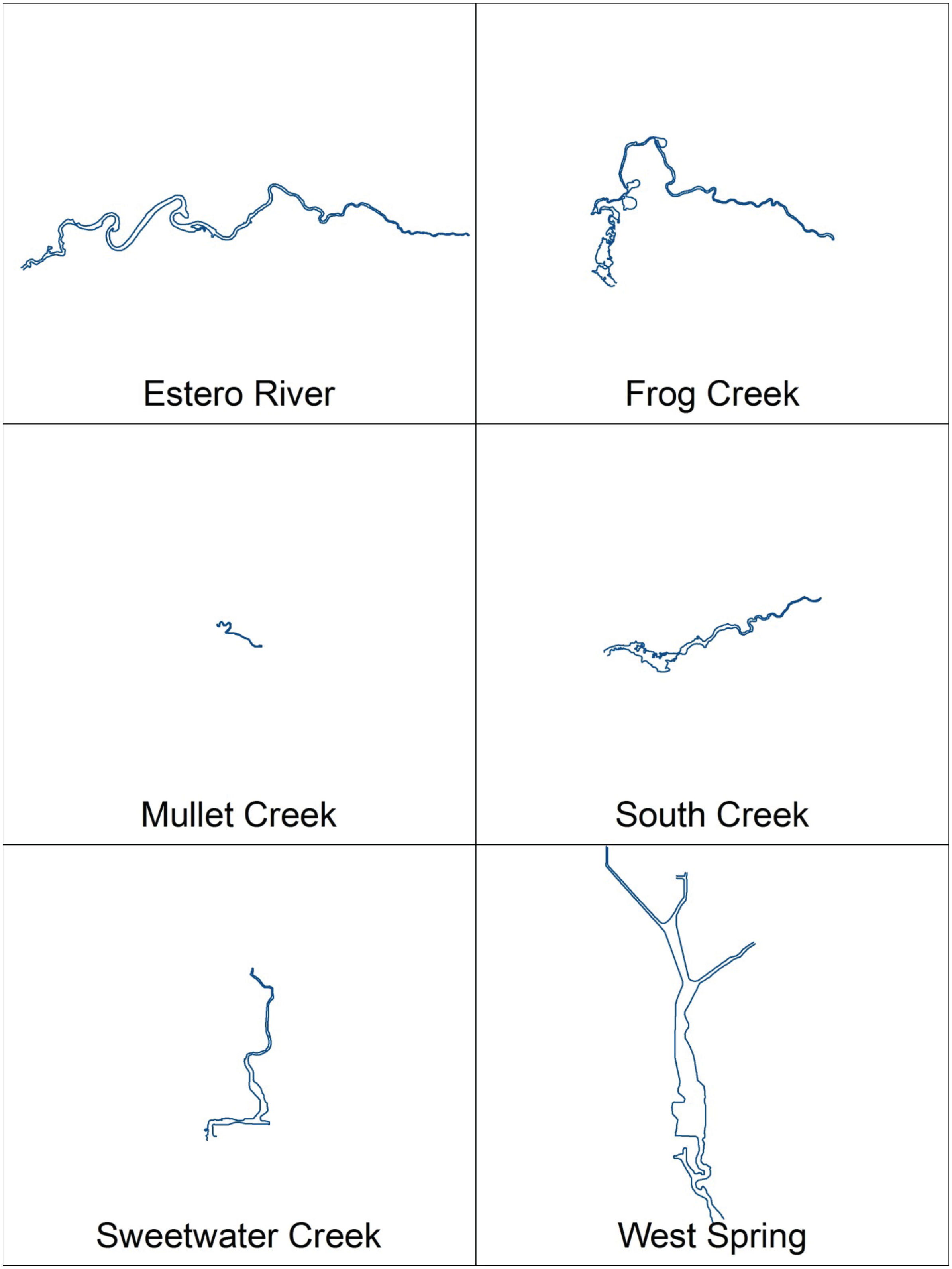


Figure 3-31. Outline of morphological creek characteristics for the 6 creeks using a common scale (1:50,000).

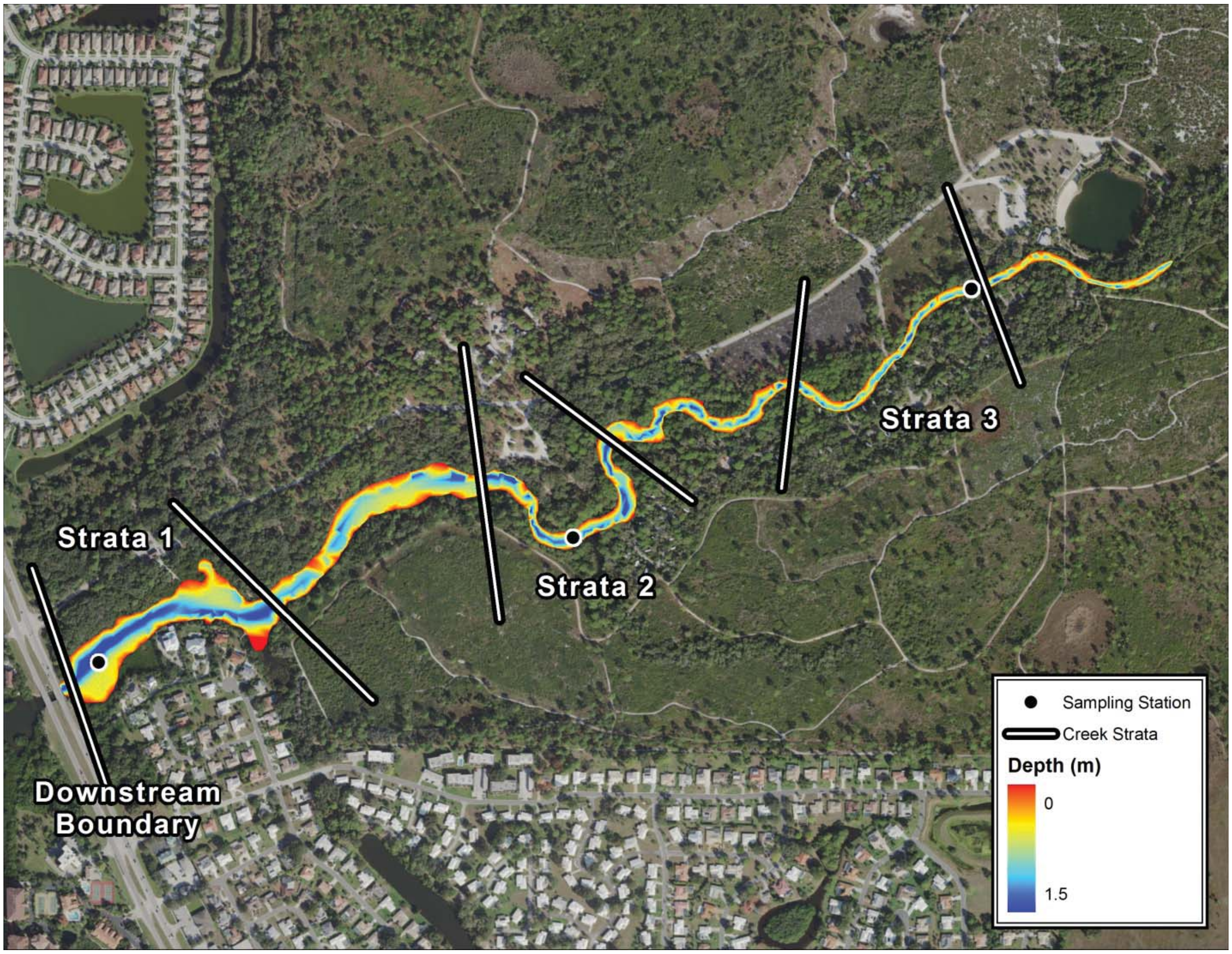


Figure 3-32. Boundary for volumetric estimates of the tidal portion of South Creek with bathymetric layer.

Table 3-5. Volumetric estimates for the sampling area of each creek in the southwest Florida tidal creeks study.

Creek	Volume (m3)	Creek	Volume (m3)
Bishop	2,263	Buck	55,429
*Mullet	2,949	Double Branch	59,167
Bear	10,957	*Estero	70,494
Sugarhouse	12,501	*Frog	84,608
*Sweetwater	23,386	Spring	94,640
Wildcat	26,177	Forked	155,731
Powell	32,565	Phillippi	202,378
*South	37,450	*West Spring	813,780

*** indicates creeks sampled during the 2017-2018 study.**

Flow data were only available for 2 creeks for the period of record so residence times could not be estimated and because these creeks take many forms, including some with intense physical alterations to a portion (generally the mouth) of the system, it is difficult to correlate these volume estimates directly with water quality observations.

Canopy cover was assessed in multiple ways using both qualitative and quantitative measurements based on in situ observation and remote sensing data. The Itree software tended to over-estimate shading of the creek centerline principally because of limitations of the pixel size (large) relative to the width of the creek (small). Qualitative field characterizations were subjective and limited only to the sampled sites. The use of the fish eye lens camera for collecting the light field shading estimates were also point based and found to be unreliable due to limitations of our small vessel necessary to traverse the creeks and the lack of confidence that the camera maintained a horizontal plane when capturing the photographs. Given these limitations, final calculations of canopy cover were made by interpreting satellite imagery using ArcGIS to develop polygons of canopy coverage estimates from recent aerial photography and categorizing coverage in 25% increments. The advantage of this method was the ability to provide a quantitative estimate for which the relative proportion of acres of each type of canopy cover could be summed, as well as a distinction of when mangrove fringe and other overhanging vegetation provided shade to only a portion of the creek but not the centerline. An example of the approach is provided in Figure 3-33 where it can be seen that below Station 1 there is an area where mangroves overhanging the creek edge but the centerline of the creek is not shaded This yielded a cover estimate for that portion of the creek that was shaded by overhanging vegetation but an open canopy (no cover) estimate for the centerline of the creek.

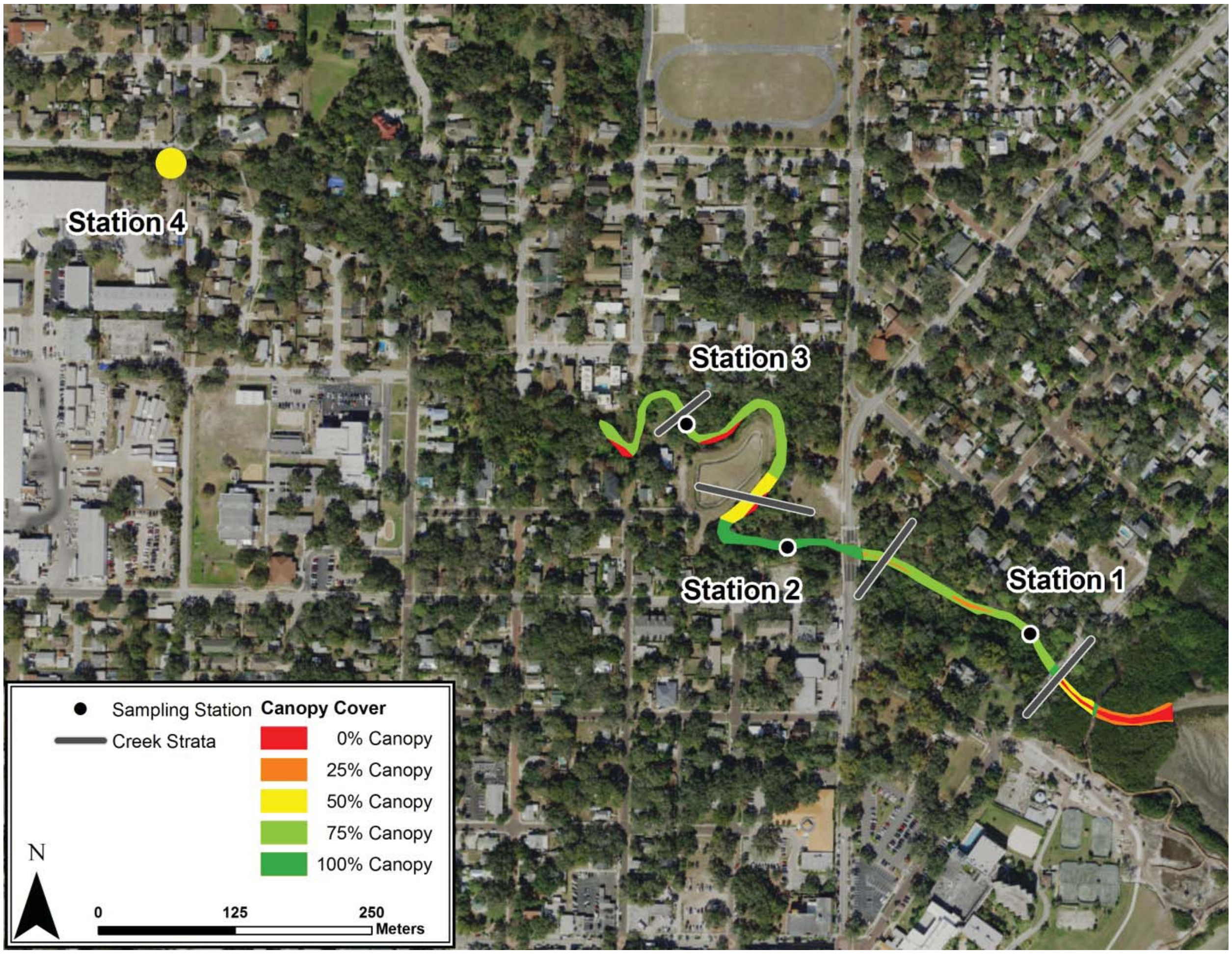


Figure 3-33. Canopy cover estimates for Mullet Creek based on interpretation of aerial imagery.

The results for each canopy coverage type were summed over the sampling area to provide an estimate of total acreage and proportion of the total acres that was consistently shaded. The values for the proportion of the total acres for each category and creek are provided in Figure 3-34. West Spring had the highest proportion of no shading while Mullet Creek had the lowest proportion of no shading and nearly 50% of the creek estimated to be 75% shaded. There also appeared to be a maximum width (ca. 10m) at which a creek could be 100% canopied.

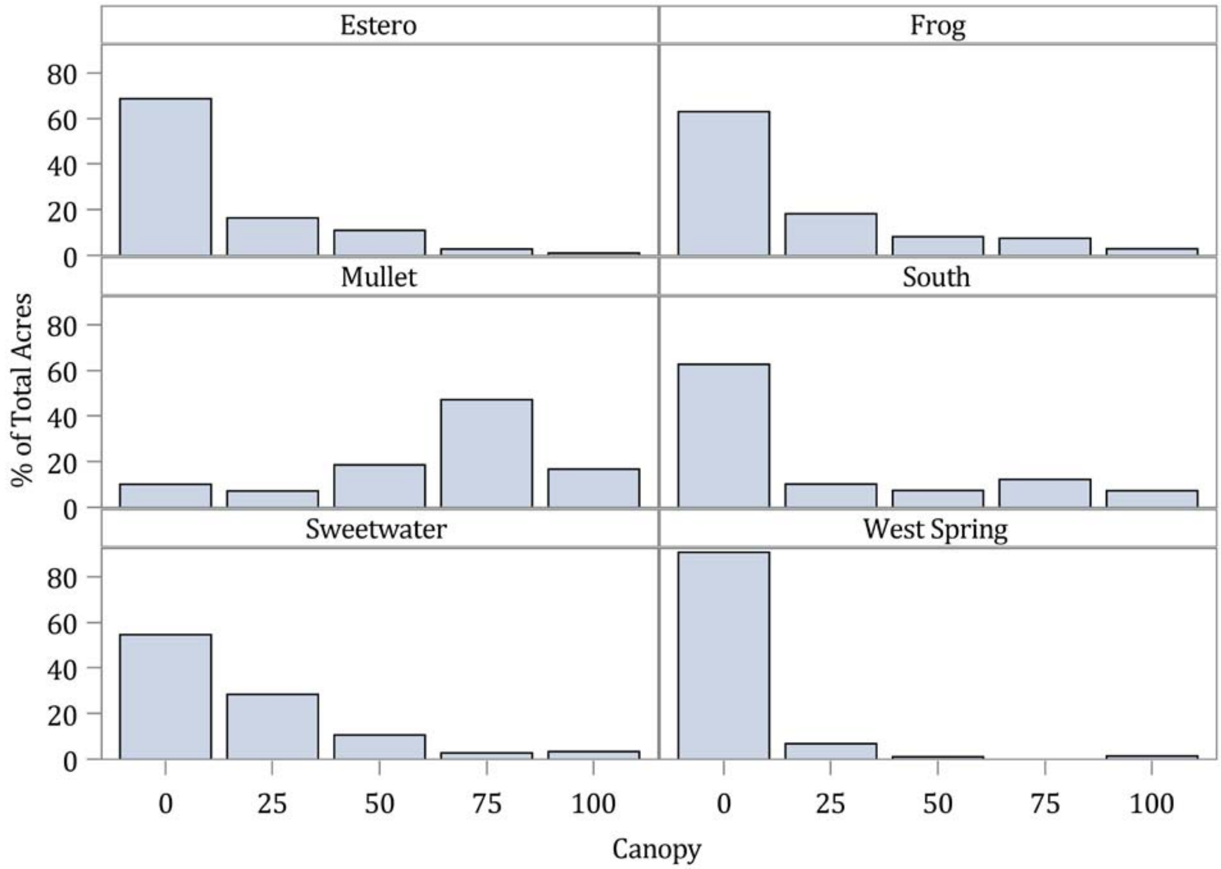


Figure 3-34. Proportion of each canopy coverage type for the sampling area of each creek.

3.7 Rainfall

The Standardized Precipitation Index (SPI: McKee et al. 1993), a probability-based index that considers only precipitation and not evapotranspiration nor other seasonal effects, is still useful to characterize drought over various time scales. The probabilities are standardized so that an index of zero indicates the average or median precipitation amount. A negative index value indicates lower than normal rainfall, and a positive index indicates above normal rainfall. The degree of separation from zero indicates the severity of conditions (Table 3-6) with values within +/- 1 indicating near normal conditions. An example of the creekshed boundary for each of the six creeks and a daily rainfall total within those boundaries is provided in Figure 3-35 (Left). Results for the 12 month SPI for the entire time series (1995-2018) is provided in Figure 3-35 (right). The vertical solid lines bracket the sampling periods 2014 and 2017-2018, respectively. There were creek-specific differences in rainfall trends relative to long term averages; precipitation was near normal during both studies (though Estero had a relatively persistent above average rainfall during the 2017-2018 study) .

Table 3-6. Ranges and characteristics of the standardized precipitation index.

SPI Category Range	Characteristic
>2.0	Extremely Wet
1.5 -2.0	Very Wet
1.0 to 1.49	Moderately Wet
-0.99 to 0.99	Near Normal
-1.0 to -1.49	Moderately Dry
-1.5 to -1.99	Severely Dry
-2.0 and less	Extremely Dry

.

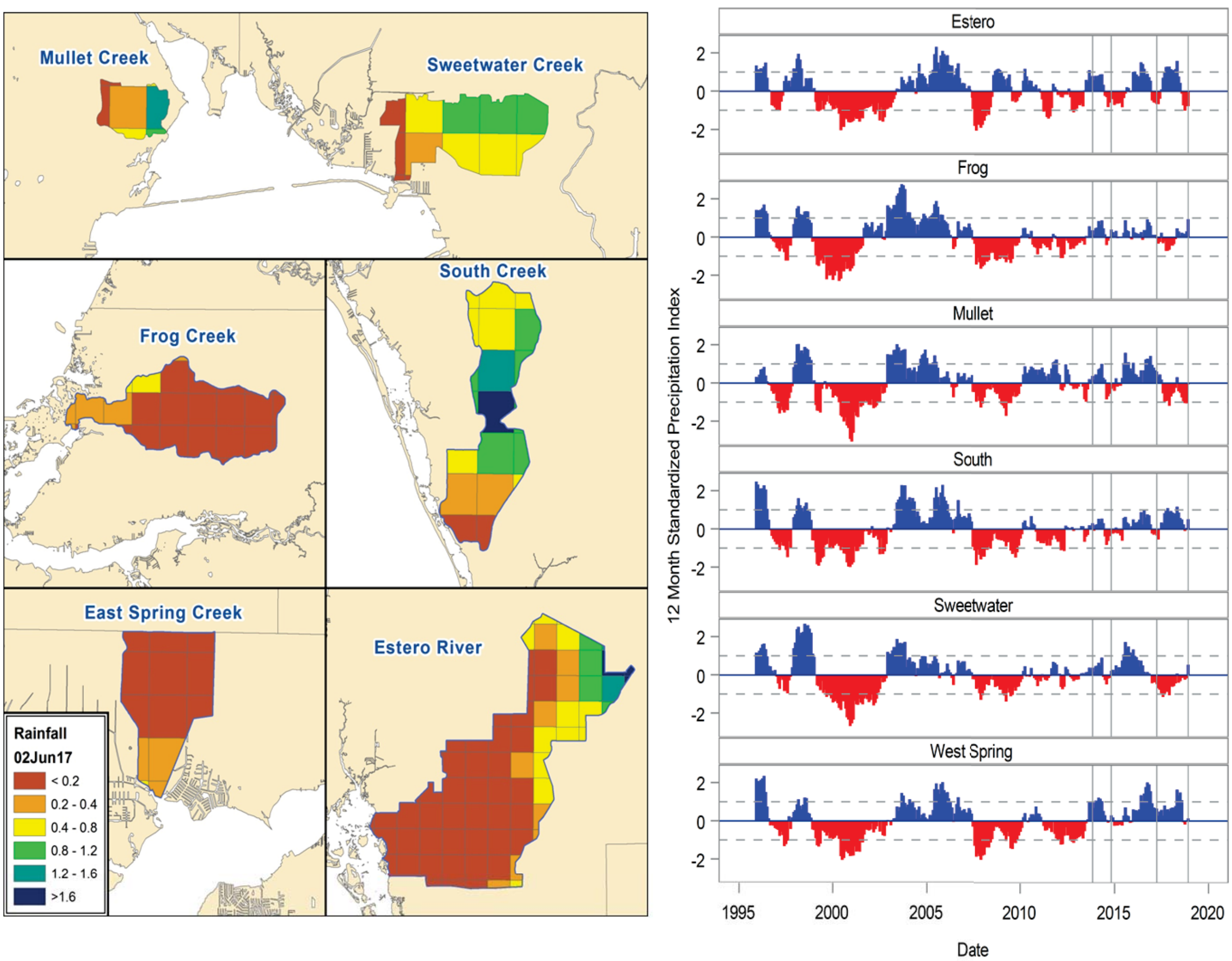


Figure 3-35. Creekshed boundaries with a daily total rain amount for each pixel within the boundary (left) and results of the 12 month Standardized Precipitation Index (SPI) for each creek.

The 12 month SPI characterizes rainfall over relatively large time scales so the data were also summed over shorter time scales (from 1 to 30 days) and standardized following an analogous routine to the SPI to describe more short term effects of rainfall on water quality observations on our sampling dates. The prevalence of days without rainfall affects the characteristics of the distribution which can result in an asymmetric distribution around the expected value. The 14-day total was chosen as an index of short-term antecedent conditions effecting instream water quality. Results for the each sampling date, including both the 14 day rainfall totals and their standardized index values, are presented in Table 3-7. Values highlighted in red and blue represent index values less than -0.5 and greater than 1, respectively. The difference in these chosen threshold values was due to the asymmetric distribution resulting from application of this process to short term rainfall conditions. Despite this, the standardized index values suggest that June 2017 and May 2018 experienced higher than expected rainfall relative to the long-term average for those dates while April 2017 was drier than normal.

Table 3-7. Sum of the previous 14 days rainfall expressed in inches (left) and as a standardized values relative to the long term average for that date (right).

	Sum of last 14 Days Rainfall (inches)						Standardized Sum 14 Days Rainfall (inches)					
Date	Estero	Frog	Mullet	South	Sweet water	West Spring	Estero	Frog	Mullet	South	Sweet water	West Spring
11/21/13	0.4	0.1	0.8	0.3	0.4	0.7	0.0	-0.6	0.2	-0.3	-0.3	0.1
1/9/14	0.9	0.9	1.0	0.3	1.0	0.9	0.4	-0.2	0.0	-0.6	-0.1	0.2
3/18/14	0.8	4.1	3.2	1.2	2.9	1.5	-0.2	2.0	2.5	-0.1	2.2	0.4
5/20/14	0.7	0.8	0.6	0.5	0.2	0.1	-0.8	-0.3	-0.3	-0.3	-0.7	-0.8
7/22/14	4.3	4.4	4.7	1.7	4.5	4.6	0.1	0.4	-0.1	-0.6	0.3	0.3
9/16/14	3.0	2.7	2.9	2.6	2.5	2.9	-0.6	-0.3	-0.2	-0.4	-0.4	-0.4
4/18/17	0.2	0.1	0.3	0.5	0.4	0.4	-0.8	-0.9	-0.8	-0.6	-0.7	-0.8
5/23/17	1.7	0.7	1.4	3.1	1.4	0.9	-0.1	-0.5	0.2	1.1	0.0	-0.4
6/20/17	12.4	5.5	6.5	8.6	4.8	8.4	3.1	1.1	1.5	2.7	0.8	2.1
10/17/17	1.2	0.4	0.3	0.3	0.9	0.5	-0.3	-0.7	-0.9	-0.7	-0.3	-0.7
11/14/17	0.0	0.1	0.0	0.0	0.0	0.0	-0.6	-0.7	-0.6	-0.5	-0.6	-0.6
12/12/17	1.2	1.3	0.5	0.9	0.7	0.7	0.4	0.4	-0.3	0.0	-0.3	-0.1
3/20/18	0.8	0.0	0.5	0.4	0.5	0.4	-0.2	-0.8	-0.6	-0.6	-0.5	-0.6
4/17/18	0.6	2.9	2.8	1.3	2.6	1.5	-0.4	1.6	1.4	0.2	1.2	0.4
5/15/18	2.2	2.9	2.1	1.3	1.7	2.2	1.4	1.5	1.4	0.4	0.8	1.2
10/16/18	1.3	0.9	1.2	1.1	1.1	1.6	-0.3	-0.4	-0.3	-0.2	-0.2	-0.2
11/13/18	1.5	0.9	0.9	0.9	0.6	1.4	0.6	0.2	0.1	0.4	-0.2	0.4
12/11/18	0.1	1.2	2.6	0.5	2.6	0.5	-0.6	0.3	0.8	-0.3	1.1	-0.3

All of the metrics described above were used to evaluate the effects of these creek attributes on nutrient dynamics and resultant water quality values within the tidal creeks in order to develop indicators of tidal creek condition for southwest Florida. The next chapter of this report describes those efforts regarding evaluation of nutrient dynamics within the system and how those dynamics affect the resultant measured water quality values.

4 Instream Nutrient Dynamics

A principal objective of this study was to gain a better understanding of the nutrient dynamics within these tidal creeks by focusing on changes in nutrients along the salinity gradient. This chapter describes results of assessments of nutrient dynamics using stoichiometric ratios of total and dissolved, inorganic and organic water quality constituents and assessing the observed mixing behavior of nutrients along the salinity gradient.

4.1 Stoichiometry

Stoichiometry is the study of elemental ratios; the most popular of which with respect to water quality is the Redfield Ratio (Redfield, A.C. 1934: 1958). The Redfield Ratio was used to characterize the elemental requirements of phytoplankton in oceanic waters and a 106:16:1 C:N:P ratio is often quoted as that which defines a breakpoint between nitrogen and phosphorus limitation. The original ratio was based on molar mass of nitrogen and phosphorus and based on inorganic forms of these elements assuming that nutrients were not limited; however, the ratio is sometimes used with total forms of nutrients and concentrations (e.g. mg/l). An example of the latter is the cutoff values for Florida's Trophic State Index calculations which is most often computed based on total forms of nutrients and their mass per unit volume. The C:N:P ratios based on median values of particulate C, N, and P (ug/l) converted to moles are provided for each creek in Table 4-1. Based on the particulate fractions of these constituents, the values are less than the Redfield ratios reported by Redfield (1934). When evaluating total forms of nutrients based on molar ratios, all creeks except Estero appear to be nitrogen limited with N:P ratios typically less than 16 (Figure 4-1). The exception was Estero which was principally phosphorus limited based on molar ratios. Estero would be considered a nutrient "balanced" system according to the Trophic State Index with a range in mass per unit volume N:P ratio between 10-30 (Figure 4-2). This outcome is likely due to the fact that there were NO23 inputs in the tidal portion of Estero in addition to the fact that the P concentrations in Estero were much lower than all other creeks in the study.

Table 4-1. Observed Redfield Ratios for creeks sampled in 2017 and 2018 using particulate carbon, nitrogen and phosphorus (ug/l) converted to molar weights.

Creek	Carbon	Nitrogen	Phosphorus
Estero	75	10	1
Frog	42	5	1
Mullet	57	8	1
South	37	5	1
Sweetwater	44	6	1
West Spring	60	8	1

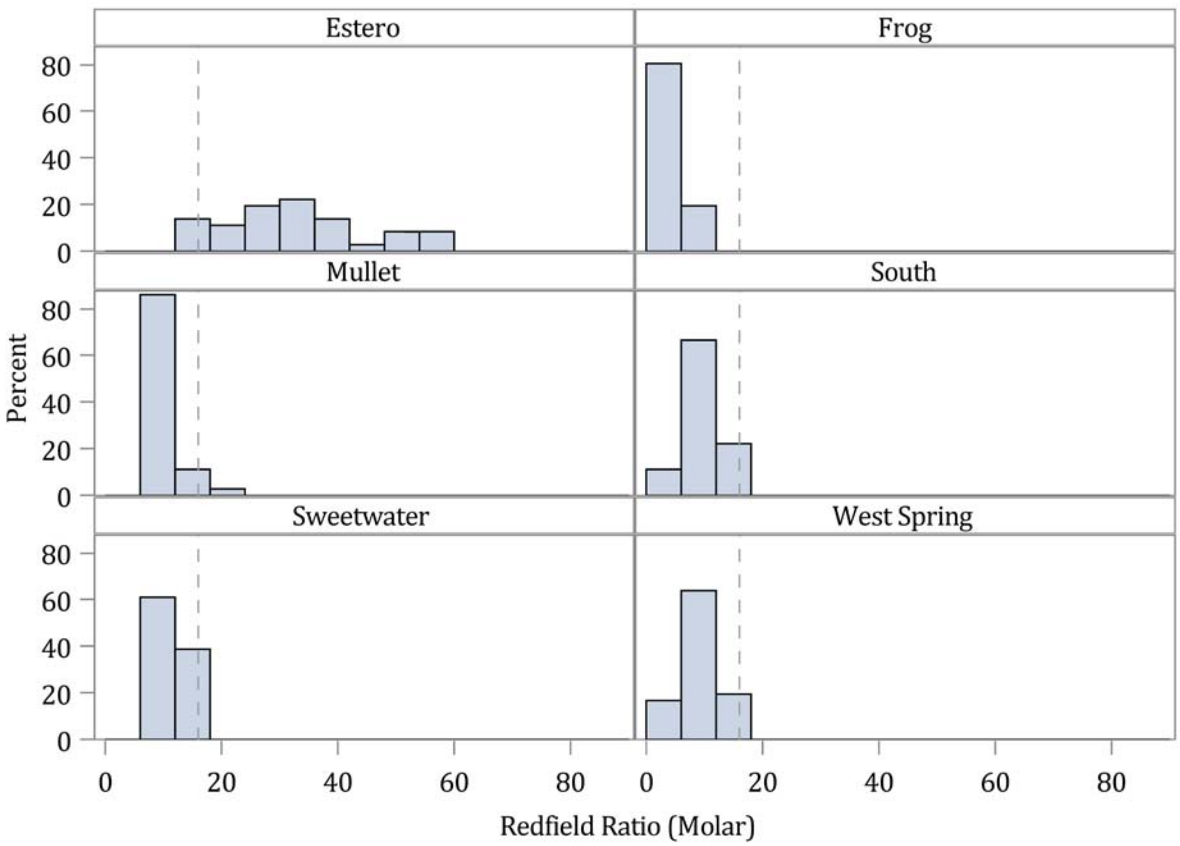


Figure 4-1. Distribution of nitrogen to phosphorus ratios based on molar weights.

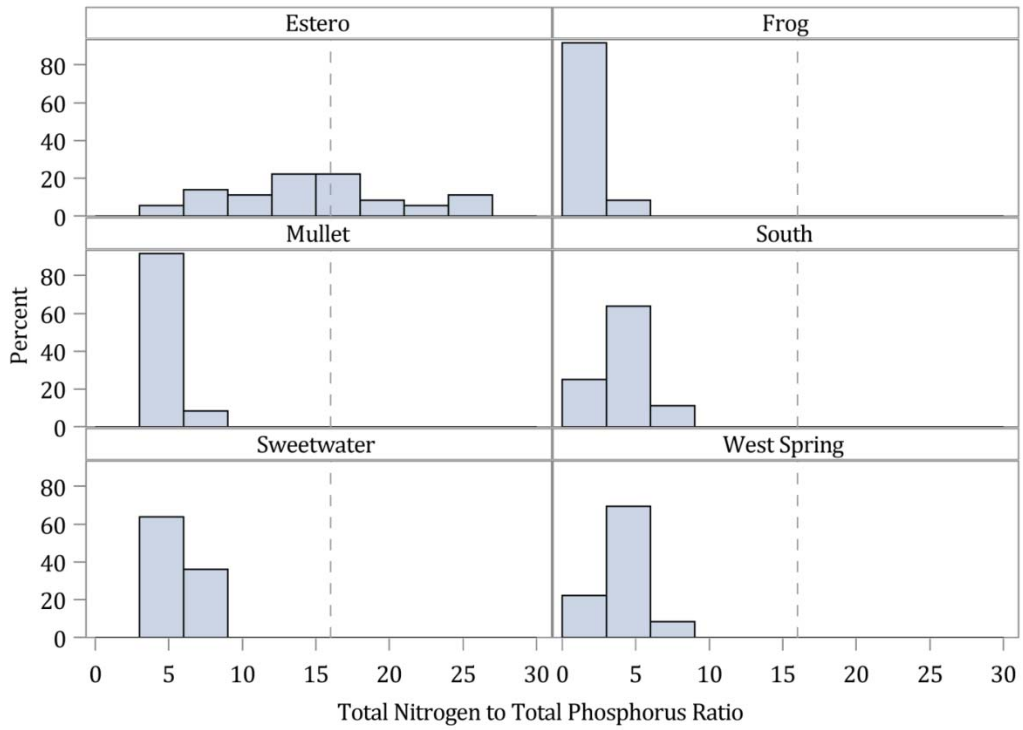


Figure 4-2. Distribution of nitrogen to phosphorus ratios based on mass per unit volume.

The ratio of dissolved nutrients based on molar weights is provided in Figure 4-3 and also suggests ratios indicative of nitrogen limitation with the exception of Estero in which half of the observations were above the Redfield Ratio of 16:1.

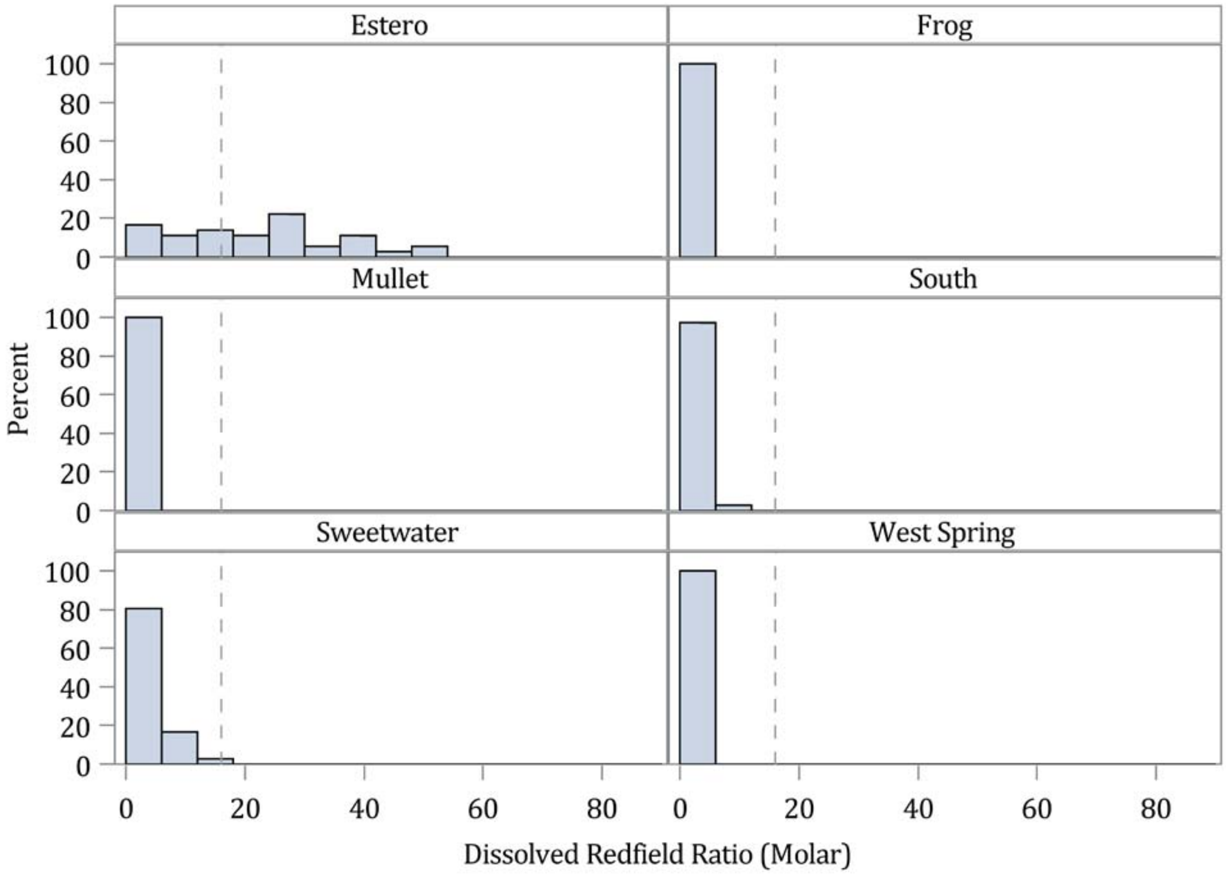


Figure 4-3. Distribution of nitrogen to phosphorus ratios based on molar weights and inorganic forms of nutrients.

Sediment nutrient ratios based on total kjeldahl nitrogen and total phosphorus also exhibited nitrogen limitation for all creeks other than Estero which was the only creek exhibiting ratio values above 16 (Figure 4-4). Together these findings suggest that most of the creeks are nitrogen limited; however, Estero may be the exception in that phosphorus values were low and addition of inorganic nitrogen inputs in the tidal portion of the system was observed resulting in ca. 50% of the samples above the Redfield Ratio threshold of 16.

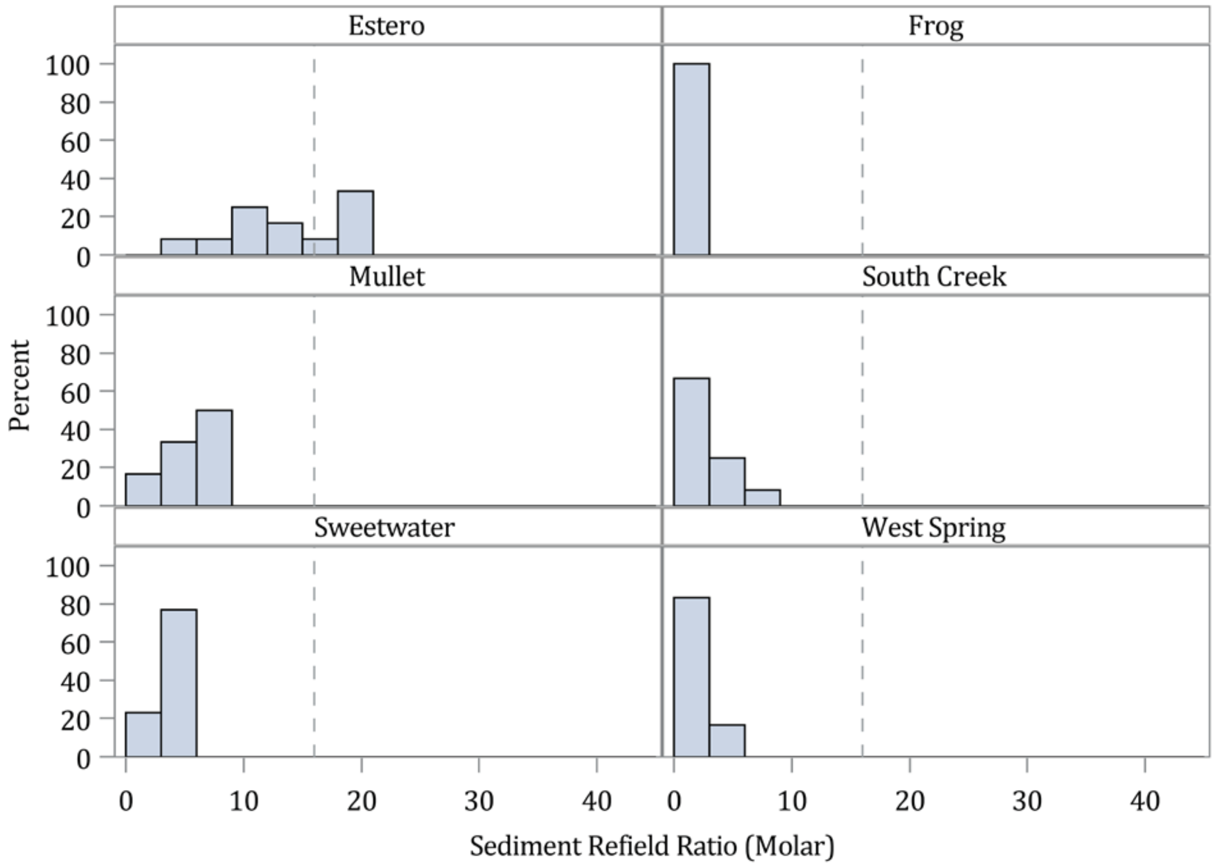


Figure 4-4. Sediment nutrient ratios based on molar weights of nutrients originally expressed as mg/kg.

4.2 Effects of Rainfall on Salinity and Nutrients

Rainfall and the resultant streamflow generated from surface water runoff is an important physical driver of instream nutrient dynamics. Direct estimates of flows were not available for all creeks in the study but the effects of rainfall as characterized in section 3.7 above were used to evaluate the effects of antecedent rainfall on salinity and nutrient delivery "events" to the tidal portion of the creeks. Salinity was highly statistically different among stations in all creeks which is an important consideration when evalauting changes in nutrient concentrations as a function of salinity. Only the lowermost stations in South Creek were not statistically different and this result may have been in part due to the low overall sample size of the study. Detailed results of the statistical analysis are provided in Appendix E.

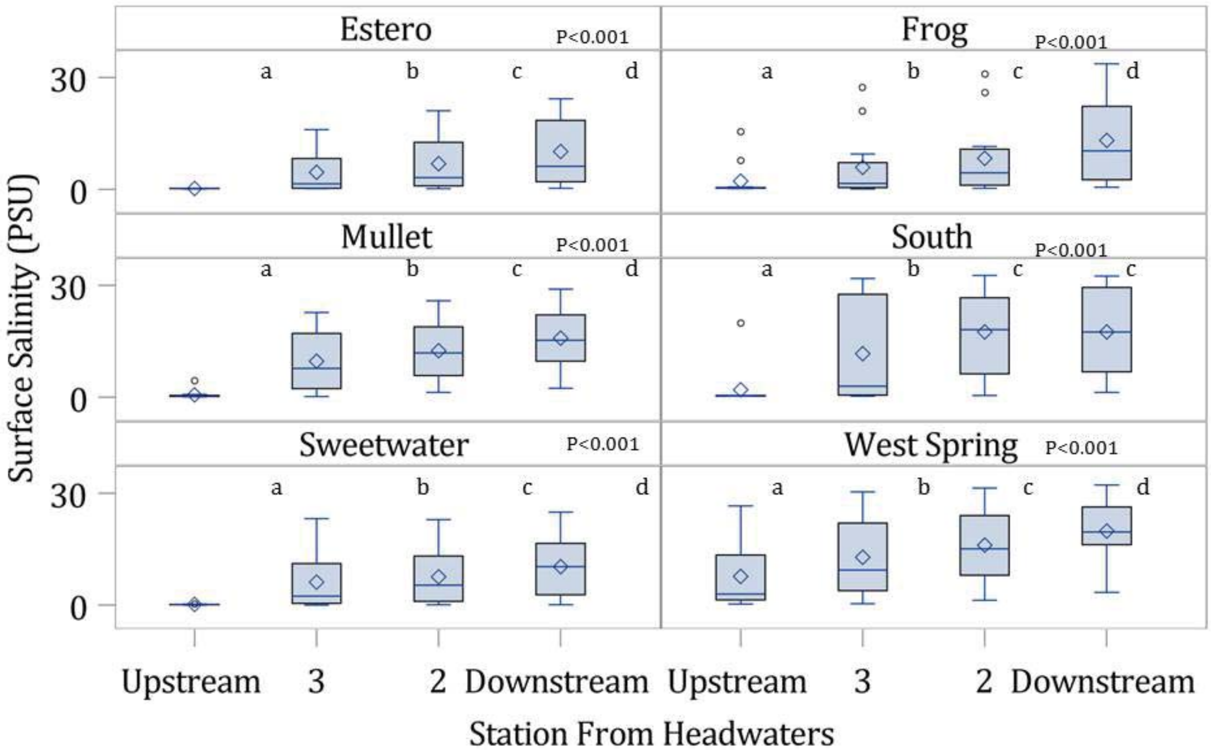


Figure 4-5. Results of analysis of variance for clustered data displaying significant differences between stations for each creek. Different letters represent significant differences.

An example of the differences in nutrient dynamics as a function of salinity is provided for Frog Creek in Figure 4-6. Each curve in each plot represents a sampling event with stations oriented from upstream to downstream and the 14 days total rainfall labeled. The June 2017 event for Frog Creek followed a total of 5.5 inches of rainfall in the 14 days prior to sampling. The entire creek was near freshwater during that June event as portrayed by the top left plot in Figure 4-6 (the green solid line). Dissolved inorganic nitrogen during the June event was 4 times the magnitude of the other two spring sampling events for that year (top right) and dissolved organic nitrogen (bottom left) was also substantially higher during the June event than during the other two events. This resulted in higher overall total nitrogen (bottom right) relative to the other events. These results point to the influence of rainfall and flows on resulting salinity and nutrient delivery in the tidal portion. Other “events” included an pulse delivery of particulate phosphorus to the tidal portion of Frog Creek during Spring 2018 which was an order of magnitude higher than any other event (Figure 4-7) and a spike in dissolved ammonia in Mullet Creek throughout the creek in May 2018 (Figure 4-8). Plots for all constituents are provided in Appendix E along with results of statistical evaluations comparing salinty, TN, TP and DNO23 among stations within each creek..

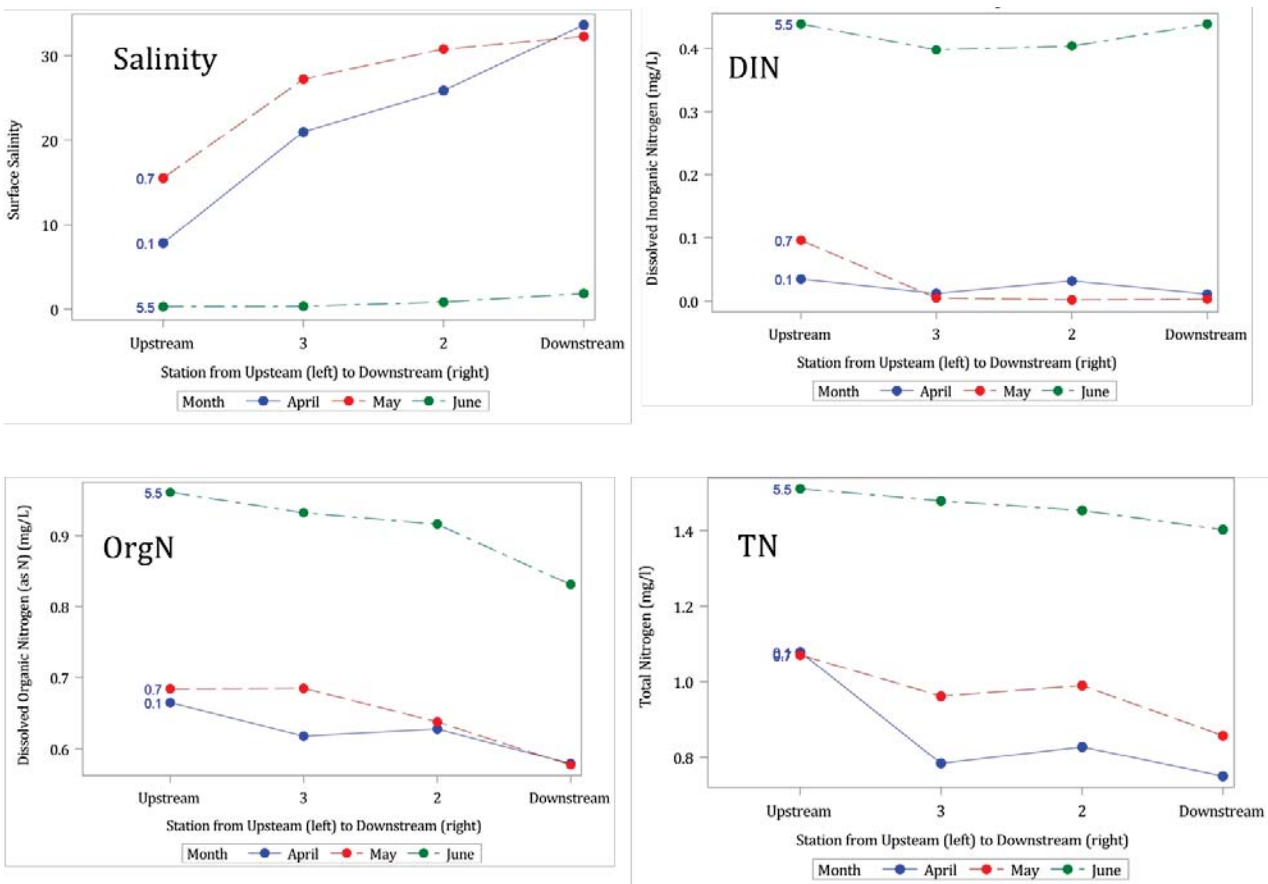


Figure 4-6. instream water quality dynamics in Frog Creek for Spring 2017 sampling events for salinity (top left), dissolved inorganic nitrogen (top right), organic nitrogen (bottom left) and total nitrogen (bottom right).

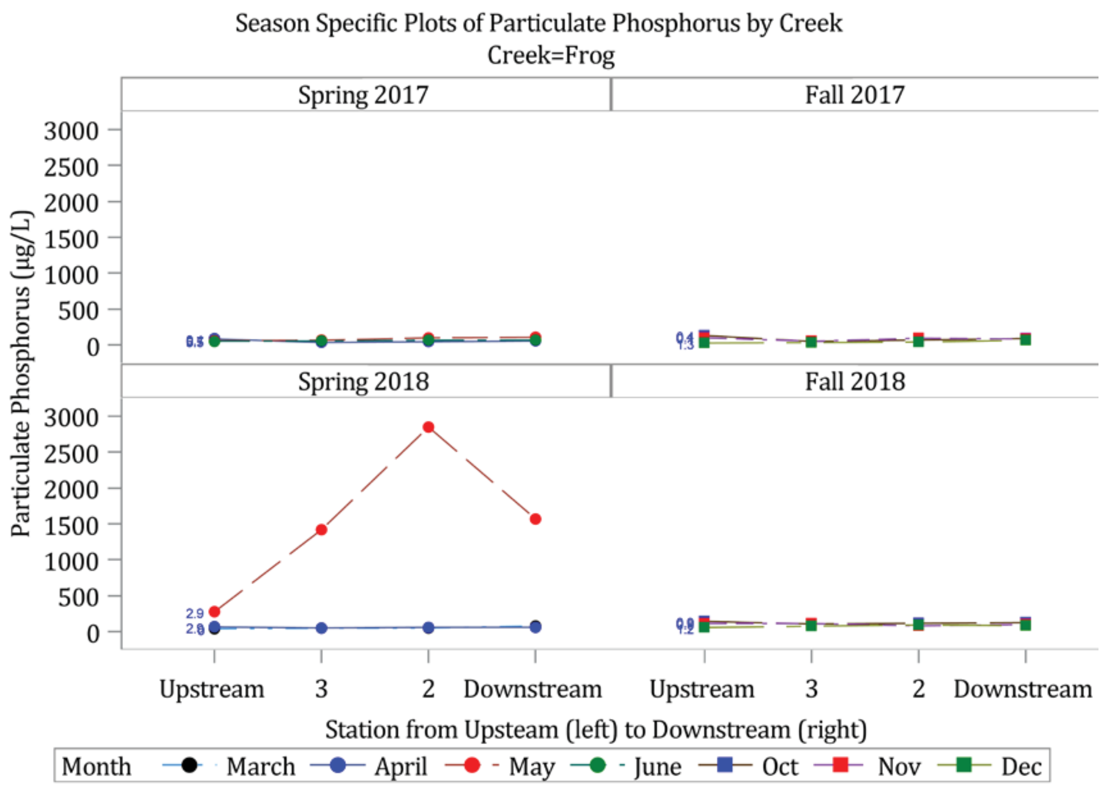


Figure 4-7. Particulate Phosphorus distribution for each sampling event in Frog Creek.

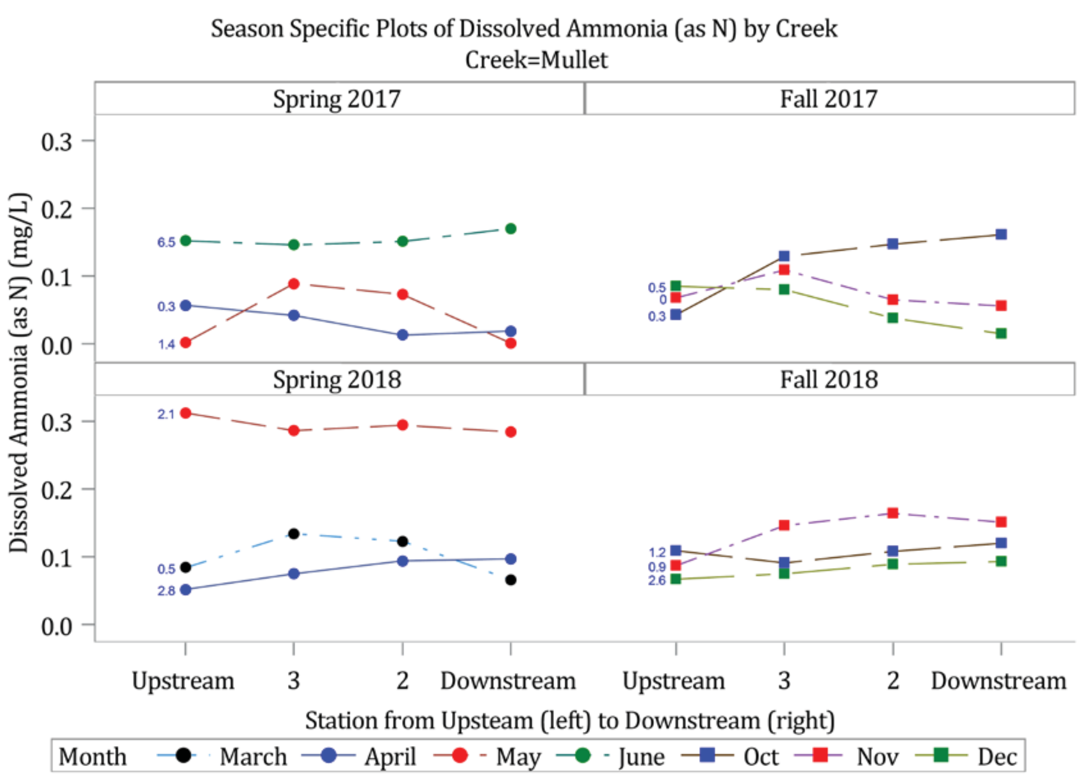


Figure 4-8. Distribution of dissolved ammonia for each sampling event in Mullet Creek.

4.3 Mixing Models and Nutrient Assimilation

The previous study found that nutrient concentrations, especially organic forms of nutrients, in the tidal portions of these creeks were generally in addition relative to the assumption of linear mixing within estuarine waters. Linear dilution lines provide a reference point from which to evaluate deviations from conservative assumptions about the dilution of nutrients along the salinity gradient. The sampling design used for this study was specifically constructed to evaluate whether nutrients acted as conservative substances within tidal creeks and to identify potential sources of nutrient addition in these creeks. By collecting water quality samples across the salinity gradient, generally within a four-hour window, the observed source water concentration was assumed to be representative of the expected inputs into the tidal creek. We used several methods based on conservative mixing principles of water quality constituents to describe the dilution of water quality in the tidal portion of the creek and evaluate different nutrient pools (e.g. organic versus inorganic nitrogen; total versus dissolved) to compare deviations from conservative mixing assumptions among these constituents. We understand that true instream dynamics are more complex than this and it is the deviations from the conservative mixing lines that is of interest in this analysis. For example, using a modification of the freshwater fraction equation (Sheldon and Alber, 2006), we calculated the expected concentration of nutrients in the tidal portion of the creek as a function of the source water concentration and the proportion of salinity relative to full strength seawater (i.e., 35‰). The expected nutrient concentration is:

$$En_c = n_f * (1 - (Sal_c / 35))$$

where :

En_c = Expected nutrient concentration at site c in tidal creek

n_f = Nutrient concentration at source water site

Sal_c = Salinity at site c in tidal creek

This equation allowed for the computation of an expected nutrient concentration at any sample location within the creek and a comparison of the expected concentration to the observed concentration at the sample location. When the salinity of the source water site was not zero, dilution was assumed to generate the expected value at zero salinity. This comparison was used to identify the behavior of nutrients in the tidal portions in the creek over various temporal and spatial scales. For example, the differences between the observed and expected nutrient concentrations (both total nitrogen and total phosphorus) from the previous study were mostly positive in all 16 creeks with the exception of TP in Doublebranch, Wildcat and Powell Creeks (Figure 4-9).

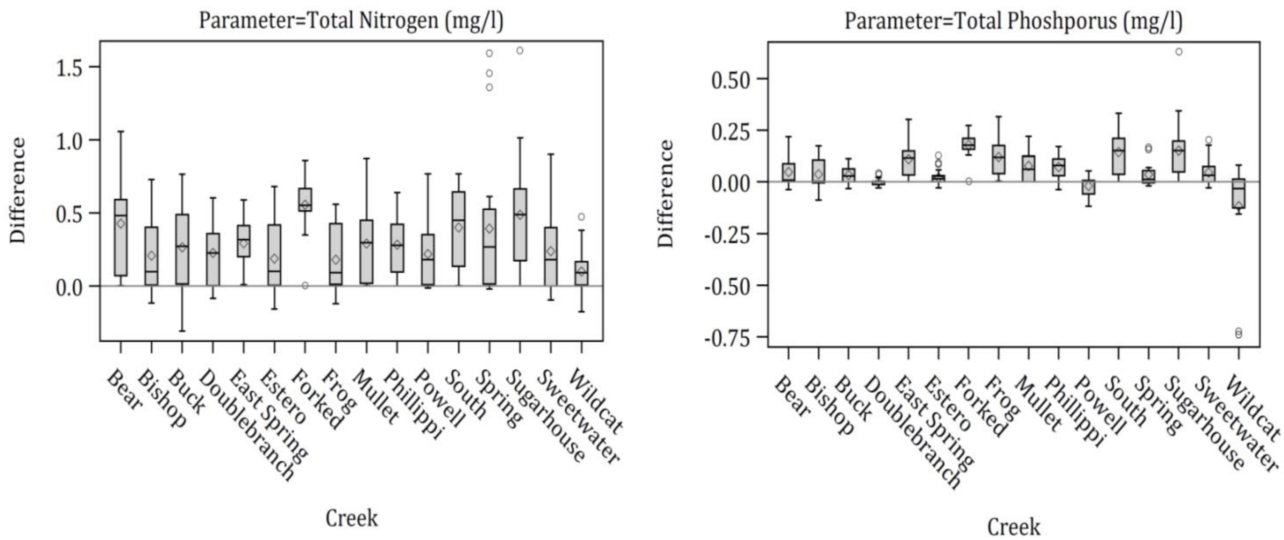


Figure 4-9. Distribution of differences between observed and expected concentrations of total nitrogen and total phosphorus for the 16 tidal creeks sampled in 2014.

The current study design included elements to help partition the sources of nutrients within the system to separate out effects of natural wetland function from anthropogenic sources based on observational data. These elements included:

- establishing the longitudinal transect to capture water quality information along the estuarine gradient within the creek;
- sampling during the typical dry season to minimize the influence of flushing and maximize the probability of capturing the organic wetland and internal cycling components of nutrient dynamics;
- evaluating the dissolved organic and inorganic pools of nutrients in the water column and sediment to provide information on nutrient cycling, and
- the collection and analysis of stable isotopes in an effort to identify nutrient sources and pathways within the tidal portion of the creeks.

To validate outcomes of the previous study, identical analysis was conducted on the samples taken from the 6 creeks surveyed for this study. This study targeted the typical dry season in southwest Florida to concentrate on instream nutrient dynamics when residence times tended to be greater and therefore biogeochemical activity more pronounced within the creek. For example, the distribution of differences for total nitrogen and total phosphorus are plotted in Figure 4-10. The nonparametric signed rank test (Zar 1984) was used to test if the differences were greater than zero and the results were highly statistically significant for most constituents (Table 4-2), with the exception being inorganic nitrogen, which was expected to be rapidly assimilated. However, Estero exhibited a different pattern than the other creeks in that inorganic forms of nutrients in Estero were in surplus in the tidal portion while CDOM, color and most organic forms of nitrogen appeared to follow the linear dilution curve. This was a peculiar finding and may indicate differential contributions from the south fork of Estero or possibly the presence of a spring vent in the upper tidal portion near Station 3.

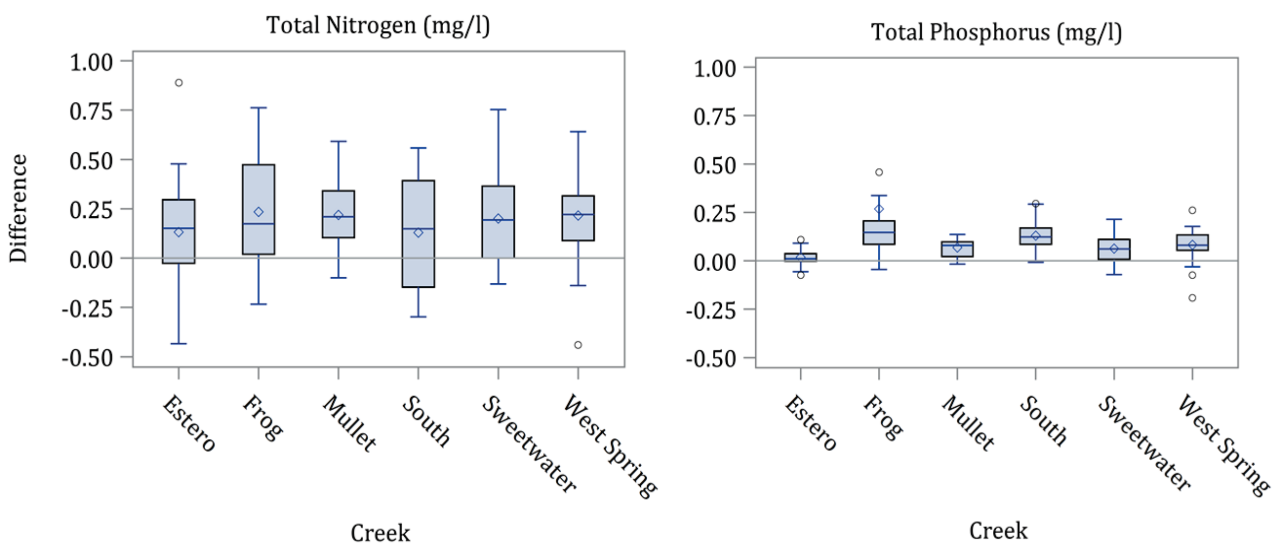


Figure 4-10. Distribution of differences between observed and expected concentrations of total nitrogen and total phosphorus for the 6 tidal creeks sampled in 2017-2018.

Table 4-2. Results of one sided signed rank test to evaluate if water quality constituent concentrations are greater than expected by dilution.

Constituent	Estero	Frog	Mullet	South	Sweetwater	West_Spring
CDOM_275	0.6441 #	<0.001	<0.001	0.0026	<0.001	<0.001
CDOM_465	0.0646 #	<0.001	<0.001	0.0049	0.0008	<0.001
Color_365	0.1976 #	<0.001	<0.001	0.0063	<0.001	<0.001
Nitrogen						
DNH3_mgl	0.0012	0.2246 #	<0.001	0.9841 #	<0.001	0.99 #
DNO23_mgl	<0.001	0.996 #	0.9099 #	0.7415 #	0.975 #	0.99 #
DIN_mgl	<0.001	0.9502 #	0.0008	0.9741 #	0.0732 #	0.99 #
DOrgN_mgl	0.8194 #	<0.001	<0.001	0.0036	<0.001	<0.001
DTKN_mgl	0.2246 #	<0.001	<0.001	0.0166	<0.001	<0.001
TKN_mgl	0.8541 #	<0.001	<0.001	0.0034	<0.001	<0.001
TDN_mgl	<0.001	0.0134	<0.001	0.0259	<0.001	<0.001
PN_ugl	0.9996 #	<0.001	0.0107	0.0023	0.0029	0.0188
TN_mgl	0.0012	<0.001	<0.001	0.0047	<0.001	<0.001
Phosphorus						
DOPO4_mgl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TDP_mgl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PP_ugl	0.9595 #	0.0034	<0.001	<0.001	0.2738 #	0.0498
TP_mgl	0.0038	<0.001	<0.001	<0.001	<0.001	<0.001

 # indicates non-significant result

The differences from those expected by dilution were then plotted as a percentage of the overall median value by station to evaluate the relative contributions spatially. For example, the differences for total nitrogen and total phosphorus were plotted for each station in Figure 4-11. The differences for the most upstream stations were zero, as expected, since these sites were located in fresh water. However, the downstream sites were all positive, with deviations from that expected by dilutions averaging ca. 40% of the median values for those stations. The same observation was made for two measures of dissolved organic matter (CDOM 465 and Color 365) as portrayed in Figure 4-12.

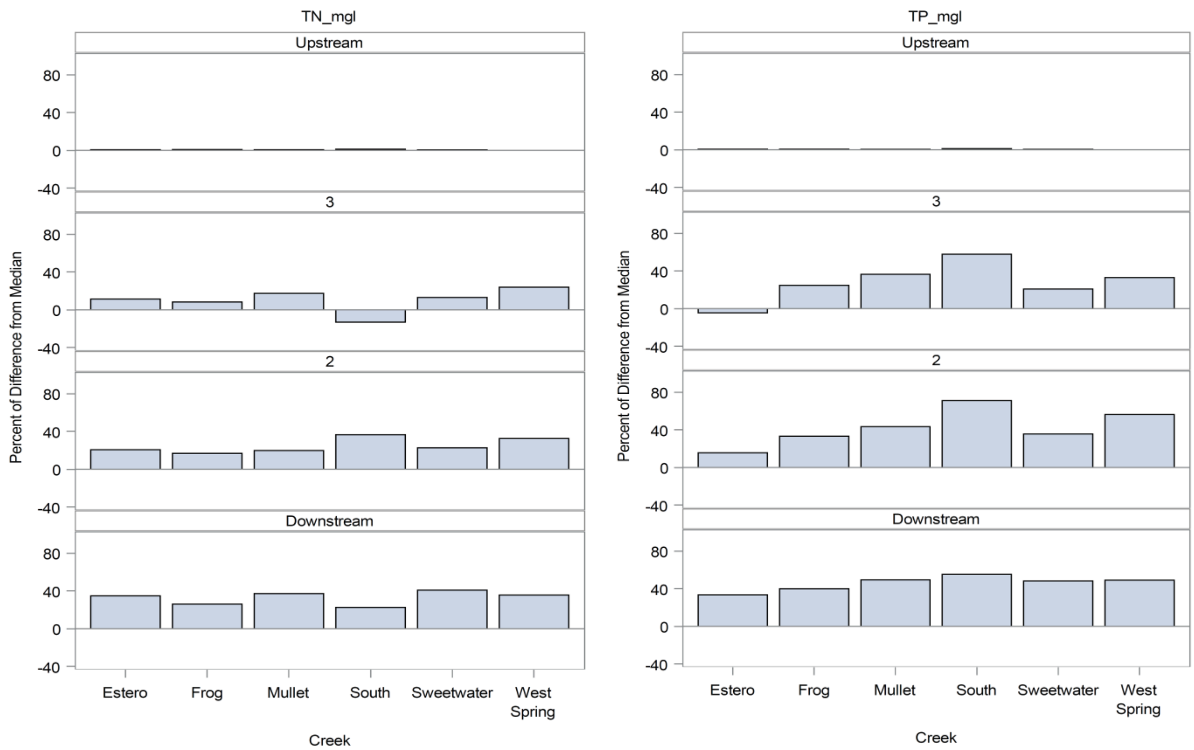


Figure 4-11. Differences from total nitrogen (left) and total phosphorus (right) concentrations expected by dilution expressed as a percentage of the overall median station value.

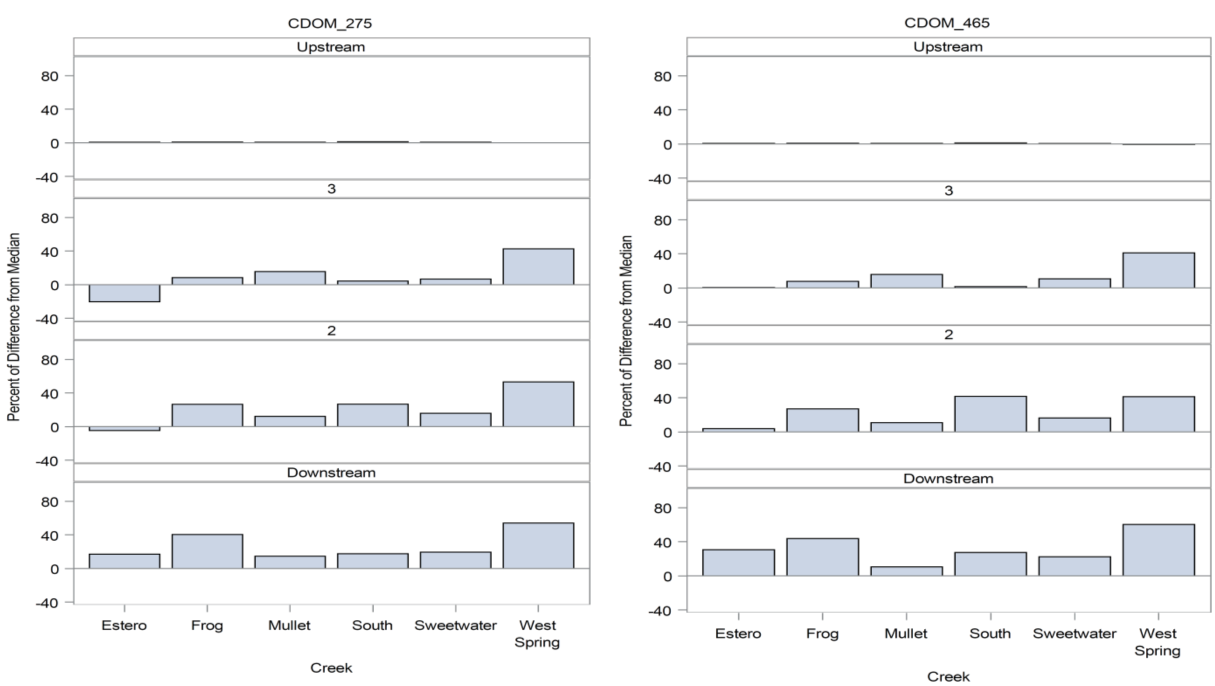


Figure 4-12. Differences in CDOM 275 (left) and CDOM 465 (right) from concentrations expected by dilution expressed as a percentage of the overall median station value.

A comparison of the principal constituents of organic and inorganic nitrogen can be seen in Figure 4-13 where organic nitrogen was in supply for most creeks while nitrite-nitrate was taken up within the system (with one notable exception) at rates higher than expected by dilution (assimilation). Nitrite-nitrate was in surplus at all three tidal stations in Estero while organic nitrogen was fairly conservative with marginal surplus at the most downstream site. This result indicates that the nitrogen delivery and assimilation in Estero is different than the other creeks. Further evidence that Estero has different nutrient dynamics than the other creeks is seen for particulate forms of nitrogen, phosphorus, and carbon. Again, it seems that organic nitrogen, phosphorus and carbon are either diluted in the upper estuarine stations in Estero while they are in surplus in the other creeks (Figure 4-14). The converse is also true; inorganic forms of nitrogen were depleted in most creeks while inorganic forms of nitrogen were in excess in Estero.

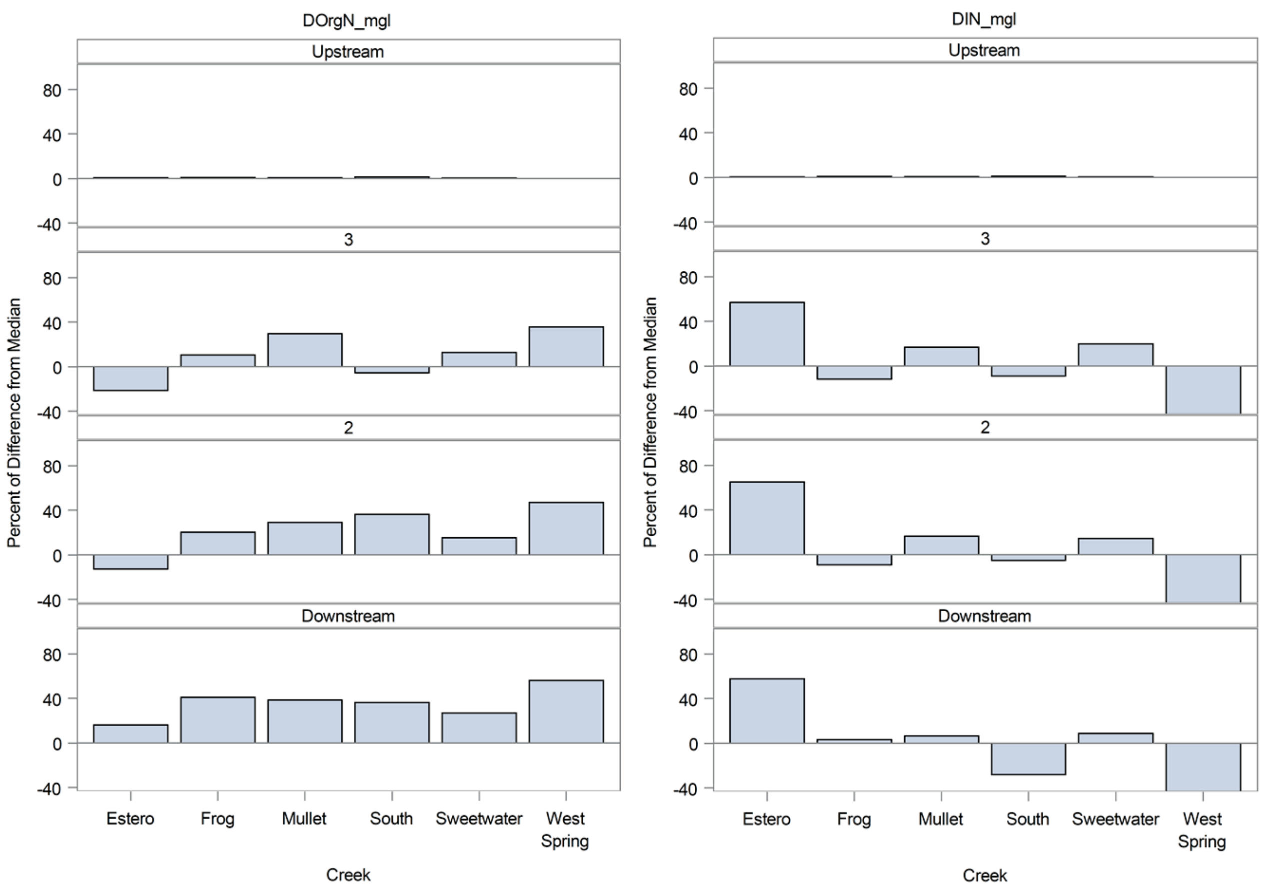


Figure 4-13. Differences in Dissolved Organic Nitrogen (left) and Dissolved Inorganic Nitrogen (right) from concentrations expected by dilution expressed as a percentage of the overall median station value.

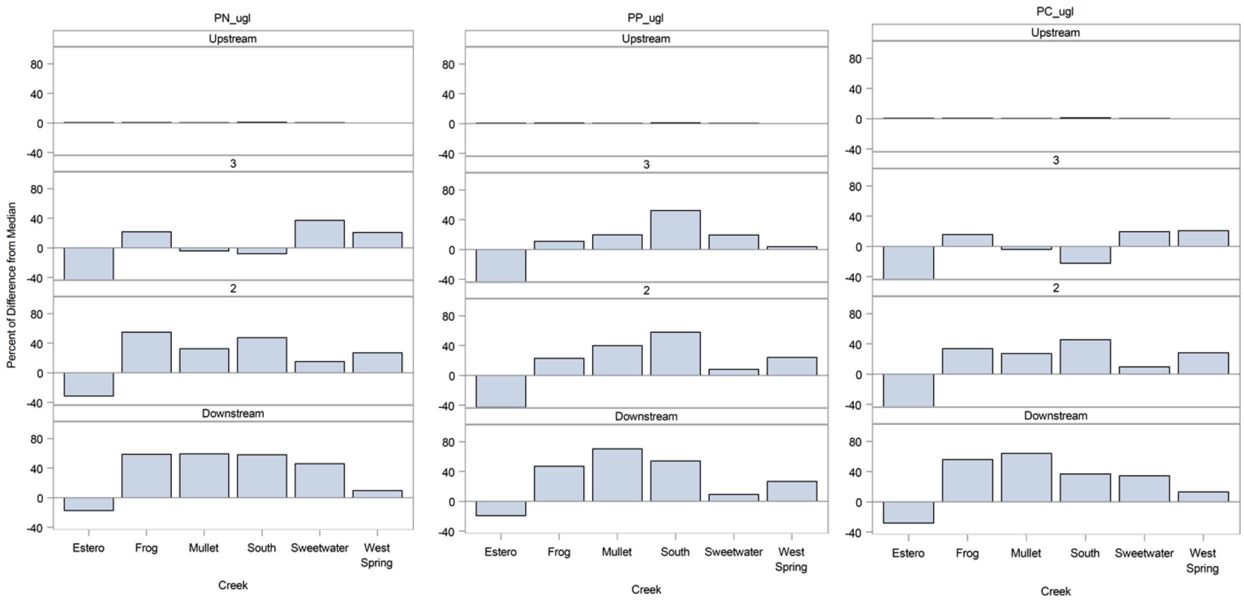


Figure 4-14. Differences from concentrations expected by dilution expressed as a percentage of the overall median station value for particulate nitrogen (left) particulate phosphorus (middle) and particulate carbon (right).

Alternative formulations of the dilution equation include: the Plew equation (Plew et al. 2018) which adds a concentration, such as the detection limit, to the downstream boundary; using the observed annual geometric average estuarine concentration of routine water quality monitoring data over the same time period (Figure 4-15); and using the EPA equation proposed to identify a downstream protective value by linear interpolation between the freshwater and estuarine numeric nutrient criteria (EPA 2012: Figure 4-16).

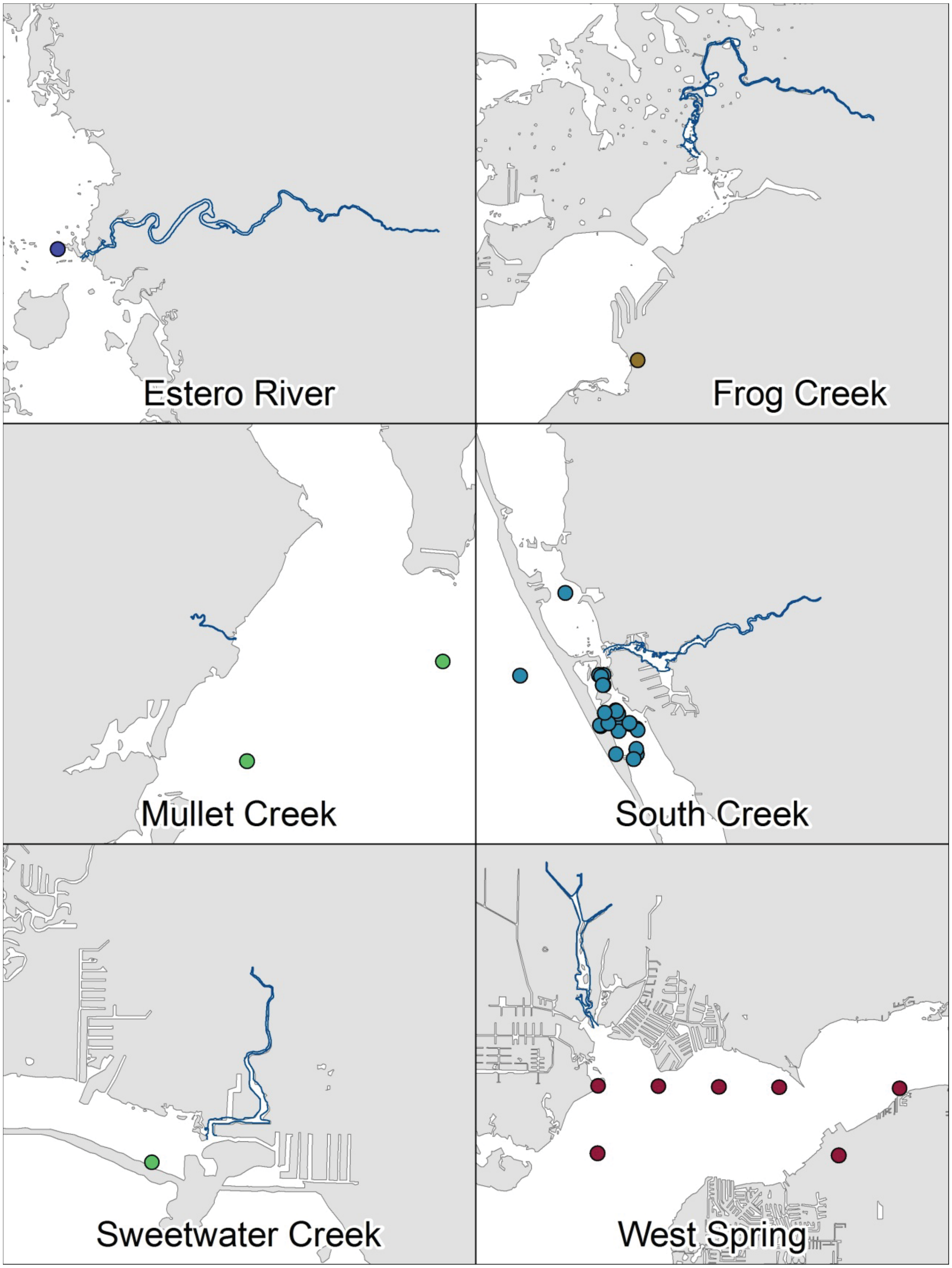


Figure 4-15. Estuarine sites used to calculate annual geometric average of estuarine nutrient concentration. Source = Impaired Waters Rule Run56 database).

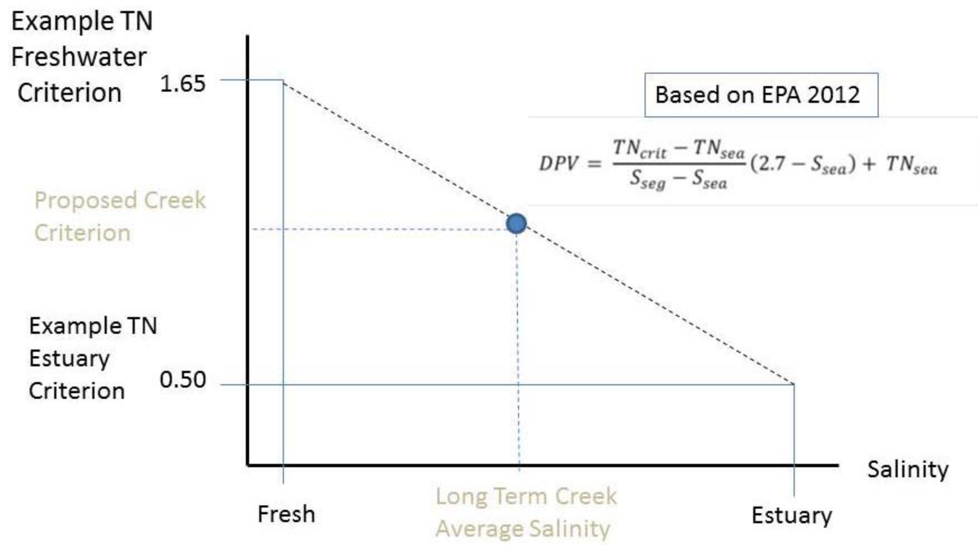


Figure 4-16. Method proposed to establish tidal creek criteria based on freshwater and estuarine criteria (EPA 2012).

While these methods produce plausible estimates of potential numeric nutrient criteria for tidal creeks, the linear dilution approach does not reflect observed or expected instream nutrient dynamics, making the reliability of this approach as protective criteria tenuous. We attempted to develop statistically based empirical nutrient-salinity relationships using nitrogen concentrations at the source water and most downstream sites as covariates to predict nutrient concentrations in the middle reaches of the tidal segment. The results suggested that salinity was not a useful predictor of nutrient concentrations in the middle reaches of the creek if the source water and downstream nutrient concentrations were known. That is, if the nitrogen concentration being delivered from the source water and at the most downstream station are both known, salinity was not a useful predictor of the nitrogen concentration for the two intermediate stations. An example prediction for each creek is provided in Figure 4-17 where salinity increases (green line) did not result in a decreased observed TN concentration in the middle sections of the creek and therefore the predicted nutrient concentrations (red line) were not a function of salinity. This confirms that, while higher salinity creeks may have lower nutrients on average, linear dilution curves do not necessarily represent within-creek nutrient dynamics and, therefore, applying linear dilution type models to establish criteria for tidal creeks would not result in site-specific protective standards. The linear mixed effects model output used for this analysis is provided in Appendix F along with resultant plots for all dates and all creeks.

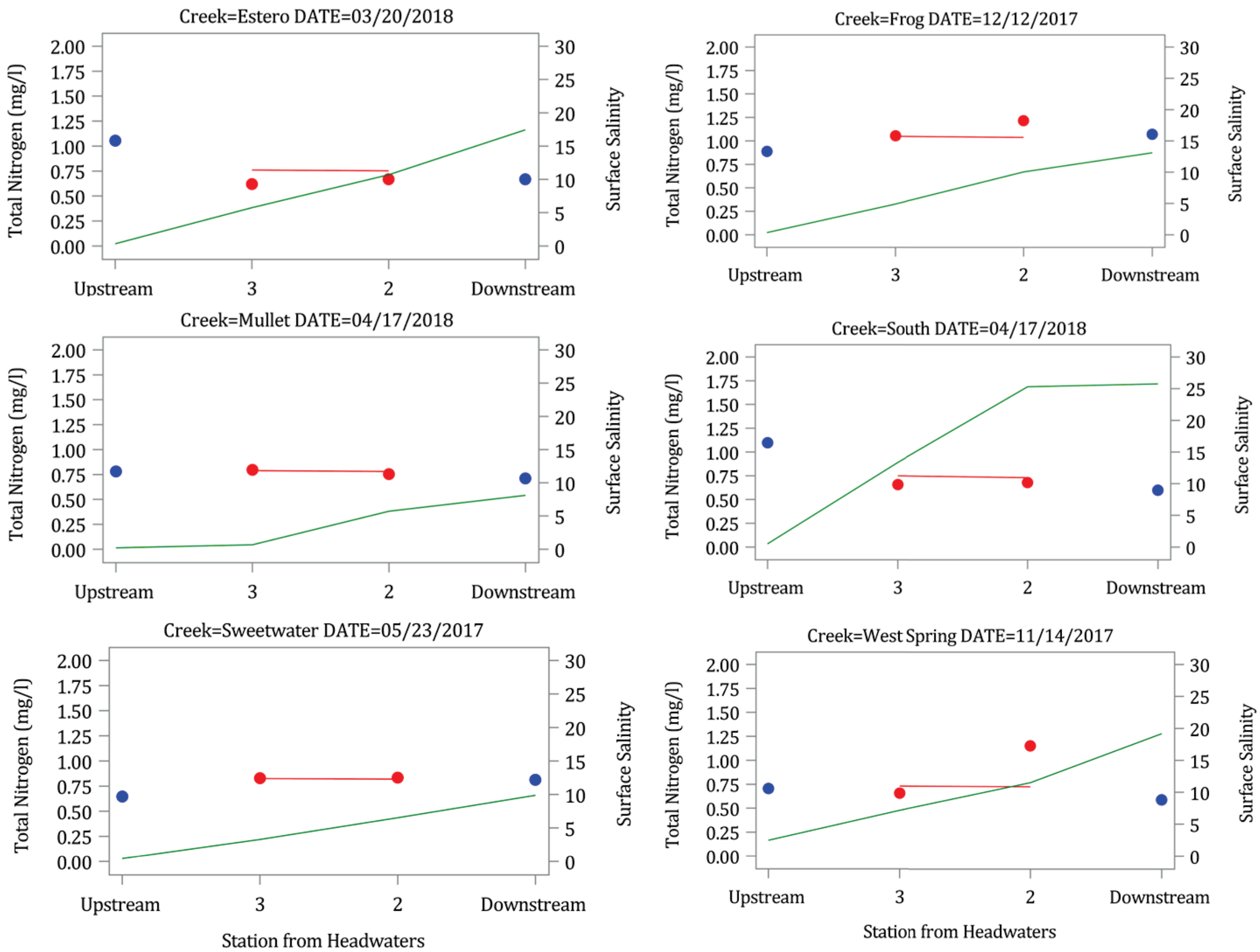


Figure 4-17. Results of linear mixed model TN predictions (red horizontal line) for middle stations based on knowing source water TN and TN at the most downstream station. Green line is surface salinity associated with each sample and dots are observed total nitrogen.

To gain a better understanding of the observed relationships between total nitrogen and salinity in creeks with long term data collection programs, we used the Impaired Water Rule database (Run 56) to identify creeks with water quality (i.e. nutrients and salinity) data collected at multiple locations within the creek on the same date to examine the date-specific nitrogen–salinity relationship in these creeks. For example, the date-specific nutrient-salinity relationships in Bullfrog Creek (Hillsborough County) are provided in Figure 4-18. The majority of curves are flat indicating little change in nitrogen as a function of changes in salinity. When TN concentrations in the freshwater portion of the creek are above 1, there is a higher probability that the curve will have a negative slope and the nutrient concentrations downstream will tend to be less than upstream concentrations.

Using all data in the IWR dataset culled for this analysis, a significant relationship between higher source water TN concentrations and the proportion of slopes that declined as a function of salinity was observed (Figure 4-19). That is, the higher the source water concentration, the more likely that those concentrations were diluted within the estuary. While this seems intuitive, it has important ramifications when drawing inferences based on dilution curves. In addition, the proportion of samples with declining slopes was indicative of the nutrient dynamics in the system as further described below.

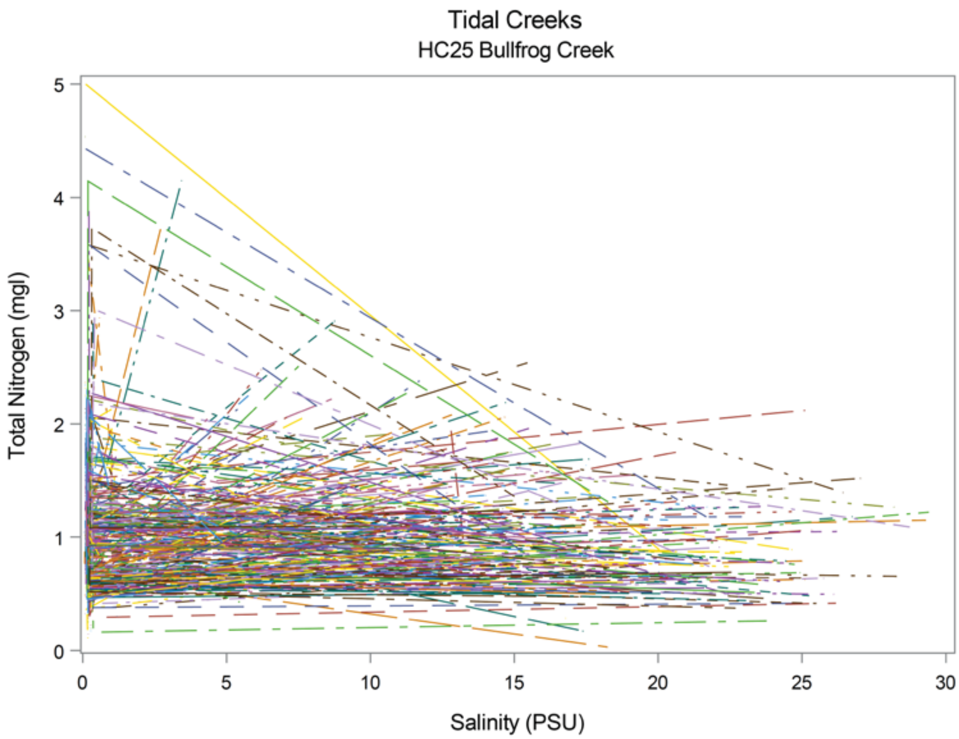


Figure 4-18. Date specific nutrient salinity curves for available data for Bullfrog Creek from IWR Run 56.

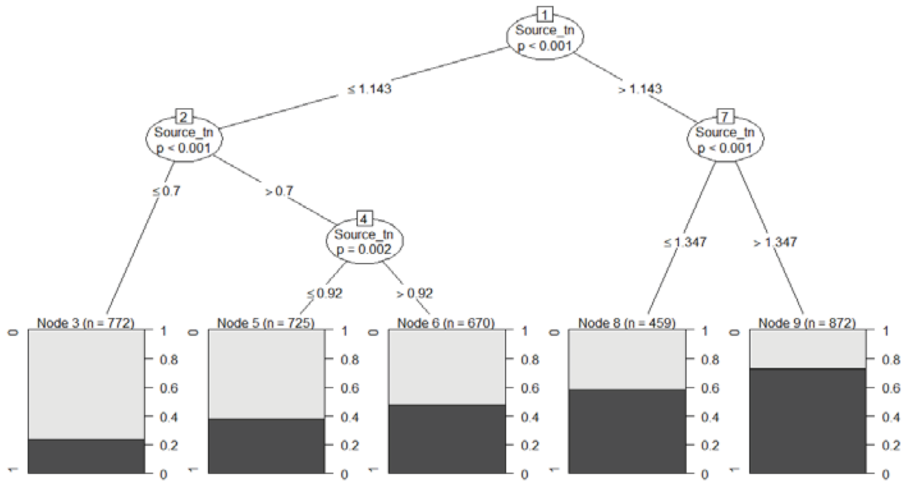


Figure 4-19. Proportion of negative date specific TN Salinity slopes as a function the source water Total nitrogen concentration showing statistically significant breakpoints separating the groups.

The proportion of curves with a declining slope for all creeks in the IWR dataset with sufficient data is listed in Table 4-3. The median percentage of negative slopes was ca. 52%. Plots of the distribution end members (i.e. Delaney and Philippi Creeks) are presented in Figure 4-20.

Table 4-3. Proportion of negative slopes between nitrogen and salinity in tidal creeks with multiple sites sampled on the same date.

Creek	Percent Declining Slopes
Delaney Creek	13.6
Mullock Creek	23.7
Spring Creek	26.8
Saint Joes Creek	38.6
Chantry Canal	47.9
Bullfrog Creek	47.9
Whisky Creek	49.1
Allen Creek	50.0
Estero River	53.8
Cross Bayou	61.2
Sweetwater Creek	63.0
Billy Creek	67.1
Rocky Creek/Channel G	68.4
Catfish/North Creek	68.8
Gottfried Creek	70.0
Hancock Creek	73.4
Phillippi Creek	87.5

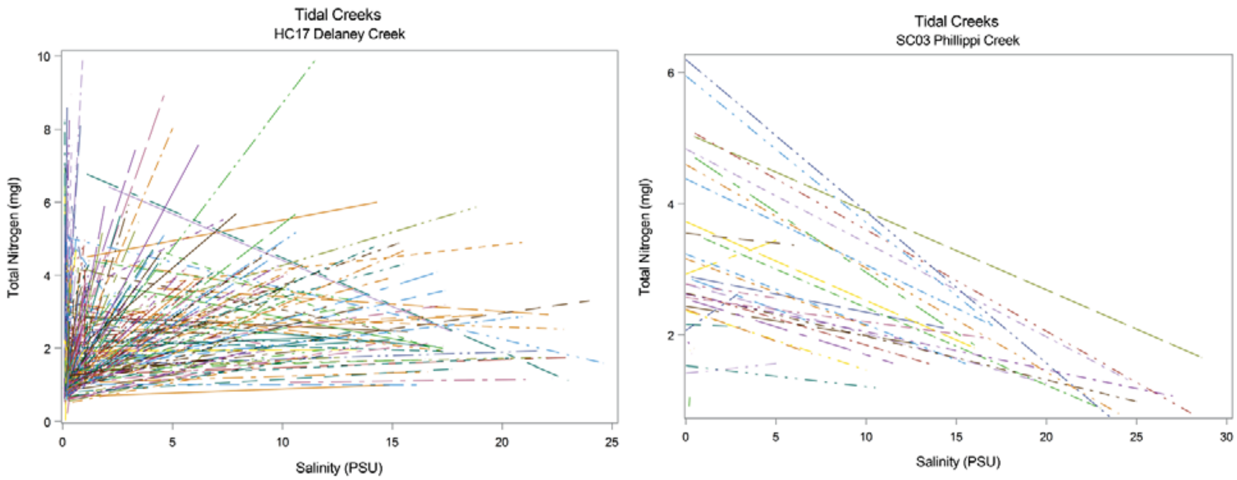


Figure 4-20. Date specific TN – salinity relationships in Delaney Creek (left) and Phillippi Creek (right).

Delaney Creek has a historical point source discharge just above the tidal portion of the creek resulting in increased TN concentrations in the tidal portion relative to its source water. The dataset for Phillippi Creek in the IWR database only has data that met the criteria for evaluation from the 1980's but demonstrates that Phillippi has historically been susceptible to high source water nitrogen concentrations. Creeks with few declining slopes may indicate either low source water concentrations or a potential source in the tidal portion of the creek. Creeks with an extremely high proportion of negative slopes indicate either high source water concentrations or rapid dilution or assimilation. In our study, South Creek had 100% of the dates sampled exhibited a declining slope and also had the highest source water TN concentrations of any creek. Isotope results did not reveal a distinct wastewater signature in South Creek though more work would need to be completed to confirm whether the source of high nitrogen inputs from the source water is natural or anthropogenic. Evaluating nutrient dynamics in this manner provides an indicator that can be further investigated to understand the spatio-temporal dynamics in nutrient delivery to these creeks. While these responses were clearly creek-specific, they can be used to generalize a conceptual expectation for the response of nitrogen in southwest Florida tidal creeks. In general, the nutrient dynamics in Southwest Florida tidal creek consist of three expected phases as described below and depicted in (Figure 4-21):

- **Dry Phase**: In this phase a low volume of freshwater is delivered to the tidal portion of the creek under "base flow" conditions. Source water nutrient concentrations tend to be lower than average and nutrient concentrations in the tidal portion of the creeks are similar to source water concentrations. The tidal concentrations represent an integration of source water inputs, assimilation, organic decomposition of both allochthonous and autochthonous material, outwelling from adjacent mangroves, and potentially sediment flux.

- **Pulse Wet Phase**: Characterized by conditions that generate storm water runoff and direct storm water infrastructure inputs. Freshwater pulses containing higher nutrient concentrations are delivered to the tidal portion of the creek and attenuated by the differential between source and tidal water concentrations. Salinity is a reasonable surrogate for estimation of the dilution of nutrients within the tidal portion of the creek under this phase.

- **Saturated Wet Phase**: Under these conditions the entire creek is essentially freshwater. Nutrient concentrations tend to be (but are not necessarily) higher and there is little to no gradient in nutrient concentrations along the length of the creek.

An observation that the slope in the nitrogen-salinity relationship is consistently positive, as was the case for Delaney Creek, should lead to the investigation for a potential source of high nutrient input in the tidal portion of the creek. Other deviations from this expectation, such as consistently negative slopes, may lead to an investigation of the source water for indications of excessive nutrients contributing to the tidal segment. This simple calculation requires proper sampling design, but can be a powerful tool to identify and eliminate potential sources of nitrogen and would serve as a useful indicator of creek condition. Further examples of this type of outcome are described in Chapter 5.

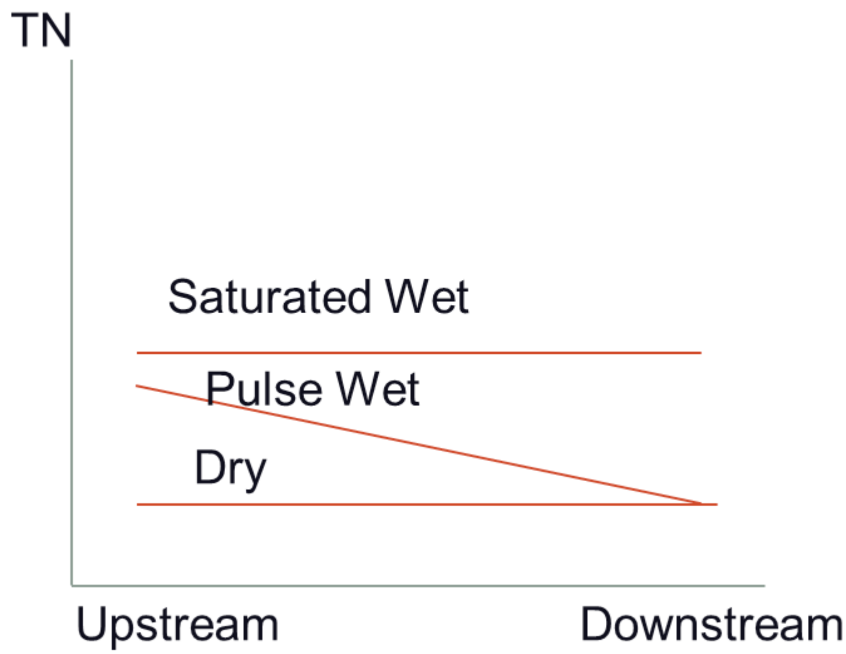


Figure 4-21. Conceptual expectation of nutrient dynamic responses to seasonal or antecedent rainfall conditions in tidal creeks.

5 Indicators of Creek Condition: Relating back to the Nitrogen Management Framework

5.1 Current Regulatory Standards for Tidal Creeks

A principal objective of the Clean Water Act (CWA) is to restore and maintain the chemical, physical and biological integrity of the Nation's waters to provide "water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water" (often referred to as "full aquatic life support"). Historically, FDEP has used a narrative standard to assess compliance with CWA objectives with respect to nutrients. The narrative standard states that "in no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural population of flora or fauna." More recently, numeric nutrient interpretations of the narrative criteria (i.e. NNC) have been established for many types of waterbodies in Florida including lakes, freshwater streams, estuaries, and springs. However, tidal creeks remain governed by the narrative standard.

Tidal creeks in Florida are considered "predominantly marine waters" and are currently evaluated by the water quality standards for DO and chlorophyll associated with the historical narrative criteria for estuaries. These criteria include an annual geometric average chlorophyll concentration of 11 ug/l and no more than 10% of dissolved oxygen values below 42% saturation. The freshwater DO saturation criterion of 38% is applied when salinity in the creek is less than 2.7 psu at the sampling location. This study recognizes that the DO and chlorophyll criteria enacted as part of the narrative criteria are likely to remain standards by which tidal creeks are assessed for regulatory impairments. This study was intended to provide additional nutrient information to derive stewardship and management goals to support planning level investigations into creeks with nutrient concentrations that represent conditions trending toward an imbalance of flora and fauna in these systems. Several tidal creeks within the study area, including ones that we sampled, are considered by the State to be "Outstanding Florida Waters" and by rule include special protections including a non-degradation threshold to preserve their historic status. **Improving the existing nutrient management framework will contribute to an improved understanding of what constitutes a "healthy" creek**. The following sections describe the evolution of the tidal creek nutrient management framework and the development of additional indicators to improve our ability to identify creeks that may need management actions. **The nutrient management framework was developed to serve as a screening tool to identify creeks with elevated nutrient concentrations for further evaluation to protect their in-stream ecology, but is not designed to explicitly identify regulatory impairments (or lack thereof) or the needs of downstream waterbodies such as larger estuarine waters that may be in need of nutrient load reductions.**

5.2 Revised Nutrient Report Card for Tidal Creeks

The original nutrient management Report Card relied on a nitrogen index score comprised of categories in which each creek with sufficient data was characterized by its distribution of annual geometric average TN concentrations. The Report Card included: target nutrient concentrations that protected individual creek types within the larger population of creeks; caution levels that were indicative of creeks trending towards a nutrient condition that was above their individual assimilative capacity; a management action level that identified a potential degredation point as an impetus to develop site-specific management actions; and thresholds which represented the potential need for nutrient reductions. The Report Card evaluation criteria were constructed to adhere as closely as possible to the criteria used by FDEP in their evaluation for impaired waters (i.e. verified period =7.5 years with a 1:3 exceedence rate allowance) with the idea that FDEP might consider the thresholds as potential numeric interpretation of the narrative criteria. However, as the previous study was being conducted, the FDEP decided that southwest Florida tidal creeks would remain under the narrative standard for the foreseeable future. Based on this decision, the Report Card was revised to be more proactive as a screening tool to inform and help NEPs and others identify management options that would better protect/restore these valuable resources. For example, in the previous grading system, the exceedence criteria required more than 1 exceedence within a 3 year time period, analogous to the routine used by FDEP. However, there are cases where a creek was only sampled during a single year and that information should not be ignored when evaluating and prioritizing creeks for investigation and planning. In addition, a longer evaluation period would incorporate more historical data for a screening level assessment of tidal creek condition. Therefore, several refinements were made to the existing nutrient management framework to include the following:

- The most recent 10 years of data were used for the evaluation, analogous to FDEPs "planning period" (Since IWR Run 56 doesn't include much 2018 data, 11 years were used for this evaluation).

- Freshwater WBIDS within the geographic boundaries established for the tidal creeks were evaluated to characterize the contributing source water to the tidal portion of the creek.

- Creeks were scored in cases where only one year of data existed.

The classification (Category) names have been changed to reflect the renewed purpose of the Report Card and now include: "Caution", "Investigate", and "Act" categories. The definitions of these categories are as follows:

- The Caution score (Yellow) would acknowledge that a creek has had higher than desirable nutrient conditions sometime in the past 10 years and represents a condition that resource managers should closely monitor.
- The Investigate score (Orange) suggests that a desktop and field investigation (e.g. "walk the WBID") should be conducted to investigate potential sources of nutrients that have been observed to be above the explicit margin of safety established for that tidal creek.
- The Act score (Coral) suggests that a creek has the potential to exceed its upstream freshwater regulatory numeric nutrient criteria threshold and a plan should be constructed to identify potential nutrient sources and remediative actions.

In cases where only one annual geometric average TN was above the Act level, but multiple other years were below the Act threshold, a creek would be assigned an orange color representing an Investigate score. If only one annual geometric average TN was above the Investigate level, but multiple other years were below the Investigate level, a creek would be assigned a yellow color representing a Caution score.

Application of the revised framework for creeks within the geographic boundaries of southwest Florida is provided in Figure 5-1. The teal color is the boundary for the Class 2 and 3 marine WBIDs. Twenty nine tidal creeks were identified as having a Coral (13), Orange (8), or Yellow (8) score (Table 5-1).

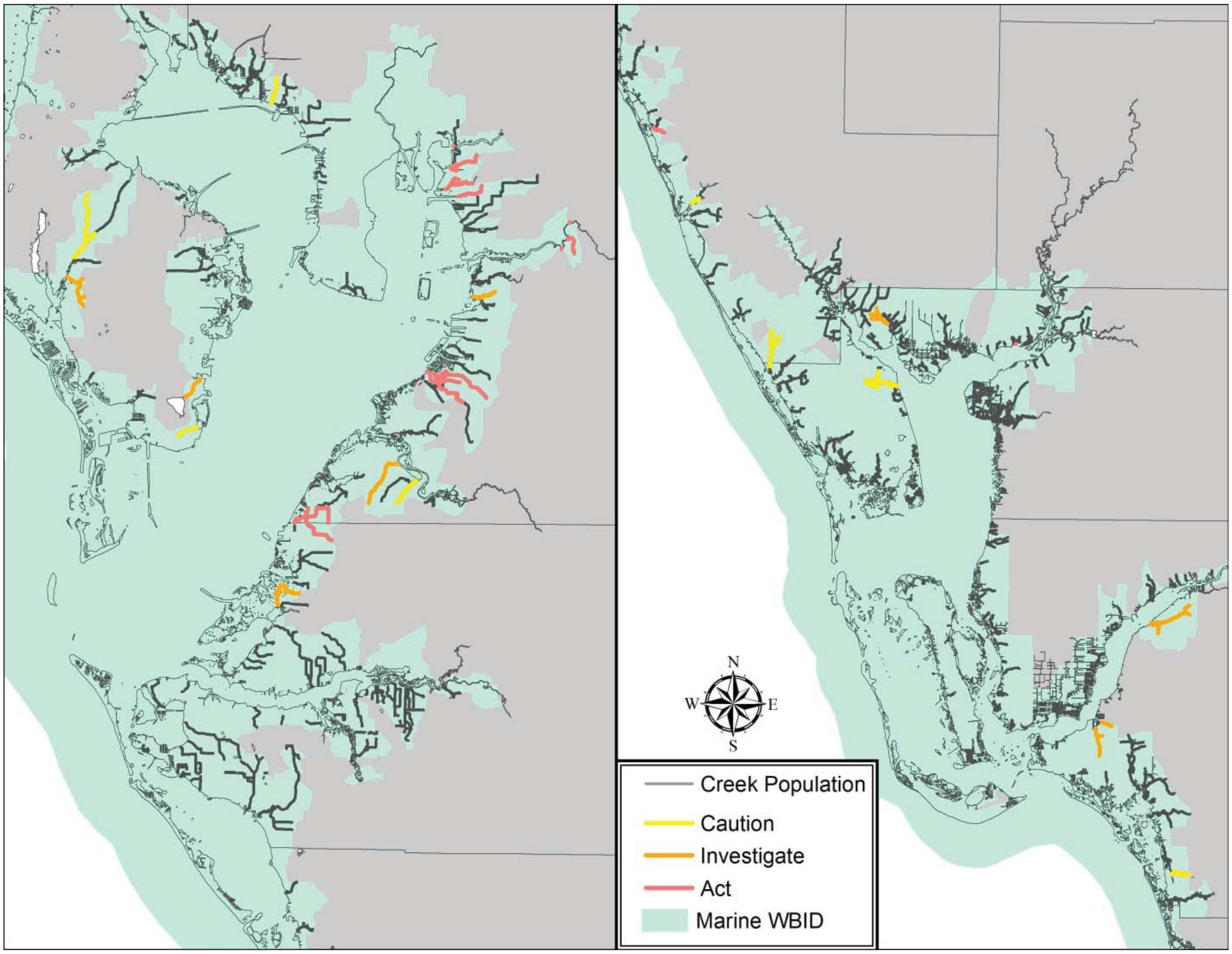


Figure 5-1. Application of revised tidal creek nitrogen management framework to the population of 306 tidal creeks.

The Report Card was also applied to the contributing source water (i.e. freshwater) segments of the creeks to identify source water segments with elevated nutrient concentrations. Eighteen 18 source water segments were identified as having either a Coral (4), or Orange (14) score (Table 5-2). In total, this application of the Report Card has identified 47 creek segments within the contiguous NEP boundaries that are immediately available for evaluation and prioritization for management actions outside of any regulatory framework.

Table 5-1. Results of evaluation using the revised Report Card to the "tidal" WBIDS with the frequency counts for each scoring class in the planning period based on annual geometric average total nitrogen concentrations.

Creek Name	Creek ID	WBID	Class	Below	Caution	Investigate	Plan	Score
Sun Coast Harbor	CC100	2056B	3M				1	Coral
East McKay Creek	HC13	1584B	3M	2			1	Coral
Ironhead Creek	HC16	1615	3M	1	2		7	Coral
Delaney Creek	HC17	1605D	3M		2	3	5	Coral
Port Sutton Ditch	HC18	1636	3M		3		7	Coral
Buckhorn Creek	HC23	1621G	3M			3	7	Coral
Rice Creek	HC24	1621G	3M		4	2	2	Coral
Masters Canal	HC29	1719	3M	1	2		7	Coral
Biscayne Canal	HC30	1725	3M	6	1		2	Coral
Piney Point Creek	HC39	1789	3M			1	3	Coral
Cummins Creek	HC41	1621G	3M			3	5	Coral
Billy Creek	LC35	3240B	3M				1	Coral
Catfish Creek	SC07	1984A	3M				1	Coral
Sam Knight Creek	CC18	2048A	3M	10			1	Orange
Sun City Slough	HC37	1771	3M	6	3		1	Orange
Dug Creek	HC49	1676	3M	4	5		1	Orange
Billy Creek	LC35	3240J	3M	4	4	3		Orange
Deep Lagoon	LC40	3240A4	3M	6	2	3		Orange
Frog Creek	MC02	1825A	3M	6		4		Orange
Saint Joes Creek	PC04	1668E	3M			1		Orange
Salt Creek	PC10	1731B	3M			1		Orange
Trout Creek	CC10	2066	3M	2	3			Yellow
Peppermound Creek	HC07	1577A	3M	5	4	1		Yellow
South Wildcat Creek	HC35	1780	3M	6	4			Yellow
Spring Creek	LC50	3258H2	3M	6	4	1		Yellow
Cross Bayou	PC02	1641	3M	2	7	1		Yellow
Bayou Creek	PC09	1709D	3M		4	1		Yellow
Shakett Creek	SC12	2002	3M	5	4	1	1	Yellow
Gottfried Creek	SC25	2049	3M	9	1	1		Yellow

Table 5-2. Results of evaluation using the revised Report Card to source water (3F) WBIDs within the geographic boundaries of the tidal creeks study with the frequency counts for each scoring class based on annual geometric average total nitrogen concentrations.

Creek Name	Creek ID	WBID	Class	Below	Caution	Investigate	Plan	Score
Riverside Creek	DC03	2028	3F				1	Coral
Curiosity Creek	HC34	1792	3F	4		1	5	Coral
Cockroach Creek	HC38	1788	3F	2		1	6	Coral
Salt Creek	PC10	1731A	3F	1			2	Coral
South Horse Creek	DC04	1623A	3F	6		4		Orange
Thornton Branch	DC09	1623A	3F	8		2		Orange
Rice Creek	HC24	1659	3F	8		2		Orange
Wolf Branch	HC32	1736	3F	5		3		Orange
E, Daughtrey Creek	LC24	3240F	3F	9		2		Orange
Bayshore Creek	LC25	3240B1	3F	8		3		Orange
Popash Creek	LC26	3240Q	3F	8		2	1	Orange
Stroud Creek	LC27	3240M	3F	9		2		Orange
Palm Creek	LC28	3240C1	3F	5		2	1	Orange
Olga Creek	LC32	3235P	3F	9		2		Orange
Mullock Creek	LC47	3258C2	3F	9		2		Orange
Gates Creek	MC14	1874	3F	4		2	1	Orange
34th Street Canal	PC07	1716A	3F	3		2		Orange
Curry Creek	SC14	2009C	3F			1		Orange

Delaney Creek is an interesting example of a tidal creek with historically elevated nutrient concentrations and recent dramatic improvements. Hillsborough County Environmental Protection Commission routinely samples two sites located within 1 km of one another. One site is located in the freshwater WBID, classified by FDEP as "Impaired" for historic chlorophyll in IWR Run 56. (The historic chlorophyll criterion has now been replaced by a statistical trend test as part of the latest triennial review and therefore this WBID is no longer considered impaired for chlorophyll). The impairment was presumed to be due to excessive nitrogen, though annual geometric average nitrogen concentrations at this site have been well below the current State standard of 1.65 mg/l. A second site downstream is located within the tidal WBID and downstream from a historical point source discharge to this waterbody. Long-term monitoring stations in both the source water and tidal portion of Delaney Creek are shown in Figure 5-2 along with timeseries plots of AGM TN concentrations for both sites. In the early 1990's, Delaney Creek had AGM source water nitrogen concentrations above the NNC of 1.65 mg/l in both the source and tidal portion of the creek. However, the trend in the timeseries changes rather dramatically between these stations with AGMs improving in the source water to well below the freshwater NNC threshold value while the downstream tidal portion of the stream increased to well over 2 mg/l in the late 1990s and 2000s. No pollutant load reduction goals associated with a TMDL have ever been established for this creek. Water quality has recently improved in the tidal WBID with AGM TN concentrations in both the source and tidal WBIDs now consistently below the freshwater nutrient threshold value in the last 4 years.

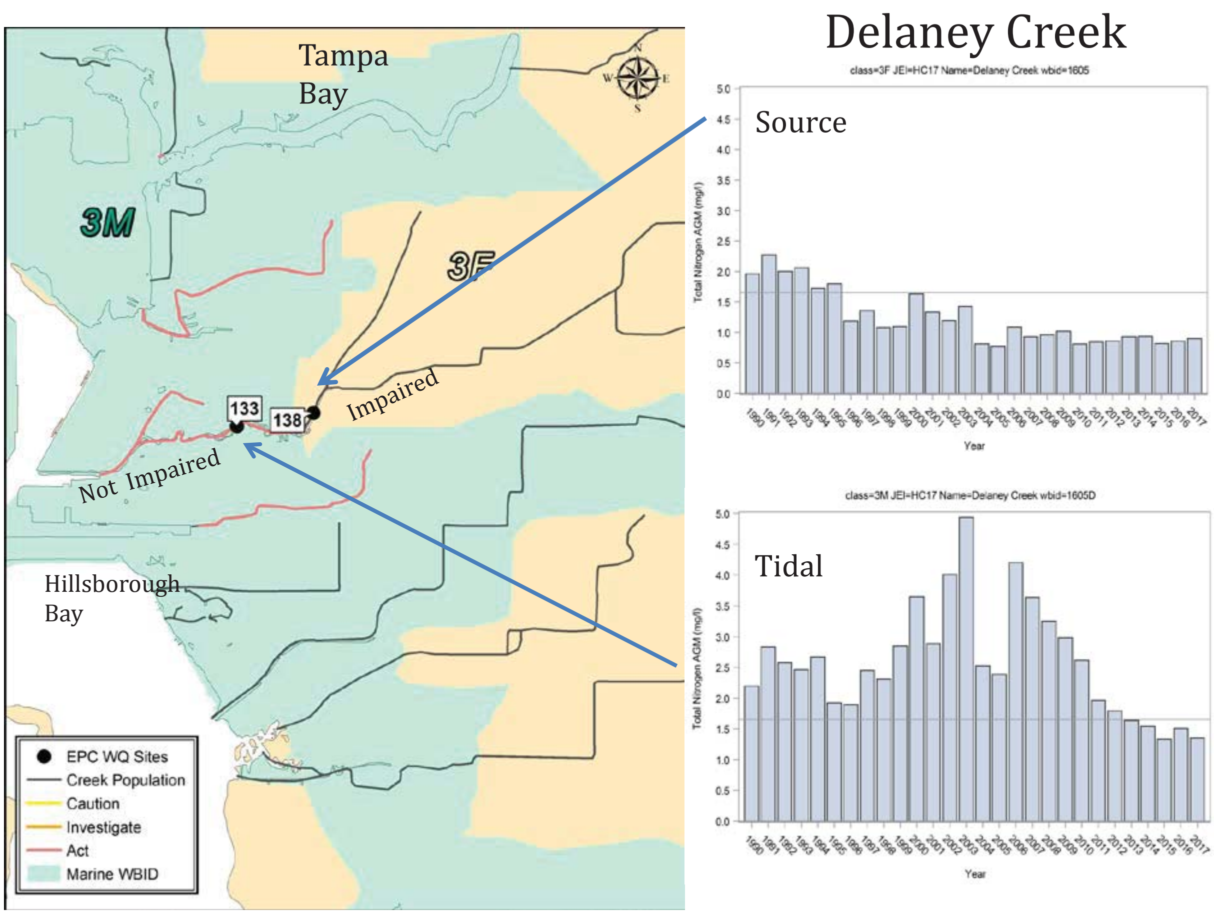


Figure 5-2. Location of Delaney Creek in Tampa Bay with times series of total nitrogen annual geometric average concentrations relative to TN NNC.

Application of the revised tidal creek Report Card has led to an “Act” score in the tidal portion of the system due to historically elevated nitrogen. The utility of the report card outcome in this case is that it has identified a creek with historically elevated nutrient concentrations for further evaluation. In this case, a management response might be to conduct a baseline biological survey to document conditions in a creek with historically high nitrogen concentrations and monitor these metrics over time to provide information on changes in the biological integrity of a creek with historically elevated nutrient concentrations that are improving over time. Our management framework has identified this creek, as well as a number of additional creeks, for which management response options can be developed and prioritized for future planning. The Report Card can also be used to direct future studies to improve the range of nutrient conditions over which biological indicators of tidal creek condition are developed and tested.

In addition to the nutrient management Report Card described above, this study devoted substantial efforts to developing additional indicators of tidal creek condition that can be used to identify potential sources of concern and inform their proper stewardship. These indicators are described in the following sections.

5.3 Using Individual Creek Results for Developing Additional Indicators

The stoichiometric ratio analysis described in Chapter 4 has revealed that the sampled creeks, except those south of the Caloosahatchee River such as Estero, are nitrogen limited. Nitrogen limitation infers that reductions in nitrogen would limit the potential for an "imbalance in flora and fauna" which is the descriptive basis for the current narrative standard used in determining impairment. For this reason, nitrogen became the focus of the nutrient management strategy and chlorophyll *a* concentrations were identified as a principal response endpoint. Considerable effort was dedicated towards identifying a quantitative relationship between nitrogen and chlorophyll; however, the conceptual relationships that define adverse effects to biological integrity; namely that higher nutrients lead to higher chlorophyll which reduces DO and thereby impairs the biological integrity of these systems was not supported by these tidal creek studies. Individual nitrogen chlorophyll regressions (based on date-averaged tidal samples) suggested that inorganic forms of nitrogen are inversely related to chlorophyll while organic forms are generally positively related to chlorophyll and these interactions only add to the noise in the observed TN-chlorophyll relationship. Dedicated analysis of these stressor response relationships can be viewed in Appendix G; however, the remainder of this chapter focuses on developing indicators based on a "weight-of-evidence" approach using exploratory analysis, literature review, and professional judgment as described below.

5.3.1 The Chlorophyll to Nitrogen Ratio as an Indicator of Physical Alteration

While stressor-response relationships between TN and chlorophyll concentrations were weak, the expression of chlorophyll per unit TN remains a valuable indicator of how phytoplankton populations in a creek respond to nutrient inputs (Carstensen et al. 2011). A high Chla:TN ratio may indicate more autotrophic conditions while extremely low ratio's may indicate dysfunction in the expected pathway between nitrogen and chlorophyll. The median chlorophyll to nitrogen ratio for the tidal portion of each creek is provided in Figure 5-3. West Spring had the highest median chlorophyll to nitrogen ratio (21.8) indicating that the tidal portion of West Spring is more sensitive to nitrogen inputs than the other creeks. West Spring is a highly physically altered system that has been deepened by dredging and has had its natural hydrology altered by a series of canals and weirs. Frog and South Creeks also had relatively high Chla:TN ratios. This study did not sample during the summer wet season; however, the previous study did and those data were combined as an additional line of evidence.

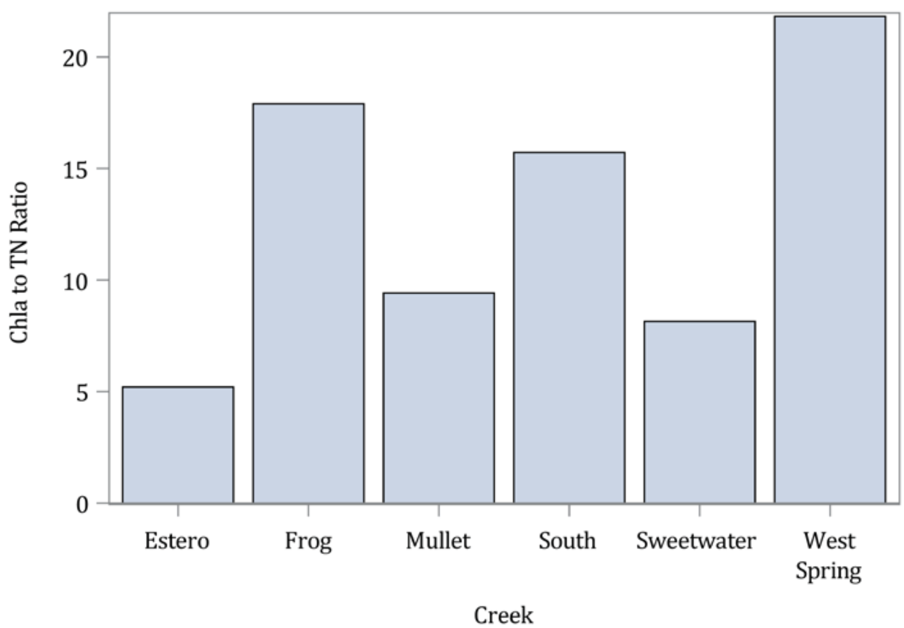


Figure 5-3. Median chlorophyll a to total nitrogen ratio for the six creeks in the current study.

The median chlorophyll to nitrogen ratios from the first study are presented in Figure 5-4. The reference line of 15 was selected to differentiate creeks with a high degree of physical alteration to either creek morphology or source water hydrology (West Spring, Forked, and Buck Creeks).

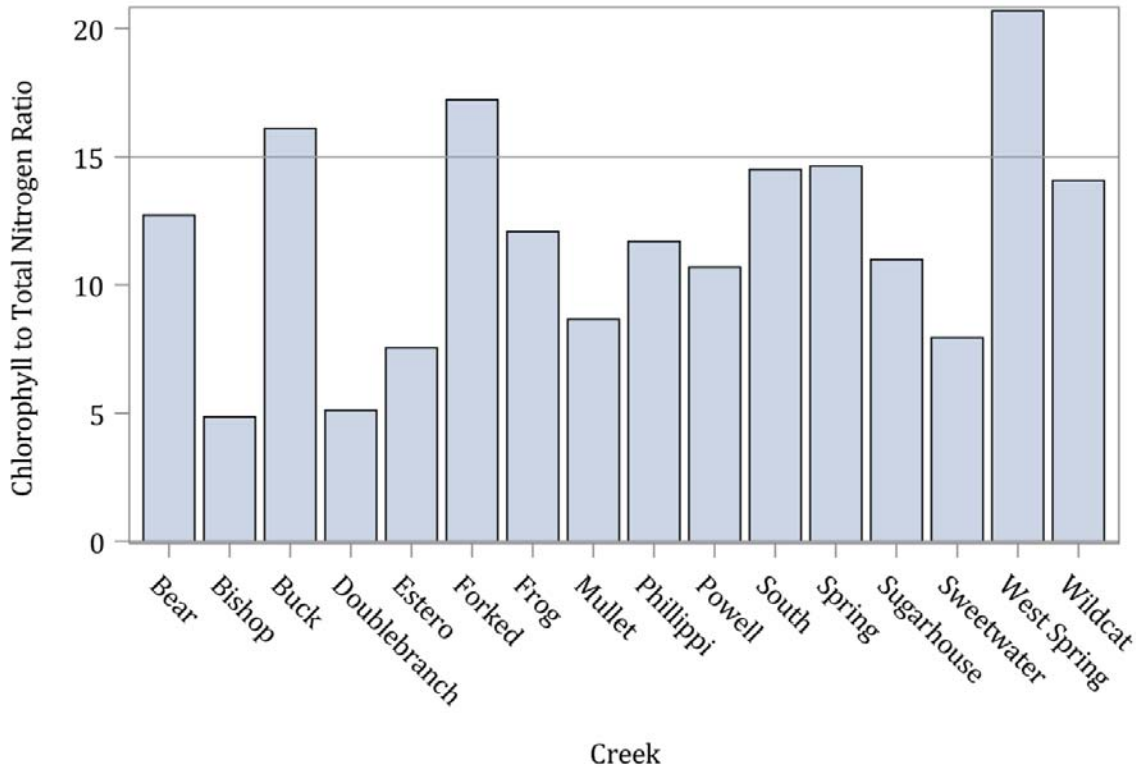


Figure 5-4. Median chlorophyll to nitrogen ratios based on all samples collected during both tidal reeks studies.

To put the Chla:TN ratios in context with numeric standards for southwest Florida estuarine waters, the NNC for estuarine waters and tidal rivers throughout the study area are provided in Table 5-3 along with the calculated Chla:TN ratio. These ratios varied considerably with values as low as 4.7 for the very open coastal estuary of St. Joseph Sound to 23.1 for the site-specific thresholds for the Alafia River Estuary associated with the TMDL for the lower river. Averaging these values resulted in a Chla:TN ratio of 12.7, while excluding the more open estuaries (denoted by asterisks in Table 4.1) from the average resulted in a value of 14.85.

Table 5-3. Site specific numeric nutrient criteria for SW FL estuarine waters with calculated chlorophyll to nitrogen ratios.				
Estuary	Total Phosphorus	Total Nitrogen	Chlorophyll a	Chla:TN Ratio
St. Joseph Sound *	0.05	0.66	3.1	4.70
Clearwater North *	0.05	0.61	5.4	8.85
Clearwater South	0.06	0.58	7.6	13.10
Alafia River Estuary	0.86	0.65	15	23.08
Palma Sola Bay	0.26	0.93	11.8	12.69
Sarasota Bay	0.19	0.49	6.1	12.45
Roberts Bay	0.23	0.54	11	20.37
Little Sarasota Bay	0.21	0.6	10.4	17.33
Blackburn Bay	0.21	0.43	8.2	19.07
Dona and Roberts Bay	0.18	0.42	4.9	11.67
Upper Lemon Bay	0.26	0.56	8.9	15.89
Lower Lemon Bay *	0.17	0.62	6.1	9.84
Charlotte Harbor *	0.19	0.67	6.1	9.10
Pine Island Sound *	0.06	0.57	6.5	11.40
San Carlos Bay *	0.045	0.44	3.7	8.41
Tidal Myakka River	0.31	1.02	11.7	11.47
Tidal Peace River	0.5	1.08	12.6	11.67
Matlacha Pass	0.08	0.58	6.1	10.52
Estero Bay *	0.07	0.63	5.9	9.37

* Indicates open water estuarine segments not used in calculation of Chla:TN ratio average to compare to tidal creeks ratio.

Therefore, a median Chla:TN ratio above 15 is recommended as an indicator that the tidal creek may become exceedingly sensitive to total nitrogen inputs.

5.3.2 Inorganic Nitrogen

Inorganic nitrogen is readily assimilated by biological processes in nitrogen limited systems and is a common indicator of anthropogenic inputs (Gowen et al. 1992). The median concentration of DIN in the tidal portion of the creeks is provided in (Figure 5-5). While TN concentrations between West Spring and Estero were similar, the proportion of TN that is comprised of DIN was quite different between these creeks. Estero and West Spring had the lowest average DIN concentrations in source water but DIN concentrations increased dramatically in the tidal waters in Estero while DIN was close to detection limits in the tidal waters in West Spring. This indicates a potential source of DIN in the tidal portion of Estero. Conversely, West Spring, which had the lowest median dissolved inorganic nitrogen concentrations, had the highest median Chla:TN ratio.

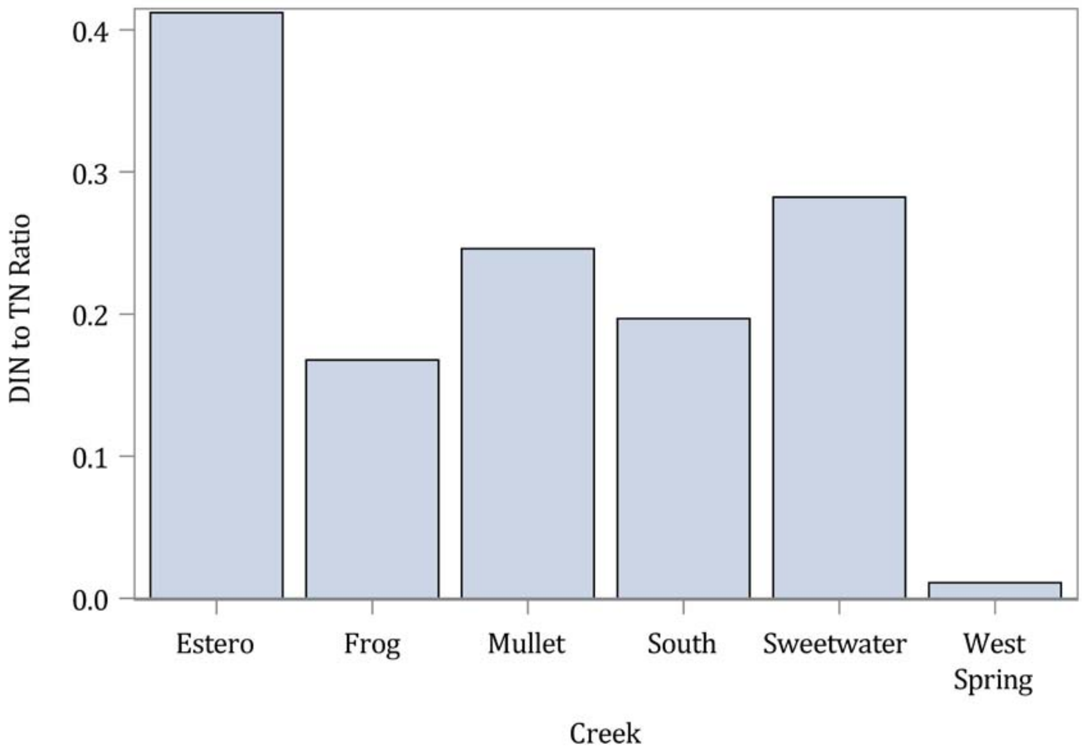


Figure 5-5. Median dissolved inorganic nitrogen to total nitrogen ratio for the six creeks in the current study.

Dissolved inorganic nitrogen is a telltale signature of anthropogenic influence via sewage, septic, processed wastewater or fertilizer inputs and thereby a good indicator of anthropogenic pollution. But DIN can also be quite challenging to use as an indicator due to its rapid uptake in tidal systems. For example, conditional probability analysis describing the relationship between DIN and the probability of exceeding a chlorophyll concentration of 11ug/l suggests that lower DIN values are associated with higher chlorophyll concentrations (Figure 5-6).

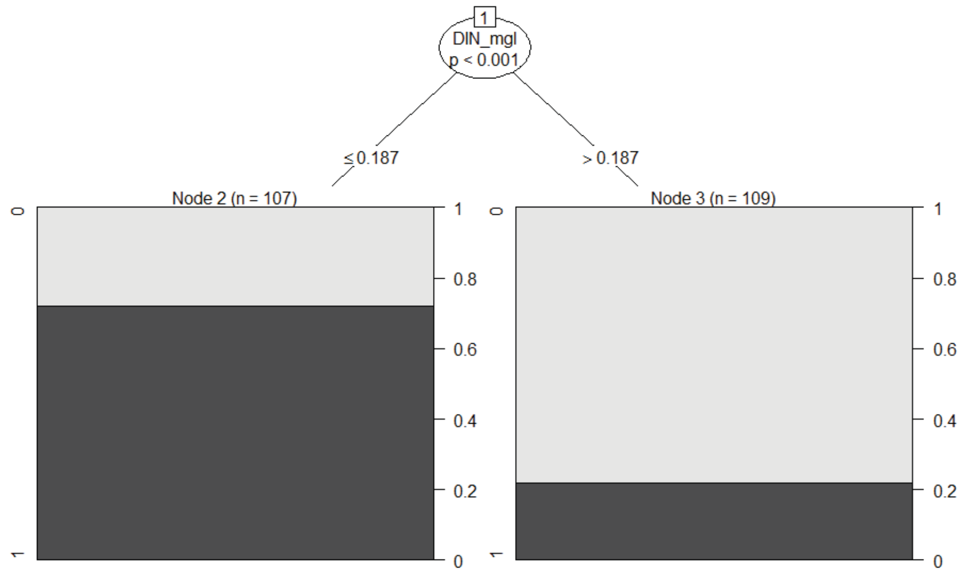


Figure 5-6. Conditional probability analysis identifying a cut point value of 0.187 for DIN that maximizes the difference in the probability of a chlorophyll concentration above 11.0 ug/l. Probability of exceedance in black.

The observation that chlorophyll concentrations were highest and DIN concentrations lowest in West Spring suggests there are feedback mechanisms between phytoplankton and available DIN in the water column. That is, as phytoplankton populations grow and reproduce, more DIN is taken up, affecting the amount of DIN remaining in the water column. While this complicates developing thresholds for DIN exceedances, nitrate in the tidal portion greater than the source water would indicate an anthropogenically derived source. This was observed in Estero where nitrates increased substantially between the source water station and the adjacent downstream station. This input may be due to any number of factors including upstream inputs from a golf course in the watershed, differential inputs between the north and south forks, or the possibility that a spring vent in the tidal portion of the system is influencing nitrate concentrations within the system. Irrespective of the source, DIN (or NO23 if dissolved fractions are not available) in the tidal portion that is higher than the source water is proposed as another indicator of tidal creek condition. An application of this approach is provided in Figure 5-7. The 1:1 line between the source water and average of the tidal water for each sampling event is provided as a reference to the paired data. Estero samples were consistently above the source water concentration, which only happened a few times in other creeks (Mullet, Frog and Sweetwater Creeks).

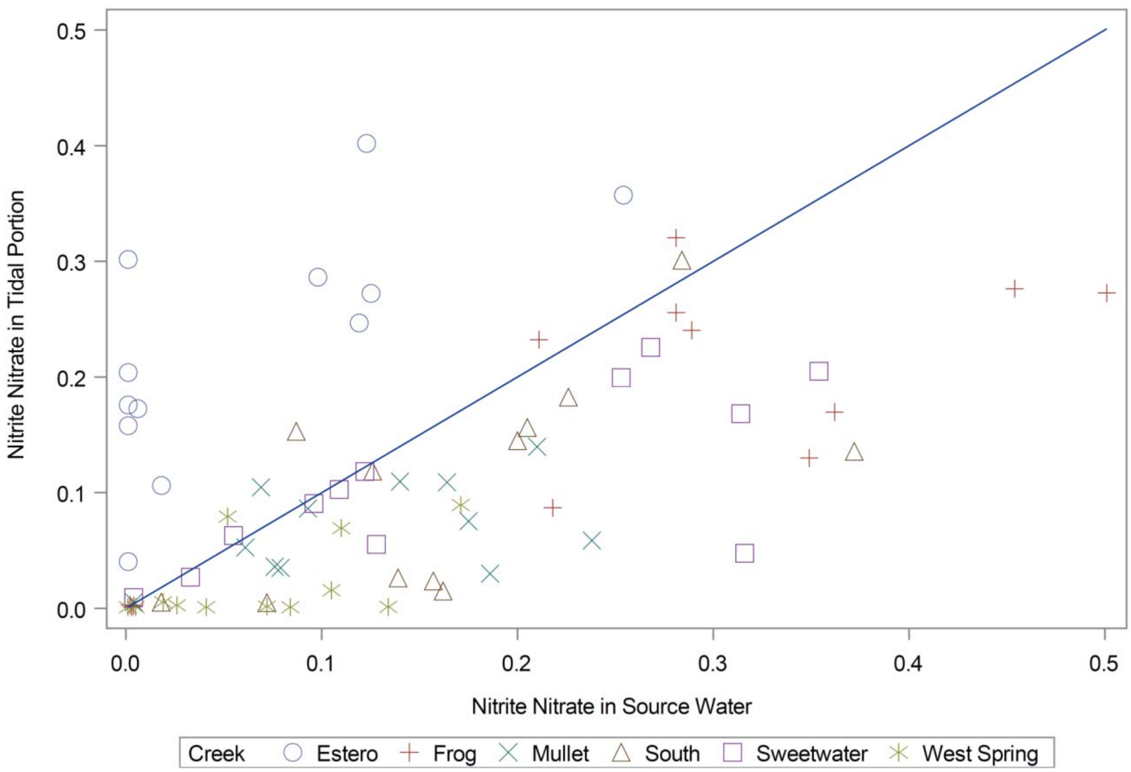


Figure 5-7. Relationship between nitrite-nitrate (NO23 mg/l) concentrations in the source water plotted against the average NO23 concentration in the tidal portion of the creek.

5.3.3 Total Nitrogen above 1.1 mg/l as an indicator

A common regulatory construct to protect water quality in nitrogen limited systems relies on total nitrogen limits to regulate phytoplankton (chlorophyll) concentrations. In open bay estuarine systems this approach has proven successful as residence times are generally sufficient for phytoplankton reproduction if a sufficient mass of the limiting nutrient is present. However, in tidal tributaries antecedent rainfall conditions can reduce residence times to the point that phytoplankton populations don't have sufficient time to reproduce. As described above, antecedent rainfall and associated seasonal signals effect both the nutrient delivery to the system and the response of phytoplankton within tidal creeks. While it was not possible to estimate residence times directly, we observed in the combined data from both studies that median TN concentrations were highest in the summer wet season, while median chlorophyll concentrations were highest during the spring Dry season Figure 5-8 suggesting the effects of residence times on phytoplankton dynamics.

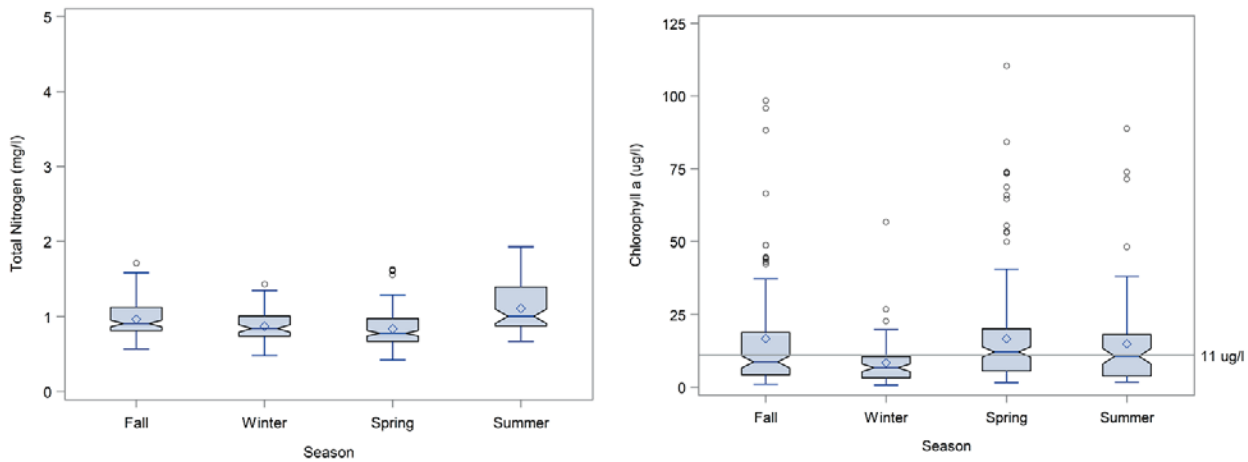


Figure 5-8. Seasonal distribution of total nitrogen (left) and chlorophyll (right) using all data from both SBEP tidal creek studies.

These seasonal dynamics confounded attempts to derive linear relationships between TN and chlorophyll. While there was a weak statistically significant relationship between total nitrogen and chlorophyll when evaluating the relationship across creeks (R^2 = 0.11), examination of the individual creek relationships suggest that the relationship is creek dependent with some creeks displaying no clear relationship (Figure 5-9). Other factors including CDOM, canopy cover and other potential covariates including the form of nitrogen (inorganic versus organic) were not helpful in improving these linear relationships.

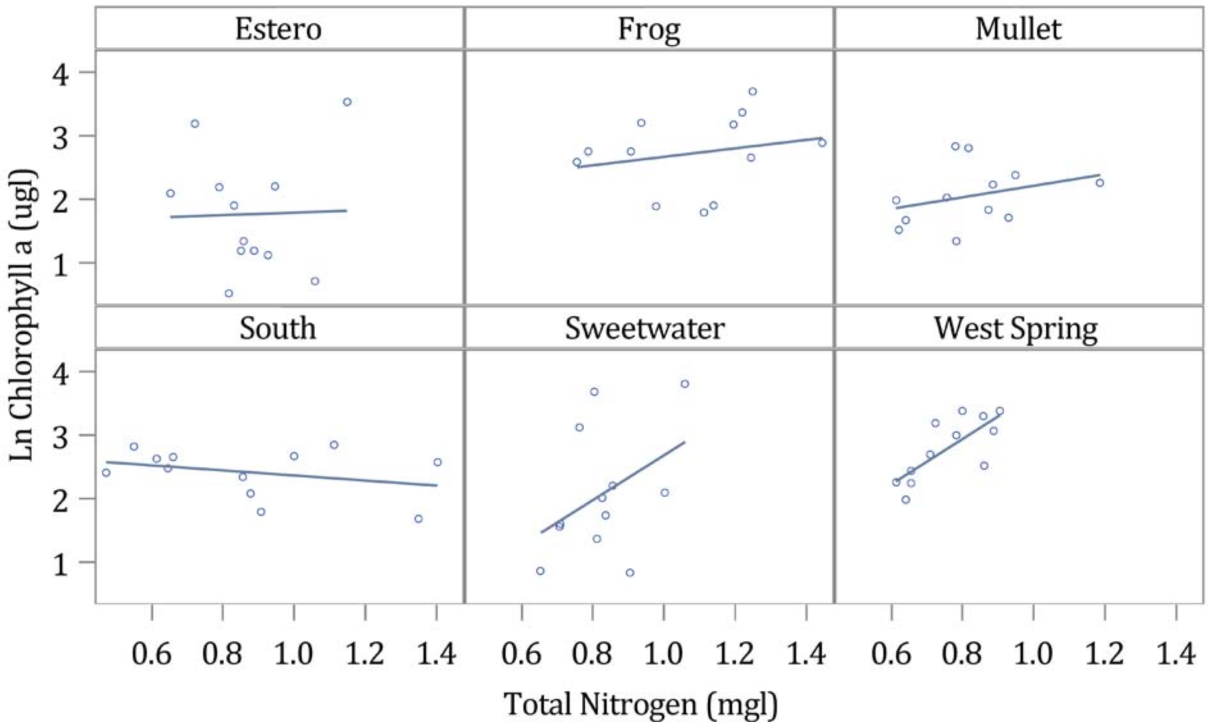


Figure 5-9. Relationship between sampling event average total nitrogen and chlorophyll (natural log transformed)

Despite the weak relationship when evaluating the data as a stressor-response relationship, thresholds in the TN chlorophyll relationship were evident. Univariate conditional probability analysis conducted on the data collected as part of this study suggested a highly statistically significant difference (i.e. $p < 0.001$) in chlorophyll concentrations (log scale) based on a threshold TN concentration of 1.1 mg/l). When TN concentrations were at or below 1.1 mg/l, the median chlorophyll was below the current chlorophyll standard of 11ug/l and when TN concentrations were above 1.1 mg/l, the median chlorophyll was above 11 ug/l (Figure 5-10). The same analysis conducted on the combined data from both creek studies confirmed the 1.1 mg/l TN threshold value (Figure 5-11) which again identified a distribution of chlorophyll values with a median above the 11 ug/l chlorophyll standard with high statistical confidence. This analysis also identified an additional TN threshold of 0.85 mg/l with values below 0.85 mg/l correlated with samples collected in the winter season.

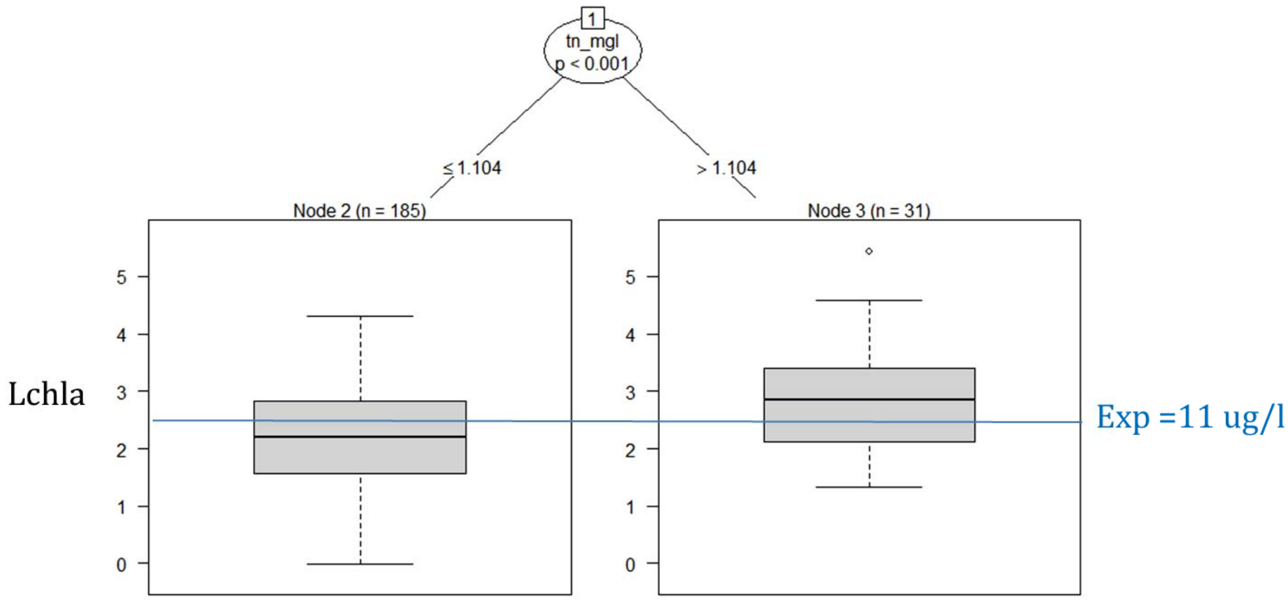


Figure 5-10. Conditional probability analysis identifying a cut point total nitrogen concentration that maximizes the difference in the distribution of chlorophyll concentrations (log scale) based on data collected in this study. Horizontal reference line represents a median (exponentiated) value of 11 ug/l.

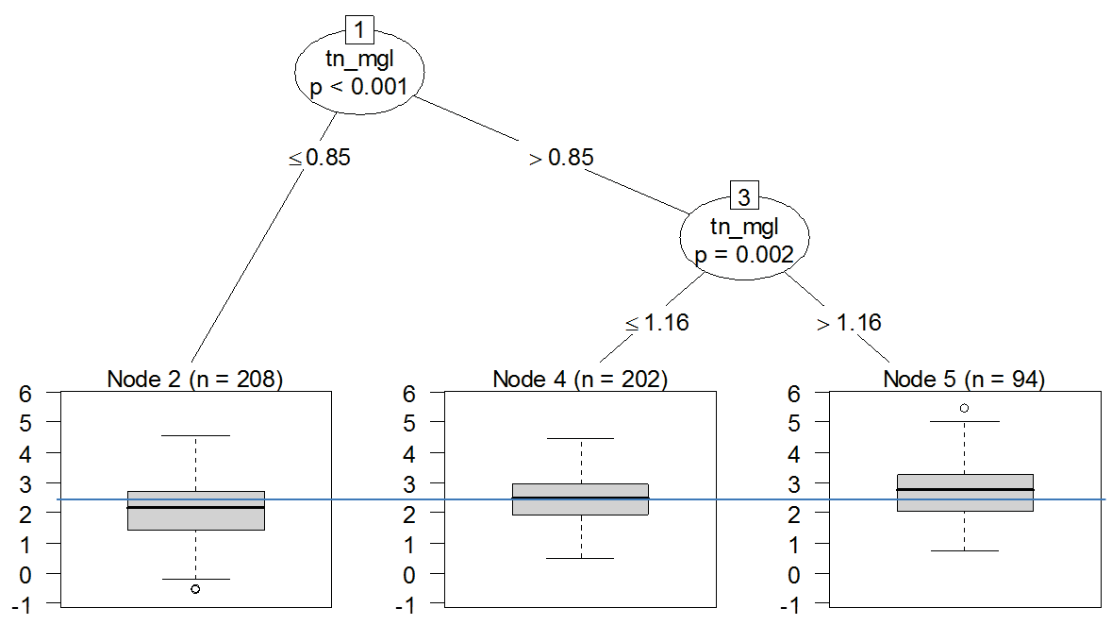


Figure 5-11. Conditional probability analysis identifying a cut point total nitrogen concentrations that maximize the difference in the distribution of chlorophyll concentrations (log scale) using all creeks data collected during both studies. Horizontal reference line represents a median (exponentiated) value of 11 ug/l.

Additional lines of evidence that a TN of 1.1 mg/l would serve as a valuable indicator include the use of a similar nitrogen value (1.2 mg/l) as a screening level indicator for Florida estuaries (Paulic et al. 1996: Table 2-10) and that 1.1 mg/l represents the median value of the lower Caution limit for all creeks based on the creek length adjustment of the original tidal creek Report Card. These additional lines of evidence support the use of a TN concentration of 1.1. mg/l as a protective value for tidal creeks. Therefore, an AGM TN concentration not greater than 1.1 mg/l is proposed as an additional indicator of creek condition.

5.3.4 The Trophic State Index

The Trophic State Index (TSI) has been used extensively as a water quality screening tool for lakes and was historically applied to estuaries until more site specific criteria were developed (Paulic et. al. 1996). The index relies on three indicators - chlorophyll, TN and TP - to describe a waterbody's trophic state. In its original form (Carlson 1977), water clarity was also considered, but the tannic nature of many waterbodies resulted in a modification that omits water clarity from the Florida calculation. The Florida Lake Index was developed from a regression analysis of data from 313 Florida lakes and uses an value of 70 to define lakes in "Poor" condition. Values from 60 to 69 represent "Fair" water quality, and values below 60 reflect "Good" water quality. The authors also applied the Trophic State Index to Florida estuaries where critical values were defined as 10 points less than those for lakes (i.e., good estuarine water quality had an index value of 0 to 49; fair quality, 50 to 59; and poor quality, 60 to 100). We considered the TSI as an additional potential method to characterize tidal creek condition relative to other Florida waterbodies.

The TSI calculation proceeds by separately calculating the TSI score for chlorophyll and nutrients and then averaging the two scores. The chlorophyll equation is simplistic and relies on the natural log transformation of chlorophyll as described in equation 1 below. The nutrients TSI score is based on the limiting nutrient, which in turn, is based on the TN/TP ratio (typically mass not molar). For southwest Florida tidal creeks, the limiting nutrient is nitrogen in all creeks with the possible exception of Estero. The result of equations two and three are used for nutrient balanced lakes (those where the TN:TP ratio is greater or equal to 10 and less or equal to 30). The result of equation four is used for phosphorus limited lakes (those where the TN:TP ratio is greater 30) and the result of equation five is used for nitrogen limited lakes (those with a TN:TP ratio of less than 10).

The final TSI is then determined by averaging these values based on the limiting nutrient as shown below.

Equation 1

Trophic State Index equations that generate the above criteria (LN = Natural Log):

$$CHLA_{TSI} = 16.8 + [14.4 \times LN(CHLA)]$$
$$TN_{TSI} = 56 + [19.8 \times LN(TN)]$$
$$TN2_{TSI} = 10 \times [5.96 + 2.15 \times LN(TN + .0001)]$$
$$TP_{TSI} = [18.6 \times LN(TP \times 1000)] - 18.4$$
$$TP2_{TSI} = 10 \times [2.36 \times LN(TP \times 1000) - 2.38]$$

** Limiting nutrient considerations for calculating $NUTR_{TSI}$:*

If $TN/TP > 30$ then $NUTR_{TSI} = TP2_{TSI}$

If $TN/TP < 10$ then $NUTR_{TSI} = TN2_{TSI}$

If $10 < TN/TP < 30$ then $NUTR_{TSI} = (TP_{TSI} + TN_{TSI})/2$

$TSI = (CHLA_{TSI} + NUTR_{TSI}{*})/2$

We applied the TSI calculations in two ways: first, by averaging all values for each year and calculating the scores, then by applying the formula to each set of values and then averaging. The second iteration was chosen to down-weight the effects of the small sample size on the calculations and to apply what seemed to be the potential for differential nutrient limitation in some creeks, especially Estero. Scores for all creeks fell below 60, which is the cut off for a "Poor" estuary and a "Fair" lake (Figure 5-12). Frog Creek had the highest TSI scores of all creeks which agreed with conclusions from the previous study that suggest that Frog Creek was at a Management Action level. However, the scores slightly improved over time when calculated on a sample-specific basis, which coincided with the improvements observed in the overall TN concentrations in Frog Creek. Estero varied considerably depending upon which calculation was used, reflecting the importance of the nutrient TSI algorithm in the calculation. Mullet and Sweetwater Creek had the lowest TSI scores on average, which also agreed with other metrics observed for these creeks.

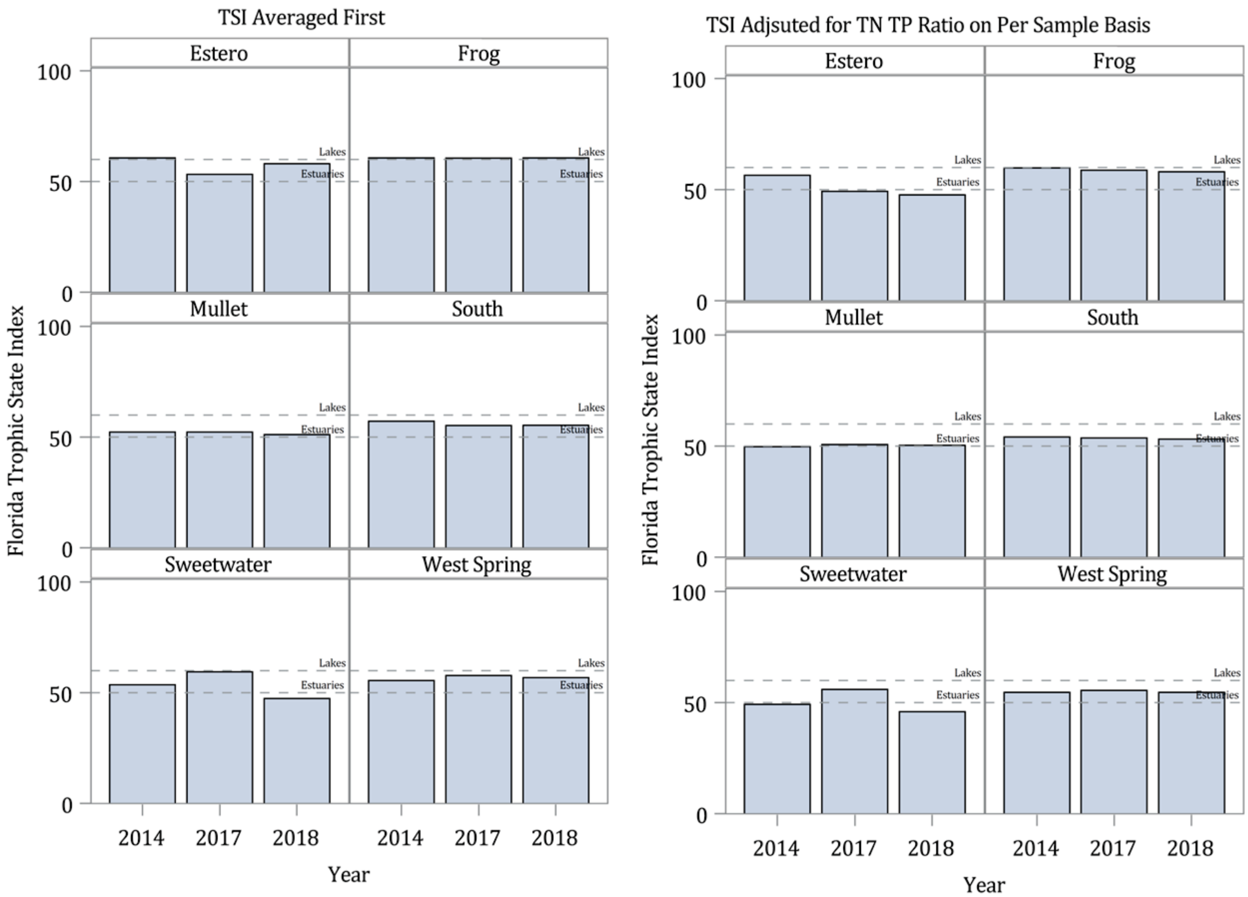


Figure 5-12. Trophic State Index scores for each year of the tidal creeks survey with reference lines indicating fair condition for estuaries (50-59) and lakes (60-69).

These results suggest that the TSI scores can serve as another valuable indicator of tidal creek condition that is associated with biological productivity in the system.

5.3.5 Algal Surveys

Rapid periphyton surveys have been implemented as a state bioassessment criterion for freshwater stream in Florida and this study undertook a first step toward relating the prevalence of periphyton to nutrients for managing southwest Florida tidal creeks. Algal survey results were somewhat counterintuitive as Frog and South Creeks had the highest TN and TP concentrations but the lowest algal presence, while Sweetwater Creek had the highest algal presence but relatively lower nutrients. Canopy cover could not explain these differences. Periphyton in tidal creeks could represent a significant sink for nutrients, thereby affecting water column nutrient concentrations used to evaluate creek condition. Therefore, the frequency of algal presence is proposed as an indicator of creek condition It is recommended that the presence of periphyton in more than 50 percent of samples is an indicator of deteriorating creek condition. However, the algal indicator should not be used as the sole indicator that a creek has reached an imbalance of flora and fauna since the two creeks with the highest nutrients in our study had little algae present.

5.4 Aggregated Index Score

It is also insightful to create an aggregated index score to report on tidal creek condition. To aggregate the individual indices, individual outcomes would be reported on an ordinal scale (Table 5-4). The aggregated index would be calculated as the average of ordinal scores for each individual index described above. Since the existing management framework is based on a four-point scale, the ordinal scale would include a 0 representing the target value, 1 representing the caution level criterion, 2 representing the management action level and 3 representing the regulatory threshold. The chlorophyll criterion of 11 ug/l is the narrative standard and is therefore included in the aggregate score but is not the sole determinant of tidal creek condition. This is based on previous findings that chlorophyll concentrations, even when above 11 ug/l were not related to any degraded condition of biological communities in tidal creeks. The remaining indices described in the sub-section above were assigned a binary value based on whether or not they were above the threshold value.

Table 5-4. Proposed Index metrics and their respective potential outcomes of the evaluation.

Index Metric	**Score**
Framework Score (Below (0),Caution(1), Investigate(2), Act(3))	0/1/2/3
Chla > 11 (ug/l) AGM	1/0
TN > 1.1 mg/l AGM	1/0
Chla/TN ratio > 15	1/0
Inorganic Tidal > Source	1/0
Trophic State Index > 60	1/0
Algae > 50% occurrence	1/0

The aggregate score would be calculated as the average of the individual scores. The average is calculated such that, if an index score is missing, its omission is not reflected in the final score. A threshold value of 0.50 is proposed as a threshold above which the creek should be considered for additional research to evaluate its biological integrity.

6 Recommendations

The southwest Florida tidal creeks nutrient management framework offers a practical and reliable mechanism to identify and prioritize tidal creeks for management responses based on commonly collected water quality data routinely reported to Florida's water quality database. Refinements to the management framework based on this study provides the NEPs with a host of tools to make informed decisions about where and how to focus remedial efforts towards restoring these waterbodies. The nitrogen management Report Card provides a list of creeks for evaluation and prioritization based on color-coded category scores. In addition, the indicators provide a mechanism for evaluating a series of analytical outcomes to aid in identifying site-specific attributes related to tidal creek condition. For example, it is possible that a creek might fail the narrative standard for chlorophyll but have total nitrogen concentrations that are not elevated for typical creeks with high biological integrity and exhibit a weak correlation between chlorophyll and nitrogen concentrations. In this case, the indicator describing the long term median Chla:TN ratio may indicate that this creek has been hydrologically altered or physically modified which has affected the creek's assimilative capacity. Alternatively, a creek may pass the narrative criteria but have elevated nutrient concentrations. This scenario highlights the value of a method that explicitly identifies a nutrient concentration as an endpoint. These are merely a few examples of how the additional indicators can supplement existing information to provide a more comprehensive evaluation of tidal creek condition with respect to water quality that will allow the NEPs to investigate and prioritize actions based on actual nutrient concentrations and respond in a proactive manner that improves stewardship of these critical wetland habitats.

There is more to identifying what constitutes a "healthy" tidal creek than evaluating water quality outcomes. The previous study evaluated biological integrity based in part on fish communities inhabiting these creeks. While stakeholders identified a healthy fish community as an important indicator of tidal creek condition, that study showed that the nutrient concentrations in these creeks did not result in deleterious effects on these fish communities. Those fish surveys demonstrated that while these creeks are critical habitats for estuarine dependent fish, there was actually some positive correspondence between of fish diversity and increasing nutrients over the range of nutrient concentrations observed in the study. It is recognized that regulatory criteria must focus on the most sensitive components of the ecosystem and fish communities, despite their ecological importance, may not be the most sensitive indicators of imbalance in southwest Florida tidal creeks. Additional biological response endpoints are critically needed. For that reason, algal surveys were explored as an alternative potential biological response endpoint. However, our results indicated that periphyton may not be the best biological endpoint, since algal frequency of occurrence was not directly positively related to source water nutrient inputs or to nutrient concentrations within the tidal portion of the creek.

Biological integrity depends on a complex interaction of static and dynamic habitat characteristics and more work is needed to develop robust indicators of biological integrity in tidal creeks. However, the water quality metrics associated with the tidal creek nutrient management framework will provide an identification and prioritization scheme that will allow managers to focus actionable efforts towards reducing one of the primary causes of water body impairments worldwide - nutrient pollution. Therefore, based on the totality of information provided in this document, the following recommendations are offered:

- The list of creeks provided in Table 5-1 and Table 5-2 provides an actionable list of creeks that can be used for future planning level investigations to identify and prioritize potential management actions for creeks with elevated nutrient concentrations.
- The indicators developed in this study lend supporting evidence when evaluating the need for a management response to identify potential causes of observed Report Card outcomes.
- The indicator score of an AGM TN concentration of 1.1 mg/l may serve as an alternative criterion to define the caution level in place of the site-specific caution levels developed as part of the previous study.
- The aggregated index score could also serve as an additional line of evidence that combines individual outcomes into a single score that represents the cumulative results of the indicator outcomes.
- Efforts should continue to develop and test additional metrics related to biological integrity and assimilative capacity of these creeks. These metrics should include evaluations of the importance of traditional habitat restoration practices in contributing to biological uplift and increased assimilative capacity within the tidal portion of the creeks.

- Collecting water quality monitoring data within stormwater ponds and collating existing data would be beneficial. These ponds often serve as headwaters to creeks or as inputs in the tidal portion. These nutrient inputs and overall water quality can be transferred to the tidal portion of the creek.

- Compiling a list of creeks with existing surface water models (such as the Water Management Districts SWMM models) would be of benefit as these models define stream morphology and hydrologic connections to the source water.

- Compiling a list of all surface water discharges with spatial attributes to characterize known anthropogenic inputs would be beneficial.

- If additional monitoring is required, every effort should be made to locate a site above the "head of tide" to characterize the source water contribution to the tidal portion of the creek.

- An efficient sampling scheme can be easily employed to characterize tidal creek condition. It is recommended that a scheme similar to the one employed here be used where necessary to evaluate water quality conditions in targeted tidal creeks.

- Probabilistic monitoring designs are the best design to obtain an unbiased estimate of the annual geometric average nutrients within tidal creeks though results of the fixed station design deployed during this study suggest that a fixed station design would return a similar AGM to the probabilistic design. The exception may be creeks like Estero where the site located at Station 3 may be identifying a localized source that may not be revealed by a probabilistic design.

- Minimum sampling requirements should include at least one site in the freshwater and tidal portion of the creek sampled quarterly on the same day within as narrow a time window as logistically possible for a duration of at least one year. Ideally, replicating this study design, including the number of stations and water quality parameters, and a sampling frequency of every other month (or monthly, if feasible) would be preferable.

This study has accelerated the research relating to the causes, effects, extent, prevention, reduction, and elimination of water pollution in Florida's wetland environments by collecting data on nutrient dynamics, stoichiometric ratios, stable isotopes, and algae in these under-sampled environments, as well as developing a nitrogen management framework that results in the identification and prioritization of creeks for management action. The revised tidal creek nutrient management Report Card and development of additional indicators of tidal creek condition will assist prioritization of investigative actions and resource management responses to increase the stewardship, management and protection of these critical coastal wetland habitats. Additionally, this study provides a scientific foundation for future research to contribute to a more holistic assessment of tidal creek condition that includes both direct evaluation of nutrient concentrations and development of additional indicators that may reflect the underlying causes of observed water quality outcomes and their effects on biological response endpoints.

7 References:

Anisfeld, S. C., R. T. Barnes, M. A. Altabet, and T. Wu. 2007. Isotopic apportionment of atmospheric and sewage nitrogen sources in two Connecticut rivers. *Environmental science & Technology* 41(18): 6363-6369.

Bateman, A. S. and S. D. Kelly. 2007. Fertilizer nitrogen isotope signatures. Isotopes in *Environmental and Health Studies* 43(3):237-247, DOI: 10.1080/10256010701550732.

Bran+Luebbe/Seal. 2005a. Ammonia in water and seawater. Method No. G-171-96 Rev. 10. Norderstedt, Germany.

Bran+Luebbe/Seal. 2005b. Phosphate in water and seawater Total P in persulfate or Kjeldahl digests. Method No. G-175-96 Rev. 12. Norderstedt, Germany.

Bran+Luebbe/Seal. 2010. Nitrate and nitrite in water and seawater; total nitrogen in persulfate digests. Method No. G-172-96 Rev. 10. Norderstedt, Germany.

Breiman, L. (2001), *Random Forests*, Machine Learning 45(1), 5-32.

Carlson, R.E. (1977) A trophic state index for lakes. *Limnology and Oceanography*. 22:2 361–369.

Carstensen, J., Sanchez-Camacho, M., Duarte, C. M., Krause-Jensen, D. & Marba, N. (2011). Connecting the dots: responses of coastal ecosystems to changing nutrient concentrations. Environmental Science and Technology 45, 9122– 9132.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117-143.

Clarke, K. R. and R. N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E Ltd: Plymouth, United Kingdom. 190 p.

Clark, J. S. 2007. Models for Ecological Data: An Introduction. Princeton University Press. Princeton N.J. 08540

Conmy, R.N. 2008. Temporal and spatial patterns in optical properties of colored dissolved organic matter on Florida's gulf coast: Shelf to stream to aquifer. Doctoral Dissertation. University of South Florida, St,. Petersburg, FL.

Coulter, 1994. Coulter LS Series Product Manual. Coulter Corporation, Miami, FL.

Demers, N. E. 2008. Using stable δ15N ratios to help differentiate nitrogen sources within a residential community- another tool for the TMDL toolbox. Watershed Council report on Delta N15 in San Carlos Park. 16 pp.

Dixon, L. K., P. J. Murphy, N. M. Becker, and C. M. Charniga. 2014a. The potential role of benthic nutrient flux in support of Karenia blooms in west Florida (USA) estuaries and the nearshore Gulf of Mexico. Harmful Algae 38:30-39.

Dixon, L. K., G. J. Kirkpatrick, E. R. Hall, and A. Nissanka. 2014b. Nitrogen, phosphorus and silica on the West Florida Shelf: Patterns and relationships with Karenia spp. occurrence. Harmful Algae 38:8-19.

Dixon, L. K. and A. Hounshell. 2013. Task 5 - Analysis of sediment diagenesis input parameters: improving TMDL and waste load allocation permit limits by determination and application of new sediment diagenesis input parameters in current water quality models. Final Report to Tetra Tech, Mote Technical Report No. 1703.
EPA (U.S. Environmental Protection Agency). 1983. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, 1979, revised March 1983.

Eilers, D. 2013. West-Central Florida Tidal Stream Assessment Study. Report to the Sarasota Bay Estuary Program.

EPA (U.S. Environmental Protection Agency). 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100 August 1993.

Environmental Protection Agency (EPA). 2012. Technical Support Document for U.S. EPA's Proposed Rule for Numeric Nutrient Criteria, Volume 1 Estuaries. Office of Water, Washington D.C. November, 2012

Greenwood, M. F. D., E. Malkin, E. B. Peebles, S. D. Stahl, and F. X. Courtney. 2008. Assessment of the value of small tidal streams, creeks, and backwaters as critical habitats for nekton in the Tampa Bay watershed. Report to the Florida State Wildlife Grants Program, Project SWG05-015.

Goffman, P.M., M. A. Altabet, J.K. Bohlke, K. Butterbach-Bahl, M.B. David, M.K. Firestone, A.E. Giblin, T.M. Kana, L.P. Nielsen, and M.A. Voytek. 2006. Methods for Measuring Denitrification: Diverse Approaches to a Difficult Problem. *Ecological Applications* 16(6): 2091-2122.

Goodwin, P., & Kamman, R. (2001). Mixing and Circulation in Tidal Wetlands. *Journal of Coastal Research,* 109-120. Retrieved from http://www.jstor.org/stable/25736167

Gowen, R. J. P. Tett, and K. J. Jones. 1992. Predicting marine eutrophication: The yield of chlorophyll from nitrogen in Scottish coastal waters. Marine Ecology Progress Series. 85:153-161

Hale, J. A., R. A. Duffey, B. Pederson, and E. R. Hall. 2008. The Sanibel River and the Impaired Waters Rule: How Stable Nitrogen Isotopes and Phytoplankton Community Analyses Can Indicate Nutrient Sources and Evaluate Nutrient Reduction Efforts. Technical Report to the Natural Resources Department City of Sanibel.

Hothorn T., K. Hornik, and A. Zeileis. 2006. Unbiased recursive partitioning: a conditional inference framework. Journal of Computational and Graphical Statistics. 15:651–674.

IAEA. 2013. Application of Isotope Techniques for Assessing Nutrient Dynamics in River Basins. International Atomic Energy Agency, Vienna. 232 pp.

Janicki Environmental, Inc. and Mote Marine Laboratory. 2016. Southwest Florida Tidal Creeks Nutrient Study. Report prepared for the Sarasota Bay Estuary Program (Technical Report Number 2013SBEP17). Sarasota, FL.

Jordan, M. J., K. J. Nadelhoffer, and B. Fry. 1997. Nitrogen cycling in forest and grass ecosystems irrigated with 15N-enriched wastewater. *Ecological Applications* 7(3): 864-881.

Kendall C. Tracing nitrogen sources and cycling in catchments. In: Isotope tracers in catchment hydrology 1998 Jan 1 Elsevier pp. 519-576.

Koenker, R., and Bassett, G. W. (1978). "Regression Quantiles." *Econometrica* 46:33–50. SAS Institute Inc. 2015. SAS/STAT® 14.1 User's Guide. Cary, NC: SAS Institute Inc.

Lapointe, B. E. and B. J. Bedford. 2007. Drift rhodophyte blooms emerge in Lee County, Florida, USA: evidence of escalating coastal eutrophication. *Harmful Algae* 6, 421–437.

Lapointe, B. E., L. W. Herren, D. D. Debortoli, and M. A. Vogel. 2015. Evidence of sewage-derived eutrophication and harmful algal blooms in Florida's Indian River Lagoon. *Harmful Algae* 43:82-102.

Lapointe, B.E., L. W. Herren, A. Paule, A. Sleeman, and R. Brewton. 2016. Charlotte County water quality assessment phase I: data analysis and recommendations for long-term monitoring. Report to the Charlotte County Board of County Commissioners, 51 pp.

Lyons, P., C. Thornber, J. Portnoy, E. Gwilliam. 2009. Dynamics of macroalgal blooms along the Cape Cod National Seashore. Northeast Nat. 16(1):53-66

Malkin, E. M. 2010. The economically important nitrogen pathways of southwest Florida. University of South Florida Graduate Theses and Dissertations. http://scholarcommons.usf.edu/etd/3635.

McKee, T.B., N.J. Doesken and J. Kleist. 1993: The relationship of drought frequency and duration to time scale. In: Proceedings of the Eighth Conference on Applied Climatology , Anaheim, California,17–22 January 1993. Boston, American Meteorological Society, 179–187

Mitchell, B. G., A. Bricaud, K. Carder, J. Cleveland, G. Ferrari, R. Gould, M. Kahru, M. Kishino, H. Maske, T. Moisan, L. Moore, N. Nelson, D. Phinney, R. Reynolds, H. Sosik, D. Stramski, S. Tassan, C. Trees, A. Weidemann, J. Wieland and A. Vodacek. 2000. Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples. Chapter 12 In: Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 2. G. S. Fargion and J. L. Mueller, Eds. NASA/TM-2000-209966. National Aeronautical and Space Administration, Goddard Space Flight Space Center, Greenbelt, Maryland.

Murphy, P., and D. Hicks. 1986. In situ method for measuring sediment oxygen demand. In: Hatcher, K. J. (Ed.), Sediment Oxygen Demand. Sediment Oxygen Demand, Methods, Modeling and Processes, University of Georgia Press, Athens, GA.

Paerl, H. W. and M. L. Fogel. 1994. Isotopic characterization of atmospheric nitrogen inputs as sources of enhanced primary production in coastal Atlantic Ocean waters. *Marine Biology* 119:635-645.

Paulic, M. J. Hand and L. Lord 1996. Water quality assessment for the State of Florida: Section 305[B]. Florida Department of Environmental Protection. December 1996.

Peebles, E. B., A. M. Hoare, E. M. Malkin, G. S. Ellis, D. J. Hollander. 2010. Nitrogenous organic matter accumulation in Safety Harbor, Florida: sources and decadal-scale trends. Pp. 15 – 26 in Cooper, S.T. (ed.) Proceedings, Tampa Bay Area Scientific Information Symposium, BASIS 5: 20-23 October 2009. St. Petersburg, FL. 538 pp.

Peterson, B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18(1987):293-320.

Plew, D. R., J. R. Zeldis, U. Shankar, and A. H. Elliot. 2018. Using simple dilution models to predict New Zealand estuarine water quality. Estuaries and Coasts 41:1643-1659.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Redfield, AC (1934). "On the proportions of organic derivatives in sea water and their relation to the composition of plankton" (PDF). James Johnstone Memorial Volume: 176. Retrieved March 1, 2019.

Redfield, A.C. (1958). The Biological control of chemical factors in the environment. American Scientist. **46** (3): 205–221, 230A. ISSN 0003-0996.

Rokach, L. and O. Maimon. 2008. Data mining with Decision Trees: Theory and Applications. Series in Machine perception artificial intelligence. Vol 69. World Scientific Publishing Co. Singapore.

SAS Institute Inc. 2014. SAS/STAT® 9.4 User's Guide. Cary, NC: SAS Institute Inc.

Savage, C. and R. Elmgren. 2004. Macroalgal (*Fucus vesiculosus*) δ15N values trace decrease in sewage influence. *Ecological Applications* 14(2):517-526.

Sheldon, J. E. and M. Alber 2006. The calculation of estuarine turnover times using freshwater fraction and tidal prism models: a critical evaluation. Estuaries and Coasts. V29(1). 133-146.

Solorzano, L. and J. H. Sharp. 1980a. Determination of total dissolved nitrogen in natural waters. *Limnol. Oceanogr*. 25(4):751–754.

Solorzano, L. and J. H. Sharp.1980b. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr*. 25(4):754–758.

Soranno, P. A., K. S. Cheruvelil, R. J. Stevenson, S. L. Rollins, S. W. Holden, S. Heaton, and E. Tong. 2008. A framework for developing ecosystem specific nutrient criteria: Integrating biological thresholds with predictive modeling. Limnol. and Oceanogr. 53:773-787.

Standard methods for the examination of water and wastewater. 1997. Federation WE, American Public Health Association. American Public Health Association (APHA): Washington, DC, USA.

Standard methods for the examination of water and wastewater. 2001. Federation WE, American Public Health Association. American Public Health Association (APHA): Washington, DC, USA.

Standard methods for the examination of water and wastewater. 2011. Federation WE, American Public Health Association. American Public Health Association (APHA): Washington, DC, USA.

Thermo Electron, 2004. FlashEA 1112 Elemental Analyzer Operating Manual, 5th ed. Thermo Electron Corp., Italy, 327 pp.

Tichy, L. 2015. GLAMA – Gap Light Analysis Mobile App: User's Manual Application Version 3.0. Brno, Czech Republic.

Valiela, I., M. G. McClell, and G. Tomasky. 2000. Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay Nitrogen Loading Model. *Biogeochemistry* 9(3):277-293.

Vincente – Serrano, S. M. , S. Begueria, and J. I. Lopez-Moreno. 2010. A multiscaler drought index sensitive to global warming: The Standardized Precipitation Evaporation Index. Journal of Climate. V23: 1696-1718.

Wigand, C., R. A. McKinney, M. L. Cole, G. B. Thursby, and J. Cummings. 2007. Varying stable nitrogen isotope ratios of different coastal marsh plants and their relationships with wastewater nitrogen and land use in New England, USA. *Environmental Monitoring and Assessment* 131:71-81.

Yang, Y. –Y. and G. S. Toor. 2016. $\delta^{15}N$ and $\delta^{18}O$ reveal the sources of nitrate-nitrogen in urban residential stormwater runoff. *Environmental Science and Technology* 50:2881-2889, DOI: 10.1021/acs.est.5b05353.

Zar, H. J. 1984. Biostatistical Analysis. Prentice Hall. Englewood Cliffs, New Jersey. 07632

Zimmerman, C.F., C.W. Keefe, and J. Bashe. 1997. Method 440.0 Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis. Revision 1.4. National Exposure Research Laboratory, Office of Research and Development , U.S. Environmental Protection Agency, Cincinnati, Ohio.