Identifying Potential Drivers of Change in Seagrass and Algal Community Composition in SWFL Aquatic Preserves

Final Report

Prepared for:

Charlotte Harbor and Estero Bay Aquatic Preserves

Prepared by:

Janicki Environmental, Inc.

March 2022

Executive Summary

Florida enacted the Aquatic Preserve Act of 1975, and there are currently 42 aquatic preserves designated across the state, managed by the Florida Department of Environmental Protection. Six of these aquatic preserves are located within or adjacent to the Charlotte Harbor estuarine complex including Lemon Bay, Gasparilla Sound-Charlotte Harbor, Cape Haze, Pine Island Sound, Matlacha Pass, and Estero Bay. A primary resource management goal of the Aquatic Preserves is to protect and enhance the health and functioning of seagrass habitats. To that end, long-term seagrass monitoring sites have been established throughout this area as an essential resource management tool to track changes in species composition, abundance, and the presence of attached and drift algae which have been observed to be at nuisance levels in recent years and are a cause for concern.

The objectives of this study were to evaluate changes in seagrass community based on transect level data using statistical analysis to identify potential drivers of change in characteristics of seagrass condition including species frequency/composition, abundance, transect length, "deep edge" depth, density (shoot count), and associated macroalgal and epiphytic coverage. Principal components analysis based on seagrass species frequency of occurrence, average total abundance (Braun-Blanquet: BB) score and mean and "deep edge" (defined by the 95th percentile of depth) suggested that sites fell across a gradient represented by those sites with a high frequency of *Halodule wrightii,* occurrence to deeper sites that tended to have a higher frequency of occurrence of *Thalassia testudinum* and *Syringodium filiforme*. Those sites with the highest average total BB scores, tended to be sites that were not monotypic in species frequency of occurrence with nine of the 10 sites containing all three major seagrass species types.

Changes in seagrass abundance scores over time suggested the drought associated years between 2007 and 2009 generally resulted in gains in seagrass while the

majority of sites displayed reduced abundance in 2012 and 2016 relative to their previous years score. In addition, noticeable declines have occurred in scores since 2017 in several strata with median abundance scores in Estero Bay reaching the lowest recorded for that stratum while Gasparilla Sound, Lower East CH, Tidal Myakka and Tidal Caloosahatchee scores returned back to circa 2004 levels. Sites EB02, ICW01, EB03, MP03, MP02, and EB01 exhibited the largest cumulative negative change in abundance. Sites with the largest negative change (i.e. < -1) tended to have a higher frequency of occurrence of Halodule though there were many sites with relatively high Halodule frequency of occurrence that had overall gains in total BB scores over the time period. Overall, more sites displayed a cumulative positive change over time than negative changes greater than 1 unit evaluated. Sites PI04, GAS01, MP04, SC01, and GAS06 exhibited the largest cumulative positive change in total BB score over the time period. Total transect length contracted over time at EB01 and EB03 in Estero Bay, but the remaining sites were either non-trending or positive over the period of record. The maximum depth of the primary seagrass species either increased or was non-trending over time with no statistically significant decreasing depth trends at any station.

Results of seasonal Kendall Tau trend test on WBID average monthly water quality data suggest approximately 50% of WBIDs exhibited increasing trends in total nitrogen and temperature between 1998 and 2020. Salinity was mostly non-trending with only two decreasing trends over time. Total phosphorus was either non-trending or decreasing over time except in Upper Charlotte Harbor and Lower Caloosahatchee which exhibited small increasing trends over time. Chlorophyll was non-trending or decreasing, especially in the southern portion of the study area, though an increasing trend in chlorophyll was observed in Upper Lemon Bay. Turbidity was decreasing or stable in all WBIDs except the WBID representing Northern Estero Bay (Ft Myers Beach). Other constituents were mostly non-trending or had trends representing improving conditions over the period of record evaluated.

Higher than average monthly flows were strongly associated with many water quality constituents including salinity which was strongly negatively correlated ($p<0.001$) with flows in all strata. The strength of the association statistic was generally aligned with proximity to the discharge source. That is, river flows were more strongly correlated to river water quality than to more open estuary segments located closer to passes. Total phosphorus, total nitrogen and its constituents (total kjeldahl nitrogen and nitrate-nitrite) were consistently positively associated with deviations in flows while water clarity (as measured by secchi disk depth) was consistently negatively correlated with flows indicating that as flows increase, water clarity tends to be reduced. Other water quality constituents such as chlorophyll, temperature, and turbidity were more weakly correlated with flows indicating more proximal influences on water quality may be mediating the relationship between flows and these constituents.

The relationship between antecedent freshwater inflows and changes in seagrass BB scores were species-specific. All significant correlations between flows and Halodule were negative indicating higher than average flows for up to 6 months prior to the sampling event were negatively correlated with seagrass BB score. That is, higher than average antecedent flows were associated with lower Halodule abundance scores at several sites in the study area. The relationship between antecedent flows and Thalassia BB scores were more site-specific with 4 sites resulting in a negative correlation and 5 sites suggesting a positive correlation. The relationship between flows and total BB scores were similar to the Halodule results suggesting higher than average flows during antecedent conditions to seagrass sampling was associated with lower total abundance scores.

Bivariate correlation analysis suggested that nutrients, salinity, and parameters associated with water clarity (e.g. chlorophyll, secchi disk, and turbidity), were significantly correlated with total BB scores. Nutrient parameter estimates suggest predominantly negative correlations when significant, indicating potential negative effects of increasing nutrients on total BB scores across the study area.

Multiple linear regression analysis across strata suggested that, after accounting for site and depth effects, total phosphorus explained the largest proportion of variation and was a negative overall effect. Total nitrogen, nitrate-nitrite and turbidity were also significant negative effects while light attenuating constituents (secchi disk and chlorophyll) were not statistically significant in the across strata model after accounting for other effects. Results of strata-specific analysis of total BB scores suggested that relationships between TP and BB scores were generally consistent among sites within a stratum and that TP was deterministic in all strata once the final model was reduced to only those parameters that explained a significant portion of the overall variation in total abundance. However, the sign of the coefficient for Estero Bay was positive while all other segments were negative which may be explained by the low overall concentrations of total phosphorus in Estero Bay. Effects of antecedent total nitrogen concentrations were more site-specific within strata with some positive and negative effects indicated. In several strata including Upper West CH, San Carlos Bay, Matlacha Pass, and Lemon Bay, antecedent nitrate – nitrite concentrations were negatively related to abundance scores indicating increasing concentrations may be related to reduced abundance over time. These strata include the sites with the highest cumulative loss in abundance over time and suggest anthropogenic inputs may be present at these locations. Synergistic effects of increased nutrients and temperatures have been posited to play a role in seagrass growth inhibition and this may have played a role in our study as well since both nutrients and water temperatures have been increasing over time in several strata.

Natural environmental patterns provide overriding forcing functions on water quality constituents that govern seagrass success, and the period between 1998 and 2010 contain several extreme events including an intense period of hurricane activity, a protracted drought and a subsequent return to a more near normal or slightly above average freshwater inflow pattern. However, even after accounting for salinity which is an integrative measure of antecedent freshwater inflows to the estuary, nutrients were identified as potential significant drivers with typically negative parameter estimates in the regression framework suggesting negative impacts of increased nutrients on seagrass success. While linking average antecedent water quality to site-specific transect information is a generalization of the conditions that a particular site might experience, these results suggest nutrients play a key role in controlling seagrass abundance in combination with other factors. While some of the change in BB score over time may be due to natural variation in environmental condition, increasing trends in total nitrogen and temperature throughout much of the study area present stressors for these seagrass communities. In addition, negative association between antecedent nitrate-nitrite concentrations and total abundance scores conform to known mechanisms of anthropogenically based pollution.

While overall the transect level data suggest a return to baseline conditions at the beginning of the study period rather than a historic decrease in seagrass that has not been observed in the past, a weight of evidence suggests that nitrogen concentrations either in the form of total or nitrate-nitrite, may have reached critical thresholds in some cases that, in combination with other environmental factors, have resulted in recent seagrass losses. It cannot be discounted that the protracted drought in 2007 through 2009 resulted in abnormally beneficial conditions for seagrass meadows that have now regressed back to their long-term averages. However, recent observations of prolific macroalgae and red tide blooms in much of the study area are a cause for great concern that the ecosystem metabolism is vacillating in ways that might indicate a future shift towards an alternative ecosystem state. To date, the lack of intense phytoplankton blooms (other than red tide blooms) provide some hopeful evidence that the system has not yet crossed a tipping point that might result in a more phytoplankton-dominated system but all efforts should be made to limit nutrient loads to these aquatic preserves to combat other environmental forces that stress these ecosystems.

Table of Contents

List of Tables

List of Figures

Background:

Florida enacted the Aquatic Preserve Act of 1975, which later allowed for the designation of 42 current aquatic preserves across the state to ensure that its exceptional submerged resources are set aside to be preserved in essentially natural conditions to be enjoyed by future generations (Chapter 18-20.001 F.A.C.). As described by Brown (2019), aquatic preserves are submerged lands with exceptional biological, aesthetic, and scientific values and are managed to sustain their natural resources for the public's continued enjoyment. There are six aquatic preserves within or adjacent to the Charlotte Harbor estuarine complex that are administered by the Florida Department of Environmental Protection (DEP) through the Office of Resilience and Coastal Protection (RCP). Five of these are managed out of the Punta Gorda office as the Charlotte Harbor Aquatic Preserves (CHAP) including Lemon Bay; Gasparilla Sound-Charlotte Harbor; Cape Haze; Pine Island Sound; and Matlacha Pass. Estero Bay Aquatic Preserve (EBAP) is located just to the south and is managed from an office in Fort Myers Beach. These six aquatic preserves (hereafter referred to as "AP") include jurisdictions of two of Florida's five water management districts--the Southwest and South Florida Water Management District.

A primary resource management goal of the AP is to protect and enhance the health and functioning of seagrass habitats. To that end, long-term monitoring sites have been established throughout the AP as an essential resource management tool (Figure 1). While aerial photography is routinely conducted in parts of the study area, site-specific evaluations are valuable for elucidating more localized changes in seagrass communities including species composition, abundance, and the presence of attached and drift algae which have been observed to be at nuisance levels in recent years and are a cause for concern. Water quality is known to be a critical driver of seagrass success with previous studies suggesting water clarity (Duarte 1991; McPherson and Miller 1994; Dixon and Kirkpatirck 1999; Dixon and Wessel 2016), nutrients (Tomasko et al. 1996, Tomasko et al. 2001) and salinity (Montague and Ley 1992; Tomasko and Hall 1999) contributing to seagrass condition as well as events such as floods, droughts, and hurricane activity (Tomasko et al. 2020).

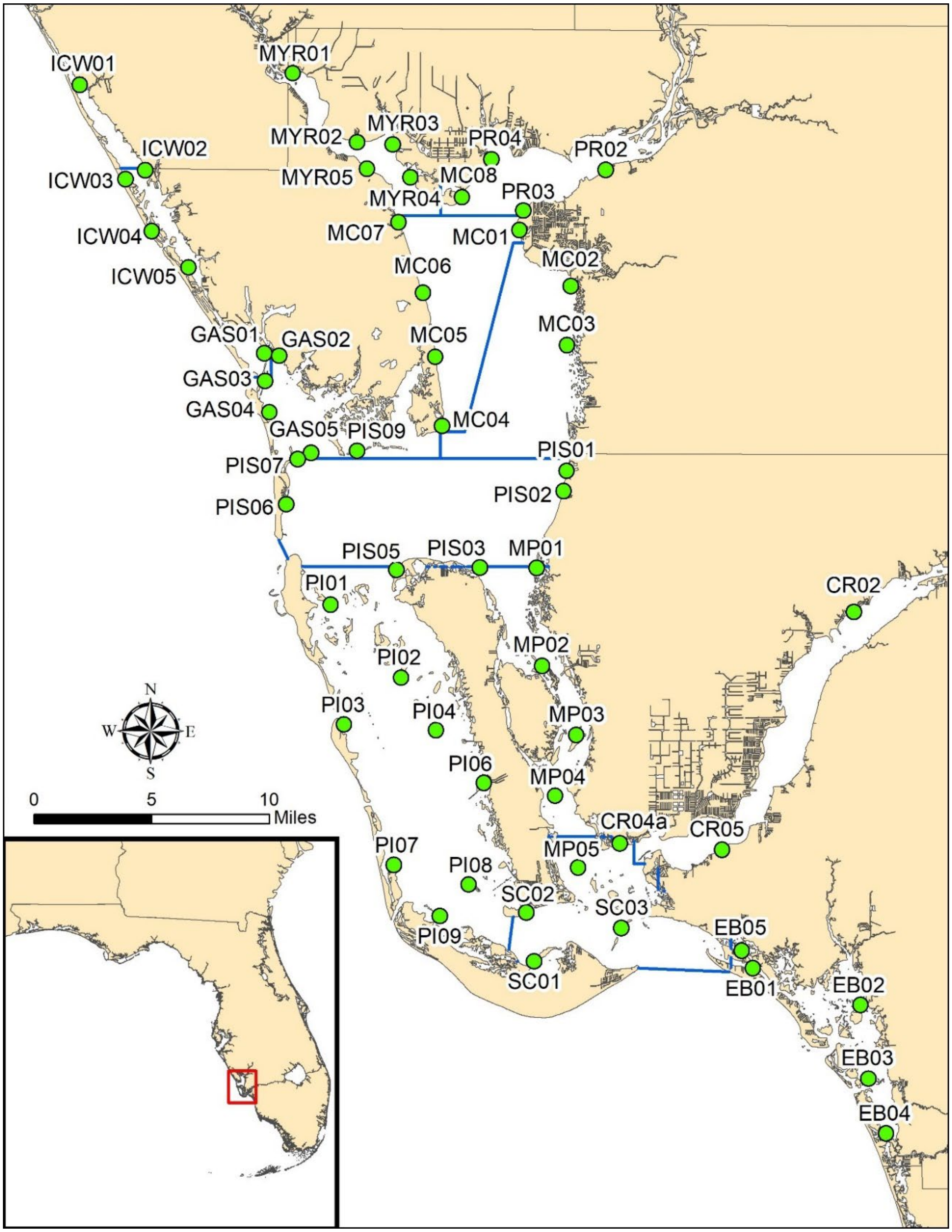

Figure 1. Aquatic preserve seagrass transect locations within the study area.

Two previous studies have been conducted specifically using the AP seagrass transect data to evaluate relationships between water quality and seagrass condition. The Charlotte Harbor Environmental Center (CHEC 2006) evaluated transect level data collected between 1998 and 2004 in Lemon Bay, Gasparilla Sound, Charlotte Harbor and the Peace and Myakka Rivers. The results of that analysis suggested declines in species-specific seagrass abundance (as characterized by Braun-Blanquet scores) in several, but not all sites between 1998 and 2004. Water quality was weakly correlated with seagrass abundance with salinity and water clarity providing the highest strength of association. Brown et al. 2013 evaluated seagrass trends between 1999 and 2009 by region for all strata defined above as well as Pine Island Sound, Matlacha Pass and San Carlos Bay. The analysis focused on describing trends among strata and among years rather than evaluating site-specific information. That report suggested seagrass appeared to be relatively stable across the study area with minor declines associated with considerably high rainfall years. In addition, two thesis studies were recently completed evaluating factors affecting seagrass abundance in Estero Bay (Rickards 2018) and within the CHAP (Taylor-Manges 2021).

Goals and Objectives:

The goal of this report was to provide a unified analysis of changes in seagrass with a focus on identifying potential drivers of seagrass and algae community change over time throughout the entire study area including both the aquatic preserves of the Charlotte Harbor complex and Estero Bay. The objectives of this study were to evaluate changes in seagrass community based on transect level data using statistical analysis to identify potential drivers of change in characteristics of seagrass condition including species frequency/composition, abundance, transect length, “deep edge” depth, density (shoot count), and associated macroalgal and epiphytic coverage.

Data and Methods:

The following paragraphs describe methods used to compile and analyze data for this project.

Data Compilation:

Seagrass data were provided as an export table from the aquatic preserve master database. Daily flow data were downloaded from the United States Geological Survey data portal (USGS) for all tributaries within the study area. Water quality data were downloaded from the FDEP Impaired Waters Rule database (Run 61) for waterbody identifiers (WBIDs) within the study area (Figure 2:Left). Data providers include Lee County, DEP Division of Environmental Assessment and Restoration, CHAP's Charlotte Harbor Estuaries Volunteer Water Quality Monitoring Program, and Coastal Charlotte Harbor Water Quality Monitoring Network; a consortium of natural resource entities that uses a stratified random sampling design with strata designations as defined on the right panel map in Figure 2.

Ancillary data included seagrass areal acreage estimates and land use landcover data collected by the Southwest Florida Water Management District for the Charlotte and Sarasota County portions of the system. Continuous data on physical water chemistry constituents for sondes located in Matlacha Pass is also included. All data used in this project have been collated into a master database delivered as part of Task 1 deliverables for this project.

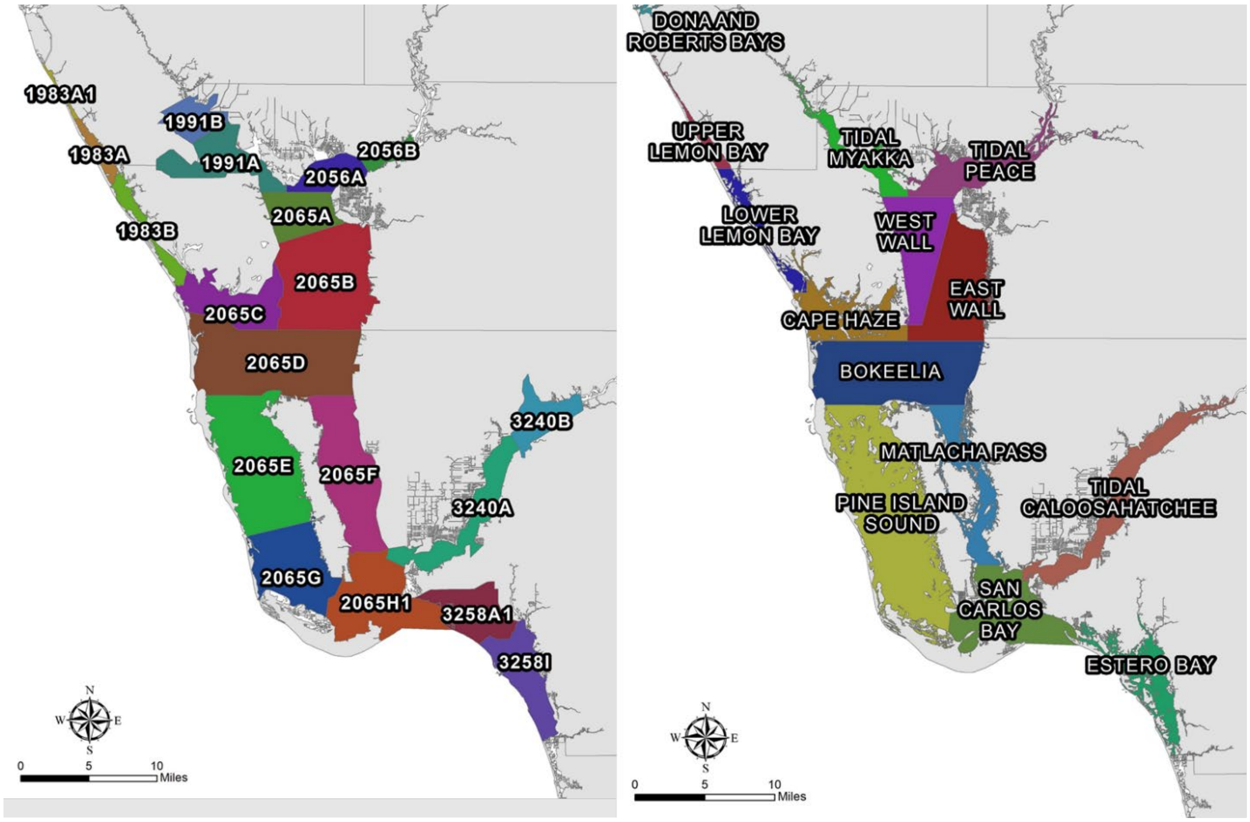

Figure 2. Florida Department of Environmental Protection Waterbody Identifiers (WBIDs: Left) and water quality sampling strata (Right) for the study area.

Seagrass Data Collection Methods:

CHAP seagrass monitoring consists of monitoring 50 transects annually, EBAP monitors 5 sites bi-annually (EB sites), and DEP Division of Environmental Assessment and Restoration monitors 3 transects in the Caloosahatchee River (CR) on a quarterly basis as well as 3 sites (MP04, MP05 and SC03) also monitored by CHAP for a total of 58 sites monitored regionally. Methods for seagrass data collection have been previously described and the reader is referred to CHEC 2006, Brown et al 2013 and Brown 2019 for details. The following paragraphs describing the seagrass data collection methodology are paraphrased with permission from those documents. The majority of the transects ("Sites") were established in 1998 and generally extend towards open water along a compass bearing from a vegetated (e.g. mangrove) shoreline (CHEC 2006). Small PVC stakes mark most quadrat ("Station") locations. The "end of bed" for each site was generally defined as the furthest distance from shore where seagrass could be found after a brief search by snorkel or SCUBA. Note that the end of bed is not always the deepest part of the transect. As described in CHEC (2006), sampling stations were generally spaced every 10 meters along transects less than 50 meters total length, and every 50 meters along transects greater than 50 meters in length. A 1-square meter PVC quadrat divided into a l0xl0 grid was placed on the bottom at each station. Two observers agreed on an estimate of abundance using the Braun-Blanquet (BB) vegetation index (See Table 2 below for explanation) for each species of seagrass present in the quadrat. Those species include: *Thalassia testudinum* (Thalassia), *Syringodium filiforme* (Syringodium), *Halodule wrightii* (Halodule), *Halophila* spp., *Vallisneria americana* (Vallisneria) *and Ruppia maritima* (Ruppia). These species make up the "Total" seagrass BB scores. Other submerged aquatic vegetation (SAV) including *Caulerpa* species were not included in the total BB scores but were recorded as species-specific abundances within each quadrat where found. Macroalgae (drift algae) is also given an abundance separate from seagrass total abundance.

Seagrass transect data through 2020 were supplied by the aquatic preserves as an exported table from their master database. These data include locational information for strata, site, and station within site where quadrats are used to estimate seagrass abundance recorded as Braun-Blanquet scores. Seagrass total abundance, species abundance, drift algae abundance, average blade length, and epiphytic abundance were recorded along with sediment type, depth, and other physical characteristics of the station.

Analytical Methods**:**

The process of evaluating data with respect to the goals and objectives of this study relied on data visualizations, descriptive statistics, exploratory data analysis, and statistical model building to generate inferential statements about potential factors explaining change in seagrass abundance. The following subsections describe analytical components of data handling to identify potential drivers of seagrass condition.

Flows:

Daily flow data were subset to the 1998-2020 period of record, and values were standardized to the period of record monthly average for that site. Flows were natural log transformed with a constant of 1 added to each value prior to transformation to account for zero flow values in the timeseries. Deviations from the long-term average were expressed in units of standard deviation based on the following equation:

$$Q_\theta = (Q - Q_\mu)/Q_\sigma$$

Where:

$Q_\theta = standardized\ flow\ value$

$Q_\mu = long\ term\ monthly\ average\ flow\ (1998 - 2020)$

$Q_\sigma\ = standard\ deviation\ of\ the\ long\ term\ monthly\ average\ flow$

The resultant values are centered at zero with a standard deviation of 1 allowing for interpretations analogous to standard normal statistical distributions.

Water Quality:

Water quality constituents evaluated for this project included primary drivers of water clarity which are known to affect light availability (directly or indirectly) to seagrass (Table 1). Monthly WBID averages were calculated for each constituent for trend testing and for correlating with seagrass change. In addition, each water quality constituent was standardized to its long term monthly average analogous to the method used to standardize flows above except values were not transformed prior to standardization. This standardization was used as an additional metric to correlate deviations in flow with deviations in water quality and seagrass abundance over time.

Table 1. List of water quality constituents used in trend analysis and to correlate with seagrass change.

Constituent	Units	Abbreviation
Chlorophyll a corrected for pheophytin	µg/l	CHLAC
Color	PCU	COLOR
Dissolved Oxygen	% saturation	DOSAT
Nitrate-Nitrite	mg/l	NO32
Salinity	PSU	SALIN
Secchi Disk depth	meters	SD
Temperature	degrees centigrade	TEMP
Total Nitrogen	mg/l	TN
Total Phosphorus	mg/l	TP
Turbidity	NTU	TURB

Seagrass

Seagrass abundance data were reported using the eight unit marine modification of the Braun-Blanquet (BB) vegetation index scale (Braun-Blanquet, 1965:Table 2) which we condensed to a 6 unit scale described by Brown et al. (2013) by combing 0.1, 0.5 and 1 into a single category (i.e. 1 representing less than 5% SAV cover). Species-specific abundance has been reported since 1998 for all strata other than Estero Bay (i.e. 2002) and Tidal Caloosahatchee (i.e. 2007) though as discussed in Brown et al. (2013), 1998 data were not considered in the analysis due to the refinements in methods that occurred during the first year of sampling. Seagrass species are referred to by their genera for this report. For example, *Halodule wrightii* is referred to as Halodule.

Table 2. Braun-Blanquet categories and representation as percent cover used for reporting seagrass abundance throughout the study area.

Braun-Blanquet Score	**Percent Cover**
0	Not present
0.1	Single or Rare
0.5	Very Few
1	< 5%
2	5% - 25%
3	25% - 50%
4	50% - 75%
5	75% - 100%

Total seagrass abundance (i.e. total abundance of either Halodule, Syringodium, Thalassia, or Ruppia) has been reported since 2004 for all strata other than Estero Bay (2006) and Tidal Caloosahatchee (2007).

As described in Brown et al. 2013, a BB score of 0 was included in the analysis of site level total average abundance but not for species average abundance, as the latter relates only to the abundance of a particular species. Total BB scores began in 2004 (2006 for Estero Bay) while species-specific scores have been reported since 1999 (2002 for Estero Bay). For species-specific scores, the averages were calculated based only when species-specific BB scores were reported. All stations were used for generating descriptive statistics while only those "repeat" stations as identified by a data table in the AP master Access Database were used for statistical modeling to identify potential drivers of seagrass change. The seagrass bed was designated by a "B" or E" to signify beginning or end of bed, respectively and the associated station numbers were used to estimate total transect length. Since the end of bed station may not reflect the deepest water depth, the "deep edge" was evaluated by calculating the maximum depth where one of the four principal seagrass species was recorded.

Combining Flows, Water Quality and Seagrass Data

Correlating flows, water quality and seagrass data required matching flow gages with WBID average water quality data using the WBID and bay segment assignments listed in Table 3 and displayed in Figure 3. It is acknowledged that these assignments are likely only to represent approximations to the true effect of hydrologic inputs to the system which are a combination of complex interactions of freshwater inputs, hydrology, and estuarine circulation, however, descriptive analysis described in the results section supports the assignment to reflect general association between flows and water quality in these systems. The Peace River Arcadia gage was assigned to all Charlotte Harbor Proper segments in the Upper Estuary, The Myakka River gage was assigned to Lemon Bay segments since no direct USGS gaged estimates were available for the entire timeseries in Lemon Bay. The Caloosahatchee gage was assigned to all Southern Charlotte Harbor segments while the Ten Mile Canal gage was assigned to the Estero Bay WBIDs since it enters the estuary in the most proximal location to both WBIDs in the estuary as well as most seagrass transects. The Ten Mile Canal gage was highly correlated with all other gages draining to Estero Bay (rho_s at least 0.74). Descriptive statistics and plots of all gages used can be found in Appendix A.

Table 3. Assignment of flow timeseries to WBID level water quality data.

WBID	Bay Segment	Flow Gage	Site Number
1983A	Lemon	Myakka River Near Sr 72 Near Sarasota	02298830
1983A1	Lemon	Myakka River Near Sr 72 Near Sarasota	02298830
1983B	Lemon	Myakka River Near Sr 72 Near Sarasota	02298830
1991A	Myakka	Myakka River Near Sr 72 Near Sarasota	02298830
1991B	Myakka	Myakka River Near Sr 72 Near Sarasota	02298830
2056A	Peace	Peace River At Sr 70 At Arcadia	02296750
2056B	Peace	Peace River At Sr 70 At Arcadia	02296750
2065A	Upper Harbor	Peace River At Sr 70 At Arcadia	02296750
2065B	Upper Harbor	Peace River At Sr 70 At Arcadia	02296750
2065C	Cape Haze	Peace River At Sr 70 At Arcadia	02296750
2065D	Bokeelia	Peace River At Sr 70 At Arcadia	02296750
2065E	Pine Island Sound (North)	Peace River At Sr 70 At Arcadia	02296750
2065F	Matlacha Pass	Peace River At Sr 70 At Arcadia	02296750
2065G	Southern Pine Island Sound	Caloosahatchee River At S-79 Nr. Olga	02292900
2065H1	San Carlos Bay	Caloosahatchee River At S-79 Nr. Olga	02292900
3240A	Lower Tidal Caloosahatchee	Caloosahatchee River At S-79 Nr. Olga	02292900
3240B	Upper Tidal Caloosahatchee	Caloosahatchee River At S-79 Nr. Olga	02292900
3258A1	Estero Bay North	Tenmile Canal At Control Near Estero	02291673
3258I	Estero Bay South	Tenmile Canal At Control Near Estero	02291673

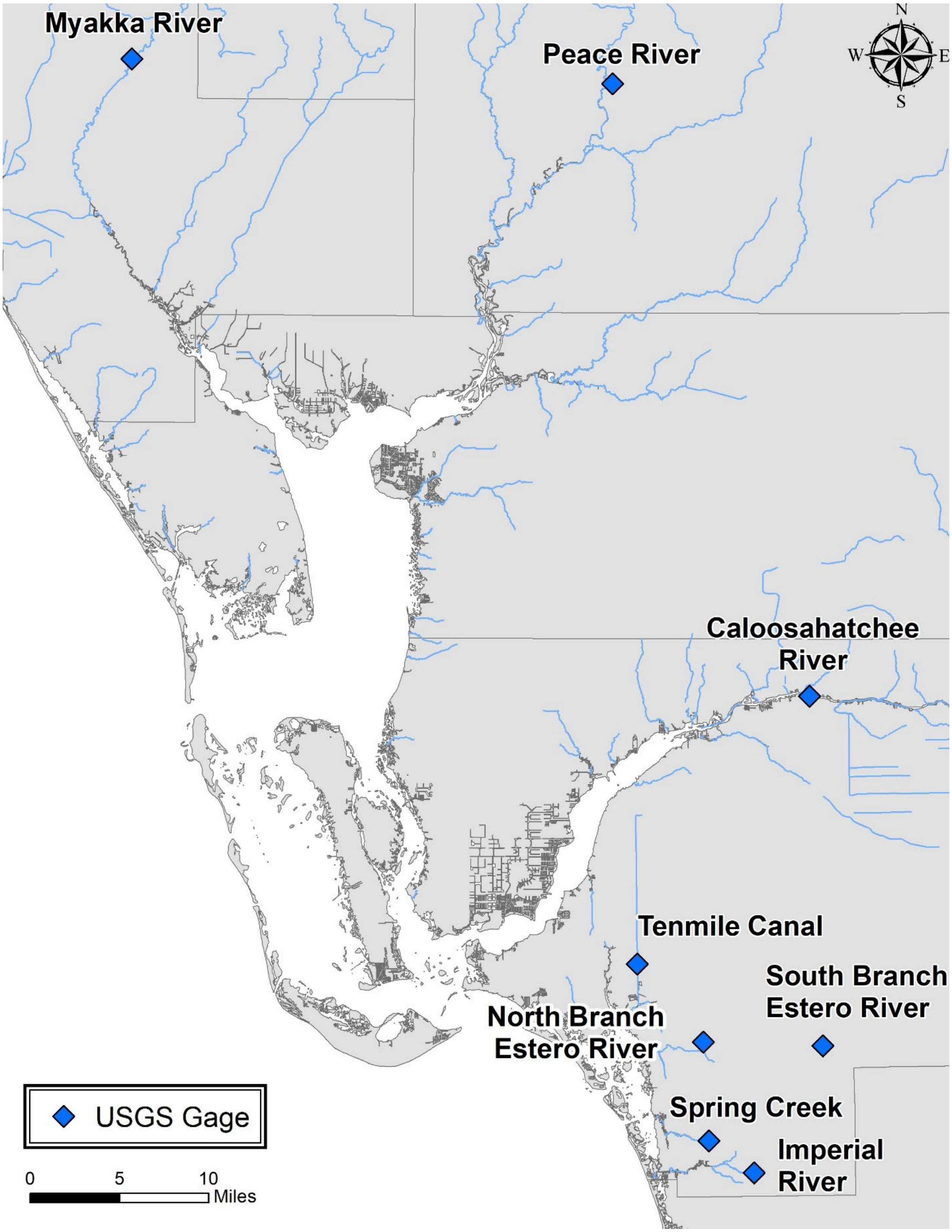

Figure 3. Location of United States Geological Survey discharge gages in the study area.

Lag averages (up to six months) in flow and water quality were calculated to describe antecedent conditions potentially affecting the flow – water quality relationship as well as the water quality – seagrass relationship. These metrics of antecedent condition were also evaluated as potential explanatory variables in statistical models.

Statistical Analysis

Non-parametric statistics were the default analytical method chosen for descriptive and correlative analysis and parametric statistics were used in statistical models to identify potential drivers of seagrass change. Nonparametric analysis included Principal Components Analysis and Hierarchical Agglomerative Clustering using the FactoMineR (Sebastian et al. 2020) and factoextra (Kassambara and Mundt 2020) packages in R software (R Core Development Team 2021). Seasonal Kendall Tau test for trend (Hirsch and Slack 1984) was used to evaluate time series trends in monthly water quality data while the Mann-Kendall test for trend was used to assess trends over time in annual site-specific total and species-specific BB scores by averaging the station (quadrat) specific scores for each site over a sampling event. The Cochrane Armitage (CA) test (Armitage 1955) was used to evaluate trends in the presence of drift algae at sites over time. For descriptive assessments, year to year changes in total transect average BB scores were calculated by differencing (i.e. $Y_t - Y_{t-1}$). Conditional inference trees (Hothorn et al., 2006) and ensemble models (Brieman 2001) were used to explore potential breakpoints and drivers in relationships between seagrass and potential drivers of seagrass condition. Descriptive assessments were carried out on all sites with more than two years of sampling. After descriptive assessments were completed, several sites including CR01, CR02a, CR02b, CR03, CR04b, PI06, PIS08, SC04, SC05, PI10, PI11, and PIS09 were omitted for regression modeling after consensus with the project team due typically to their short duration of data collection.

Linear regression modeling was initially conducted using a stepwise selection procedure on transect average total BB scores to inform the parameter set used in more sophisticated analysis using mixed-effects general linear regression models. The advent of mixed-effects regression modeling and its use in hierarchical or multi-level modeling is a way to evaluate potential drivers of water quality on seagrass BB scores that allows for the use of all data collected in its rawest form while providing robust statistical inference on the significance of potential drivers in explaining BB scores. As applied to seagrass transect data, mixed-effects modeling is a way to estimate the effects at higher order levels (e.g. sites or strata) while incorporating the station information as a "random" effect to account for variation that exists between stations. That is, instead of averaging stations within a site to obtain the site level score which substantially reduces the true number of observations collected, the mixed-effects model uses all the raw data to obtain better estimates of the site average while separating the station variation from the error term in the model. The mixed-effects model formulation was defined as:

Mixed-effects Model:

$$Y_{ijk} = \beta_0 + \beta_{0_j} + \beta X_{ijk} + e_{ijk}$$

Where:

$Y_{ijk} =$ total Braun Blanquet score at each site (i), and station (j) and date(k)

$X_{ijk} =$ water quality parameter set values each site (i), station (j), and date(k)

$\beta_0 =$ overall intercept

$\beta_{0_j} =$ random intercept for station nested within site

$\beta X = D$eterministic effect of water quality parameter set

$e_{ijk} =$ residual $(N_{(0)\ \mathrm{iid}})$

The model building process started with a full model including TN, TP, NO32, Temperature, Chla, Turb and Secchi disk (SD) with depth and site covariates as main effects. Salinity was not included in the initial modeling effort given the broad distribution of sites across the estuarine gradient and because the focus of the analysis was to identify potential drivers that could be influenced by management actions. Instead, salinity was considered as a post hoc assessment of the strata specific model results. The mixed-effects model treats the station effect as "random" nested within each site and provides a separate variance component for that term. Evaluating the benefits of the random component to the model was judged using the covariance parameter test (SAS Institute, Inc. 2018) since it is not reasonable to calculate a coefficient of determination (R^2) for a mixed-effects model due to its additional variance component and the value of incorporating different fixed effects terms in the model was evaluated using the penalized Akaike Information Criteria (AICC). The models were first developed across strata, then within strata using the full parameter set, and finally each strata was evaluated separately to determine the final parameter set identified as potential drivers of total BB scores in that segment. The reader is referred to Littell et al. (1996) for an introduction into mixed-effects modeling using SAS software and to Furman et al. (2018), and references therein, for a description of the evolution of the Braun-Blanquet scores and methodological approaches to analyzing the resulting data using parametric statistics within the ANOVA framework. When identifying potential drivers, the p value was relaxed to 0.10 and the term "marginally significant" was used to identify the term as a potential driver when the p value was between 0.01 and 0.10. Statistical Analysis Systems (SAS Institute, Inc. 2021) was used for these analyses as well as for producing many of the graphics provided in this report.

Generalized linear models were also constructed to evaluate potential drivers of BB scores using the raw BB data as the response variable instead of modeling the average BB scores. Generalized linear models are a family of models including logistic regression that allow for the response variable to be expressed as something other than a continuous normal distribution. As applied here, the response variable (BB score) was treated as polychotomous, and models were fit as generalized logistic regression models which compare each total BB score category to the 0 category (as a reference group). Generalized linear models were implemented in much the same way as the linear regression models described above to identify potential drivers of change in seagrass abundance. These models were also implemented using SAS software (SAS Institute, Inc. 2021).

Appendices are referenced throughout the results section and supplemental materials describing various aspects of the analysis, including responses to comments received during peer review, are provided as the last appendix (J) to this document.

Results:

Descriptive Assessment

Fifty-six seagrass transects met the initial inclusion criteria for analysis including sites from all strata within the study area. Principal components analysis based on SAV species frequency of occurrence, average total BB score and mean and "deep edge" (defined by the 95th percentile of depth) suggested that four sites with a high frequency of occurrence of either no seagrass ("None") or Ruppia were distinguished from the remaining sites (Figure 4). These sites (PR01, PR02, MYR01, and CR02) were located in the major river systems contributing to the study area. The remaining sites fell across a gradient represented by those sites with a high frequency of Halodule occurrence which negatively weighted the dimension 2 axis on one end of the gradient and deeper sites that tended to have a higher frequency of occurrence of Thalassia and Syringodium that positively weighted the dimension 1 axis.

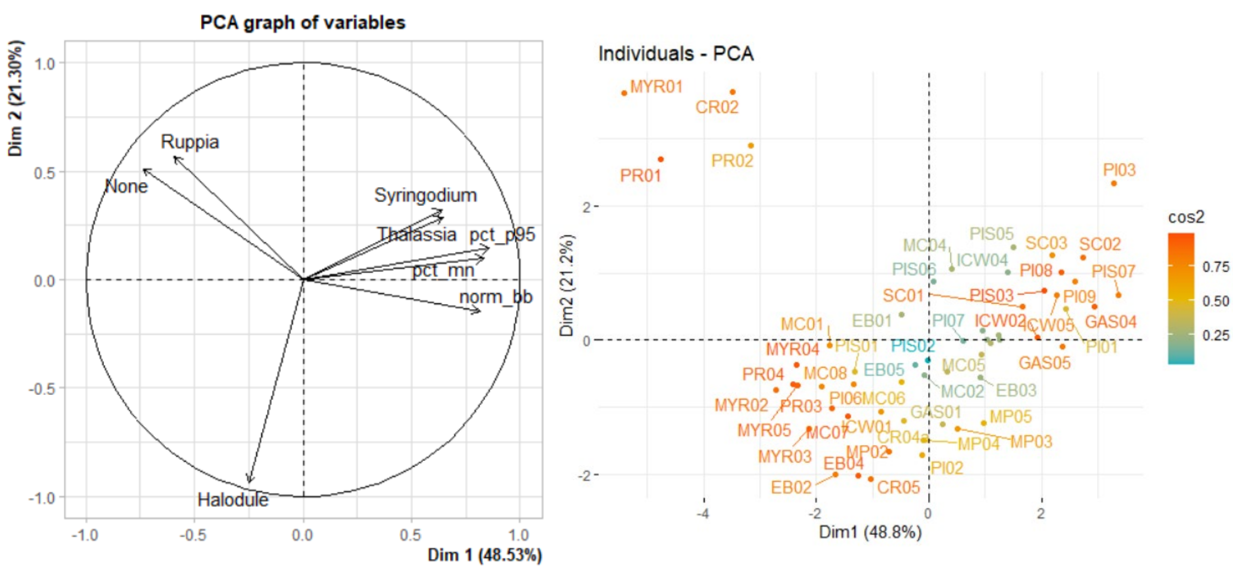

Figure 4. Results of principal components analysis using species frequency of occurrence and depth characteristics (pct_p95 = 95th percentile of depth (cm); pct_mn = mean depth (cm); norm_bb = average total braun blanquet scores).

A heatmap of the normalized values for each variable that contributed to the PCA results is provided in Figure 5 and is a convenient way to assess the distribution of site attributes for all 56 sites. The heatmap displays color coded scores from blue to red which represent a scale relative to the highest value for each variable. For example, a value of 1 represents the highest recorded value for frequency of occurrence of a particular species and all other values are a fraction of that highest value. For Ruppia, the highest frequency of occurrence was found in MYR01 followed by CR02 and all other sites had low frequency of occurrence relative to those sites. An intriguing result of this plot was the observation that those sites with the highest average total BB scores (right column in red), tended to be sites that were not monotypic in species frequency of occurrence. The 10 sites with the highest average total BB score are listed in Table 4. All of these sites had at least 20% occurrence of Halodule and only one site (GAS01) had over 60% occurrence of any species. Nine of the 10 sites contained all three major seagrass species types though PI01 and GAS01 contained 2% or less of Thalassia and EB03 contained only 1% Syringodium.

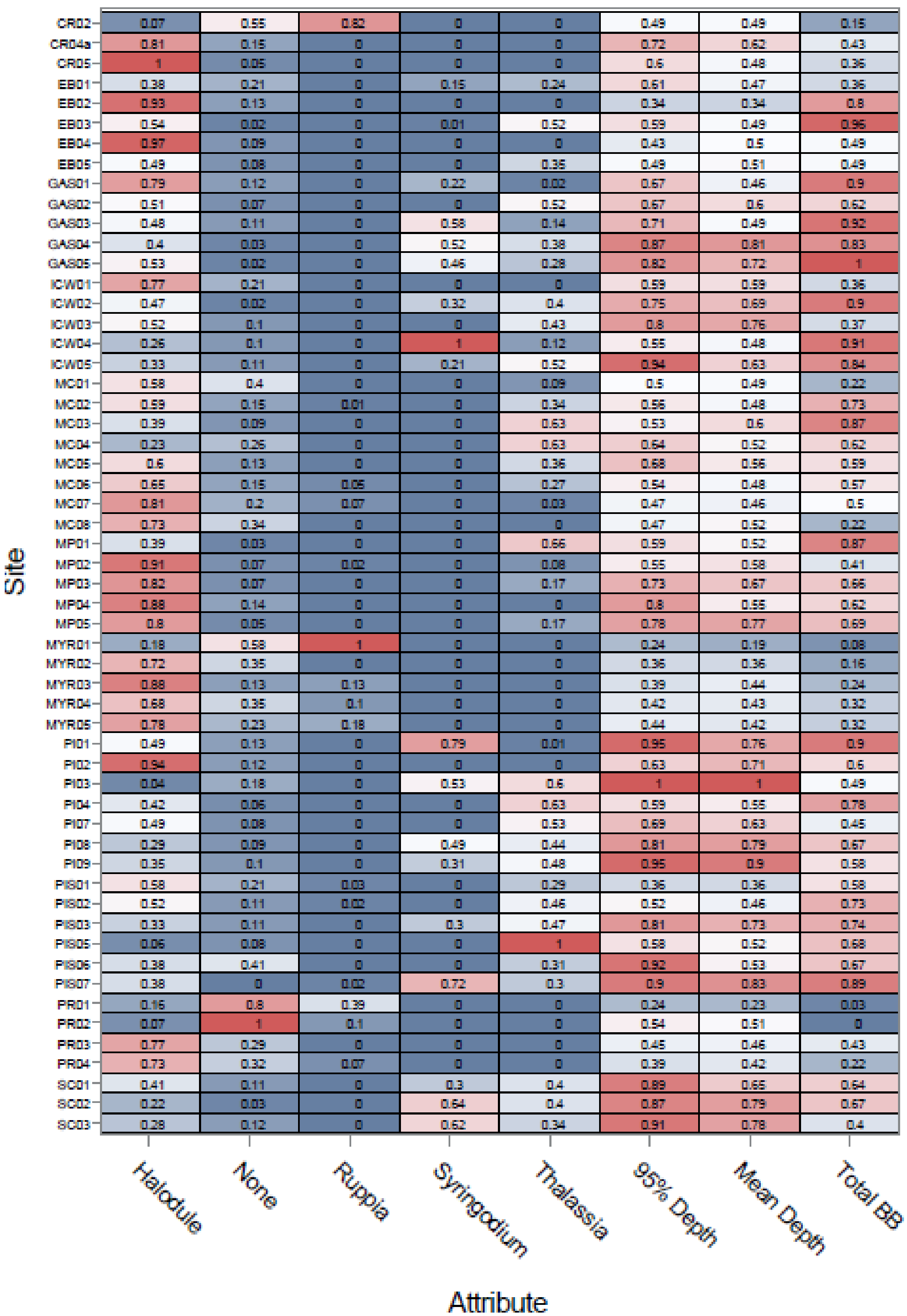

Figure 5. Heatmap displaying seagrass frequency of occurrence, depth and total abundance statistics normalized as a proportion of the highest observed value for each column.

Table 4. Top ten highest ranking sites based on average total Braun-Blanquet scores with percent frequency of occurrence of each of the dominant species.

Site	Halodule Percent Occurrence	No Seagrass Percent Occurrence	Syringodium Percent Occurrence	Thalassia Percent Occurrence	Total Average Braun-Blanquet Scores
GAS05	50.88	1.41	23.67	24.03	3.11
EB03	51.6	1.46	0.58	44.31	2.98
GAS03	46.12	9.48	30.17	12.07	2.87
ICW04	24.32	8.78	52.03	10.14	2.84
GAS01	75	10.61	11.36	1.52	2.81
ICW02	45.02	2.16	16.88	34.2	2.81
PI01	46.72	11.68	40.88	0.73	2.8
PIS07	36.46	0	37.5	25.52	2.75
MC03	36.75	8.02	0.22	53.9	2.71
MP01	37.1	2.71	0	57.01	2.7

Results of hierarchical cluster analysis resulted in five groups of sites with the riverine sites described above forming one group, another group containing generally more hydrologically constrained sites and the final group generally representing more near coastal open water sites (Figure 6). These are general descriptions based on the results of the cluster analysis though it should be noted that the sites represent a gradient of conditions as depicted by the PCA plots. All Gasparilla Sound and Pine Island Sound sites were contained within a single grouping; however, the other strata had sites fall into different groupings based on their site and seagrass characteristics. This suggests that different factors within strata may be affecting site conditions in some strata.

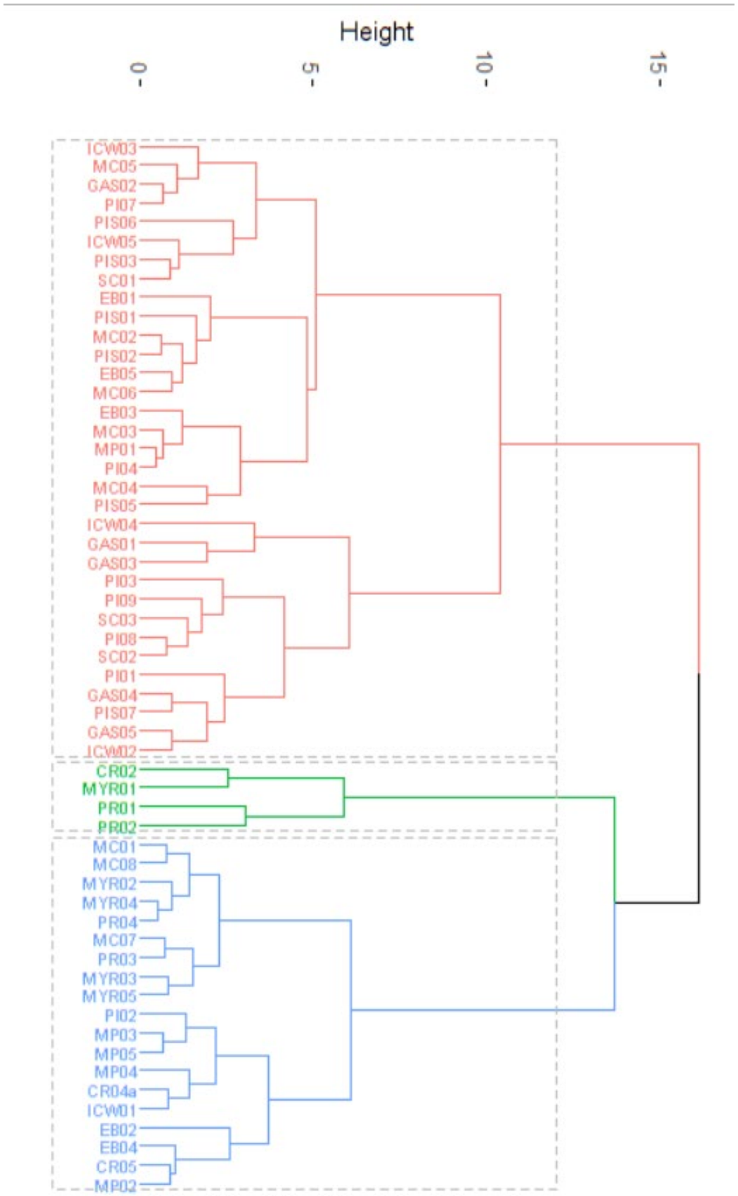

Figure 6. Hierarchical cluster analysis of the site characteristics delineating sites into three color coded groups corresponding to their overall similarity in seagrass characteristics.

Identifying Change in Braun-Blanquet Scores

The difference in total BB score from one year to the next was calculated for each year and site. The distribution of these differences were then plotted for each year across strata as boxplots (Figure 7). Results of one-way ANOVA with Tukey adjustment for multiple comparisons suggested a significant Year effect with 2006, 2007, 2009 and 2014 having a mean difference greater than zero while 2012, 2016, had a mean difference significantly less than zero. The year 2019 was marginally significant (p=0.071) and negative. A non-parametric test of the median values supported the ANOVA results but suggested that the median change in 2019 was not significantly different from zero.

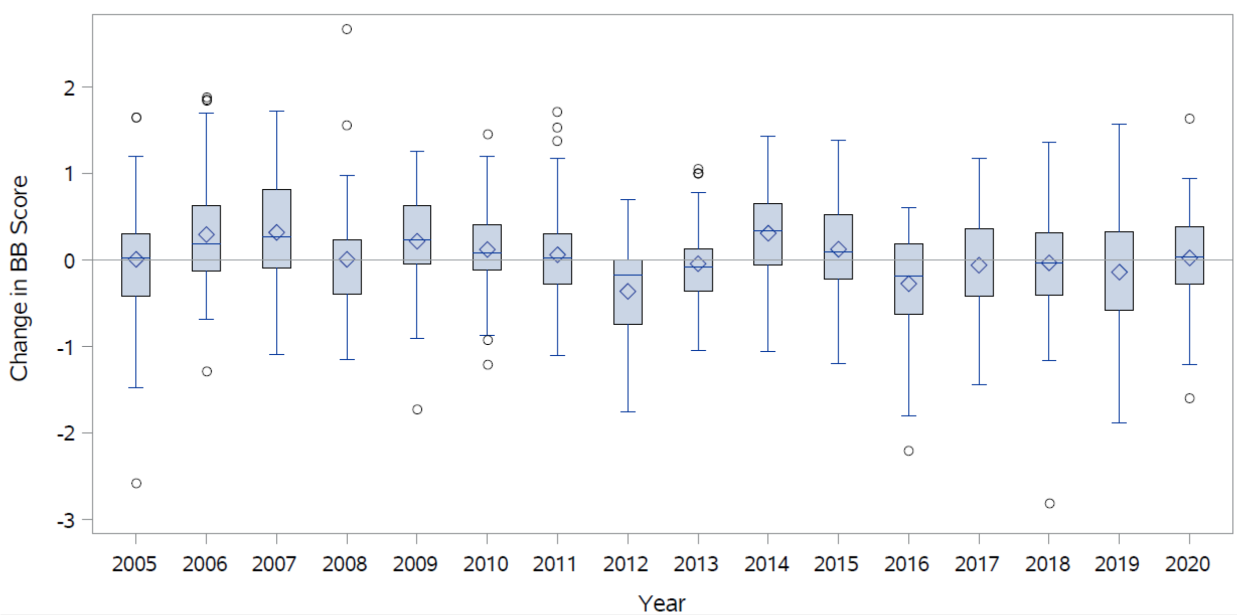

Figure 7. Distribution of year to year changes in total Braun-Blanquet scores across strata for the 56 sites in the study area. The horizontal solid line represents the median value and the diamond represents the average value. The box represents the interquartile range (IQR) and the whiskers represent 1.75 the IQR. Open circles represent values outside the whiskers.*

At the strata level, interannual variation in total BB scores was evident in all strata (Figure 8). Most strata displayed increasing trends in median BB scores between their lows in 2004-2007 to their highs in 2009-2011 with either stable or declining trends thereafter. Note that Estero Bay and tidal Caloosahatchee did not begin reporting total BB scores until 2006 and 2007, respectively. Noticeable declines have occurred in scores in several strata since 2017 with median scores in Estero Bay reaching the lowest recorded for that stratum while Gasparilla Sound, Lower East CH, Tidal Myakka and Tidal Caloosahatchee scores returned back to circa 2004 levels. However, Lemon Bay, Matlacha Pass and Pine Island Sound have been comparatively stable over the period of record (at the strata level). It is important to note that this description applies to median BB scores across sites within a stratum and therefore this summary of trends in total BB scores should not be applied to any individual transect within the strata. Enlarged individual strata plots are provided (Appendix B).

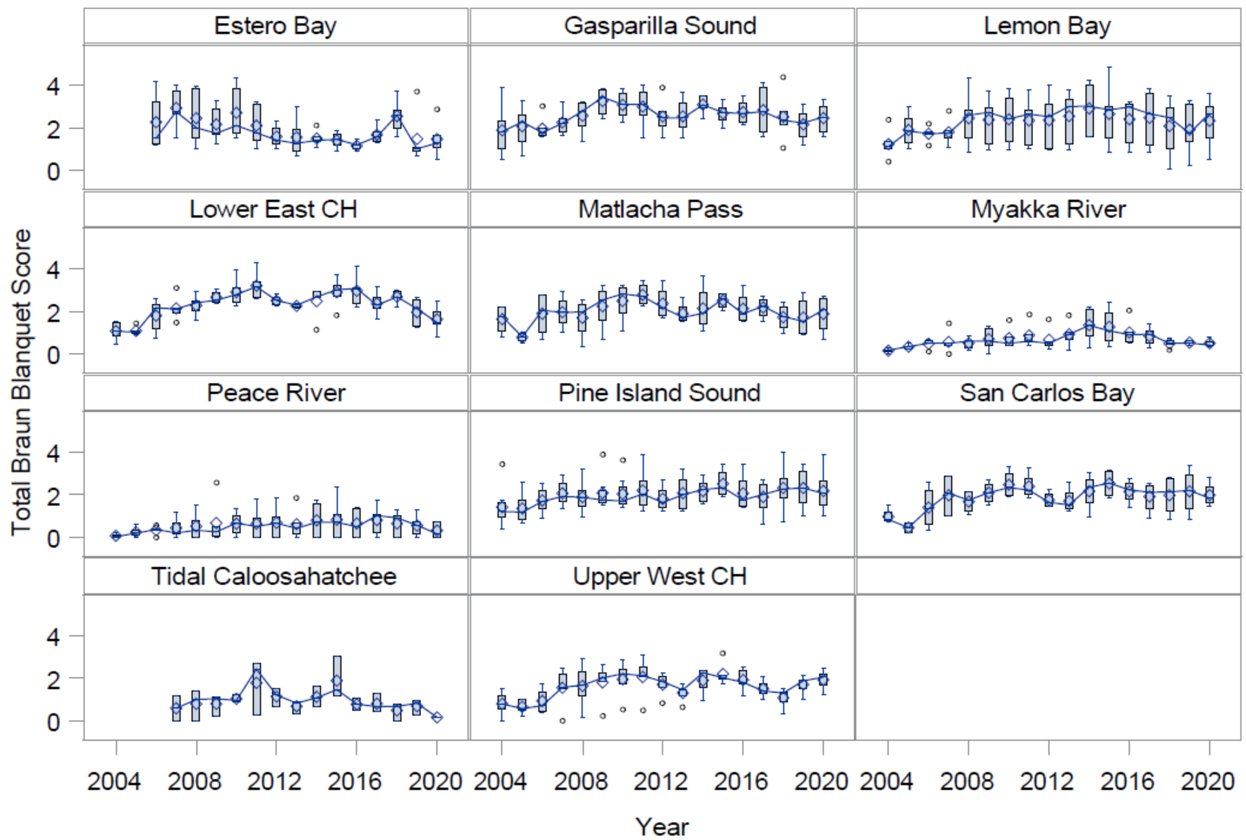

Figure 8. Stratum specific distribution of total BB scores over time with line connecting the median value of total BB score for each year.

Seagrass Trends over Time

Site-specific period of record trends over time were evaluated using the Mann-Kendall test for individual species as well as total BB score. In Estero Bay, trends were either stable or negative for both total and species-specific BB scores (Table 5). Trends were mixed in Lemon Bay, Pine Island Sound and Matlacha Pass with both positive and negative changes in species-specific scores while trends were generally stable or positive in Lower East CH, Peace River, Myakka River, Gasparilla Sound, San Carlos Bay, and the Tidal Caloosahatchee. Trends were either stable or positive over the period of record in Upper West CH. Sometimes individual species trends could offset the results for the total BB score resulting in different outcomes within a site.

Table 5. Results of Mann-Kendall test for trend in species-specific Braun-Blanquet (1999-2020) and total Braun-Blanquet scores (2004-2020) organized by seagrass strata. Asterisk denotes significance level while sign (+ or --) denotes the direction of the trend as positive or negative, respectively. A lack of information in a table cell denoted sites and species where either the null hypothesis was not rejected or there were insufficient data to evaluate the trend.

Strata	Site	Halodule	Syringodium	Thalassia	Total BB
Estero Bay	EB01			--**	
	EB02	--**			--**
	EB03			--*	
	EB04				
	EB05			--**	--*
Gasparilla Sound	GAS02				+*
	GAS03				
	GAS04				
	GAS05				
	PIS06				
	PIS07				
Lemon Bay	GAS01		+*	+*	+***
	ICW01	--**			
	ICW02	+*	+**	--***	
	ICW03			--**	
	ICW04			+***	
	ICW05				+*
Lower East CH	MC02			+**	+***
	MC03				
	PIS01				
	PIS02				+*
	PIS03				
Matlacha Pass	MP01				
	MP02			--***	
	MP03				
	MP04	+***			
Myakka River	MYR01				+*
	MYR02				
	MYR03				
	MYR04				
	MYR05				
Peace River	MC08	+*			
	PR01				
	PR02				
	PR03				
	PR04				+***

Strata	Site	Halodule	Syringodium	Thalassia	Total BB
Pine Island Sound	PI01				
	PI02	+***			+**
	PI03				
	PI04	+**		+***	+***
	PI07			--***	--*
	PI08		+*		+**
	PI09	+**	+**		
	PIS05			+*	
San Carlos Bay	MP05				
	SC01	+**			+**
	SC02	+***			
	SC03				
Tidal Caloosahatchee	CR02	+***			
	CR04a				
	CR05				
Upper West CH	MC01	+**		+***	+***
	MC04			+*	
	MC05				
	MC06			+**	+*
	MC07			+*	

*=p<0.05; **=p<0.01; ***=p<0.001

The Mann-Kendall trend test does not consider the magnitude of the scores only the direction of pairwise comparisons. To include magnitude, the sum of year-to-year differences in average total BB scores was calculated for each site. Since Estero Bay did not begin reporting total BB scores until 2006, only changes since 2006 were used for this comparison of all sites. Similar to the results presented above, the change in BB score over time was site dependent with approximately half of the sites showing some negative change and half showing some positive change. More sites displayed a cumulative positive change (greater than 1) than negative change (less than –1). Sites EB02, ICW01, EB03, MP03, MP02, and EB01 exhibited the largest cumulative negative change in BB score while PI04, GAS01, MP04, SC01, and GAS06 exhibited the largest cumulative positive change in total BB score over the time period (Figure 9). Similar plots for individual years were generated in response to peer review and can be found in the supplemental materials associated with this project.

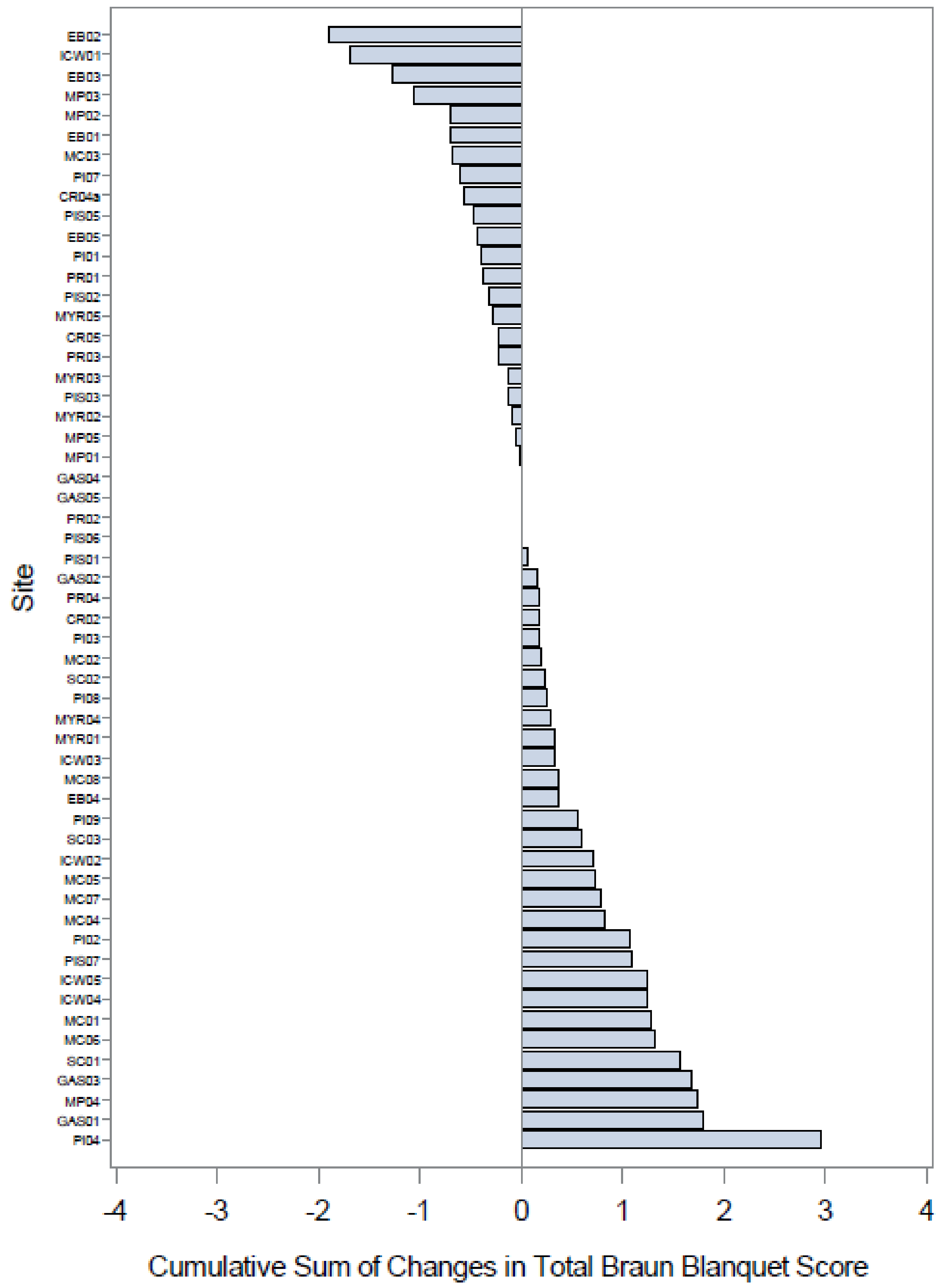

Figure 9. Cumulative sum of year to year change in total Braun-Blanquet score for each site between 2007 and 2020.

A plot comparing outcomes between using 2006 as the start date and 2004 as the start date (noting that Estero Bay still only included data since 2006) suggested that most sites were consistent in the direction of trend being either negative (lower left: Figure 10) or positive (upper right: Figure 10) for both evaluations. Site PIS07 was the only site that suggested an overall positive score above 1 since 2006 but a negative score since 2004 (lower right: Figure 10) indicating losses between 2004 and 2006. Seagrass change since 2004 at four stations (MP01, PIS02, PIS03 and GAS05) were net positive since 2004 but zero or slightly negative since 2006 (upper left panel Figure 10) indicating gains occurred between 2004 and 2006. Sites with the largest cumulative negative change over time (i.e. < -1) tended to have a higher frequency of occurrence of Halodule (not shown).

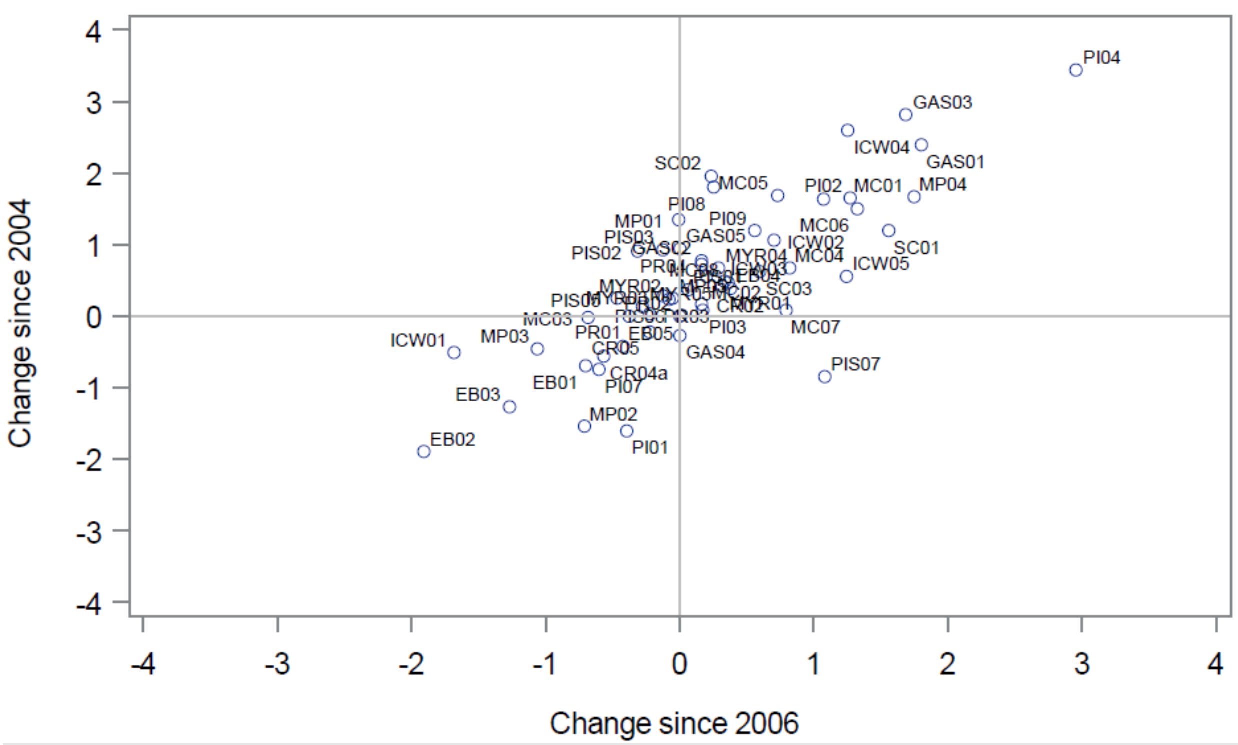

Figure 10. Cumulative change in BB scores since 2004 (x axis) and since 2006 (y axis).

A timeseries of site average total BB scores for the 6 sites with the greatest cumulative loss (left panel) and greatest cumulative gains (right panel) is provided in Figure 11. Sites with cumulative losses tended to be more Halodule dominant sites (Figure 12) though there were also many sites with relatively high Halodule frequency of occurrence that had overall gains in total BB scores over the time period.

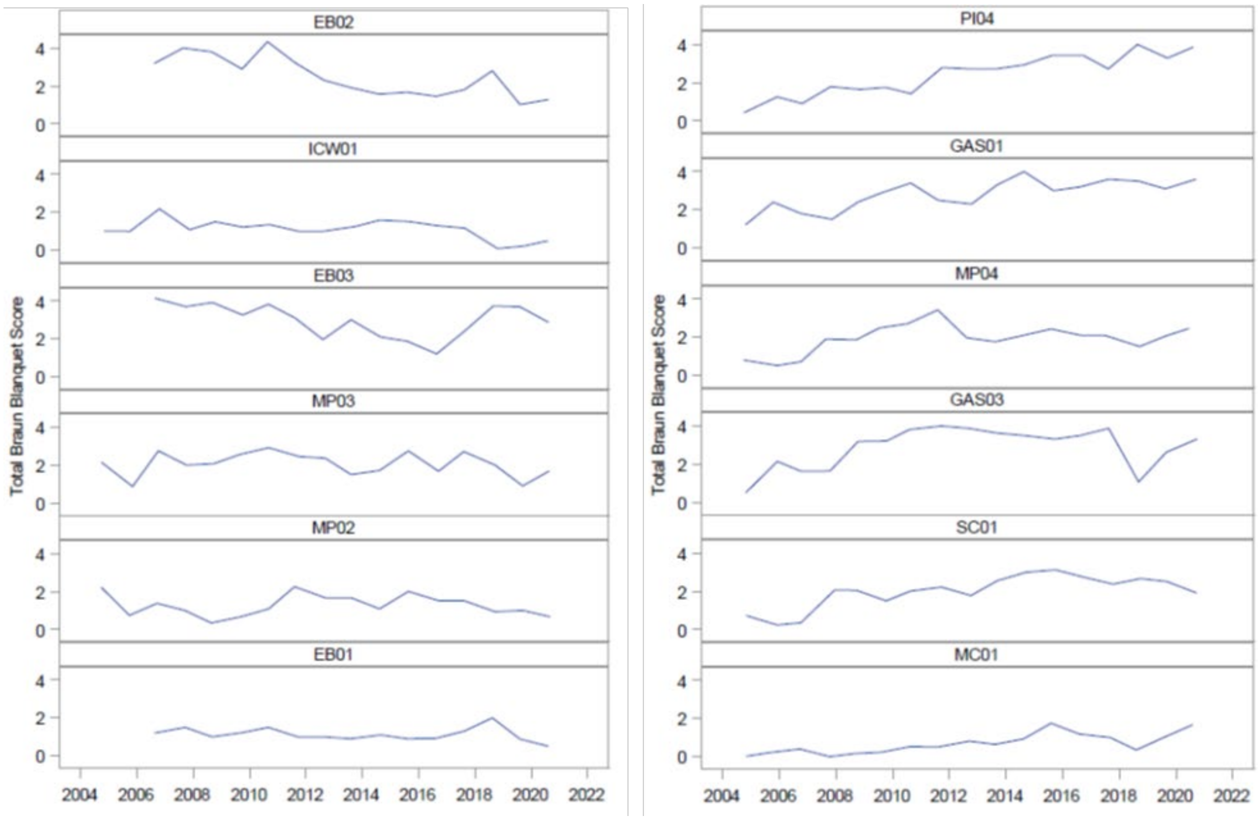

Figure 11. Sites with greatest cumulative negative change (left panel) and greatest cumulative positive change (right panel).

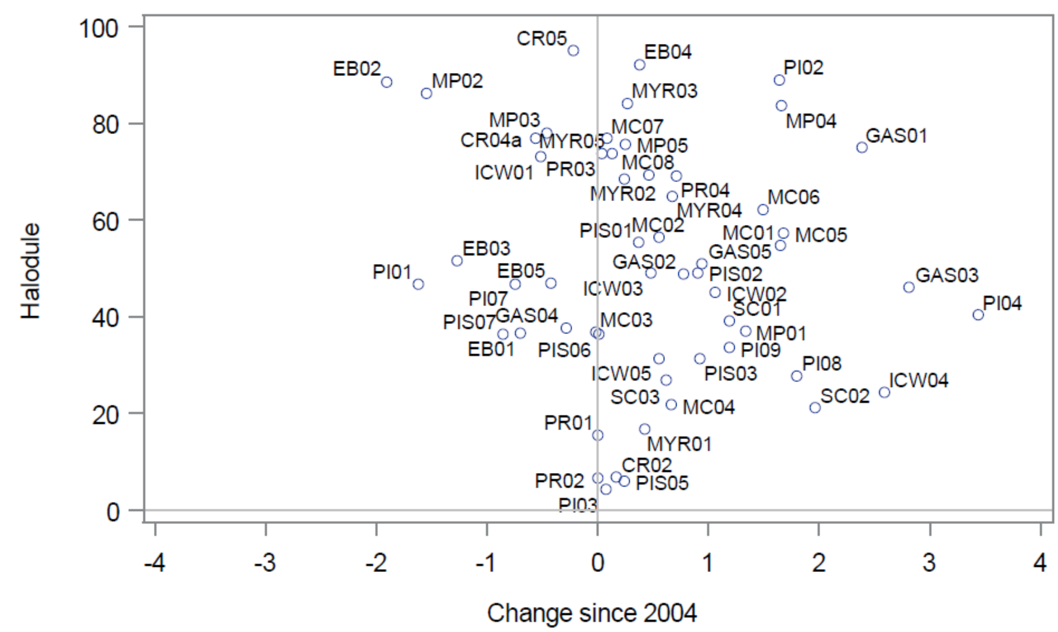

Figure 12. Total BB change since 2004 (x axis) and overall percent frequency of occurrence of Halodule.

Changes in seagrass distributions over time were not always monotonic and could be more subtle and complex. Bubble plots were a convenient method to describe species occurrence and relative abundance over time at a particular site and station by displaying each species as a different color with the size of the circle ("bubble") reflecting its BB score. An example is provided for the site GAS05 in Gasparilla Sound (left) and Lemon Bay site ICW01 (right) in Figure 13. At the GAS05 site, Halodule is dominant and abundant at the beginning of the transect (Station 0), the site transitions to Thalassia dominant by Station 100, becomes a mix of seagrass types at Station 150, and finally transitions back to a more Halodule dominant system (with Syringodium observed in the most recent years) towards the end of the transect. The Lemon Bay site (ICW01: right panel) is dominated by Halodule with decreasing abundance at station 100 and 150 over time. Little or no seagrass reported at all stations since 2017 contributed to this site being one with the largest negative cumulative change in BB score over time. Bubble plots like this for each site are provided in Appendix C.

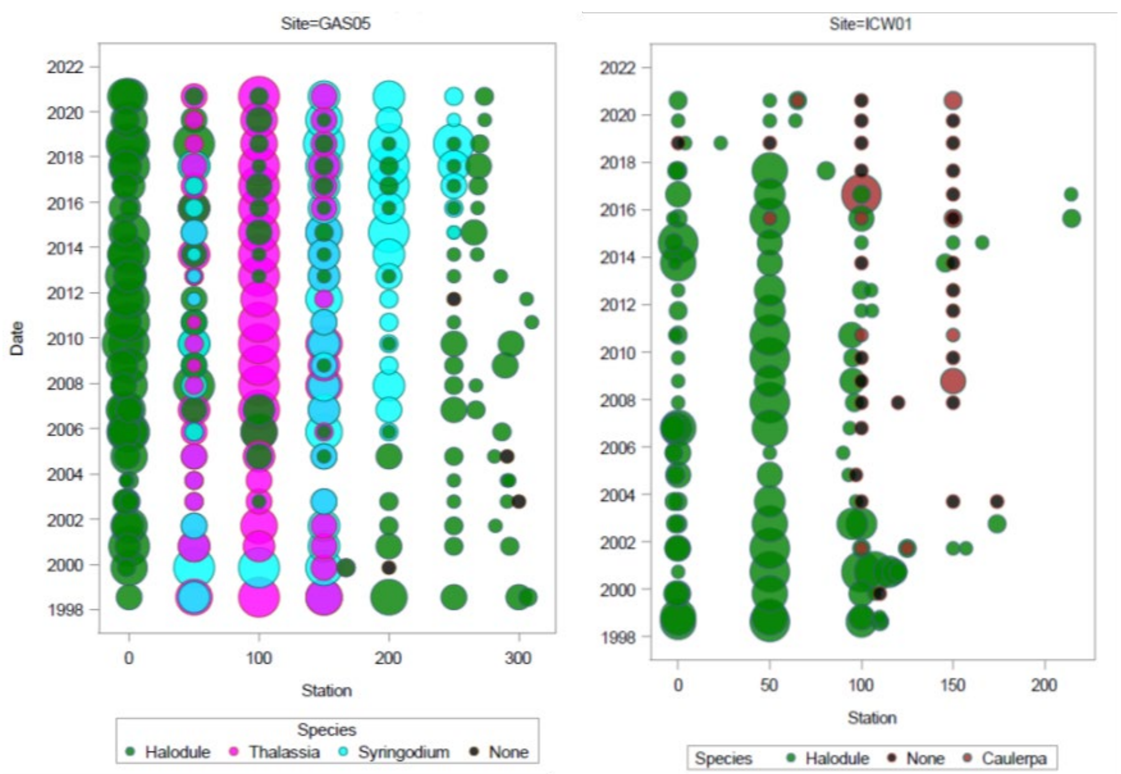

Figure 13. Bubble plots of species-specific Braun-Blanquet scores for each surveyed station over time.

Another example of the subtleties of seagrass change at these sites is provided by the species-specific timeseries plots. For example, in Figure 14 (left panel) there was a general increasing trend in Thalassia BB scores at sites MC02 and MC03 over time which was offset by decreases from very high abundance scores since 2019. This rapid decrease, while dramatic, was not sufficient to suggest an overall decreasing trend in total BB scores over the entire time period. In both MC02 and MC03, Halodule and Thalassia scores in 2020 were similar to scores reported in 1999. A change analysis of the Water Management District seagrass coverages from aerial surveys for 2018 and 2020 suggest substantial losses of seagrass acreage in the “East Wall” strata where MC02 and MC03 are located with most losses occurring on the shelf extending westward from the East Wall shoreline (Figure 14: Right panel).

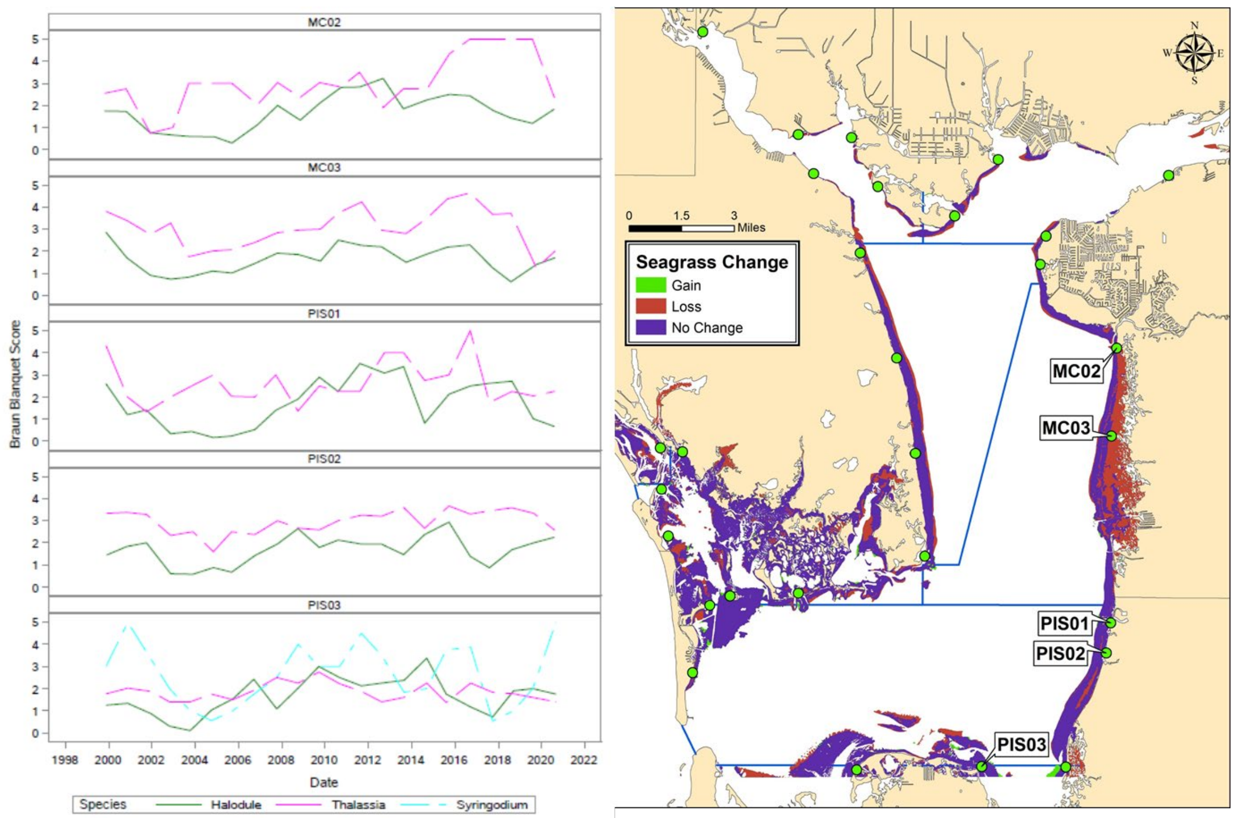

Figure 14. Timeseries of species-specific Braun-Blanquet scores for 5 sites in Lower East strata (left panel). MC02 and MC03 are located in the “East Wall” strata used by the SWFWMD for reporting areal acreage of seagrass from biennial surveys (right panel).

Depth Distribution and Transect Length:

The depth distribution of each total BB category across strata is provided in Figure 15. The highest BB scores tended to occur most frequently in depths less than 1 meter while the highest proportion of scores representing between 50% and 75% coverage occurred in depths between 1m -1.5m. The largest proportion of scores representing the <5% category occurred in depths greater than 2m while sites without seagrass were most commonly recorded in the deepest and shallowest depths.

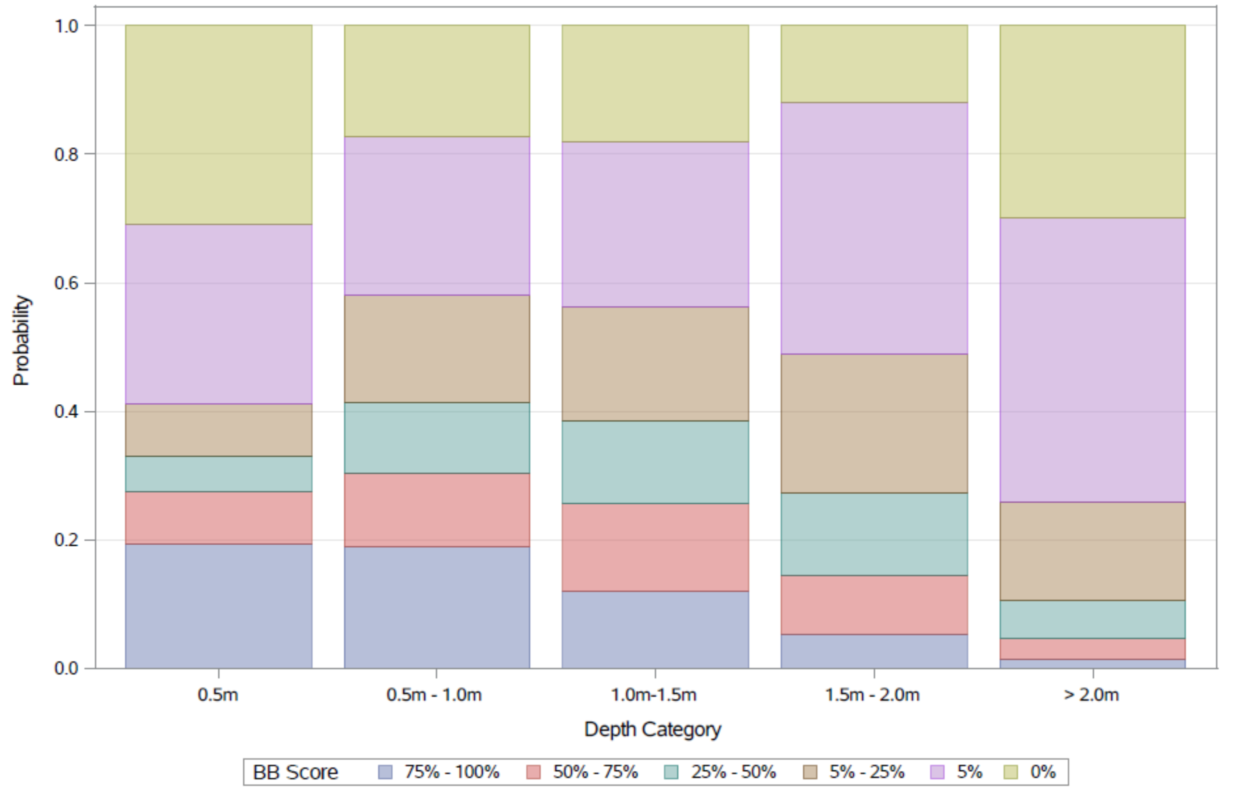

Figure 15. Proportion of samples recorded for each total Braun-Blanquet category by depth category.

The maximum depth of the primary seagrass species either increased or was non-trending over time with no statistically significant decreasing depth trends at any site. Total transect length contracted over time at EB01 and EB03 in Estero Bay, but the remaining 54 sites were either non-trending or positive over the period of record. A table of results of Mann-Kendall trend test for transect length and maximum depth is provided in Appendix D along with plots of each metric for each site.

Macroalgae/Drift Algal Occurrence and Abundance:

Drift algae (which includes various species of red, green and brown macroalgae) BB abundance was recorded when present at the station and was otherwise represented by a missing field in the database. For analysis, these missing values were assumed to be defined as the absence of algae at the site, assigned a 0, and the proportion of samples with algae present were evaluated for trend using the Cochrane Armitage Test. The presence of drift algae increased over time at three strata including Estero Bay, Gasparilla Sound and Pine Island Sound (Figure 16). Otherwise, drift algae occurrence was stable or decreasing over the time period. It should be noted that the increase in presence of drift algae from 2016 onward in Estero Bay may, in part, be influenced by a program initiated in 2016 in Estero Bay to collect and assess drift algal biomass along their transects.

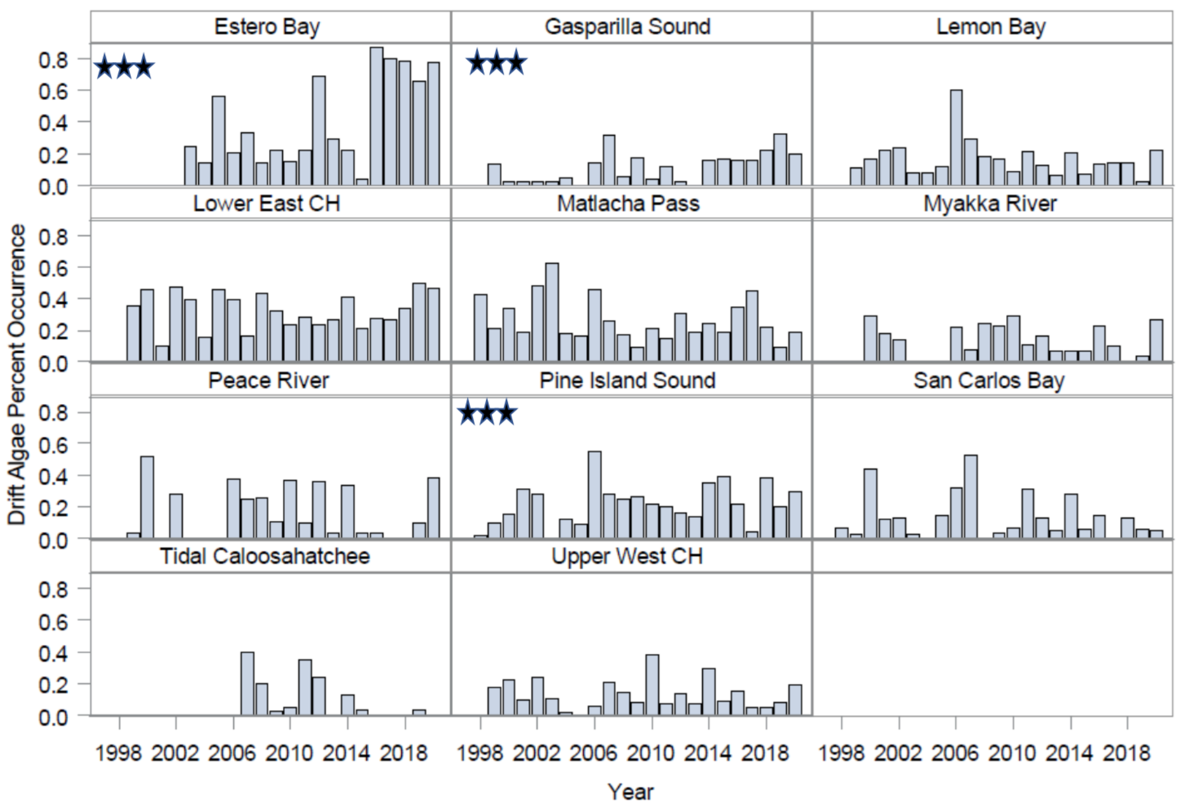

Figure 16. Proportion of samples where drift algae was present by year between 1998 and 2020. Stars represent statistically significant increasing trend as evaluated using the Cochrane Armitage trend test.

Regression of percent drift algae occurrence and total BB score suggested no statistically significant negative relationships (Figure 17) despite Matlacha Pass and Lemon Bay exhibiting a negative trendline. The only statistically significant relationships were positive and these results were affected by the presence of a few outliers that weighted the regression towards samples with high occurrence scores.

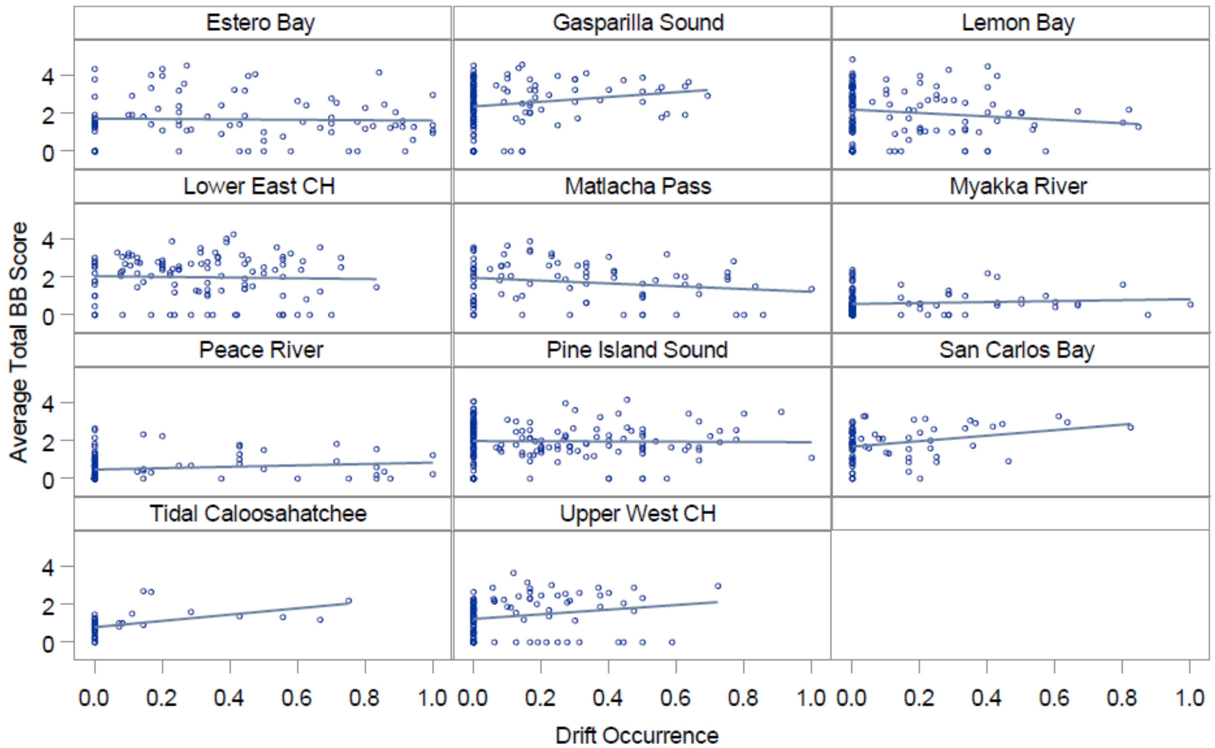

Figure 17. Bivariate plot of the proportion of samples where drift algae occurred and total seagrass Braun-Blanquet scores with regression line.

Regression of drift algae abundance and total BB score also suggested no statistically significant negative relationships (Figure 18) despite Lemon Bay, Estero Bay and Matlacha Pass displaying a negative trendline. The only statistically significant relationships were positive in San Carlos Bay and the Tidal Caloosahatchee River which had low average total BB scores when drift algae abundance was near zero.

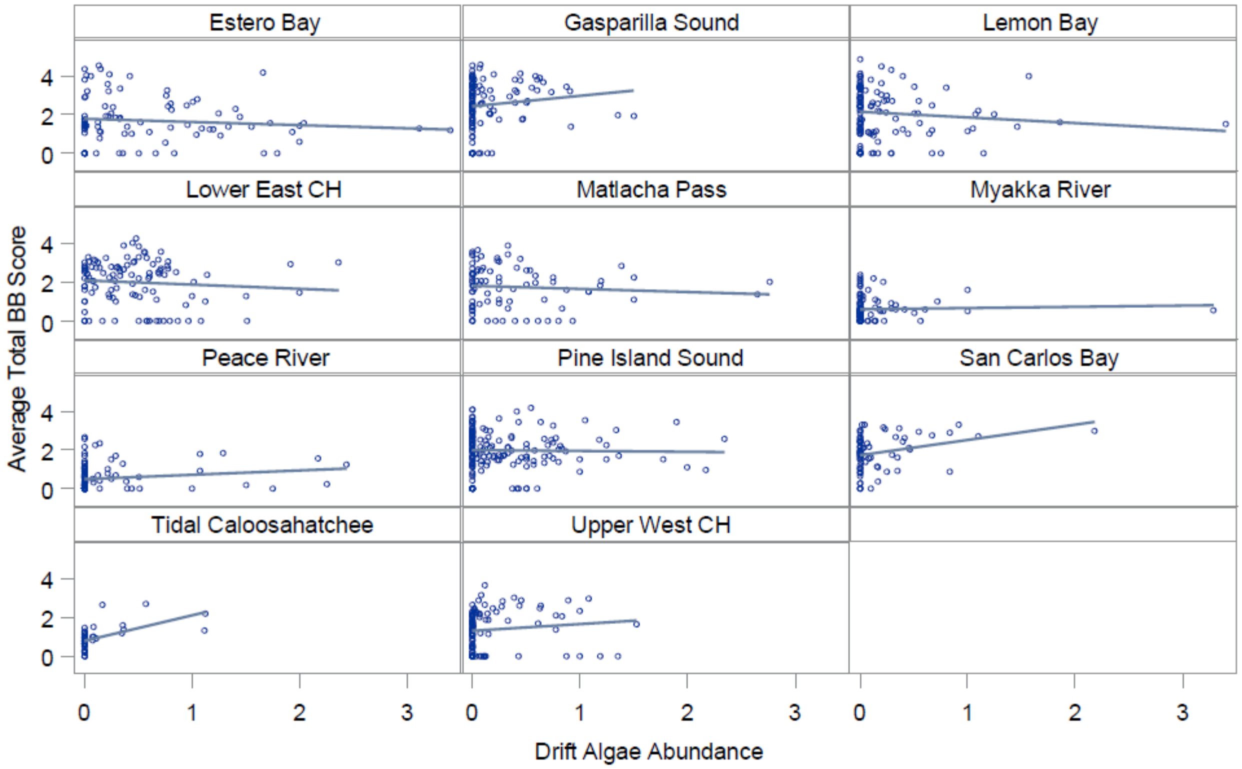

Figure 18. Bivariate plot of drift algae abundance and total seagrass Braun-Blanquet scores with regression line displaying trend.

Evaluating Status and Trends in WQ

Approximately 50% of WBID's evaluated within the AP exhibited increasing trends in total nitrogen and temperature between 1998 and 2020 (Table 6). Salinity was mostly non-trending though large fluctuations in salinity were apparent and there were two decreasing trends (Gasparilla and Upper Caloosahatchee) over time. Total phosphorus was either non-trending or decreasing over time except in Upper Charlotte Harbor and Lower Caloosahatchee which exhibited small increasing trends over time. Chlorophyll was non-trending or decreasing, especially in the southern portion of the study area, while an increasing significant trend in chlorophyll was observed in Upper Lemon Bay. Turbidity was decreasing or stable in all WBIDs except Northern Estero Bay (Ft Myers Beach) where it was significantly increasing over time. Other constituents were mostly non-trending or had trends representing improving conditions over the period of record evaluated.

Table 6. Results of seasonal Kendall Tau trend test on WBID average monthly water quality data between 1998 and 2020.

Segment	WBID	COLOR	CHLAC	DOSAT	NO23	SALIN	SD	TEMP	TN	TP	TURB
Upper Lemon Bay	1983A1	na	↑	0	0	0	↑	0	↑	↓	↓
Middle Lemon Bay	1983A	↑	0	0	0	0	↑	↑	0	↓	0
Lower Lemon Bay	1983B	0	0	0	↓	0	0	↑	↓	↓	0
Myakka River	1991A	0	↓	↓	0	0	0	0	0	0	↓
Myakka River	1991B	0	0	0	0	0	0	0	↑	0	0
Peace River	2056A	0	0	0	0	0	0	0	0	0	↓
Peace River	2056B	0	0	0	0	0	0	0	0	0	↓
Upper Charlotte Harbor	2065A	0	0	0	↓	0	0	0	↑	↑	0
East/West Wall	2065B	0	0	↑	↓	0	0	0	↑	0	0
Gasparilla/Cape Haze	2065C	↓	0	0	↓	↓	↓	↑	0	↓	0
Bokeelia	2065D	0	0	0	↓	0	0	↑	↑	0	↓
Northern Pine Island	2065E	↓	0	↑	0	0	↑	↑	0	↓	0
Matlacha Pass	2065F	0	↓	↑	↓	0	↓	↑	↑	0	0
Southern Pine Island	2065G	↓	↓	↑	0	0	0	↑	0	↓	0
San Carlos Bay	2065H1	0	0	0	0	0	↑	0	0	↓	0
Lower Caloosahatchee	3240A	0	↓	↑	0	0	↑	0	↑	↑	↓
Upper Caloosahatchee	3240B	0	↓	↑	↑	↓	↑	0	↑	0	↓
Ft Myers Beach	3258A1	↓	↓	0	0	0	0	↑	↑	↓	↑
Estero Bay	3258I	0	↓	0	0	0	0	↑	↑	↓	0

↑ = statistically significant (α=0.10) increasing trend over time
↓= statistically significant (α=0.10) decreasing trend over time
0 = No trend

According to the latest draft assessment using FDEP's Run61 database, southern Pine Island Sound, San Carlos Bay, Upper Lemon Bay and Upper Charlotte Harbor are impaired for both chlorophyll and total nitrogen exceedances (Figure 19). In addition, all of Estero Bay, San Carlos Bay, Pine Island Sound and Matlacha Pass are impaired due to total nitrogen exceedances. The most upstream WBIDs within the study area in the Myakka (WBID 1991B) and Peace River (2056B) are also listed as impaired for total nitrogen. The remaining sites are either not listed, or in the case of the tidal

Caloosahatchee, already under a Basin Management Action plan for nutrient load reductions.

Sites with statistically significant trends (45% of sites, or 25 out of 56) in species-specific BB scores are overlaid on the impaired waterbodies in Figure 20 and suggest a mix of positive and negative trends even among segments with impairments for total nitrogen. Estero Bay exhibited mostly decreasing trends in Thalassia over its period of record while Thalassia trends were mostly increasing (where significant) within the strata comprising Charlotte Harbor Proper. It should be noted that most of the sites with increasing trends were outside the area of East Wall where aerial photography suggested dramatic losses in seagrass acreage in recent years.

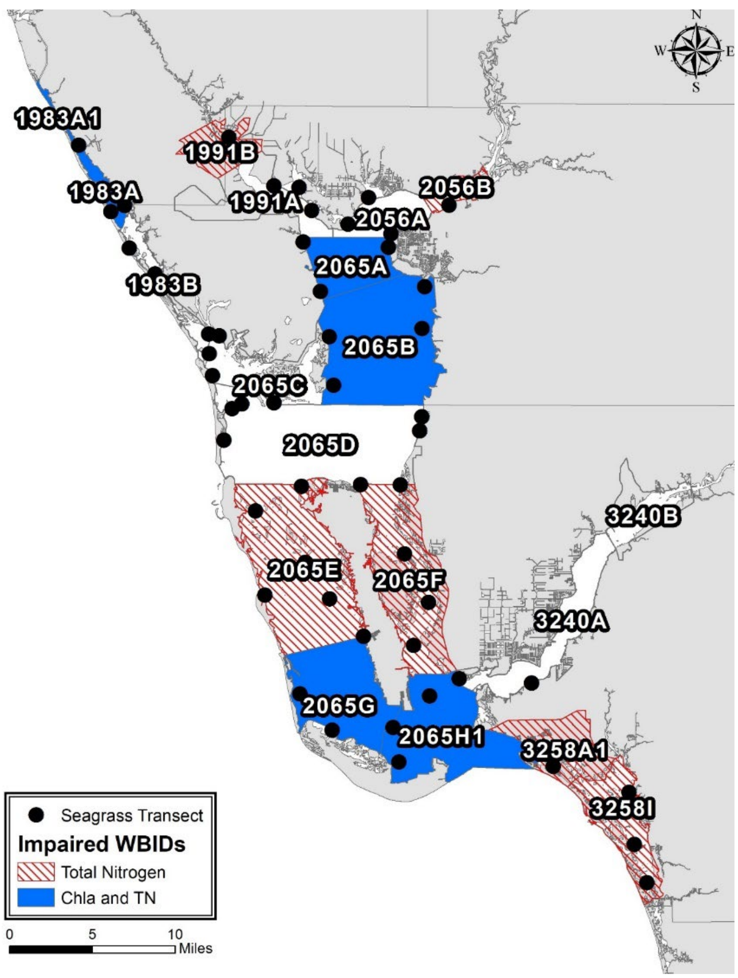

Figure 19. Waterbodies listed as "Impaired" for total nitrogen and/or chlorophyll a (uncorrected) within the study area.

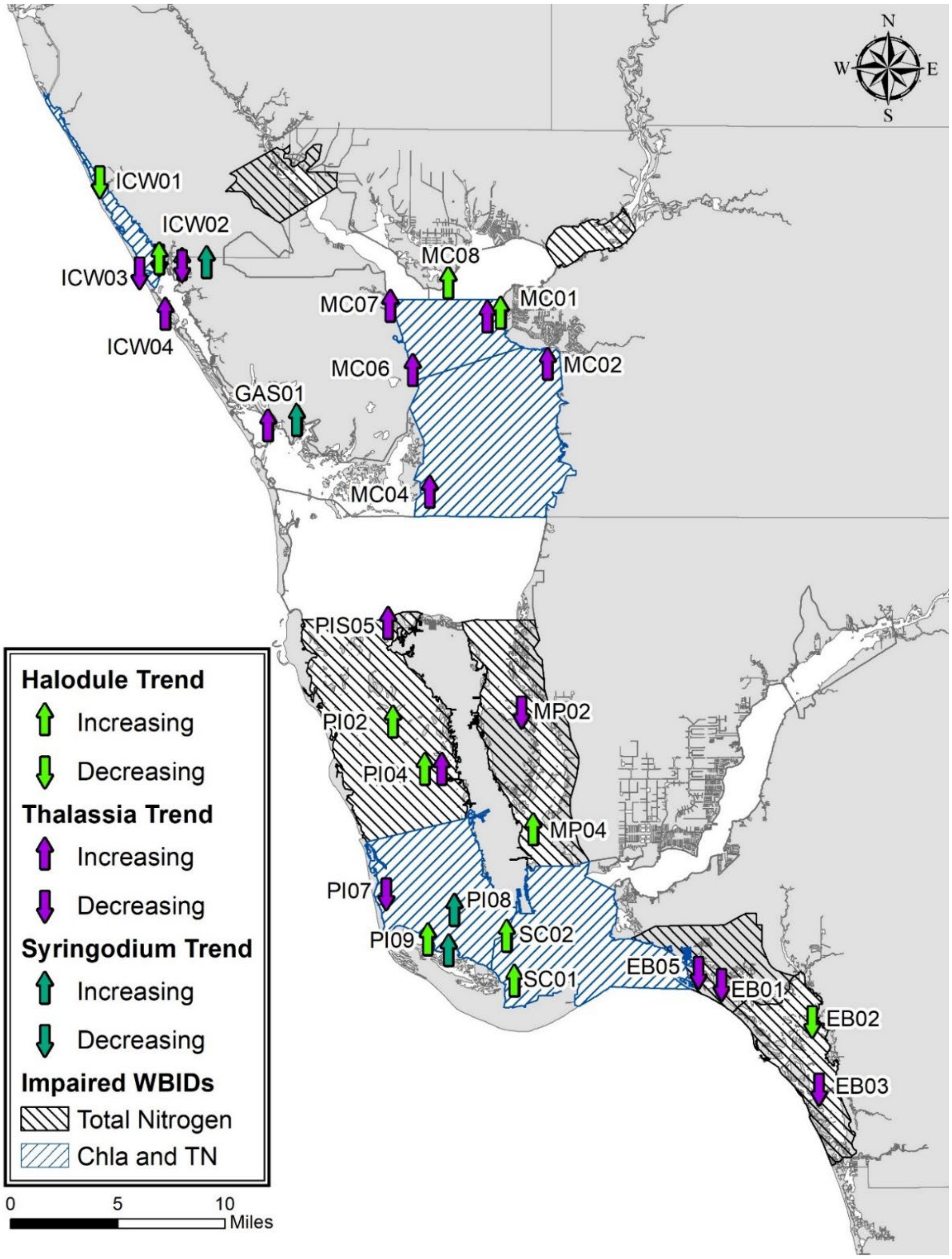

Figure 20. Statistically significant species-specific Braun-Blanquet trends overlaid on Impaired waterbodies.

Flows and Water Quality

The period of record contained extreme deviations in flows. As displayed in the timeseries provided in Figure 21 for the Peace River near Arcadia gage (USGS 02296750), large negative deviations in daily flows between 2000 and 2002 relative to long term monthly averages coinciding with a historic drought in southwest Florida; higher than average flows between 2003 and 2005, and another protracted, though somewhat less intensive, drought between 2006 and 2010.

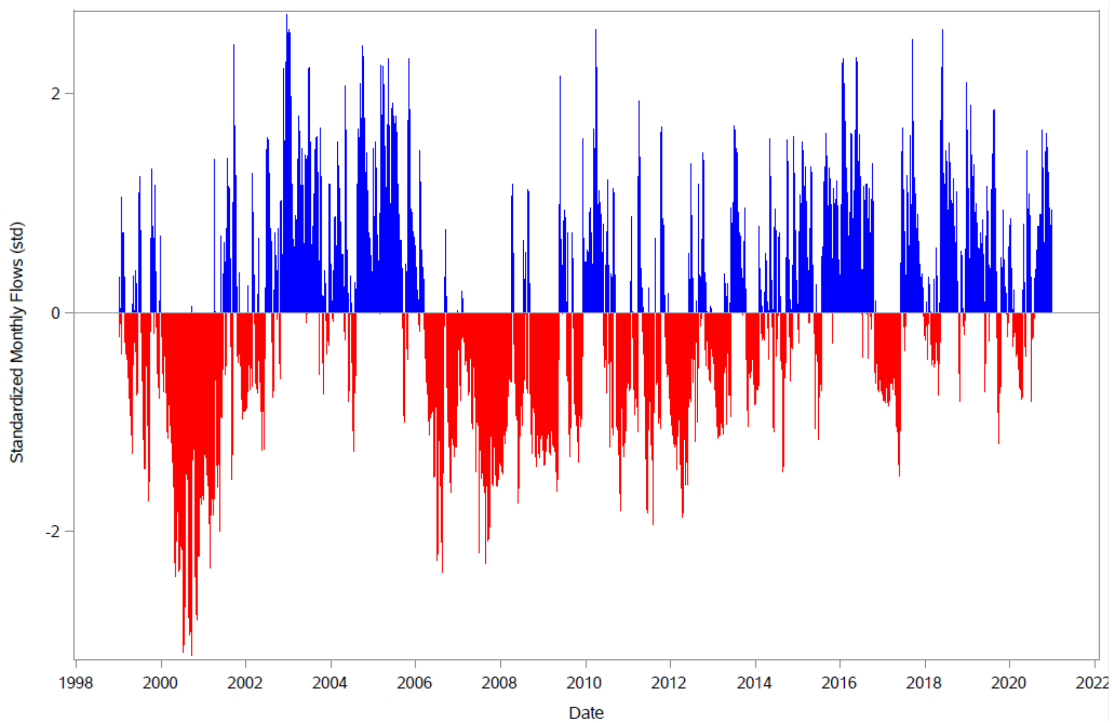

Figure 21. Standardized flows from Peace River at Arcadia based on 1998-2020 period of record.

Higher than average antecedent flows were strongly associated with many water quality constituents including salinity which was strongly negatively correlated ($p<0.001$) with flows in all strata (Table 7). The strength of the association (defined by Spearman's (Rho_s) statistic) was generally aligned with proximity to the discharge source. That is, river flows were more strongly correlated to river water quality than to more open estuary segments located closer to passes. Total phosphorus, TN and its constituents (TKN and NO32) as well as Color were consistently positively associated with deviations in flows while salinity and measures of water clarity as measured by secchi disk depth was consistently negatively correlated with flows indicating that as flows increase, water clarity tends to be reduced. Other water quality constituents such as Chla, Temp, and Turb were more weakly correlated with flows indicating more proximal influences on water quality may be mediating the relationship between flows and these constituents. Bivariate plots of these relationships can be found in Appendix E.

Table 7. Results of Spearman rank correlation between principal water quality constituents and the 2 month standardized deviation in flow for the corresponding gage. Missing cells represent the lack of a statistically significant correlation.

Segment	Gage	Chla	Color	NO23	Salinity	Secchi	Temp	TKN	TN	TP	TURB
Lower Caloosahatchee	Caloosahatchee River At S-79 Nr. Olga		0.69***	0.51***	-0.83***	-0.50***		0.57***	0.61***	0.36***	
San Carlos Bay	Caloosahatchee River At S-79 Nr. Olga	0.24***	0.48***	0.44***	-0.48***			0.46***	0.54***	0.31***	
Southern Pine Island	Caloosahatchee River At S-79 Nr. Olga	0.15*	0.29***	0.13*	-0.59***			0.20**	0.23***		
Upper Caloosahatchee	Caloosahatchee River At S-79 Nr. Olga	-0.32***	0.48***	0.43***	-0.84***	-0.37***		0.29**	0.39***	-0.36**	0.13*
Lemon Bay	Myakka River Near Sr 72 Near Sarasota	0.084*	0.42***	0.14***	-0.62***			0.09*	0.11**	0.10**	
Myakka River	Myakka River Near Sr 72 Near Sarasota		0.61***	0.26***	-0.71***	-0.26***	-0.10*	0.31***	0.31***	0.21***	
Bokeelia	Peace River At Sr 70 At Arcadia	0.20**	0.48***	0.22***	-0.73***	-0.32***		0.34***	0.39***	0.46***	-0.17**
East/West Wall	Peace River At Sr 70 At Arcadia	0.25***	0.712***	0.16*	-0.81***	-0.41***		0.33***	0.36***	0.49***	
Gasparilla/Cape Haze	Peace River At Sr 70 At Arcadia	-0.23***			-0.59***					0.17**	
Matlacha Pass	Peace River At Sr 70 At Arcadia		0.46***	0.186**	-0.73***	-0.14*		0.15*	0.26***	0.25***	
Northern Pine Island	Peace River At Sr 70 At Arcadia	0.31***	0.32***		-0.59***			0.23***	0.26***	0.19**	
Peace River	Peace River At Sr 70 At Arcadia		0.75***	0.52***	-0.78***	-0.33***		0.48***	0.55***	0.39***	
Upper Charlotte Harbor	Peace River At Sr 70 At Arcadia	0.20**	0.67***	0.34***	-0.74***	-0.35***		0.45***	0.49***	0.44***	
Estero Bay	Tenmile Canal At Control Near Estero		0.18**	0.18**	-0.54***	-0.18**		0.15*	0.16**	-0.13*	
Ft Myers Beach	Tenmile Canal At Control Near Estero		0.19**	0.25***	-0.50***			0.21**	0.22***		

*=p<0.05; **=p<0.01; ***=p<0.001

Flows and Seagrass Change

The relationship between antecedent freshwater inflows and changes in seagrass BB scores were species-specific. This section reports only those assessments that resulted in a statistically significant spearman rank correlation coefficient. All significant correlations between flows and Halodule were negative indicating higher than average flows for up to 6 months prior to the sampling event were negatively correlated with seagrass BB score (Table 8). That is, higher than average antecedent flows were associated with lower Halodule BB scores at several sites in the study area.

Table 8. Results of spearman rank correlations between Halodule wrightii Braun-Blanquet scores and lag average deviations in freshwater inflow. The list represents the lag average flow resulting in the highest correlation coefficient of lag averages tested between 2 and 6 months.

Strata	Site	Flow lag average	Spearman's Rho
Pine Island Sound	PI08	4 month	-0.853**
Pine Island Sound	PI03	6 month	-0.486*
San Carlos Bay	MP05	4 month	-0.514*
Matlacha Pass	MP03	4 month	-0.515*
Upper West CH	MC06	3 month	-0.601*
Upper West CH	MC05	4 month	-0.69**
Gasparilla Sound	GAS05	3 month	-0.586*
Tidal Caloosahatchee	CR05	3 month	-0.795**
Tidal Caloosahatchee	CR04a	3 month	-0.753**

*=p<0.05; **=p<0.01; ***=p<0.001

The relationship between antecedent flows and Thalassia BB scores were more site-specific with 4 sites resulting in a negative correlation and 5 sites suggesting a positive correlation (Table 9).

Table 9. Results of spearman rank correlations between Thalassia testudinum Braun-Blanquet scores and lag average deviations in freshwater inflow. The list represents the lag average flow resulting in the highest correlation coefficient of lag averages tested between 2 and 6 months.

Strata	Site	Flow lag average	Spearman's Rho
Gasparilla Sound	PIS07	6 month	-0.776**
Pine Island Sound	PIS05	2 month	0.482*
Lower East CH	PIS03	2 month	-0.536*
Lower East CH	MC02	6 month	0.56*
Upper West CH	MC01	4 month	0.72**
Lemon Bay	ICW05	5 month	0.668**
Lemon Bay	ICW04	5 month	0.581*
Gasparilla Sound	GAS04	6 month	-0.503*
Estero Bay	EB03	3 month	-0.693**

*=p<0.05; **=p<0.01; ***=p<0.001

The relationship between flows and total BB scores were similar to the Halodule results suggesting higher than average flows during antecedent conditions to seagrass sampling was associated with lower total BB scores. This result also suggests the Halodule-flow relationship is driving the relationship between flows and total BB score.

Table 10. Results of spearman rank correlations between total Braun-Blanquet scores and lag average deviations in freshwater inflow. The list represents the lag average flow resulting in the highest correlation coefficient of lag averages tested between 2 and 6 months

Strata	Site	Flow lag average	Spearman's Rho
Pine Island Sound	PI08	3 month	-0.655**
Pine Island Sound	PI07	6 month	-0.54*
San Carlos Bay	MP05	4 month	-0.548*
Matlacha Pass	MP03	4 month	-0.561*
Matlacha Pass	MP01	3 month	-0.576*
Upper West CH	MC05	4 month	-0.722**
Gasparilla Sound	GAS04	2 month	-0.482*
Estero Bay	EB04	2 month	-0.554*
Tidal Caloosahatchee	CR05	3 month	-0.746**
Tidal Caloosahatchee	CR04a	3 month	-0.823**
Tidal Caloosahatchee	CR02	6 month	0.567*

*=p<0.05; **=p<0.01; ***=p<0.001

Event Based Evaluations

Extreme natural events such as droughts and floods can affect estuarine ecological dynamics. Some authors (e,g, Brown et al. 2013, Tomasko et al. 2020) have suggested that tropical storms affecting Florida's Gulf Coast may impact seagrass distribution and abundance and indeed within the period of record of this study, several hurricanes have resulted in extreme freshwater inputs into the study area. A timeseries plot of discharge from the three principal tributaries to Charlotte Harbor is presented in Figure 22 with red stars indicating the dates that Hurricane Charley made a direct landfall in Charlotte Harbor in August of 2004 and Hurricane Irma made landfall south of the study area in September of 2017. Flows during Hurricane Irma represented the highest flows on record for the Caloosahatchee River gage and the highest flows in the previous 60 years at the Peace River gage. While these two hurricanes also represented high flow conditions in the Myakka River, those flows were appreciably less extreme than even flows in June of the previous year in the Myakka, highlighting the very geographical distinctiveness of rainfall patterns within the study area. However, the seagrass response as evaluated by a year over year change in total BB scores (Figure 23; duplicate of Figure 8 above) was not consistently negative in either 2017 or 2018 across the study area. While the largest negative change in score occurred in 2018 at site GAS03, approximately half the sites were stable or increased in total BB score in 2017 and 2018.

Extreme flows can occur not only due to tropical storms but can be thought of as deviations from expected conditions in either direction in any month of the year. The standardized flows portray deviations in flows relative to their expected values which highlights both extreme flows as well as extreme drought conditions in the timeseries. For example, in Figure 24, extreme conditions are denoted by black rectangles representing by the 1st and 99th percentile values of monthly deviations from average flow. A vertical reference line identifies Hurricane Irma. While this plot confirms that Hurricane Irma resulted in an extreme flow event, there were also many other cases with extreme deviations from average condition in either direction within the timeseries.

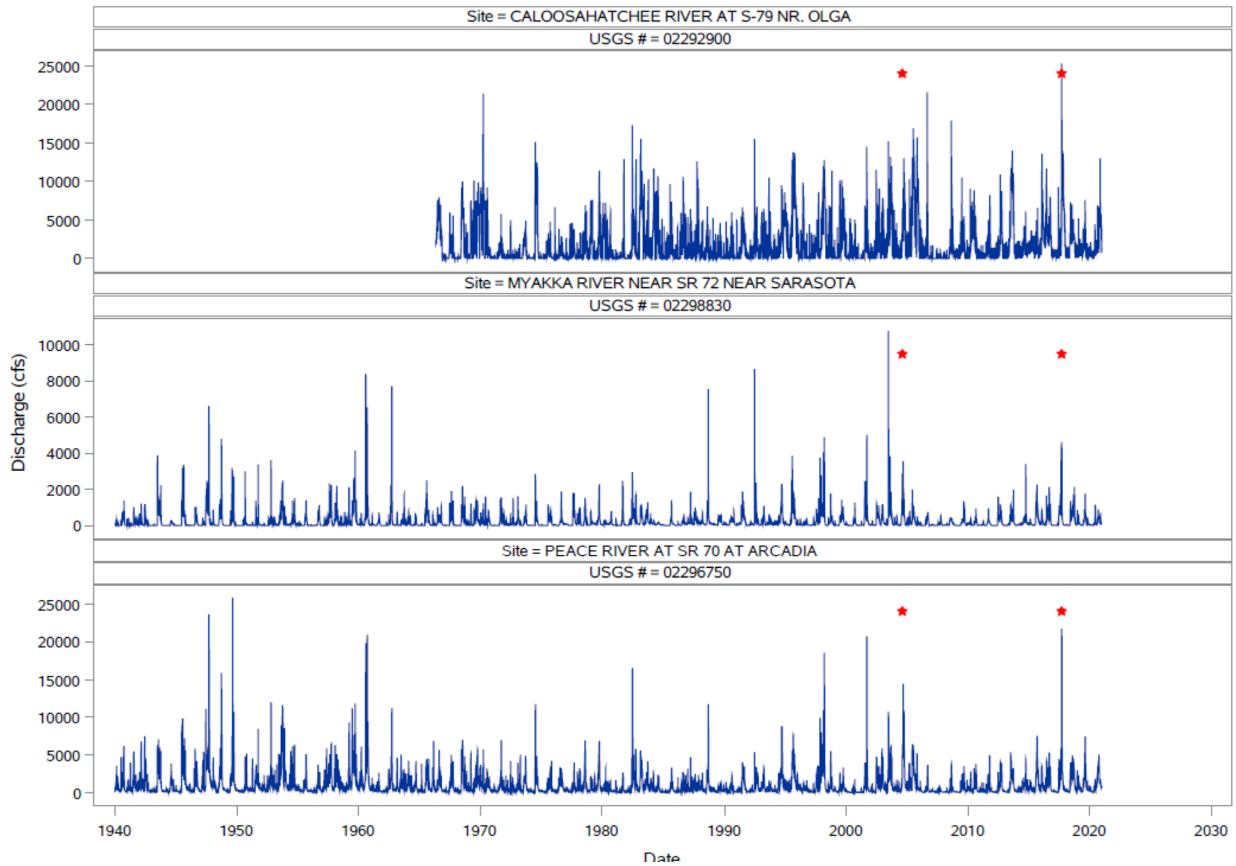

Figure 22. Flow timeseries for the three principal tributaries to the study area. Red stars indicate the dates of Hurricanes Charley in 2004 and Irma in 2017. Timeseries plots for all gages evaluated in the study are provided in Appendix A.

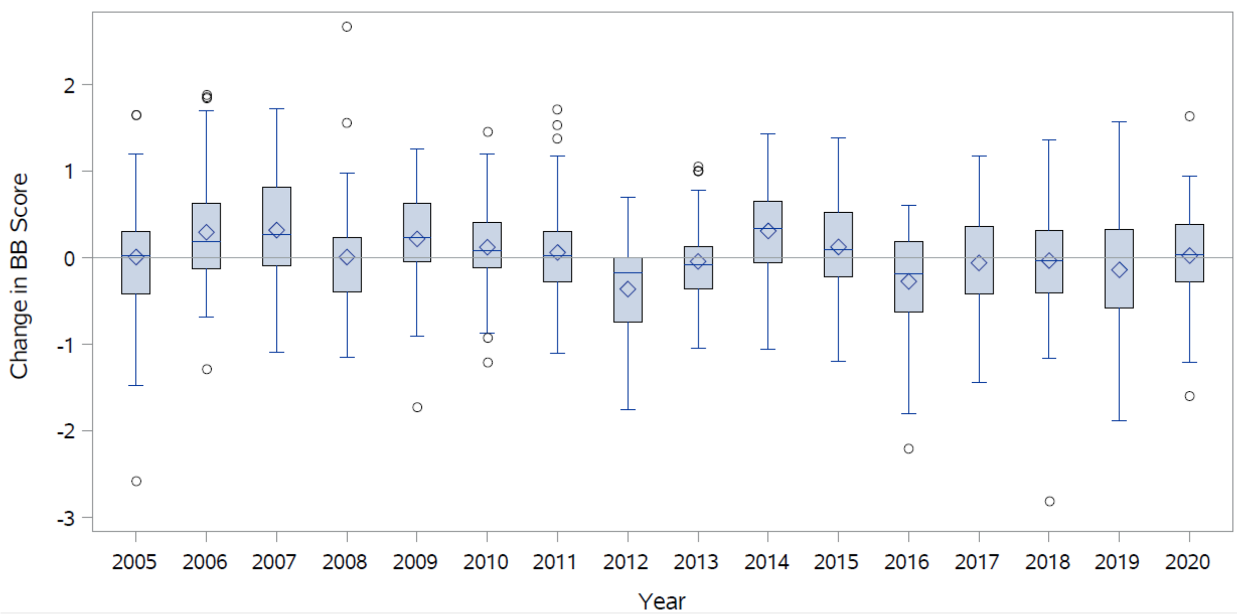

Figure 23. Distribution of year to year changes in total Braun-Blanquet scores across strata for the 56 sites in the study area.

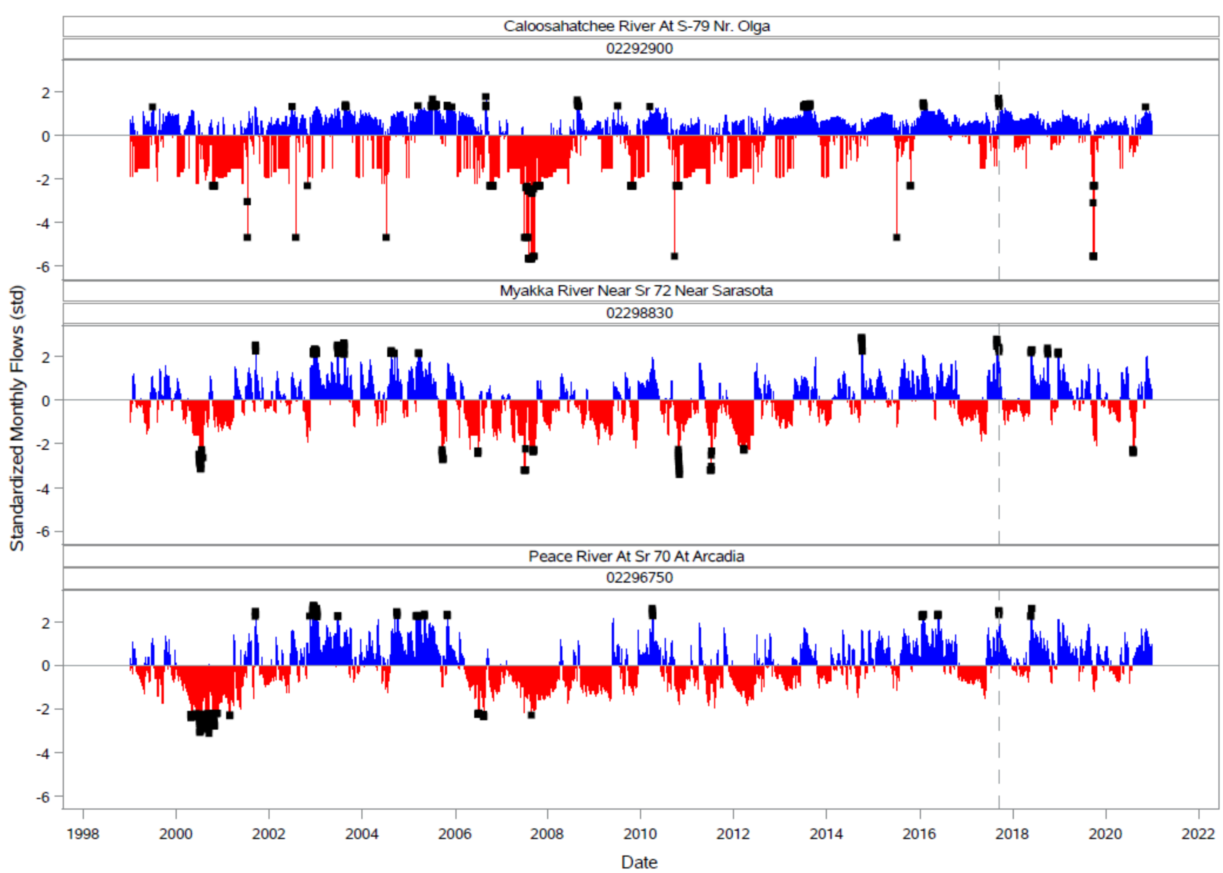

Figure 24. Flow timeseries for the three major rivers to the study area expressed as deviations from long term (1998-2020) monthly averages. Vertical broken line represents date of Hurricane Irma.

Water Quality and Seagrass and Algae Change

While there was not complete uniformity in simple bivariate relationship between water quality and seagrass, changes in total BB scores were most commonly associated with antecedent total nitrogen, total phosphorus, salinity, color, and water temperature. For total nitrogen, the 5 month average TN concentration prior to seagrass sampling typically returned the highest correlation coefficient of those tested (i.e. conditions between 2 and 6 months prior to sampling). TN was significantly negatively correlated with total BB scores at 9 of 56 sites including PI01 in Pine Island Sound, SC03 and MP05 in San Carlos Bay, MYR05 in Myakka River, PIS03 in Lower East CH, GAS03 in Gasparilla Sound and CR05 and CR04 in the Tidal Caloosahatchee which had the strongest correlation coefficients of any sites. No sites were significantly positively correlated with TN based on this univariate analysis. A plot of this relationship presented by seagrass segment is provided in Figure 25 where site-specific relationships within segment are depicted by a solid line. The plots suggest site-specific relationships within strata where some sites were non-trending while others were negatively associated with TN.

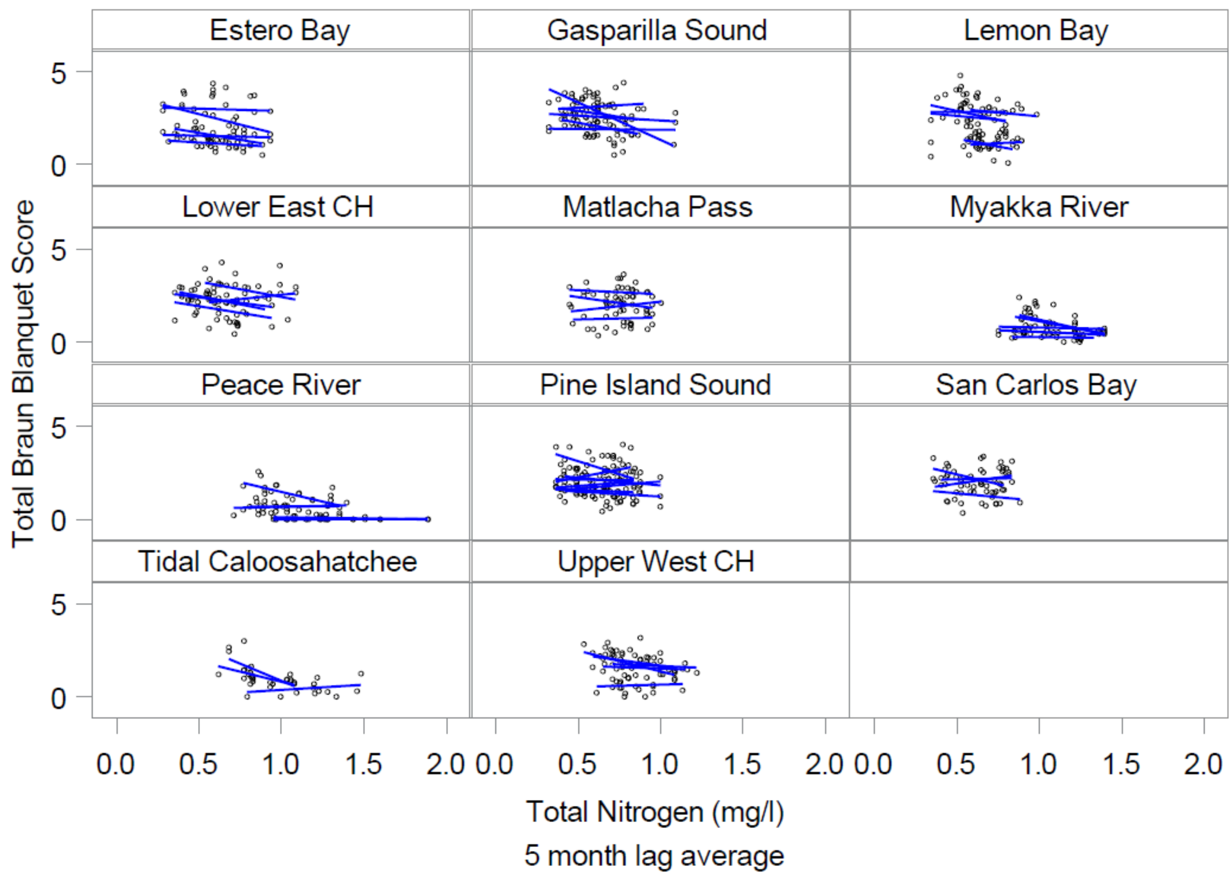

Figure 25. Scatterplot of 5 month antecedent total nitrogen concentrations and total Braun-Blanquet scores by seagrass segment. Solid lines represent site-specific trends using a linear regression line.

Associations between drift algae abundance and TN were both positive and negative. In Estero Bay most regression lines suggested positive associations though only one site (EB04) was statistically significant (Figure 26). The other positive association was at site PIS02 in Lower East CH. The remaining 9 significant correlations were negative which may suggest that uptake of nitrogen by drift algae is increasing drift algal abundance while decreasing water column concentrations.

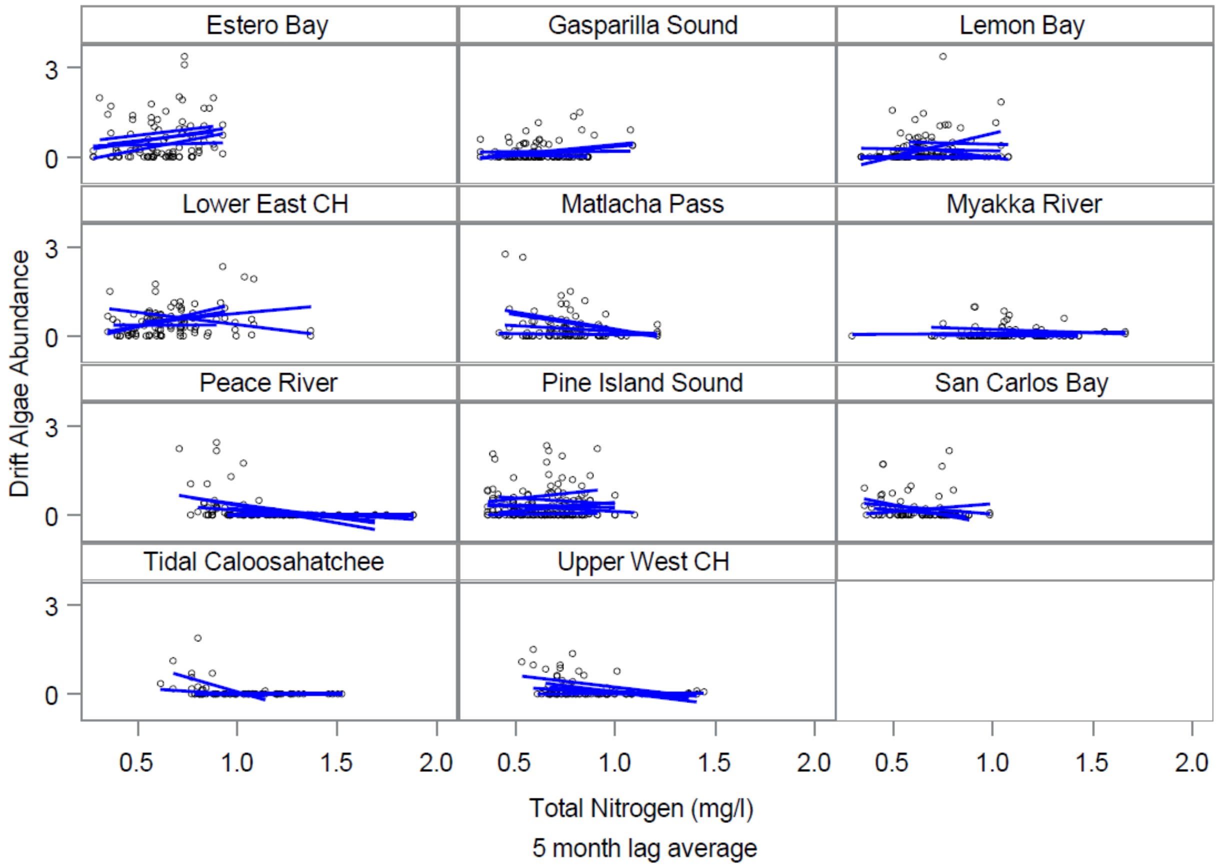

Figure 26. Scatterplot of 5 month antecedent total nitrogen concentrations and drift algae abundance scores by seagrass segment. Solid lines represent site-specific trends using a linear regression line.

Total phosphorus was also commonly negatively correlated with total BB scores with 13 of the 14 sites with significant correlation yielding negative results. Only at site EB01 in Estero Bay was total phosphorus positively associated with total BB scores as a simple bivariate relationship. Interestingly, Estero Bay had the lowest distribution of TP concentrations of all segments though the Tidal Caloosahatchee, San Carlos Bay and Matlacha Pass also had lower distributions than the remaining segments (Figure 27).

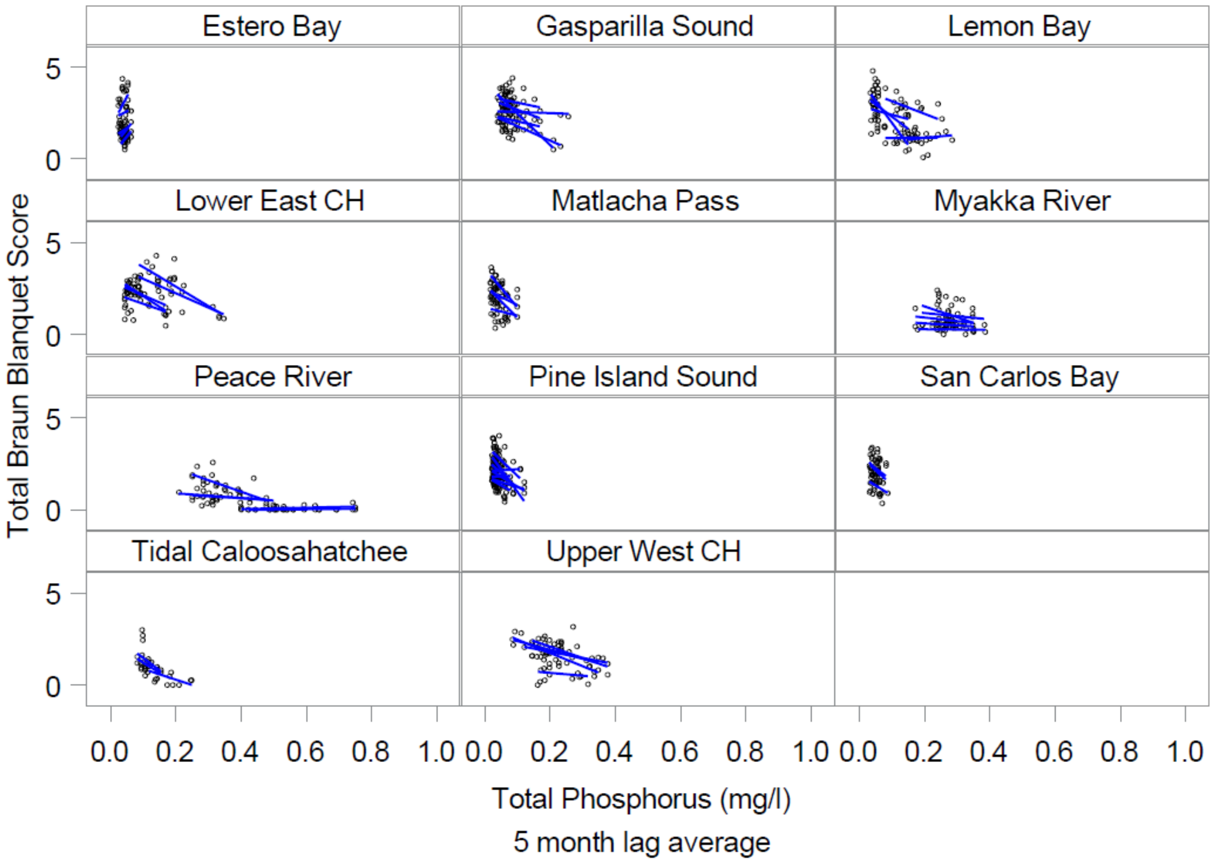

Figure 27. Scatterplot of 5 month antecedent total phosphorus concentrations and total Braun-Blanquet scores by seagrass segment. Solid lines represent site-specific trends using a linear regression line.

Associations between drift algae abundance and TP were typically non-significant with only 5 of 56 statistically significant relationships observed (Figure 28). Four of the 5 significant relationships were negative with the single positive association observed at site ICW05 in Lemon Bay.

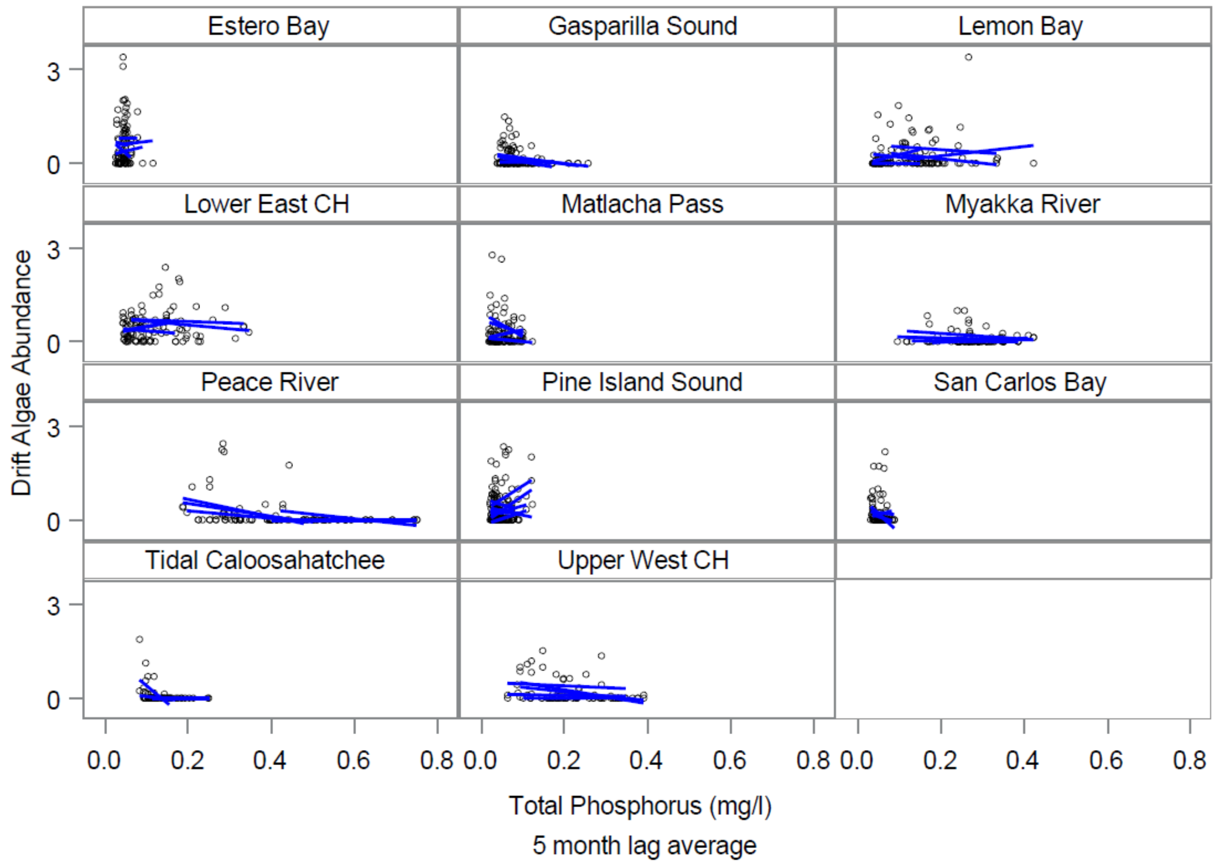

Figure 28. Scatterplot of 5 month antecedent total phosphorus concentrations and drift algae abundance scores by seagrass segment. Solid lines represent site-specific trends using a linear regression line.

Other water quality associations included salinity which was mostly positively associated with total BB scores with 9 of 10 statistically significant correlations suggesting a positive relationship. Only at site CR02 was a negative association with total BB scores and salinity statistically significant. Secchi disk depth was also positively associated with total BB scores at most (10 of 11 sites with significant results). Water temperature was significantly correlated with total BB scores at 23 of 56 sites with all correlations suggesting a positive association between antecedent temperatures and BB scores though the range in antecedent water temperatures was quite small (typically between 27 degrees and 29 degrees C). The two-month antecedent water temperature condition was most commonly the strongest association. The remaining water quality constituents examined had fewer and often conflicting results among sites. For example, chlorophyll concentrations were weakly associated with total BB scores at 5 sites with 3 negative and two positive results. Turbidity was significantly associated with total BB scores at 8 sites with 4 positive and 4 negative results. A table of all results of this analysis is provided in Appendix F.

Statistical Modeling

Statistical modeling was employed as a multistep process beginning with application of two automated selection procedures (stepwise linear regression and Random Forest ensemble models) to screen among a host of potential drivers of transect average seagrass BB scores at the segment level. The results suggested that nutrients, salinity and parameters associated with water clarity (e.g. chlorophyll, secchi disk, and turbidity), were the principal determinants of total BB scores at the segment level and that the 5 month lag average antecedent condition was most commonly identified to represent the antecedent condition. Once the general parameter set was identified, mixed-effects models were used to evaluate the full parameter set and then parsimoniously reduce the parameter set until only significant terms remained in the model. First a model was constructed to evaluate the parameter set across strata, then within strata, and finally at the site level.

The results of the full model across strata suggested that including the random effects component significantly improved the model according to the covariance parameter test ($p<0.001$: Appendix G). The site and depth covariates were significant contributors to explaining variation in total BB scores and that after accounting for these effects, nutrients remained a significant factor affecting total BB scores (Table 11). All nutrient parameter estimates were negative, indicating negative effects of increasing nutrients on total BB scores across the study area. TP had the largest F value indicating it explained the largest proportion of variation of the fixed effects in this model. Light attenuating constituents (secchi disk and chlorophyll) were not statistically significant in this model while turbidity was significant ($p=0.027$).

Table 11. Results of mixed-effects model on data across all sites in the study area.

Effect	Parameter Estimate	F Value	Pr > F
Site	Cat	2.02	<.0001
Depth	Cat	17.63	<.0001
TN[5]	-0.5076	11.05	0.0009
TP[5]	-5.0228	148.23	<.0001
SD	0.02799	0.44	0.5063
CHLAC	-0.00121	0.93	0.3340
NO3O2[5]	-3.4044	17.24	<.0001
TURB[5]	-0.5076	4.91	0.0268
TN*Site	Cat	2.95	<.0001

The same analysis was then applied at the strata level where incorporating a nested random station effect again improved the model fit for all strata as seen by the AICC scores in the bar charts of Figure 29 where smaller numbers represent improved model fit. The Site*TN interaction term was an improvement for some strata and not others at this initial screening level.

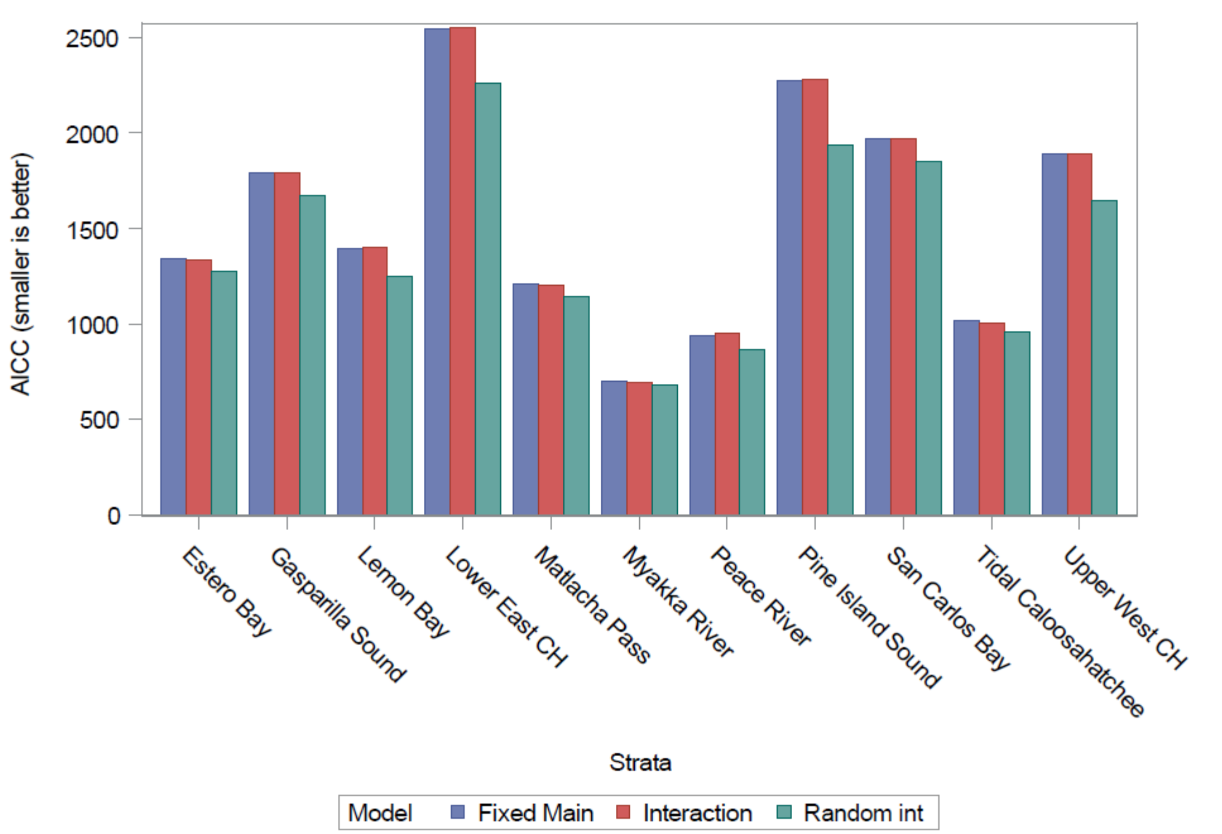

Figure 29. Model AICC comparison for strata based models including main effect, interaction, and random intercept model.

Based on the full parameter set mixed effect model results, TN, NO32, and TP were, with one exception, negatively related to total BB scores where significant in the model framework. The one exception was for TP in Estero Bay where a positive association was suggested which supports the bivariate results. Again, Estero Bay had the lowest TP concentrations of any strata. Light attenuating constituents did not have consistent effects on BB scores, either not explaining significant amounts of variation in BB scores (turbidity for most segments) or with mixed signs of the coefficient among segments (chlorophyll and secchi disk). The interaction between TN and site was significant for 7 of 11 strata evaluated.

Table 12. Full mixed effect model results for stratum based analysis.

Seagrass Strata	Chla5	SD5	NO3O25	TN5	TP5	TURB5	Site	Depth	Inter
Estero Bay	+	ns	ns	--	+	ns	ns	ns	***
Gasparilla Sound	ns	ns	ns	--	--	ns	*	***	*
Lemon Bay	ns	ns	ns	ns	--	ns	ns	ns	ns
Lower East CH	ns	--	--	ns	--	ns	ns	***	ns
Matlacha Pass	--	--	--	ns	--	ns	ns	ns	**
Myakka River	ns	ns	ns	--	ns	ns	ns	*	ns
Peace River	ns	ns	ns	ns	ns	--	*	ns	ns
Pine Island Sound	+	+	ns	ns	--	ns	ns	***	**
San Carlos Bay	ns	ns	--	ns	ns	--	ns	***	*
Tidal Caloosahatchee	ns	+	--	--	--	ns	*	*	***
Upper West CH	ns	--	ns	ns	--	ns	*	ns	***

Note Inter = site*TN interaction term

Based on these initial model results, non-significant water quality constituents were sequentially removed from the model using the F statistic to guide removal until all remaining variables were significant at alpha=0.10. Final mixed-effects models for each stratum were developed and are described in the following paragraphs. Details of the model results, including residual plots and statistics can be found in Appendix H.

Estero Bay:

After removing secchi and turbidity, the depth category was no longer a significant effect in the Estero Bay model (Table 13) and was also removed. The final model suggested that both TN and NO32 were negatively associated with total BB scores and that total BB scores were significantly different among sites. The TP and Chlac coefficients remained positive suggesting increased chlorophyll and TP concentrations preceding sampling were associated with higher total BB scores. This outcome was not directly intuitive until examining the magnitude of their distributions relative to other strata where it was realized that the concentrations of both these constituents were the lowest among all strata examined. The results suggest that increasing nitrogen concentrations have a negative effect on total BB scores but that there may also be some TP limitation occurring in this system which is discussed further in the discussion section.

Table 13. Fixed effects model estimates for final model in Estero Bay.

Effect	Parameter Estimate	F Value	Pr > F
Site		3.40	0.0099
TN^5	-4.3383	72.16	<.0001
TP^5	5.85	56.19	<.0001
$Chla^5$	0.2319	49.23	<.0001
$NO32^5$	-43.4301	11.51	0.0008
TN^5*Site		4.81	0.0003

Gasparilla Sound:

The final Gasparilla Sound model contained both TN and TP with negative coefficients though the TN term in the model was only marginally significant after removing extraneous constituents from the full model (Table 14). The TN*site interaction remained highly statistically significant indicating that some particular sites were more susceptible to the effects of TN than others as observed in Figure 30 where sites GAS03, PIS06 and PIS07 had substantially more negative associations with increasing TN concentrations than other sites.

Table 14. Fixed effects model estimates for final model in Gasparilla Sound.

Effect	Parameter Estimate	F Value	Pr > F
Site		5.68	<.0001
Depth		5.05	0.0006
TN^5	-1.32	3.36	0.0673
TP^5	-7.36	26.82	<.0001
TN^5*Site		6.41	<.0001

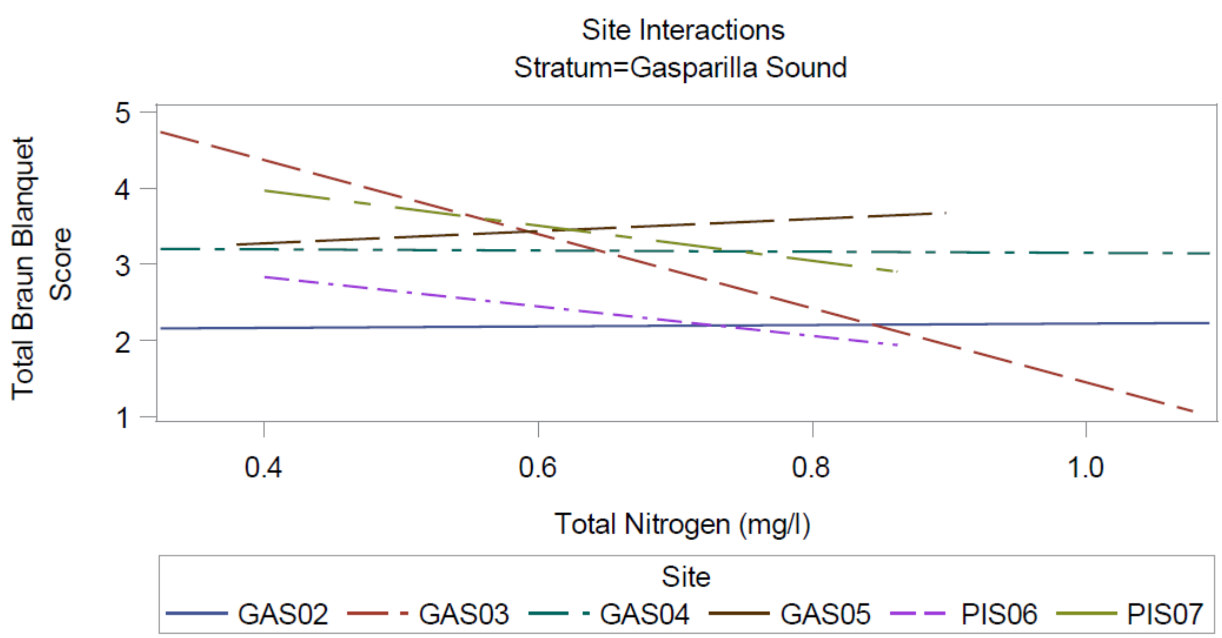

Figure 30. Bivariate plot of relationship between total nitrogen and total Braun-Blanquet scores in the Gasparilla Sound segment.

Lemon Bay:

In Lemon Bay, the TN and TN*site interactions were not statistically significant but antecedent nitrate-nitrite conditions explained a significant portion of variation in total BB scores. Both TP and NO2O3 resulted in negative coefficients. The site effect was significant but the depth term was not significant in the model.

Table 15. Fixed effects model estimates for final model in Lemon Bay.

Effect	Parameter Estimate	F Value	Pr > F
Site		2.86	0.0327
TP[5]	-4.43	8.77	0.0033
NO3O2[5]	-52.54	10.61	0.0012

Lower East CH:

In Lower East CH, only site, depth and antecedent TP conditions were significant in the final model (Table 16). While the main effect of TN was not significant in the model, a closer examination of bivariate plots of TN and BB scores suggests a negative association between BB scores and TN for most sites but a confounding positive association for site MC02 (solid blue line in Figure 31. These opposing trends likely obfuscated the statistical assessment of significance of the main effect in the regression model and therefore the TN*site interaction term was retained given its marginally significant result (p=0.08). Bivariate plots for TP suggested that all sites had a negative response to increasing antecedent TP conditions (Figure 31: Bottom) with a highly significant associated p value.

Table 16. Fixed effects model estimates for final model in Lower East CH.

Effect	Parameter Estimate	F Value	Pr > F
Site		3.30	0.0118
Depth		3.20	0.0128
TP^5	-8.67	73.96	<.0001
TN^5	0.02	0.01	0.9154
TN^5*Site		2.10	0.0794

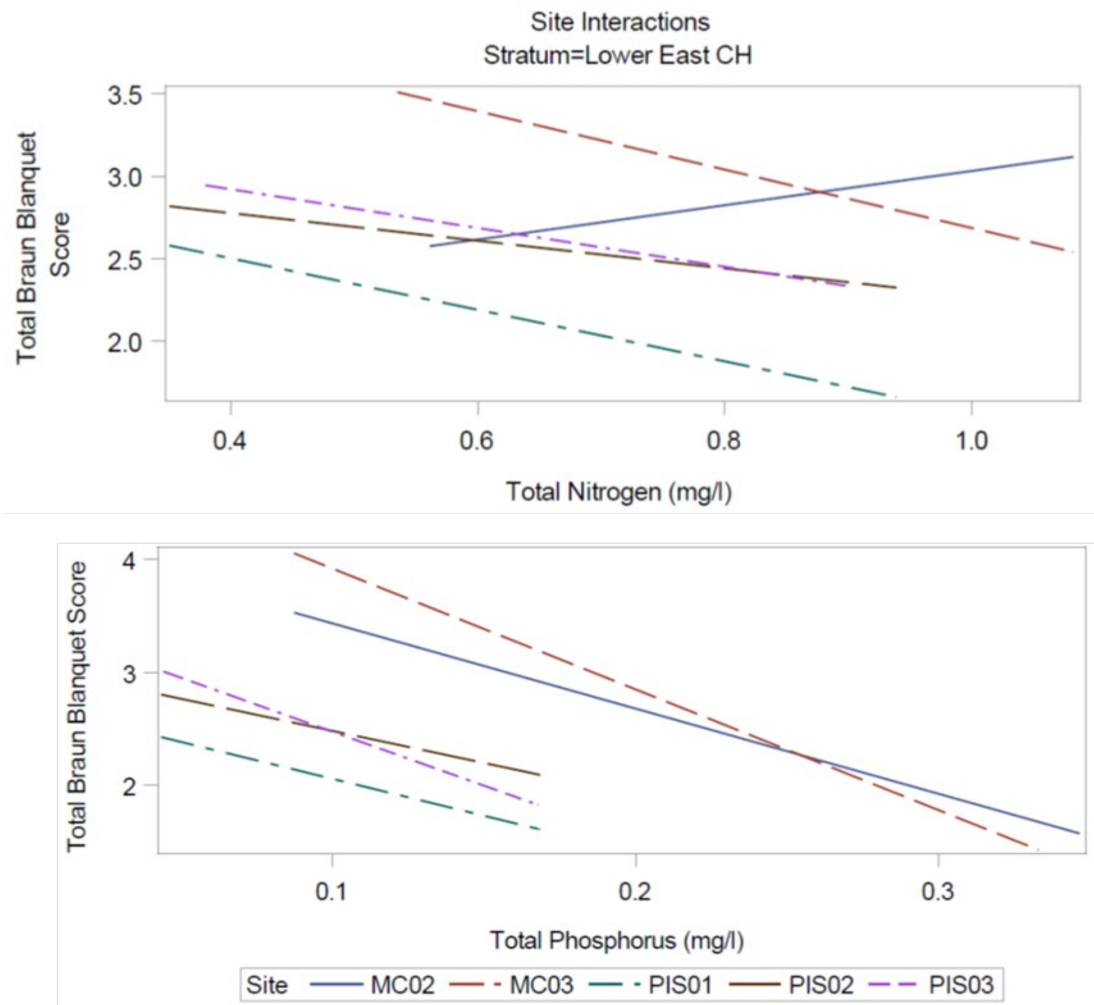

Figure 31. Linear bivariate plots of the site-specific relationships between nutrients (x axis) and total Braun-Blanquet scores in Lower East CH.

Matlacha Pass

The final Matlacha Pass model included only TP and NO32 which both displayed a negative relationship with total BB scores. The remaining terms were found to be correlated in Matlacha Pass to the degree that it affected parameter estimation for turbidity and Secchi disk which were both reported to be positive. Therefore, those terms were dropped from the final model. The final model suggests that reductions in both TP and NO32 in Matlacha Pass would improve total BB scores in Matlacha Pass.

Table 17. Fixed effects model estimates for final model in Matlacha Pass.

Effect	Parameter Estimate	F Value	Pr > F
Site		3.90	0.0250
TP^5	-13.2111	21.62	<.0001
$NO32^5$	-6.9357	7.01	0.0085

Pine Island Sound

The final Pine Island Sound model suggested a highly significant negative relationship with TP and marginally significant negative relationship with TN (Table 18). Since the site*TN interaction was highly significant, the main effect was again retained in the model. The model also predicted a positive effect of water clarity as measured by secchi disk in addition to significant differences among sites and depth categories.

Table 18. Fixed effects model estimates for final model in Pine Island Sound.

Effect	Parameter Estimate	F Value	Pr > F
Site		2.33	0.0279
Depth		7.17	<.0001
TN^5	-0.049	2.81	0.0942
TP^5	-9.90	13.15	0.0003
SD^5	0.40	8.67	0.0034
TN^5*Site		3.83	0.0004

San Carlos Bay

The final model for San Carlos Bay suggested a negative effect of NO32 and turbidity with a marginally significant negative effect of TP (Table 19). The TN main effect was not significant; however, the interaction term was again significant indicating that the relationship between TN and BB score was site dependent. Adding a chla*site interaction term resolved an initial positive coefficient and suggested that the relationship between chlorophyll and BB scores was site dependent. While the TP main effect was only marginally significant, the bivariate plot of the TP-BB score relationship suggests all slopes were negative (Figure 32).

Table 19. Fixed effects model estimates for final model in San Carlos Bay.

Effect	Parameter Estimate	F Value	Pr > F
Site		3.43	0.0193
Depth		7.38	<.0001
TP^5	-9.85	3.09	0.0794
$NO3O2^5$	-16.06	23.99	<.0001
$TURB^5$	-0.029	5.64	0.0179
TN^5*Site		5.46	0.0003
Chla*site		3.16	0.0242

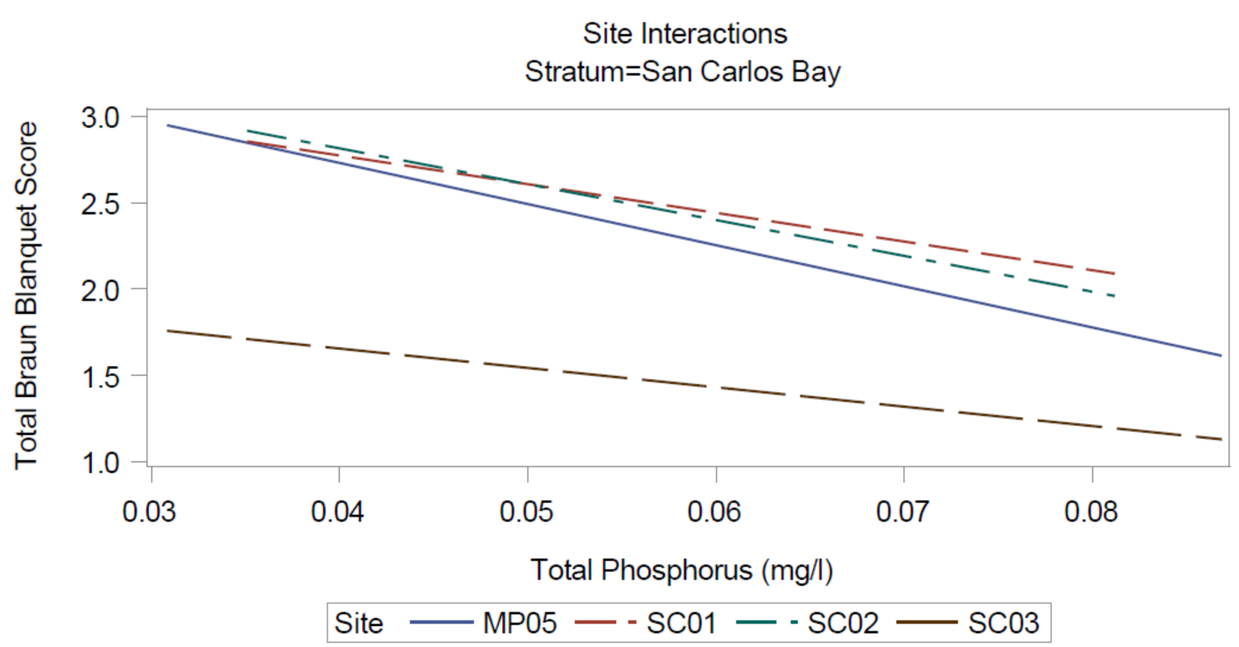

Figure 32. Linear bivariate plots of the site-specific relationships between total phosphorus and total Braun-Blanquet scores in San Carlos Bay.

Upper West CH

The final Upper West CH model included TP, Chla, and NO32 with negative coefficients for all constituents. The TN main effect was not significant but the TN*site interaction term was highly significant indicating site-specific relationships with antecedent TN concentrations. Because NO32 was retained in the model the TN main effect was omitted.

Table 20. Fixed effects model estimates for final model in Upper West CH.

Effect	Parameter Estimate	F Value	Pr > F
Site		3.06	0.0173
TP^5	-5.92	55.25	<.0001
$Chla^5$	-0.01	5.55	0.0189
$NO3O2^5$	-9.88	5.74	0.0170
TN^5*Site		3.11	0.0090

Generalized Linear Models:

The results above reflect efforts to model the mean total BB response as a function of potential explanatory terms in the model. An alternative approach is to maintain the polychotomous nature of the total BB scores and evaluate the probability of observing a certain category as a function of either continuous or categorical variables. We used a stratum specific generalized logistic regression model to evaluate the probability of a total BB score in a particular category for all categories relative to zero (i.e. no grass). Only continuous effects were modeled as predictors since the site and depth categories may not contain all classes of responses which can negatively affect model parameterization. In addition, the modeling effort was restricted to only main effects (i.e. no interaction term) for the same reason. A stepwise section routine was used to keep only those terms that had a statistically significant effect in at least one comparison within the stratum.

The results allow one to look at the response of each BB score category in relation to potential explanatory terms. For example, in Estero Bay, TN had a significant negative effect on the probability of observing BB scores in the 3 highest categories (Table 21) while TP was only a significant factor affecting the probability of occurrence in the 2 lowest categories relative to the reference (zero) category. As was observed with the linear regression modeling, TP was consistently negatively related to BB scores across strata while TN results were mixed with several strata suggesting an increased probability of occurrence with increasing TN; however, many of these results also contained predicted negative effects of NO23 suggesting multicollinearity may be playing a role in these results. Interaction terms were also not included in this model which likely affected the sign of the TN results as described above. The detailed results of this modeling effort can be found in Appendix I.

Table 21. Results of stratum level stepwise generalized logit model evaluating the effect of potential drivers on each total Braun-Blanquet score category relative to the "no grass" category. Blank cells represent insignificant terms in the model for all levels of response while "ns" is present where at least one level was significant, but the others were not. A "–" indicates a negative significant relationship (alpha=0.10) while "+" indicates a significant positive relationship.

Strata	BB Score Category	CHLAC[5]	NO3O2[5]	TN[5]	TP[5]	SD[5]	TURB[5]
Estero Bay	5%	ns	ns	ns	--		
	5% - 25%	ns	ns	ns	--		
	25% - 50%	ns	ns	--	ns		
	50% - 75%	ns	ns	--	ns		
	75% - 100%	+	--	--	ns		
Gasparilla Sound	50% - 75%				--		
	75% - 100%				--		
Lemon Bay	5%		--	+	ns		
	5% - 25%		--	+	ns		
	25% - 50%		ns	ns	--		
	75% - 100%		ns	ns	--		
Lower East CH	5%	ns	ns	+	--		
	5% - 25%	ns	ns	ns	--		
	25% - 50%	ns	ns	ns	--		
	50% - 75%	+	ns	ns	--		
	75% - 100%	+	--	+	--		
Matlacha Pass	5%	ns				+	
	5% - 25%	ns				+	
	50% - 75%		ns	+	--		
	75% - 100%		--	ns	--		
Peace River	5%			ns	--		
	5% - 25%			+	--		
	25% - 50%			ns	--		
	50% - 75%			ns	--		
Pine Island Sound	ns				ns	ns	ns
San Carlos Bay	5%		+			ns	
	75% - 100%		--			ns	
Tidal Caloosahatchee	5%		ns		--	+	
	5% - 25%		ns		--	+	
	25% - 50%		--		ns	+	
	75% - 100%		--		ns	ns	
Upper West CH	75% - 100%				--		

Discussion

Overall, seagrass Braun-Blanquet scores seem to be influenced by both large scale interannual variation driven by meteorological patterns associated with droughts and surplus flows as well as the associated nutrient loads, concentrations and more localized effects. Over the full period of record, most sites displayed a stable or positive change in total BB scores; however, the beginning of the time series of total BB scores for most sites (i.e. 2004) represents a near minimum for seagrass abundance scores coinciding with a protracted period of above average flows between 2003 and 2005. Many of those sites gained seagrass between 2006 and 2009 but have now returned to scores near their 2004 averages. Those sites with the highest average total BB scores, tended to be sites that were not monotypic in species frequency of occurrence with nine of the 10 sites containing all three major seagrass species types while those sites with the largest cumulative loss in abundance scores tended to have a higher percent occurrence of Halodule indicating Halodule losses may be responsible for observed decreases in total BB scores.

Hurricane Irma in 2017 imparted large scale forcing functions on the environmental conditions necessary for seagrass success, including some of the highest tributary flows on record. A subsequent, protracted *Karenia brevis* ("Red Tide") bloom resulted in massive fish kills within the study area. While these events may have contributed to adverse environmental conditions for seagrass in some strata in recent years, there was a lack of uniformity in the response of total BB scores to these events across sites within the study area suggesting other factors are likely also contributing to changes in seagrass abundance within the system.

Timeseries water quality trends suggested TN concentrations have been increasing throughout much of the study area at the same time TP concentrations have been decreasing in several segment. Results of the segment-specific regression analysis of total BB scores suggested that TP was consistently a deterministic constituent explaining variation in total BB scores once the final model was reduced to only those constituents that explained a statistically significant portion of the overall variation in total BB scores. The direction of the effect was typically negative both within and between segments which suggests a negative effect of increasing TP on total BB scores.

The exception was Estero Bay where significant positive associations were found between TP and total BB scores. The effects of antecedent TN concentrations were more site-specific as explained by the significant TN*site interaction term in most models. The TN main effect was often masked by opposing site-specific relationships as described by the interaction term presenting as highly statistically significant. In several strata including Upper West CH, San Carlos Bay, Matlacha Pass, and Lemon Bay, antecedent NO32 concentrations were deterministically linked to negative effects on total BB scores. Measures of water clarity and light attenuating constituents were less consistently related to total BB scores in the regression framework.

Total BB scores at most sites have been either stable or increasing over the period of record and these increases correspond to observed improving trends in TP concentrations over the period of record. However, increasing nitrogen trends throughout much of the study area are disturbing and the site-specific nature of the relationship between total BB score and TN may indicate that TN concentrations have reached a threshold value above which may result in a negative overall effect on seagrass condition. A univariate regression tree of the TN BB scores relationship across strata suggests that antecedent TN thresholds may exist that define key condition for total BB scores (Figure 33). The proximity to exchange with Gulf waters and the natural dilution of freshwater inputs may confound these results as a causal mechanism but it is clear that waters with higher antecedent TN conditions are correlated with lower total BB scores and that several breakpoints may exist in that relationship. These thresholds held for stratum-specific analysis for Gasparilla Sound (ca. 0.7 mg/l), Lemon Bay (0.6 mg/l), the Myakka and Peace River (1 mg/l) and the Upper West CH strata (0.8 mg/l) and were in general agreement with numeric nutrient criteria (NNC) established by FDEP to protect these waters' designated uses for the propagation of fish and wildlife, shellfish harvesting, as well as for recreation in and on the water (F.A.C 62-302).

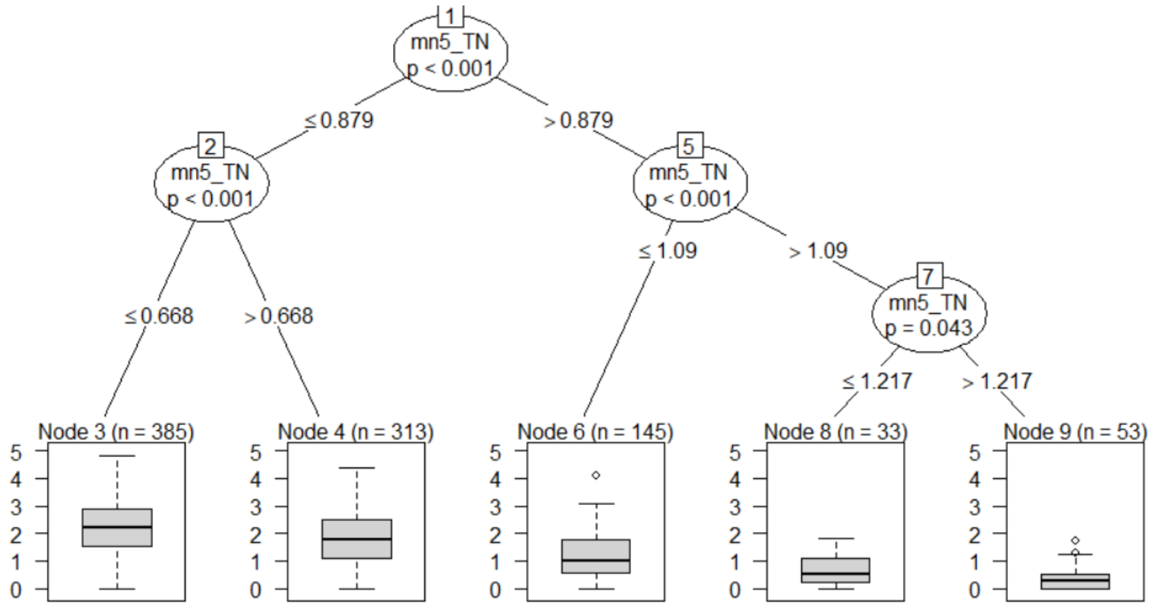

Figure 33. Conditional Inference Tree identifying potential breakpoints in total nitrogen concentration that maximize the difference in groups of total Braun-Blanquet scores.

The finding that NO32 concentrations were negatively related to total BB scores in some segments suggests anthropogenic inputs may be present at these locations. Nitrates were a significant negative factor explaining variation in total BB scores in the three strata containing sites with the highest loss in BB score over time. As described in Touchette and Burkholder 2000, most nitrate is regarded as 'new' nitrogen for the system, contributed from anthropogenic rather than regenerated inputs (Glibert, 1988; Henriksen and Kemp, 1988). Mesocosm experiments conducted at the southernmost extent of the range for *Zostera marina* have repeatedly documented significant lower survival of that species when grown in sandy sediments under water-column nitrate enrichment unrelated to light reduction from algal overgrowth or other source (Burkholder et al., 1992, 1994; Touchette, 1999). These studies also highlighted the potential negative synergistic effects of increased nutrients and temperatures which may play a role in our study as water temperatures were observed to be increasing over time in many strata.

The consistent total phosphorus results suggesting a negative effect on total BB scores was somewhat counterintuitive in that phosphorus addition has been shown to increase seagrass production in several estuaries (Touchette and Burkholder 2000 and references therein). As a generalization, seagrasses are regarded as N-limited when growing in sandy or organic sediments, and as P-limited in carbonate sediments (Touchette and Burkholder, 2000 and references therein). The TP results in Estero Bay could be considered to conform to this traditional understanding as TP was positively associated with total BB scores in Estero Bay which may be generally considered to contain a large fraction of carbonate sediments (Missimer 1970). The strata in the northern part of the study area contained higher concentrations of TP and are generally considered to be nitrogen limited systems but the southern portion of the study area appears to be a transition area to lower TP concentrations. While there is little evidence in the literature to support the hypothesis that excess phosphorus can, as a direct effect, result in negative outcomes for seagrass species, excess phosphorus in phosphorus limited estuarine systems may be deleterious as an indirect effect. Based on outcomes of this study, two hypotheses are presented in the following paragraphs to explain the relationships we observed between nutrients ant seagrass BB scores.

The first hypothesis posits that the large pool of TP delivered to the estuary between 2004 and 2006, followed by subsequent pervasive drought conditions which increased water clarity and reduced other light attenuating factors may have led to a period of sustained seagrass growth in the study area corresponding to TP uptake in the system. The decreasing timeseries trend in TP was correlated with increasing total BB scores over time with median and average TP concentrations higher in 2004 and 2005 than at any time later in the timeseries (Figure 34a). The trend in TP concentrations remained even after adjusting out the effects of salinity as observed in the residual plot from a linear regression between TP and salinity (Figure 34b). The 2003-2005 time period was characterized as a wet period of record with several hurricanes affecting the area including direct landfall of Hurricane Charley in 2004. This part of the timeseries corresponded to a period of lower BB scores which subsequently increased for many sites after 2006 when extensive drought condition beset the region beginning in late 2006 and lasting through much of 2009.

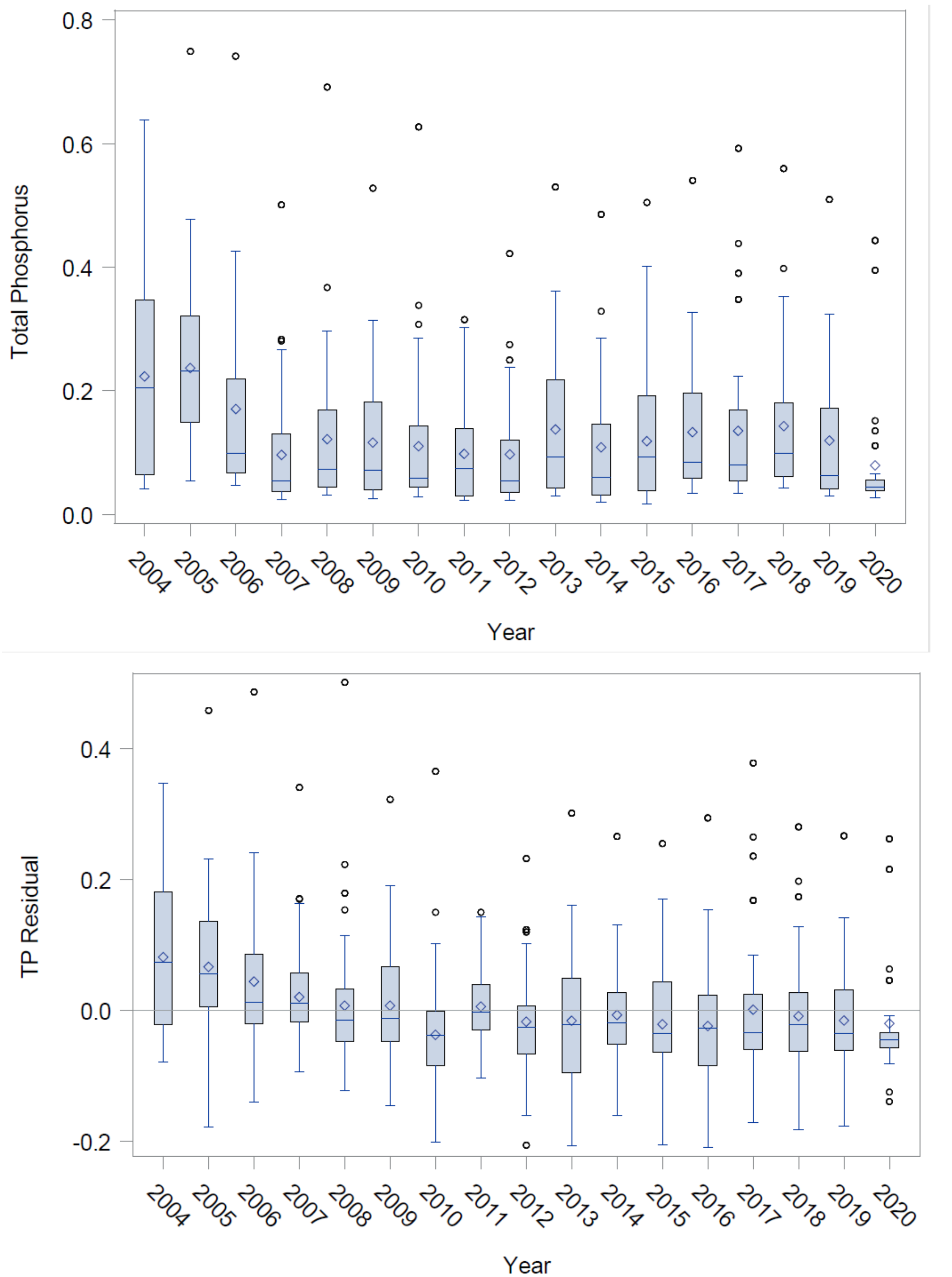

Figure 34. Annual distiribution of total phosphous (top) and total phosphorus residuals (bottom) after regressing on salinity.

The second hypothesis posits that increasing nitrogen concentrations combined with increasing temperatures in some site-specific locations has reached a critical threshold in recent years resulting in a die back in seagrass at those sites. 2018 was an anomalously high year for TN relative to the remainder of the timeseries over which total BB scores were reported (Figure 35:Top) and after correcting for the effects of salinity, the TN residuals remained biased positive between 2017 and 2020 suggesting more TN was observed than would be expected given the salinity (Figure 35: Bottom). The red tide bloom in 2018 and associated fish kills may have contributed to this observation.

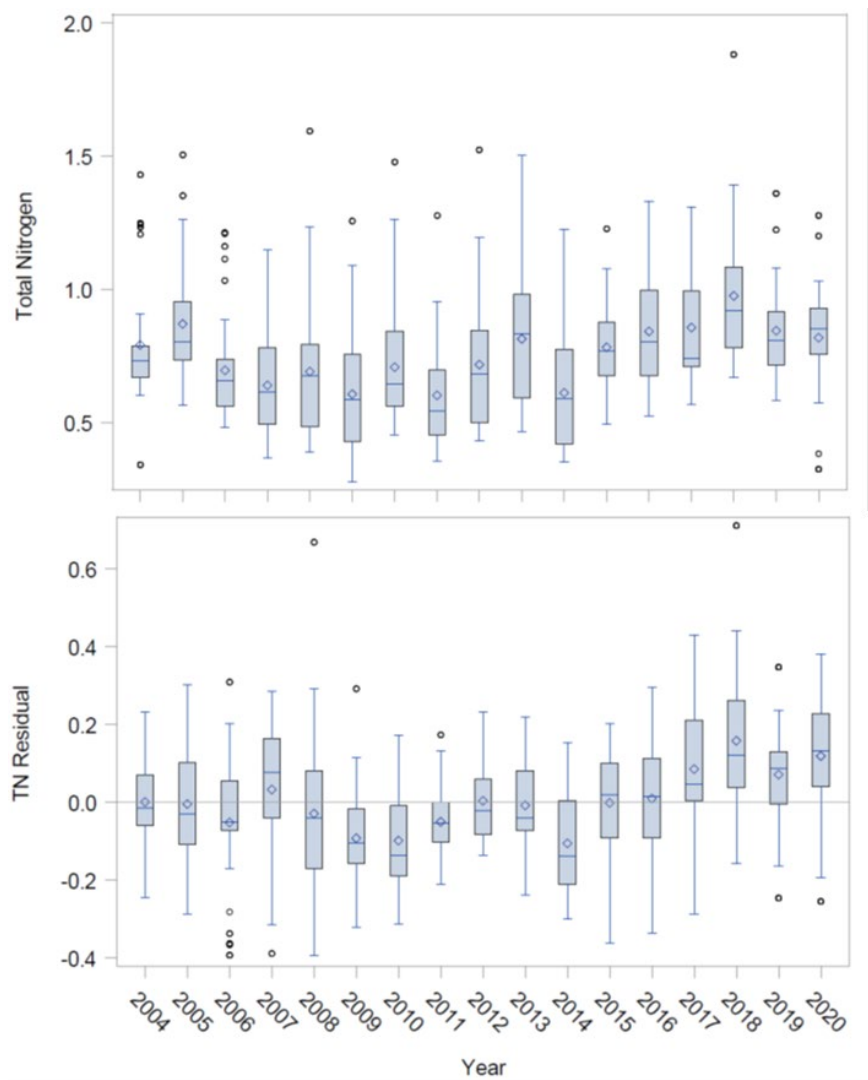

Figure 35 Annual distiribution of total nitrogen (top) and total nitrogen residuals (bottom) after regressing on salinity.

In Estero Bay, these higher TN concentrations corresponded with a higher frequency of occurrence of drift algae which can smother seagrasses and result in lower BB scores. These segments are now declared impaired by FDEP for total nitrogen corresponding to observed increases in anthropogenic effects in the watershed including increased impervious surface (EBABM 2019). However, in other strata increasing frequency of drift algae occurrence was not evident in the data and it remains possible that in Estero Bay, an increased focus on drift algae identification may have led to the observed increase in frequency of occurrence. In addition, in the case of TP, it is possible that changes in the minimum detection limit may have affected the results in some locations though supplemental analysis based on peer review comments (provided in Appendix J) suggested that this effect would likely be limited to a few WBIDs and generally not affect inference from this study.

Natural environmental patterns provide overriding conditions on water quality constituents that govern seagrass response and cannot be ruled out as drivers of seagrass change over time. The period between 1998 and 2010 contain several extreme events including an intense period of hurricane activity, a protracted drought and a subsequent return to a more near normal or slightly above average freshwater inflow pattern. Since 2014, there has been a noticeable lack of protracted drought conditions and a fairly consistent above average flow pattern. These inter-annual variations are superimposed on predictable seasonality in flows and water quality. Therefore, water quality constituents covary in a predictable manner within a stochastic interannual pattern. Several methods were used to account for these factors. Standardizing flows and water quality removed seasonality prior to correlating those variables, evaluating relatively long-term antecedent conditions (i.e. 5 month lag averages) to seagrass sampling resolved seasonality within the regression framework and differencing the timeseries of transect average BB scores provided a more deterministic evaluation of variation in the timeseries. The analysis first focused on those constituents that could be managed either directly (e.g. nutrients) or indirectly (water clarity related constituents) and found nutrients to be significant potential drivers of seagrass Braun-Blanquet condition. Even after accounting for salinity which is an integrative measure of antecedent freshwater inflows to the estuary, nutrients were identified as potential significant drivers with typically negative parameter estimates in the regression framework suggesting negative impacts of increased nutrients on seagrass success. Accounting for site and depth characteristics improved the model fit in most cases while the inclusion of interaction terms in the regression framework, as well as from descriptive plots, suggested that some relationships are highly site-specific. Light attenuating parameters such as chlorophyll, secchi disk and turbidity were not reliable predictors of total BB scores across strata though they had effects in some strata. The lack of significant changes in "deep edge" depth over time also suggests that losses in seagrass may not be due strictly to light limitation. Analysis of shoot density, blade length and epiphytes suggested positive correlations with total BB scores indicating that those metrics are interrelated with the principal response endpoint (i.e. BB scores) used for the analysis of seagrass transect data. Summary analysis of those relationships are also provided as supplemental materials in Appendix J.

Limitations of this study included that the seagrass data contained a large degree of natural variation. To account for this artifact, the mixed-effects modeling approach implemented for this analysis used the hierarchical structure of the data to properly account for the station-specific variability in BB scores while providing best linear unbiased estimates of effects of potential drivers at the site and strata level. For example, even where consistent trends were found among sites within a stratum (e.g. Estero Bay), station-specific variation in scores remained quite large (Figure 36: top) and in other segments where large seagrass losses have been reported by aerial mapping efforts, station BB scores vacillate considerably from year to year in some cases (Figure 36: Bottom). While these attributes limit the use of the models as direct predictive equations to estimate future BB scores, they do not invalidate model inference regarding the identification of drivers of change in BB scores.

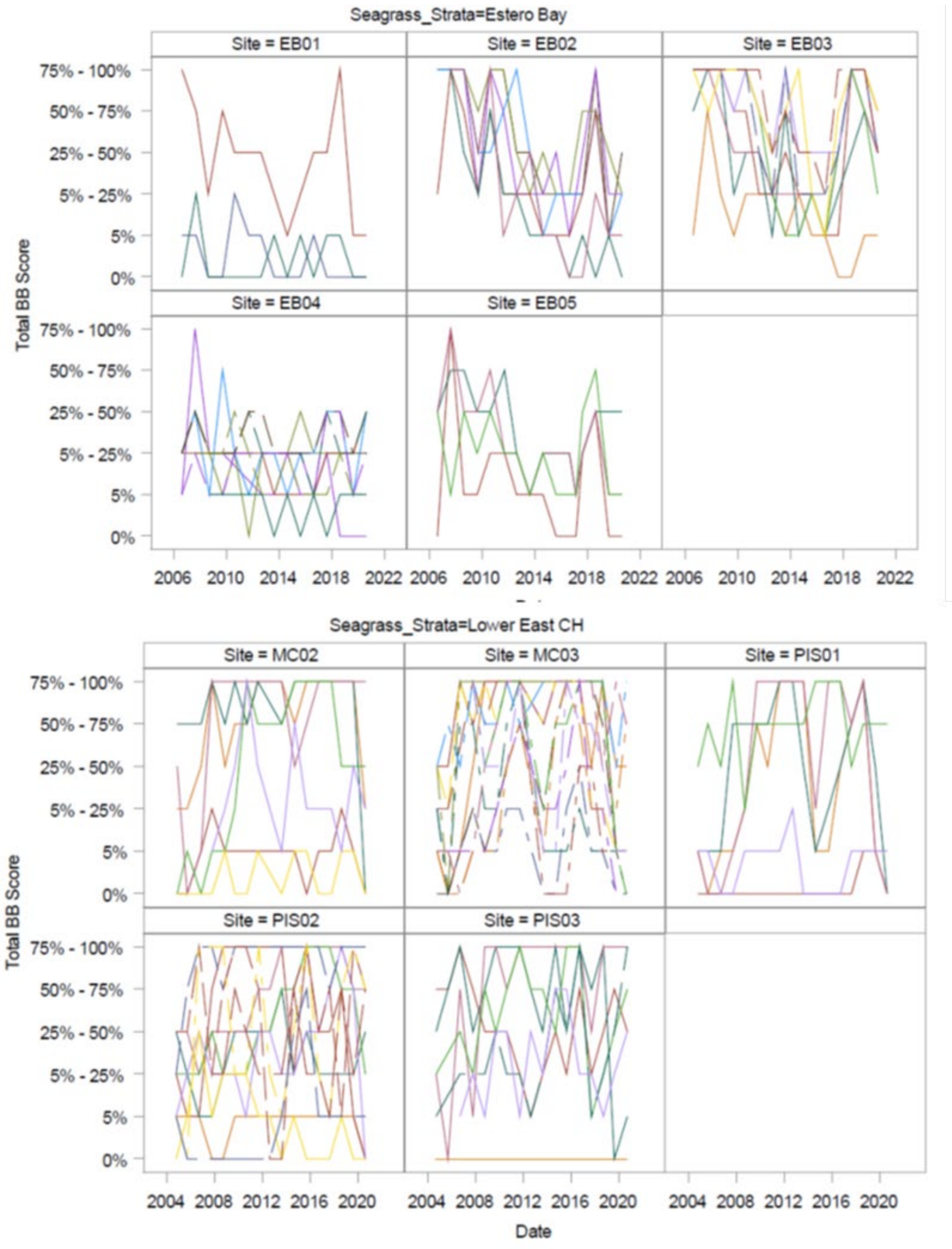

Figure 36. Station-specific timeseries trends in total BB scores for sites in Estero Bay (top) and Lower East CH (bottom).

Other limitations included linking WBID average water quality to site-specific transect information which is an obvious generalization of the conditions that a particular site might experience. While the stratified random sampling design used by the water quality monitoring network that made up the majority of the data used to correlate with seagrass information was designed to represent relatively homogeneous spatial areas, the spatial representation of the data within any particular year could have induced variability into the true water quality to seagrass relationship. Annual data for seagrass BB score limited the number of observations from which to make inferences on changes over time or to develop relationships with potential drivers based on transect average scores. The limited number of observations may have reduced the power of the statistical tests in that regard. The incorporation of station-specific random intercepts in the model allowed for the inclusion of more data points than simply site averaging and was seen as a beneficial component to assess relationships among potential drivers using a hierarchical method. Quantification of the effects of macroalgae was difficult given the overall low probability of occurrence over the study period, though Estero Bay had the most consistent occurrence of algae since 2016. The occurrence tended to be most noticeable in the depth categories between 0.5 and 1.5 m which is also both where the highest Braun-Blanquet scores were found and where the greatest reduction in seagrass have occurred. The timeseries trend tests did not incorporate the magnitude of the values but rather the sign of the pairwise comparison in a timeseries. Therefore, it is possible though unlikely for a particular site to have an overall increasing trend over time but end the timeseries with a lower overall BB score than at any point in the timeseries. Species-specific seagrass BB scores had a longer period of record but the convention to only estimate BB scores when the species occurs limited the ability to characterize species-specific changes over time. Despite these limitations, the evidence suggests that nutrients, in combination with other environmental factors, are influencing seagrass species distribution patterns in a predictable manner. While some of the change in BB score over time may be due to natural variation in environmental condition, increasing trends in TN and temperature throughout much of the study area present stressors for these seagrass communities. In addition, negative association between antecedent NO32 concentrations and total BB scores conform to known mechanisms of anthropogenically based pollution. While the analysis presented herein does not constitute causal inference, the weight of evidence suggests that nitrogen concentrations either in the form of TN or NO32 may have reached critical thresholds that, in combination with other environmental factors have resulted in recent seagrass losses at some locations in the study area. Overall, the transect level data suggest a return to baseline conditions at the beginning of the study period rather than a historic decrease in seagrass that have not been observed in the past. It cannot be discounted that the protracted drought in 2007 through 2009 resulted in abnormally beneficial conditions for seagrass meadows that have now regressed back to their long-term averages. However, recent observations of prolific macroalgae blooms are a cause for great concern that the ecosystem metabolism is vacillating in ways that might indicate a shift to an alternative state and changes in the TN/TP ratio as evidenced by opposing timeseries trends in these constituents suggests that the system may be trending in a directional manner. To date, the lack of intense phytoplankton blooms (other than *Karenia brevis* blooms) provide some hopeful evidence that the system has not yet crossed a tipping point that might result in a more phytoplankton dominated system but all efforts should be made to limit nutrient loads to these aquatic preserves to combat other environmental forces that stress these ecosystems.

References:

Armitage, P. (1955) Tests for linear trends in proportions and frequencies. *Biometrics* 11: 375-386.

Braun-Blanquet, J. 1965. Plant sociology: the study of plant communities. Authorized English translation of Pflanzensoziologie 1932. Translated, revised, and edited by George D. Fuller and Henry S. Conard, 1 st ed. McGraw-Hill, New York, NY.

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

Brown, M., R. Leary, N. Langford, M. McMurry, and H. Stafford 2013. Results of the Florida Department of Environmental Protection, Charlotte Harbor aquatic preserves' seagrass monitoring program from 1999-2009. Florida Scientist 76(2): 92–106.

Brown, M. 2019. Charlotte Harbor Aquatic Preserves: 18-Year Results of the Seagrass Transect Monitoring Program 1999-2016. Report prepared by the Charlotte Harbor Aquatic Preserves. Punta Gorda, FL 33955

CHEC 2006. Charlotte Harbor Environmental Center, Inc and Charlotte Harbor Aquatic Preserves Florida Department of Environmental Protection seagrass transect data summary and analysis from a six-year period: 1999 - 2004. Reports. Paper 18. http://scholarcommons.usf.edu/basgp_report/18

Dixon, L. Kellie and Gary J. Kirkpatrick. 1999. Causes of light attenuation with respect to seagrasses in upper and lower Charlotte Harbor. Report to Southwest Florida Water Management District, SWIM Program and Charlotte Harbor National Estuary Program. Report. available from SWFWMD, SWIM Program, Tampa, Florida, and CHNEP, North Ft Myers, Florida.

Dixon, L. K and M.R. Wessel 2016. A spectral optical model and updated water clarity reporting tool for Charlotte Harbor seagrasses. Florida Scientist. 79:61-92

Duarte, C. M. 1991. Seagrass depth limits. Aquatic Botany 40(4): 363-377.

EBABM (Estero Bay Agency on Bay Management). 2019. State of the Bay Update. Report prepared by the Southwest Florida Regional Planning Council and the Charlotte Harbor National Estuary Program. Ft. Myers, 2019.

Furman BT, Leone EH, Bell SS, Durako MJ, Hall MO (2018) Braun-Blanquet data in ANOVA designs: comparisons with percent cover and transformations using simulated data. Mar Ecol Prog Ser 597:13-22. https://doi.org/10.3354/meps12604

Glibert, P., 1988. Primary productivity and pelagic nitrogen cycling. In: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen Cycling in Coastal Marine Environments. Scientific Committee on Problems in the Environment (SCOPE) 33. Wiley, New York, pp. 3–31.

Henriksen, K., Kemp,W.M., 1988. Nitrification in estuarine and coastal marine sediments. In: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen Cycling in Coastal Marine Environments. Scientific Committee On Problems in the Environment (SCOPE) 33. Wiley, New York, pp. 207–249.

Hirsch, R.M., and J.R. Slack.1984. A nonparametric trend test for seasonal data with serial dependence: Water Resources Research v. 20, p. 727–732

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

Kassambara, Alboukadel and Fabian Mundt (2020). factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7.999. http://www.sthda.com/english/rpkgs/factoextra

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. *SAS System for Mixed Models.* Cary, NC: SAS Institute, Inc.

McPherson BF, and Miller RL. 1994. Causes of light attenuation in Tampa Bay and Charlotte Harbor, southwestern Florida. Water Resources Bulletin 30:43-53

Missimer, T. M. 1970. A preliminary study of the sedimentary processes and depositional environments of Estero Bay, Florida. Unpublished.
DOI: 10.13140/RG.2.2.11901.26088

Montague, C. L. and J. A. Ley. 1993. A possible effect of salinity fluctuation on abundance of benthic vegetation and associated fauna in Northeaster Florida Bay. Estuaries 16(4): 703-717.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rickards, L. 2018. Seagrass abundance and distribution in relation to changing environmental factors in Estero Bay, Florida. Florida Gulf Coast University Thesis. https://fgcu.digital.flvc.org/islandora/object/fgcu%3A31528

SAS Institute Inc. 2018. SAS Stat Users Guide V9.4. Cary, NC: SAS Institute Inc.

Sebastien Le, Julie Josse, Francois Husson (2008). FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software, 25(1), 1-18. 10.18637/jss.v025.i01

Taylor-Manges, A. Spatial and temporal relationships between water quality and seagrasses: a case study of the Charlotte Harbor estuary. University of Florida Thesis. https://ufl-flvc.primo.exlibrisgroup.com/permalink/01FALSC_UFL/175ga98/alma99383249531706597

Tomasko, D.A., Dawes, C.J., Hall, M.O., 1996. The effects of anthropogenic nutrient enrichment on turtle grass (*Thalassia testudinum*) in Sarasota Bay, Florida. Estuaries 19, 448–456.

Tomasko DA, and Hall MO. 1999. Productivity and biomass of the seagrass *Thalassia testudinum* along a gradient of freshwater influence in Charlotte Harbor, Florida. Estuaries 22:592-602.

Tomasko, David A., Denise L. Bristol, and Judith A. Ott. 2001. Assessment of present and future nitrogen loads, water quality, and seagrass *(Thalassia testudinum*) depth distribution in Lemon Bay, Florida. Estuaries 24(6A):926-938.

Tomasko, D., M. Alderson, R. Burnes, J. Hecker, N. Iadevaia, J. Leverone, G. Raulerson, and E. Sherwood, 2020. The effects of Hurricane Irma on seagrass meadows in previously eutrophic estuaries in Southwest Florida (USA). Marine Pollution Bulletin 156 111247.

Touchette, B. W. and J. M. Burkholder 2000. Review of nitrogen and phosphorus metabolism in seagrass. Journal of Experimental Marine Biology and Ecology. 250, 133-167.