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Executive Summary

Sarasota County and the Sarasota Bay Estuary Program were interested in a review of the annual sea grass data collected in Sarasota Bay in order to identify potential temporal trends in frequency of occurrence and cover of seagrasses, drift algae and epiphytes, and whether there have been trends in sea grass composition within eight distinct areas of the Bay. In addition, there was an interest in identifying possible relationships among the five biota (three seagrass species, drift algae, and epiphytes) and their relationships with water quality parameters measured on a regular basis throughout the Bay. These results and additional analyses to look at variation among sub-areas within each of the 8 areas of the Bay were then used to review the current sampling design.

In the following summary, “frequency of occurrence” refers to the proportion of sites having the biota of interest. So, for example, a predicted frequency of occurrence for drift algae of 0.65 is interpreted to mean that approximately 65% of the sites in an area are expected to have drift algae present. “Cover” refers to either the percentage of the bottom covered by a seagrass or the Braun-Blanquet\(^1\) score for drift algae or epiphytes. Hence a predicted cover of 35 for Halodule is interpreted to mean that approximately 35% of the bottom area within a randomly sited quadrat is expected to be Halodule. A predicted mean cover of 1.5 for drift algae, say, is interpreted to mean that the average Braun-Blanquet score for drift algae at a site is 1.5, or equivalently approximately 8% coverage of the bottom area within a sampled quadrat. Details on the datasets used, the statistical methods applied, and the results of the analyses are to be found in the main body of the report.

Findings

Thalassia

Mean Thalassia frequency of occurrence showed very little change in time (Figure 10) in most segments the exceptions being segments 14, 16, and DR, all of which have very low average frequency of occurrence of Thalassia (Figure 11). The statistically significant differences among annual means in these 3 segments are due to the fact that even small changes in the expected percentages are considered statistically different. Segments 10, 11, and US all have high mean frequency of occurrence; segments US and 11 average around 60% and segment 10, 80% (Figure 11).

Cover of Thalassia was found to be correlated with some of the water quality parameters measured by Sarasota County Environmental Services. The variables most related to cover of

\(^1\) Braun-Blanquet scores are 0 = no cover, 1=<5% cover, 2=5-25% cover, 3=26-60% cover, 4=51-75% cover, 5=76-100% cover.
Thalassia (Tables 46 and 47) included salinity ($r = +0.478$, p-value $< 0.0001$), depth ($r = +0.341$, p-value $< 0.0001$), nitrates ($r = -0.286$, p-value $= 0.0036$), and color ($r = -0.281$, p-value $< 0.0001$). Thalassia is negatively correlated with cover of Halodule ($r = -0.426$, p-value $< 0.0001$) and positively correlated with cover of Syringonium ($r = +0.233$, p-value $< 0.0001$).

Halodule

Mean Halodule frequency of occurrence showed a temporal change in all segments (Figures 12 and 13b) where average frequency of occurrence was approximately 43% in years 2013 – 2015 versus an average of 57% in earlier years (Table 28). Although there is some evidence of a decline, the statistical tests for differences in frequency of occurrence among years were not significant (Table 29). The lack of statistical significance could be due to natural variability in Halodule frequency of occurrence or insufficient statistical power to discern a difference.

Halodule cover was found to be correlated with some of the water quality parameters measured by Sarasota County Environmental Services. The variables most related to cover of Halodule (Tables 46 and 47) included water depth ($r = -0.363$, p-value $< 0.0001$), color ($r = +0.306$, p-value $< 0.0001$), salinity ($r = -0.278$, p-value $< 0.0001$), and secchi disk depth ($r = -0.247$, p-value $< 0.0001$). Halodule cover is negatively correlated with cover of Thalassia ($r = -0.426$, p-value $< 0.0001$) and Syringonium ($r = -0.280$, p-value $< 0.0001$).

Syringonium

Mean Syringonium frequency of occurrence was always low but showed an increase in time (from 1% to 8%; Figure 15 and Tables 33 and 34) in most segments except segments 14 and LB. These 2 segments have very low average frequency of occurrence of Syringonium in all years (Figure 15b).

Syringonium cover was found to be correlated with some of the water quality parameters measured by Sarasota County Environmental Services. The variables most related to cover of Syringonium (Tables 46 and 47) included color ($r = -0.307$, p-value $< 0.0001$), depth ($r = +0.300$, p-value $< 0.0001$), phosphorus ($r = -0.292$, p-value $< 0.0001$), secchi disk depth ($r = +0.280$, p-value $< 0.0001$), salinity ($r = +0.278$, p-value $< 0.0001$), and nitrates ($r = -0.267$, p-value $= 0.0063$). Syringonium is negatively correlated with cover of Halodule ($r = -0.280$, p-value $< 0.0001$) and positively correlated with cover of Thalassia ($r = +0.233$, p-value $< 0.0001$).

Composition of the Three Sea Grass Species

Above we described the pairwise relationships between cover of the three species, e.g. Halodule cover is inversely related to Thalassia cover. The focus now is the likelihood of different combinations of the three species to be found together within a segment and whether the composition changed over time. To that end, a 3-character categorical variable was created.
in which the presence or absence of a seagrass species is identified: “THS” (also called PAsgrass in the text) where the first character is 0 if Thalassia is absent and 1 if present, the second character is presence (1) or absence (0) of Halodule and the third is presence (1) or absence (0) of Syringonium. Hence, THS = 011 indicates that Halodule and Syringonium were observed at a sample location but not Thalassia.

As expected, the composition of the three frequencies of occurrence (THS) varies among segments (Figures 22, 23, and 24). Segments 11, 13, and US have approximately equal percentages of the three species (Figure 24); segments 14, 16, DR and LB have high percentages of sites with Halodule compared to the other two species; and Thalassia is most common in segment 10.

All segments have some fraction of sites with no seagrass observed (THS = 000, Figure 22), although the proportion varies among segments. On the other hand, the proportion of empty sites Bay-wide is fairly consistent over time (25 – 30%; Figure 21). The proportion of sites with only Halodule (THS = 010) is inversely related to the proportion of empty sites (THS = 000) in several segments, namely Segments 13, 14, 16, DR, and LB (Figure 23). As can be seen in these segments, when Halodule frequency of occurrence decreased, the proportion of empty sites increased. In other segments, there is no evidence of changes in the overall compositions of the three species. The temporal changes of seagrass frequency of occurrence composition appeared to be due mainly to replacing Halodule sites with empty sites. This result is in keeping with the earlier conclusion that Halodule frequency of occurrence was decreasing in recent years – it appears that the Halodule is not being replaced with another species but instead the sea grass beds are either contracting areally or possibly thinning out.

Drift Algae

Overall, drift algae is increasing in frequency of occurrence over time (from ~37% in 2010 to ~70% in 2015; Figures 1 and 2) in all segments. The average frequency of occurrence of drift algae varies by segment with segments 10, 11 and DR having the lowest proportions (25 – 40%) and segments 14, 16 and LB having the highest proportions of frequency of occurrence (80 – 100%).

Cover also shows some evidence of an increase over time (Figure 4) but the trend depends on the segment in which data were collected. All segments show a trend except for segments 11 and 13 which do not show any temporal changes (Table 10). Overall though, cover is low (mean BB scores mostly less than 2; Table 9).

Frequency of occurrence of drift algae did depend on the specific combination of seagrass species, with higher frequency of occurrence associated with observing two or three species of sea grass (Figures 16 and 17; Table 41). This relationship did appear to depend on segment but
was likely due to the variability of sea grass compositions among segments. It is hard to know whether higher frequency of occurrence proportions for drift algae on multi-species sites is a function of the structural complexity of multiple seagrasses or to the combination of species at a site. Frequency of occurrence of drift algae was always above 50%, even on sites with no seagrass (Figure 17); hence, high values may be related solely to some physical process that entangles drift algae.

The cover of drift algae was found to be correlated with some of the water quality parameters measured regularly by Sarasota County Environmental Services. The variables most related to cover of drift algae (Tables 46 and 47) included chlorophyll a (r = –0.336, p-value<0.0001), dissolved oxygen (r = +0.327, p-value < 0.0001), and water temperature (r = –0.318, p-value<0.0001). Drift algae showed a slight negative correlation with cover of Thalassia (r = –0.118, p-value=0.0147) but was uncorrelated with cover of either Halodule (r = +0.065, p-value=0.1843), or Syringonium (r = +0.036, p-value=0.4639).

Epiphytes

Epiphyte frequency of occurrence is consistent in time (except for 2014) for most segments (Figures 5 and 6) but there is some evidence that the 2014 decline (Figure 5 and Table 14) is due to a decline in segments 14, 16, and LB (Table 15). Some segments have very high proportions of frequency of occurrence, for example segments 10 and 16 have average annual frequency of occurrence greater than 0.8 for all years, while others have lower values, for example segment 13 which has annual averages ranging from 0.3 to 0.6.

Cover of epiphytes has a general decreasing trend in time since 2012 (Figures 8 and 9; Tables 19 and 21) in all segments except DR. The lack of significant decline in DR is likely due to the large variability in mean values and the low sample sizes. Overall, the epiphyte abundances are higher than those for drift algae averaging ~1.75 prior to 2013 and decreasing to a low of 0.8 in 2014.

Presence of epiphytes was predicted to be high (> 80%) when at least one species of seagrass was observed (Tables 43 and 45; Figure 18). The particular combination of seagrasses did not change the expected frequency of occurrence of epiphytes.

The cover of epiphytes was found to be uncorrelated with the water quality parameters measured by Sarasota County Environmental Services. On the other hand, epiphytes showed positive correlations with cover of two of the three sea grass species (Thalassia: r = +0.330, p-value<0.0001; Halodule: r = +0.373, p-value<0.0001; Syringonium: r = +0.117, p-value=0.0162).
Sampling

Given the current small sample sizes within sub-segments and segments in Sarasota Bay, it is difficult to make any recommendations for decreasing the sampling effort. There are instances where it appears that there is a trend in time or differences among segments but the tests are not statistically significant. This could be due to lack of power or truly no differences.

In addition, it was requested that consideration be given to consolidating sub-segments within segments where they were similar in their characteristics. These characteristics range from temporal trends in individual biotic variables such as Thalassia cover to a cluster analysis to identify sub-segments that are similar in the means of the five biotic cover variables. Overall, the only segment that is eligible for consolidation is 14, where all the sub-segments could be combined. A quick review of the effect of reducing the sample size from the current 20 stations in the segment indicated that a sample size of 15 would be needed to observe changes in drift algae and epiphyte abundances. Hence, there is no advantage to a change in the current strategy.

Background

Currently, SWFWMD maps seagrass distribution in Sarasota Bay using photo-interpreted aerial photography. These are the main source for seagrass condition data used for nutrient and resource management decisions within Sarasota Bay. However, aerial imagery alone doesn’t address questions about more sensitive and insightful changes over time at a local scale that may likely be precursors to the larger changes in seagrass acreage observed in the aerial imagery. These changes include species composition, seagrass density, changes at the deep edges of grassbeds, and parameters of ecological health (epiphytes, macroalgae, etc.). These changes could also be related to water quality and environmental characteristics and so it is of interest that the relationship of the environmental parameters to sea grass measures be explored in so far as the data provide in order to provide more information about possible drivers of species composition and density.

Sarasota County has been collecting in situ data semi-annually for several years in order to obtain information that could be used to address these issues. There is a need though to analyze the data using appropriate modern statistical methodology so that the results can be properly communicated to managers in a prompt and timely manner. Analytical results can serve a variety of end uses, including: determining compliance with seagrass targets related to nutrient management and TMDLs, improving data collection methods to better serve the finer-
scale objectives, and developing new analytical approaches for detecting and quantifying impacts to managed resources.

This project capitalizes on the data collected in Sarasota County bay waters by County staff since 2009. These data have been collected using a combination of fixed and random sites sampled semi-annually although the analyses are focused on the data collected in the winter season (Jan – Mar). All sites were distributed evenly within the Sarasota County’s Ambient Water Quality Monitoring Program segments and sub-segments.

This project addresses two main areas of interest. The first relates to the spatial and temporal distribution of species composition, epiphytes, and drift algae. Here we consider year and segment as possible drivers of the observed variability. This approach does not answer fine scale questions at the level of individual sea grass beds but does provide fairly fine resolution of variability among the segments.

As part of the study of the temporal trends is the relationship of the sea grass metrics with environmental, water quality, and biological attributes that are available for the same dates as the sea grass data collection. Study of the relationships might give insight as to the underlying causes of any trends that are observed.

This approach allows us to also consider whether the current sampling design can be made more efficient. Initial answers to these questions can aid county staff in developing sampling efforts that can more clearly aid in effective monitoring of the seagrass community in Sarasota Bay. The approach will consider whether the stratification currently used can be modified to optimize the overall information by increasing the accuracy and precision of the results. A secondary result is that these results can also be used to determine whether the current sample sizes should be modified.

The result of the analyses will guide the second major aspect of the project, which is reviewing the sampling strategy for efficiency and precision. Here we will compare sub-segments within segments to determine if there are any differences in the means or temporal trends. If adjacent sub-segments do not differ, we will estimate the accuracy and precision of modified stratification schemes that combine segments/sub-segments based on similarities in the trends and means/abundances of the seagrass composition, epiphytes and drift algae. A set of recommendations based on this review will be developed.

The statistical analyses are:

1) trends in frequency of occurrence and cover of epiphytes and drift algae and whether the trends are segment-specific;
2) whether there is a relationship between type or presence/absence of seagrass and the frequency of occurrence of epiphytes/drift algae;
3) whether there are relationships among the environmental parameters, water quality metrics, biological parameters and presence/absence of sea grass species and species composition; and,
4) changes in seagrass species composition over time in various segments of the bay.

Data

Two datasets were provided for use in the statistical analyses. The first includes the sampling data for the seagrass survey conducted from 2008 through winter 2015. There are 8 segments in Sarasota Bay with 5 sub-segments each and until 2010 were sampled twice a year, in winter and in summer. Since 2011, the basic sampling strategy was to sample during a single season (winter) using the following strategy: a single fixed station within each segment which was visited every season or year plus an additional 3 or 4 randomly located stations within each sub-segment\(^2\). Prior to 2011, some sub-segments had up to 10 stations sampled in a season. Currently, the total number of stations at which seagrass is measured is 160 (Table 1).

The data collected at each site include presence/absence of seagrass, composition of the seagrass as percentage/species for the three main species found in the Bay, and Braun-Blanquet scores for epiphyte and drift algae cover (0 = not present,…, 5 = 100% coverage at the site). Additional information such as blade height, percent bottom covered, water temperature and sediment type were also collected. Percent species composition was not reliably reported prior to 2009 and so there is a shorter time period for composition than for presence/absence of seagrass. The data provided include only data collected from 2009 on.

The second dataset included monthly water quality data (Table 2) collected in the segments and sub-segments of Sarasota Bay. Some variables are weather indicators while others are water quality or condition. One station was sampled per month per sub-segment (Table 3) except for 2015 where only 2 months are available. Data were provided from 2008 through winter 2015.

All data were used as provided. No observations were removed except as indicated in the methods section.

\(^2\) Except for the segment DR which has several sub-segments that have only 1 station per season.
**Statistical Methods**

For all analyses except the correlation of environmental information with seagrass, only the winter season was analyzed due to the discontinuation of summer sampling after 2012\(^3\).

**Trends in Frequency of Occurrence and Cover of Epiphytes and Drift Algae**

Occupancy (i.e. presence or absence at a sample location) of drift algae and epiphytes were analyzed using generalized linear mixed models with a binary distribution for the presence/absence of algae or epiphytes, fixed effects of year and segment and their interaction, and a random effect for repeated observations at fixed stations within sub-segments within segments. A logit link was used. A \( \chi^2 \) test was conducted to determine if the random effect should be included in the model.

If the F-test for a fixed effect was statistically significant, pairwise comparisons among the levels of the effect were conducted. The Tukey-Kramer method for controlling the family-wise error rate was used. F-tests for differences among segments within each year as well as F-tests for differences among years within each segment were conducted if warranted.

To test cover of epiphytes or drift algae, we first calculated the mean and standard errors of the Braun-Blanquet scores for samples in each sub-segment, segment, and year combination. This approach provides 5 observations per segment per year for each variable.

First, a general linear model with year and segment was fitted to the drift algae means in order to check that the residuals were approximately normally distributed. Since the residuals were approximately normally distributed, weighted general linear models assuming normality with fixed effects of year, segment, and their interaction were fit to the drift algae means and the epiphyte means with weights equal to the inverse of the squared standard errors of the sample means used in the analyses. This weighted approach adjusts for the fact that we used means with unequal variances (due to unequal sample sizes) and not individual observations.

\(^3\) We tested for seasonal effects by fitting a general linear mixed model to the percentages of coverage of the three sea grasses and found that the year by season interaction was not significant, implying that there were no seasonal effects. We were required to use an interaction term since summer was not sampled in the later years. Similar analyses for the Braun-Blanquet scores for drift algae and epiphytes showed that in some years the mean for summer differed from the mean for winter but the statistically significant differences ranged from \(-0.4\) to \(+0.3\) implying that the effect of season was not consistent across years. Hence we focused on the winter data.
For all models the following tests were performed. F-tests for differences among segments within each year as well as F-tests for differences among years within each segment were conducted. In addition, if the F-test for a fixed effect was statistically significant, pairwise comparisons among the levels of the effect were conducted. The Tukey-Kramer method for controlling the family-wise error rate was used.

All tests were conducted using a Type I error rate of $\alpha = 0.05$.

**Individual Trends in Frequency of Occurrence of Sea Grasses**

Occupancies of each seagrass species, Thalassia, Halodule, and Syringonium, at sampled stations were analyzed separately using generalized linear mixed models with a binary distribution for the presence/absence of the species, fixed effects of year and segment and their interaction, and a random effect for repeated observations at fixed stations within sub-segments within segments. A logit link was used. A $\chi^2$ test was conducted to determine if the random effect should be included in the model.

If the F-test(s) for a fixed effect was statistically significant, pairwise comparisons among the levels of the effect were conducted. The Tukey-Kramer method for controlling the family-wise error rate was used. F-tests for differences among segments within each year as well as F-tests for differences among years within each segment were conducted if warranted. All tests were conducted using a Type I error rate of $\alpha = 0.05$.

These analyses do not address the relationships among the species but instead focus on a more simple question of whether the probability of encountering the species during sampling is changing in time. Relationships are addressed separately in a later section of this document.

**Relationship of Drift Algae and Epiphytes to Seagrasses**

In order to study the relationship of the epiphytes and drift algae to the seagrass compositions, a 3–digit code was constructed indicating presence or absence of each species at each sample location where each digit is either a 0 or 1 to indicate absence (0) or presence (1) of a seagrass species. The first digit is for Thalassia, the second for Halodule and the third for Syringonium. Hence, 011 indicates that Thalassia is absent at that site but both Halodule and Syringonium are present.

Occupancy of drift algae or epiphytes as a function of the composition of seagrass at a site (the 3–digit code) was analyzed using a generalized linear mixed with a binary distribution for the
presence/absence of algae or epiphytes, fixed effects of year and of segment interacted with
the 3–digit categorical variable denoting composition of seagrass (“PAseagrass”), and a random
effect for repeated observations at fixed stations within sub-segments within segments. A logit
link was used. The interaction term segment*PAseagrass was required as some compositions
of seagrass are not observed in some segments. Hence any effect of segment can only be
determined for specific combinations of PAseagrass. A χ^2 test was conducted to determine if
the random effect should be included in the model. The test failed to reject the null hypothesis
of no random effects for any model fitted and so it was removed from the analyses.

If the F-test(s) for a fixed effect was statistically significant, pairwise comparisons among the
levels of the effect were conducted. The Tukey-Kramer method for controlling the family-wise
error rate was used. F-tests for differences among segments within each year as well as F-tests
for differences among years within each segment were conducted if warranted. All tests were
conducted using a Type I error rate of α = 0.05.

Relationships among Seagrass Species

Several seagrass and epiphyte and drift algae variables were averaged within each sub-segment
within segment and year. The specific variables were percentage of Thalassia, percentage of
Halodule, percentage of Syringonium, Braun-Blanquet score for drift algae and Braun-Blanquet
score for epiphytes. In addition, the proportion of samples collected in each sub-segment
within each segment and year containing Thalassia (“PAThalassia”), Halodule (“PAHalodule”),
Syringonium (“PASyringonium”), epiphytes (“PAepiphytes”), and drift algae (“PAdrift”) were
calculated. For example, PAHalodule = 0.4 indicates that 40% of the sub-segment stations had
Halodule present. Pairwise Spearman rank-order correlations of these values were then
calculated in order to determine whether there were any associations among these variables.

In addition to the descriptive relationships, occupancy of seagrass at a site (the 3–digit code
THS) was modeled using a generalized linear model with a multinomial distribution and a
generalized logit link and with fixed effects of year and segment and their interaction. No
random effect was included as it was not significant. In addition, the interaction of year and
segment was removed as it also was not significant. This model was fitted in order to determine
if the composition of seagrass species at a site depended in the past on segment or year. Plots
of the predicted values were used to explore the relationships in time and within segment.
More formal tests were not conducted as the data are too sparse for rigorous analysis at this
time.

The areal percentages for each seagrass species are correlated since they are by definition the
area covered by the species within a quadrat and so are constrained to sum to 100% or less.
The data are not normally distributed and so are not easily analyzed. Further, classic methods for analysis of compositional data (cf. Aitchison\(^4\)) cannot be used here since in such methods all percentages must be greater than 0. As a result, we used plots of the mean areal percentage by segment and year for each species to confirm the relationships observed in the analyses of occupancy.

**Relationship of Seagrass Composition and Occupancy with Water Quality Parameters**

Here we used data from both summer and winter seasons in order to ensure a variety of values for the water quality parameters. The data for the seagrass samples within sub-segments within segments within month and year were averaged as described above in order to obtain a single value by sub-segment, segment, year and month. These derived quantities could be correlated with the single water quality and environmental sample collected within each sub-segment, segment, month and year. Pair-wise spearman rank-order correlations were performed between the seagrass and epiphyte and drift algae data (mean percentage of Thalassia, mean percentage of Halodule, mean percentage of Syringonium, mean Braun-Blanquet score for drift algae and mean Braun-Blanquet score for epiphytes; PAThalassia, PAHalodule, PASyringonium, PAepiphytes, and PAdrift) and the water quality parameters.

**Changes to the Sampling Strategy**

The result of the above analyses guide a review of the sampling strategy for efficiency and precision. These results will be used in combination with the following analyses to determine whether it is appropriate to combine sub-segments within segments.

We compared sub-segments within segments using general linear models to determine if there are any differences in the means or temporal trends. The general linear models were fitted to each of five variables, the three seagrass percentages and drift algae and epiphyte Braun-Blanquet scores, with sub-segment and year and their interaction as the fixed effects.

If the F-test(s) for a fixed effect was statistically significant, pairwise comparisons among the levels of the effect were conducted. The Tukey-Kramer method for controlling the family-wise error rate was used. F-tests for differences among segments within each year as well as F-tests

for differences among years within each segment were conducted if warranted. All tests were conducted using a Type I error rate of $\alpha = 0.05$.

An alternative approach that used the interrelationships among the five variables was also conducted in which the subsegment LSmeans within a segment were used in a cluster analysis. Ward’s method for clustering was used. If the summary statistics for the sub-segments tend to show clustering, it would indicate that these sub-segments can be combined into a single sub-segment.

Adjacent sub-segments were considered for combining based on similarities in the trends and means/abundances of the seagrass composition, epiphytes and drift algae.

If adjacent sub-segments are considered for combining, we compared the accuracy and precision of the modified stratification schemes that combine segments/sub-segments for estimating trend across years using smaller sample sizes. This was accomplished by randomly selecting a subset of the current sub-segments and running analyses of drift algae and epiphyte cover on the reduced dataset, testing for trend. We used all possible subsets of sub-segments to verify that the conclusions were not due to particular subset that was chosen.

Results

Trends in Frequency of Occurrence and Cover of Epiphytes and Drift Algae

Drift Algae

Frequency of Occurrence

Overall, year and segment were found to be statistically significant whereas the interaction of the two effects was not (Table 4). In general, the likelihood of observing drift algae at a sample site is increasing across years (Figure 1; Table 5). The lack of a significant interaction between segment and year indicates that the trend across years is consistent generally among segments (Figure 2).

Pair-wise tests of differences between pairs of years indicate that the mean for 2010 is less than all later years, the mean for 2011 is less than the mean for 2014, the mean for 2012 is less than all subsequent years, the mean for 2013 is less than the mean for 2014 and the mean for 2014 is less than the mean for 2015. The tests comparing the estimated mean for 2009 to those for the other years were not conducted since the number of observations in two of the segments (Table 1) were so low that the standard error for the annual mean probability of presence was excessively high.
The mean probability of presence of drift algae in a segment ranges from a low of approximately 0.25 (segments 11 and DR) to a high of 1 (segment 16) (Figure 3; Table 6). The results of pair-wise tests of differences between mean probability of presence in segments are listed in Table 7.

**Cover**

The tests of fixed effects show that the interaction of year and segment is statistically significant indicating that the cover of drift algae on a Braun-Blanquet scale of 0 to 5 in a given year depends on the segment (Table 8). The estimated mean Braun-Blanquet scores range from a low of 0 in segment DR in 2009 (based on a sample size of 1) to a high of 2.6 in segment LB in 2011 (Figure 4; Table 9). Tests for differences among annual mean cover within each segment (Table 10) indicate that the annual mean cover of drift algae is not the same across years for all segments except segment 11. Tests for differences among segment mean cover within each year (Table 11) indicate that the mean abundances of drift algae in segments differ in each year of the study except for 2012.

**Epiphytes**

**Frequency of Occurrence**

Overall, year and segment were found to be statistically significant whereas the interaction of the two effects was not (Table 12). There does not appear to be a pattern or trend in the likelihood of observing epiphytes at a site across years (Figure 5; Tables 13, 14). The mean probability of presence of epiphytes in a year ranges from a low of 0.5 (2014) to a high of 1 (2009 and 2013). The results of pair-wise tests of differences between mean probabilities of presence in segments are listed in Table 15. The tests comparing the estimated mean for 2009 to those for the other years should not be used since the number of observations in two of the segments (Table 1) were so low that the standard error for the annual mean probability of presence was excessively high.

The lack of a significant interaction between segment and year indicates that the variability across years generally is consistent among segments (Figure 6) but some segments appear more consistent in their mean probabilities across time of encountering epiphytes than others. This is borne out in the tests for differences among the yearly means within each segment (Table 15).

The mean probability of presence of epiphytes in a segment ranges from a low of approximately 0.40 (segment 13) to a high of 1 (segment 16) (Figure 7; Table 16). The results of pair-wise tests of differences between mean probabilities of presence in segments are listed in Table 17.
Cover
The tests of fixed effects show that the interaction of year and segment is statistically significant indicating that the cover of epiphytes on a scale of 0 to 5 in a given year depends on the segment (Table 18). The estimated mean Braun-Blanquet scores range from a low of 0 in segment DR in 2009 (based on a sample size of 1) to a high of 2.6 in segment LB in 2011 (Figure 8). Tests for differences among annual mean cover within each segment (Table 19) indicate that annual mean cover of epiphytes differs across years for all segments except segment DR. Tests for differences among segment mean cover within each year (Table 20) indicate that the mean abundances of epiphytes in segments differ in each year of the study except for 2012.

Review of Figure 8 indicates that there is a trend in annual mean cover of epiphytes that is also observable in the plot of the main effect annual mean abundances (Figure 9; Table 21). In general, there is no evidence that the means for 2009 – 2012 differ among themselves but do differ from the more recent years. The mean cover for 2014 differs from those for 2013 and 2015 but the mean for 2013 does not differ from that for 2015.

Individual Trends in Frequency of Occurrence of Sea Grasses
Thalassia
The interaction of year and segment is statistically significant indicating that the probability of encountering Thalassia in a given year depends on the segment (Table 22). The estimated mean probability of presence of Thalassia is high in two of the segments in all years (segments 10 and US), is low in many segments in all years (segments 13, 14, 16, DR, and LB), and variable in segment 11 (Figure 10). This is also highlighted in the main effect of segment (Figure 11). Pairwise comparisons among segment mean probabilities (Table 23) indicates that segments 10, 11 and US have high probabilities for Thalassia presence that are significantly larger than the means for all other segments except DR. The latter is due to the large SE associated with the DR mean due to the small sample sizes in DR. The other segments have much lower probabilities of observing Thalassia but do differ statistically significantly (Table 23).

Although the main effect of year is statistically significant, the annual mean probability of presence is relatively low varying between 0 and 0.12 (Table 24) with no evidence of a general trend. Given the extremely small estimated probabilities for all years except 2010 and 2013, the only useful test is the one comparing the mean for 2010 to 2013. This test fails to reject the null hypothesis of no difference (adjusted p = 0.9998).
Halodule

The tests of fixed effects show that neither the interaction of year and segment nor the main effect of year is statistically significant indicating that the probability of encountering Halodule does not vary across years (Table 25a; Figure 12). When the model is run without the interaction effect though, year does become significant (Table 25b).

The statistically significant main effect of segment indicates that the mean probability does vary across segments (Figure 13a; Table 26). In fact, the estimated mean probability of presence of Halodule is low in those segments where Thalassia has a high probability of being present (segments 10, 11, and US) and is high in the other segments except for segment 13. Pairwise comparisons among segment mean probabilities (Table 27) indicates that the estimated probabilities do not differ statistically among segments 10, 11, 13 and US but do differ from the other segments.

The statistically significant main effect of year indicates that the mean probability does vary across years (Figure 13b; Table 28) with higher estimated probabilities in earlier years of the study. On the other hand, the mean probability of presence of Halodule ranged around 0.55 for 2009 through 2012 and dropped to approximately 0.45 in more recent years. As a result, pairwise comparisons did not indicate any statistically significant differences between years in the study (Table 29).

Syringonium

The tests of fixed effects show that neither the interaction of year and segment nor the main effect of year is statistically significant indicating that the probability of encountering Syringonium does not vary across years (Table 30a). When the model is run without the interaction effect though, year does become significant (Table 30b).

The statistically significant main effect of segment indicates that the mean probability does vary across segments (Figure 14; Table 31). In fact, the estimated mean probability of presence of Syringonium is never very high being highest in segments 11 and US at approximately 0.16. It is much lower otherwise. Pairwise comparisons among segment mean probabilities (Table 32) indicates that the estimated probabilities do not differ statistically among almost all of the segments except when compared to segments 11 and US.

The statistically significant main effect of year indicates that the mean probability does vary across years (Figure 15; Table 33) with higher estimated probabilities in more recent years of the study. On the other hand, the mean probability of presence of Syringonium ranged from 0.01 to 0.08. The pairwise comparisons indicate statistically significant differences between the
earlier years of the study when compared to the mean probabilities in years 2013 to 2015 (Table 34).

**Relationship of Drift Algae and Epiphytes to Seagrasses**

**Drift Algae**

Before fitting any models, a frequency table of presence absence of drift algae versus composition of seagrass was constructed (Table 35). There does appear to be some variation in the presence of drift algae among the various combinations of seagrass compositions but this table does not account for year or segment. As can be seen in the row totals, seagrass composition varies strongly. Also, a frequency table showing the number of observations by segment and presence/absence of three seagrasses indicates that the composition of seagrasses is itself dependent on segment (Table 36). For that reason all models used the interaction of segment and seagrass composition as a fixed effect. This allows us to test whether the relationship of drift algae to seagrass composition depends on where in the Bay the sampling occurred.

The model with year and the interaction of segment and composition of seagrass indicated that the probability of presence of drift algae depended on year and on the combination of segment and seagrass composition (Table 37). Since the year effect was discussed earlier, it is not repeated here; the inclusion of year was to remove a known source of variability. The effect of segment*PAseagrass can be seen in Figure 16. The tests of differences among segments within each type of seagrass composition (Table 38) indicates that differences in the probability of presence of drift algae among segments was statistically significant for the levels of no seagrass (PAseagrass = 0 0 0), Halodule only (PAseagrass = 0 1 0), and Thalassia and Halodule observed (PAseagrass = 1 1 0). Pairwise comparisons for these levels are given in Table 38. Segments 14 and 16 have high levels of estimated probability (0.8 and above) of drift algae being present for all combinations of seagrass species whereas segments 10 and 11 have lower values (Table 39; Figure 16).

The tests of differences among PAseagrass within each segment were statistically significant for segments 10 and US (Table 40). Pairwise comparisons for these levels are given in Table 41.

---

5 Occasionally an estimated probability is either 0 or 1 for a level of the fixed effect being tested. When that occurs, the estimate variance of the estimator is unusually large due to the estimation of a value on the boundary of possible values. Hence, pairwise tests of these estimates versus others for other levels of the effect fail to reject the null hypothesis of no difference due to the large variance. Clearly, these levels usually are different but the tests cannot be relied on to show that.
Because the segments generally did not differ very much (Table 39), the model with just PAseagrass as an effect was also run. The effect was statistically significant (Figure 17) and pairwise comparisons indicated that mean probability of presence of drift algae for the case with no seagrass present (PAseagrass = 0 0 0) differed from the case with Halodule present (PAseagrass = 010) and when Thalassia and Halodule are both present (PAseagrass = 110) (Table 42). The overall conclusion though is that generally having more than one species of seagrass at a site is associated with a higher probability of drift algae being present.

Epiphytes

Before fitting any models, a frequency table of presence absence of drift algae versus composition of seagrass was constructed (Table 43). There appears to be almost complete separation of presence or absence of drift algae for those sites with 1) no seagrasses (PAseagrass = 0 0 0) with almost all sites without epiphytes and 2) with all seagrasses present (PAseagrass = 111) where almost all sites have epiphytes. This is of course not surprising given the definition of epiphyte.

The model for probability of presence of epiphytes with fixed effects of year and the interaction of segment and composition of seagrass indicated that the probability depended on year but not on the combination of segment and seagrass composition (Table 44a). A model with just PAseagrass and year was fitted and both effects were found to be significant (Table 44b). the effect of seagrass composition can be seen in Figure 18. Since the year effect was discussed earlier, it is not discussed here. Pairwise comparisons of probabilities of epiphytes being observed among different compositions of seagrass are listed in Table 45. As can be seen, the main difference is that the probability of observing epiphytes is close to 0 for the case of no seagrass and very high (above 0.8) for all other levels of seagrass composition. The pairwise tests indicate that only PAseagrass = 0 0 0 differs from the other levels. No other pair is significantly different in the estimated probability.

Relationships among Seagrass Species

Seagrasses, epiphyte and drift algae abundances are highly positively correlated with their occupancy (presence/absence; Table 46) indicating that there is a higher proportion of samples with the taxon present when there is a higher areal percentage/quadrat of the taxon.

For other relationships we see that Thalassia cover is positively correlated with epiphytes, negatively correlated with Halodule cover, and more-or-less uncorrelated with Syringonium cover. Syringonium cover though does not seem to be strongly correlated with any other taxon. Finally, drift algae is uncorrelated with all other seagrasses and epiphytes.
Another way of viewing the relationships among the three seagrass species is a ternary plot (Figure 19) that shows the observed composition in areal percentages at each sample location. Plots for each segment are shown in Figure 20. As can be seen, the composition, as expected, varies among segments. Many of the segments contain combinations of two of the species, except for segments DR and US which have some samples with all three species.

The multinomial model for the 3-digit categorical variable of presence indicated that both year and segment were statistically significant (P < 0.0001 for both factors). The interaction could not be tested with main effects in the model due to some segments with missing combinations of seagrass presence. In order to look at the year effect a contingency table analysis was performed assuming that all observations were independent (the fixed stations are a small portion of the overall sample sizes and the random effect of fixed within segment was not statistically significant). There is no clear trend in the proportions of the different compositions of seagrass occupancy over time (Figure 21). On the other hand, the proportion of samples with Halodule alone (PA occupancy = 0 1 0) was higher in 2010 – 2013 than the other years sampled with a difference of 35% vs 26% on average between the 2 time periods.

In order to look at the segment effect another contingency table analysis was performed. There are very obvious differences among segments as expected (Figure 22). Segments 14, 16, and LB have the largest proportion of samples with Halodule alone (PA occupancy = 0 1 0) whereas segments 13, DR and US had a plurality or majority of samples with no seagrass (PA occupancy = 0 0 0). The remaining segments had various combinations of occupancy of 2 or more seagrasses.

Although the interaction of year and segment could not be tested, we plotted the estimated proportions from the multinomial model in order to show the variability within segment and year (Figure 23). As can be seen, in most of the segments two combinations tend to be linked such that as one combination decreases another combination increases. For example, in segments 13 and 14, presence of Halodule (0 1 0) changes inversely to the proportion of empty quadrats (0 0 0). These results are not statistically significant but do show the trends that should be monitored.

Since there is a strong correlation between the presence/absence of seagrass species and the areal percentages of the same species, we also plotted the mean areal percentages of each species by segment and year (Figure 24). Except for segment 10, the patterns in annual mean areal percentages of all three species trends similarly to the trends seen in the percentages of occupancy of each species. In segment ten. Halodule and Syringonium tend to trend in opposite directions with one increasing and the other decreasing. The Spearman correlation for these two species is −0.28 over all observations but this correlation is most obvious in segment 10.
Relationship of Seagrass Composition and Occupancy with Water Quality Parameters

Spearman correlations between the seagrass variables and the water quality variables indicate that *P. breve* is correlated with all three seagrass species and drift algae (Table 47). Interestingly, the relationship is positive for *Halodule* and drift algae cover metrics but negatively correlated for *Thalassia*, *Syringonium*, and Epiphyte cover metrics. Salinity, depth, nitrates, phosphorus, color and DO are other parameters that are correlated to varying degrees with the biotic variables.

Sampling Effects

The current sampling design is based on annual sampling of strata created by dividing the Bay into 8 segments which are then each subdivided into 5 sub-segments. Each of these is sampled annually at approximately 4-5 locations/sub-segment (see Table 1) except for segment DR which has a single observation within sub-segments 2 – 5, inclusive.

The results of the mixed models for effect of sub-segment, year, and their interaction are listed in Table 48. The means for each of the five variables by sub-segment within each segment are plotted in Figure 25; the results of the cluster analyses are shown in Figure 26. A summary of the findings for each segment are provided in Table 49.

There are two possible approaches to determining whether the sampling design should be modified. First, is whether to change the stratification while keeping the same sample sizes. That implies changing the current set of sub-segments which spread the sampling areally to a smaller set of sub-segments where the samples would be selected at random but with no guarantee that the samples would be spread over the entire area of the combined sub-segments. Based on the results in Figure 25 ad Table 49, this is not a recommended approach.

The second approach would be to combine sub-segments and sample randomly at a reduced rate within the sub-segment. An example of this would be to say combine sub-segments 1 and 2 with a current total of 10 sampling locations and instead sample this new sub-segment with a sample size of 5 locations. In consideration of this, a cluster analysis was performed to determine if the segments were sufficiently similar in abundances of the five biotic species that they could be combined. Only three segments (10, 14, and 16) showed potential for sub-segment consolidation.

A possible recommendation for Segment 10 would be to combine sub-segments 1 with 2 and 3 and combine sub-segments 4 and 5 (Figures 25 and 26) but there are several reasons to not do this. First, there is evidence that sub-segment 1 differs from sub-segments 2 and 3 and sub-
segment 4 differs from 5 (Table 50) in Syringonium cover. Hence a more reasonable approach would be to combine sub-segments 2 and 3 in Segment 10. This is not recommended either because there is some evidence that the two sub-segments have different temporal trends for two of the biotic variables, Thalassia and Halodule cover (Figure 27).

For Segment 16, possible consolidations include sub-segment 1 with 5 and sub-segment 2 with 3, and 4 (Figures 25 and 26). Since sub-segments 1 and 5 are not adjacent, we did not consider it further. For the other possible cluster, sub-segment 2 should not be combined with sub-segments 3 and 4 due to the statistically significant differences in epiphyte cover (Table 51). We do not recommend combining sub-segments 3 and 4 either due to the large differences in Thalassia cover means (Table 51) and differences in time in Syringonium means (Figure 28).

Perhaps the sub-segments in Segment 14 can be combined where the variation among sub-segments is quite small (Figure 25) and the temporal changes of the biota abundances are consistent among sub-segments (Figure 26). The current number of stations in Segment 14 is 20 spread over 5 sub-segments. A simple set of comparisons of the results of non-parametric testing of drift algae and epiphyte abundances using reduced number of samples indicated that in order to find statistically significant changes in the annual means of drift algae and epiphyte cover, a sample size of at least 15 is required. Hence there is very little advantage to reducing the number of stations in Segment 14.

The overall conclusion is that the current sampling design should not be modified to reduce the amount of sampling effort in any of the segments. There is evidence that there are spatially local differences in the temporal trends in the sea grass beds and as a consequence, some of the fine scale temporal responses would not be observed if the design is modified.
Statistical Analysis of Sarasota Bay Seagrass Programs: Detecting and Quantifying Change

Figures

Submitted by: Mary C. Christman, MCC Statistical Consulting, Gainesville, FL 32605
marycchristman@gmail.com

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![Figure 3](image)

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![Figure 4](image)
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![Graph showing estimated probability of observing epiphytes by segment.](image)

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Figure 23. Predicted percentages of occupancy for each combination of seagrass by year for each segment based on a multinomial logistic model using the winter data.
Figure 23 continued.

SEGMENT=13

Predicted Percent

0 10 20 30 40 50 60 70 80 90 100

Year


PREDICTED PROBABILITY OF PA SEAGRASS BY SEGMENT AND YEAR

SEGMENT=14

Predicted Percent

0 10 20 30 40 50 60 70 80 90 100

Year


PREDICTED PROBABILITY OF PA SEAGRASS BY SEGMENT AND YEAR
Figure 23 continued.

SEGMENT=16

Predicted Percent


Year

SEGMENT=DR

Predicted Percent


Year

Level

0 0 0
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0 1 0
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Figure 23 continued.

**SEGMENT=LB**

Predicted Percent

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<th>2013</th>
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**SEGMENT=US**

Predicted Percent

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<th>2011</th>
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<th>2015</th>
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<tbody>
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<td>0 1 1</td>
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</tbody>
</table>
Figure 24. Observed mean areal percentages (cover) of each seagrass species by segment and year. Blue line = Thalassia; Red = Halodule; Green = Syringonium.
Figure 24 continued.

OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR
SEGMENT=11

OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR
SEGMENT=13
Figure 24 continued.

OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR
SEGMENT=14

OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR
SEGMENT=16
Figure 24 continued.

**OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR**

**SEGMENT=DR**

**OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR**

**SEGMENT=LB**
Figure 24 continued.

**OBSERVED MEAN PERCENTAGE OF EACH SEAGRASS BY SEGMENT AND YEAR**
SEGMENT=US

The figure shows the observed mean percentage of each seagrass by segment and year from 2009 to 2015. The x-axis represents the years, and the y-axis represents the observed mean percentage. The data indicates fluctuations in seagrass abundance across the years.
Figure 25. Plots of the least squares means for each of the five variables by subsegment ID within each segment.
Figure 25 continued.

![Graphs showing the distribution of Thalassia, Halodule, Syringonium, Drift Algae, and Epiphytes for segments 11 and 13.](image-url)
Figure 25 continued.

**segment = 14**

![Graphs showing data for Thalassia, Halodule, Syringonium, Drift Algae, and Epiphytes for Subsegment ID 1 to 5.](image)

**segment = 16**

![Graphs showing data for Thalassia, Halodule, Syringonium, Drift Algae, and Epiphytes for Subsegment ID -2 to 5.](image)
Figure 25 continued.

**segment = DR**

Graphs showing the distribution of Thalassia, Halodule, and Syringonium across subsegments ID 1 to 5.

**segment = LB**

Graphs showing the distribution of Drift Algae and Epiphytes across subsegments ID 1 to 5.
Figure 26. Constellation plots showing relationships among subsegments based on the cluster analyses for each segment. Black dot with a circle is the center of the clusters; each cluster and subcluster is identified by a black dot.
Figure 27. Plots of LSmeans of cover of the 5 biotic variables by subsegment (numbered 1 to 5) and year for Segment 10 in Sarasota Bay.
Figure 27 continued (Segment 10).
Figure 28. Plots of LSmeans of cover of the 5 biotic variables by subsegment (numbered 1 to 5) and year for Segment 16 in Sarasota Bay.
Figure 28 continued (Segment 16).
Figure 29. Plots of LSmeans of cover of the 5 biotic variables by subsegment (numbered 1 to 5) and year for Segment 14 in Sarasota Bay.
Figure 29 continued (Segment 14).