Technical Memorandum:

To: Sarasota Bay Estuary Program

From: Janicki Environmental, Inc.

Date: January 07, 2019

Subject: Final Report: NNC for Sarasota Bay Estuarine Nutrient Region

Background:

The Sarasota Bay Estuarine Nutrient Region (ENR: Figure 1) extends from the Manatee Avenue Bridge in Manatee County to the Siesta Key Bridge in Sarasota County. The Florida Department of Environmental Protection (FDEP) defined the bounds of this ENR as part of their work to develop numeric nutrient criteria (NNC) for Florida estuaries. The NNC for Sarasota Bay were developed based on a "Reference Period" approach using the 2001 -2005 time frame as a period of time when seagrasses, a key ecological indicator and biological response endpoint identified for protection in the estuary, were stable and near their historical acreage. Phytoplankton abundance, measured as chlorophyll a concentrations, was identified as the key limiting water quality parameter for seagrasses and the overall average of annual averages for the 2001-2005 was identified as a target concentration (5.3 ug/l) . A threshold chlorophyll concentration of 6.1 ug/l was identified by adding one standard deviation to the target concentration (Janicki Environmental 2011).

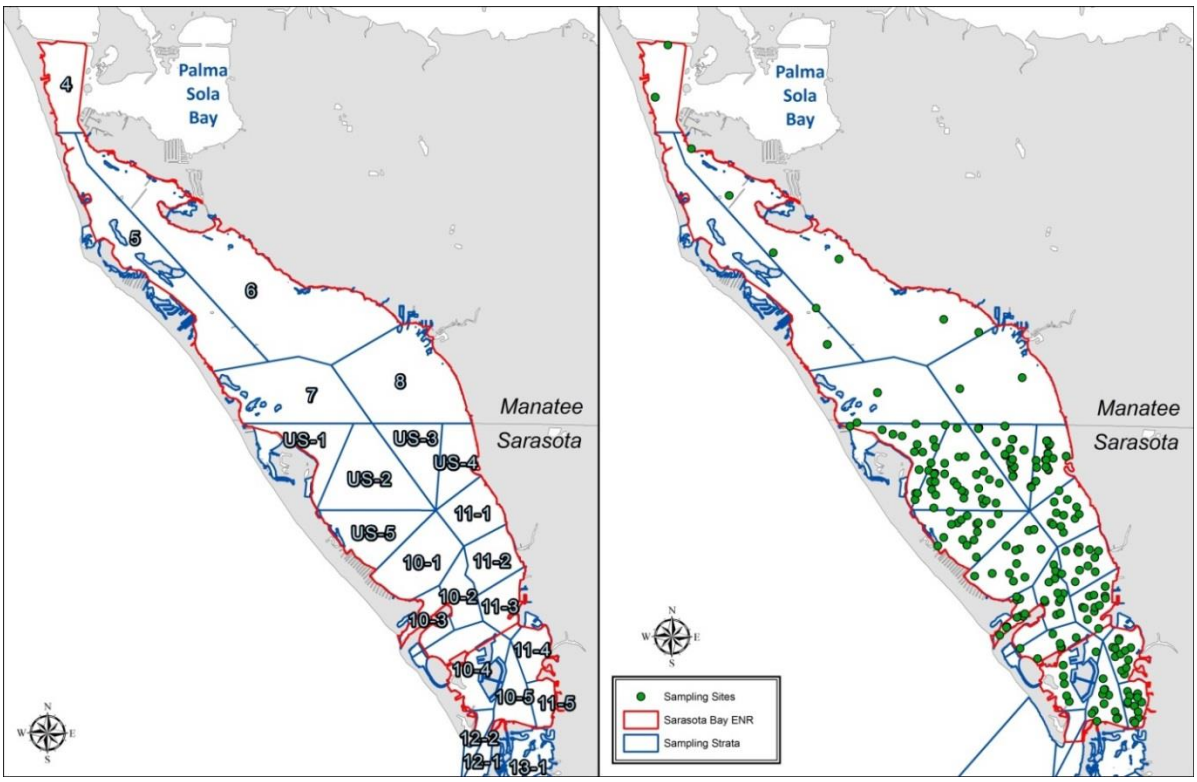


Figure 1. Location of the Sarasota Bay Estuarine Nutrient Region showing the sampling segmentation scheme (left) and the sampling points (right).

Current NNC Assessment Method:

The current NNC for this ENR is expressed in rule 62.302.532 (3)(i) FAC as the sum of a set of mathematical equations based on the results of regression analysis. The paragraph of the rule is excerpted and provided in Table 1 below for reference. The regression relationship was developed to predict the TN concentration that would result in a chlorophyll threshold exceedence based on monthly data average by region (County). The regression predicted chlorophyll concentrations as a function of TN concentrations as well as covariates including color (pcu), season, and region. Based on the regression, and this re arrangement of the regression, the threshold annual geometric mean (AGM) TN (i.e. the NNC) for a particular year is dependent solely on the color concentrations collected for that year.

Table 1. Florida Administrative code 62.302.530(3)i) describing calculation of Sarasota Bay total nitrogen NNC.

(i) Sarasota Bay	For TN, the annual geometric mean target is calculated from monthly arithmetic mean color by region and season. Annual geometric means that shall not be exceeded more than once in a three year period. The Sarasota Bay regions are defined as north (Manatee County) and south (Sarasota County). The wet season for Sarasota Bay is defined as July through October and the dry season is defined as all other months of the year. The seasonal region targets are calculated using monthly color data and shall be calculated as follows: NW_i=Ln[(13.35-(0.32*CN_i))/3.58] ND_i=Ln[(10.39-(0.32*CN_i))/3.58] SW_i=Ln[(8.51-(0.32*CS_i,)/3.58] SD_i=Ln[(5.55-(0.32*CS_i))/3.58] Where, NW_i is the TN target for i^{th} month calculated for the north region during the wet season ND_i is the TN target for i^{th} month calculated for the north region during the dry season SW_i is the TN target for i^{th} month calculated for the south region during the wet season SD_i is the TN target for i^{th} month calculated for the south region during the dry season CN_i is the arithmetic mean color during the i^{th} month within the north region CS_i is the arithmetic mean color during the i^{th} month within the south region The annual TN target is calculated as the geometric mean of all monthly regional and season targets as follows: $\underline{e^{\Sigma_i^{12}\left(\frac{NWi+NDi+SWi+SDi}{24}\right)}}$ Nutrient and nutrient response values do not apply to tidally influenced areas that fluctuate between predominantly marine and predominantly fresh waters during typical climatic and hydrologic conditions.

Implementation of the NNC for Sarasota Bay has been confounded by the fact that the method by which Manatee County has measured Color has changed over time from Visual Color method, to Apparent Spectrophotometric, to True Spectrophotometric while Sarasota Region has consistently reported Visual Color. The effects of these changes in method is visible in the time series plot of Figure 2 where

Manatee County reported Color estimates have varied quite dramatically, especially between 2006 and 2012, while Sarasota County reported Color has been stable over time (a detailed comparison of these data can be found in Dixon and Wessel 2017). The changes in method in Manatee County have yielded Color values that are outside of the range of data used to develop the regression. For example, when Color values are greater than 40 pcu in the North (Manatee County) region during the wet season, the product inside the parentheses results in a negative number which results in an invalid final product since the natural log of a negative value is undefined. This can also happen at lower Color values during the dry season; as low as 17.4 pcu in the South (Sarasota County) region during the dry season. These artifacts of the multiple regression approach have affected the implementation of the rule and resulted in annual TN NNC thresholds that range from 0.50 mg/l to 1.4 mg/l once averaged across regions. Prior to averaging, the Manatee County region varied even more due to the larger variation in Color resulting in a range between 0.28 mg/l and 2.5 mg/l. Both the FDEP and the SBEP have found implementation of the criteria for Sarasota Bay to be problematic in that it is alternatively either over, or under protective and therefore worthy of more investigation. Meanwhile, the seagrass communities in Sarasota Bay are stable and increasing in areal extent since the reference period of 2001-2005.

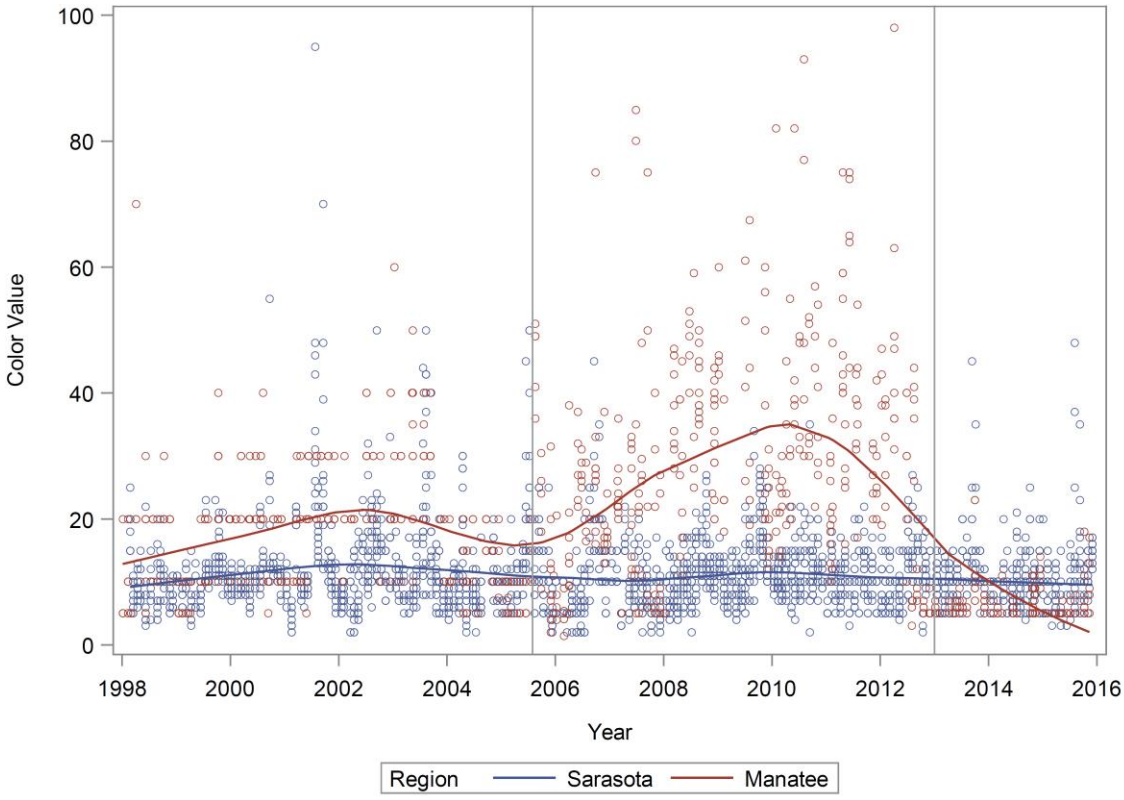


Figure 2. Timeseries plot of Color concentrations reported from Manatee and Sarasota counties.

Current Estuarine Conditions:

The areal extent of seagrass in Sarasota Bay has increased ca. 40% since the reference period (Figure 3). Additional analysis was conducted to investigate the potential for any spatially explicit location within

the larger area to have experienced seagrass decline. The areal extent of the 2004 seagrass coverage was compared to all coverages since 2004 (Figure 4) and revealed there was no evidence of seagrass losses in any location with significant areal coverage since 2004 and many areas of gain in seagrass areal extent throughout the ENR.

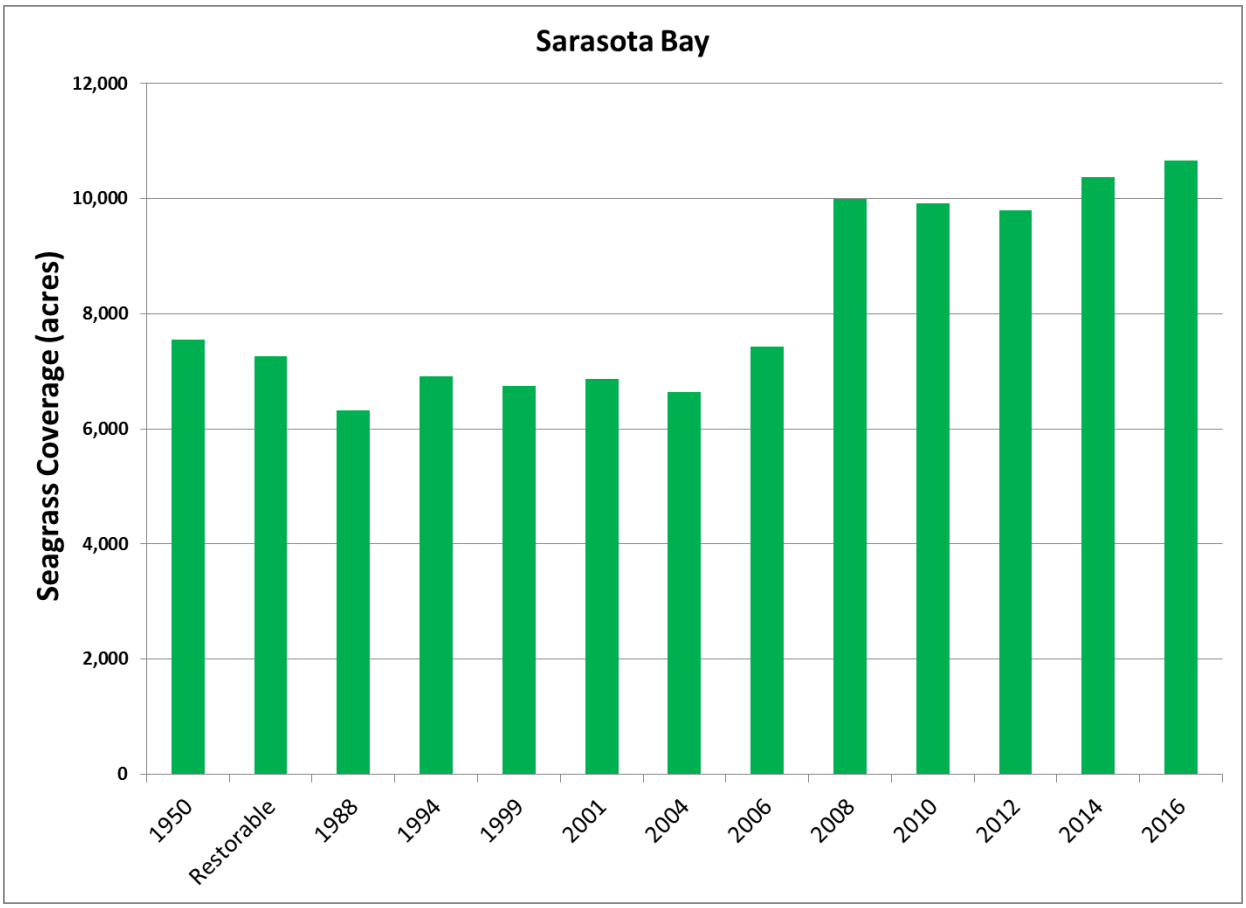


Figure 3. areal extent of seagrass in the Sarasota Bay Estuarine Nutrient Region.

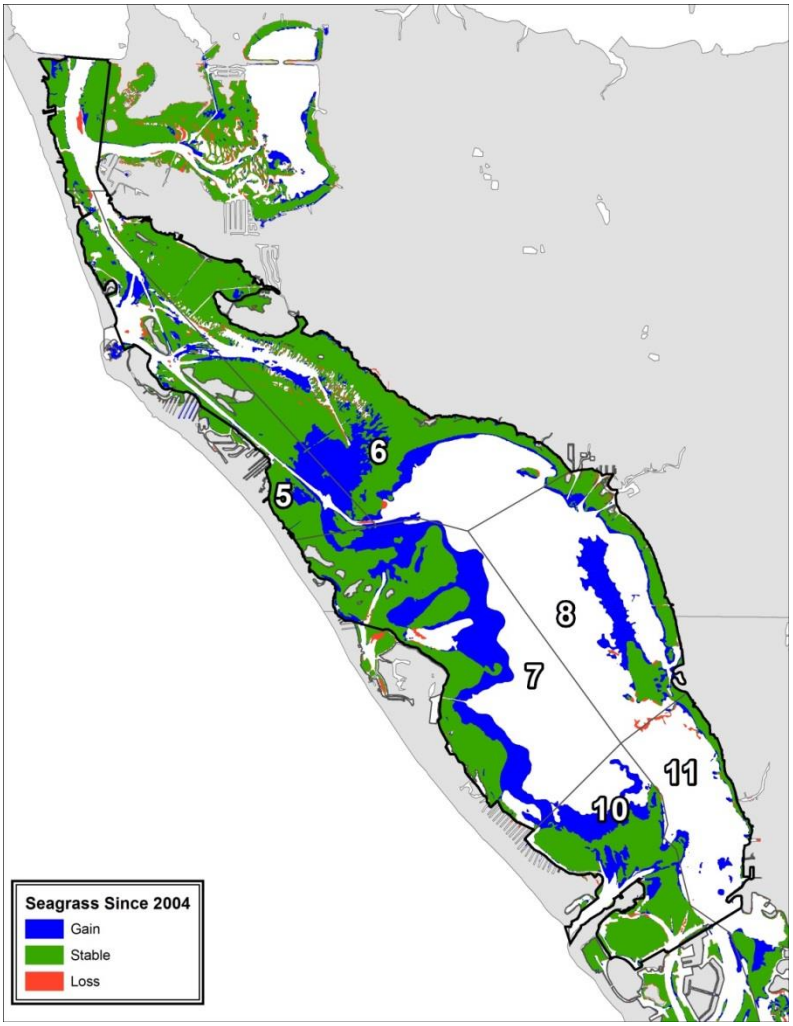


Figure 4. Comparison of 2004 (Left) and 2006(right) seagrass coverage with all subsequent coverages to evaluate areas of loss and gain.

Annual geometric average TN and Chlorophyll concentrations were calculated by taking monthly regional averages, natural log transforming those values, calculating the mean (either by region [Figure 5] or across regions [Figure 6]) and exponentiating to derive the AGM. Annual geometric average TN concentrations have been increasing over time in the Sarasota County portion of the ENR from ca. 2 mg/l in 1998 to ca. 4 mg/l in 2015 (Figure 5). Over the same time period, TN concentrations in the Manatee County portion of the ENR have been decreasing from ca. 0.7 mg/l in the late 1990's to ca. 4 mg/l in 2015. The combination of increasing trends in TN in Sarasota and decreasing trends in TN in Manatee has resulted in stable (i.e. non trending) AGM TN concentrations when combining these two regions of the Sarasota Bay ENR (Figure 6). Chlorophyll concentrations over the same time period have decreased slightly in Manatee County and have remained relatively stable in Sarasota County (Figure 7).

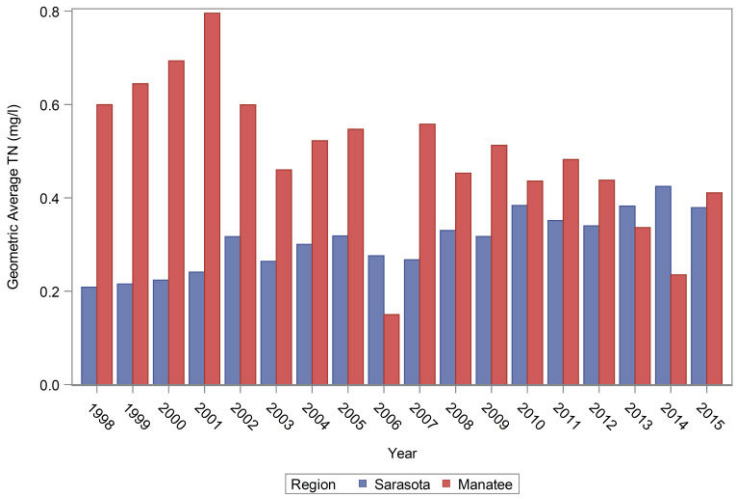


Figure 5. Annual geometric mean total nitrogen concentrations for Sarasota and Manatee Counties.

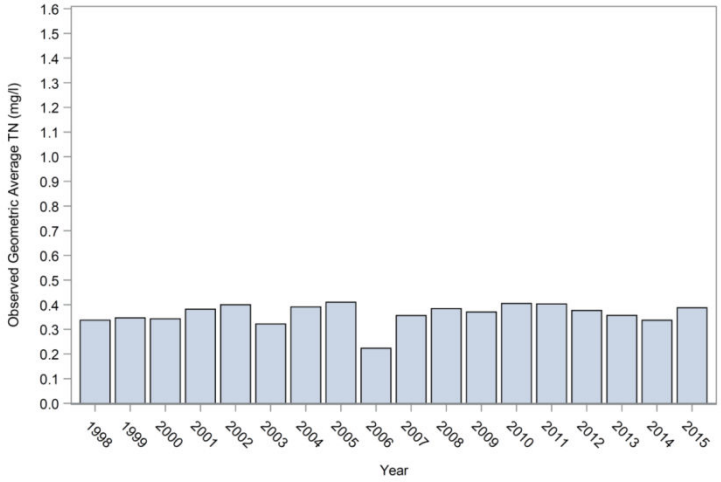


Figure 6. Annual geometric mean total nitrogen concentrations for the entire Sarasota Bay estuarine nutrient region.

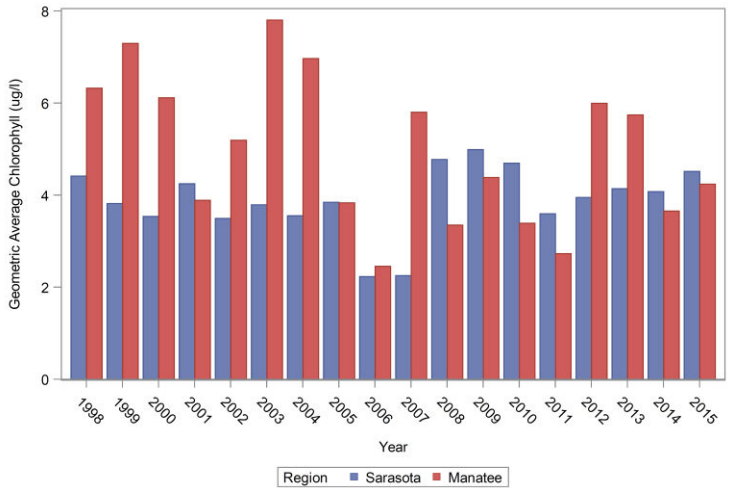


Figure 7. Annual geometric mean chlorophyll a concentrations for Sarasota and Manatee Counties.

Revised Regression Approach:

A master database was created using the FDEP's Impaired Water Rule (Run 53) for Manatee County data and Sarasota County's vender (Mote Marine Laboratory) for Sarasota County data. Data were available through June 2016 and subset to data between 1998 and 2015. This resulted in an additional 6 full years of sampling relative to the database used for the original regression analysis. While the original regression was based on monthly averaged data, the revised approach considered annual geometric average concentrations for the following reasons:

- The original chlorophyll target and threshold were based on annual averages over reference period (2001-2005)

- Implementation and evaluation of the NNC are based on annual averages (AGMs)

- While, the number of years remain low in terms of number of observations required for regressions analysis, there are several strata and many segments within the ENR from which individual AGMs can be calculated.

Manatee County samples approximately quarterly while Sarasota County samples monthly. To more evenly match the number of samples used to calculate the annual geometric means, the multiple fixed stations within a segment in Manatee County were combined. In addition, the 2 sites in Segment 4 at the very north end of the ENR were removed for regression analysis due to their close proximity and influence of Palma Sola Bay and Anna Maria Sound. While these sites had little influence on the overall AGMs for the ENR, the data points negatively affected the residual diagnostics of the regression analysis. The final dataset had 324 annual geometric means for the time period between 1998 and 2015. A plot of the annual geometric mean TN and Chlorophyll relationship by segment is provided in Figure 8. The horizontal reference line in the plot is the chlorophyll threshold value expressed as an AGM based on the same methodology as the original work. The vertical reference line in the plot represents current TN AGM conditions in the Sarasota Bay ENR. It is clear from the plots that most segments display an increasing trend in AGM chlorophyll as AGM TN increases but the sample size within any individual segment is small. Segments were grouped by strata and a linear mixed effects regression model (Littell et al. 1996) was used to assess the relationship between AGM TN and AGM chlorophyll as a hierarchical relationship. That is, the regression models the relationship between TN and chlorophyll while allowing for separate intercepts for each strata and modeling the covariance that exists among segments. The regression formulation is expressed in equation 1.

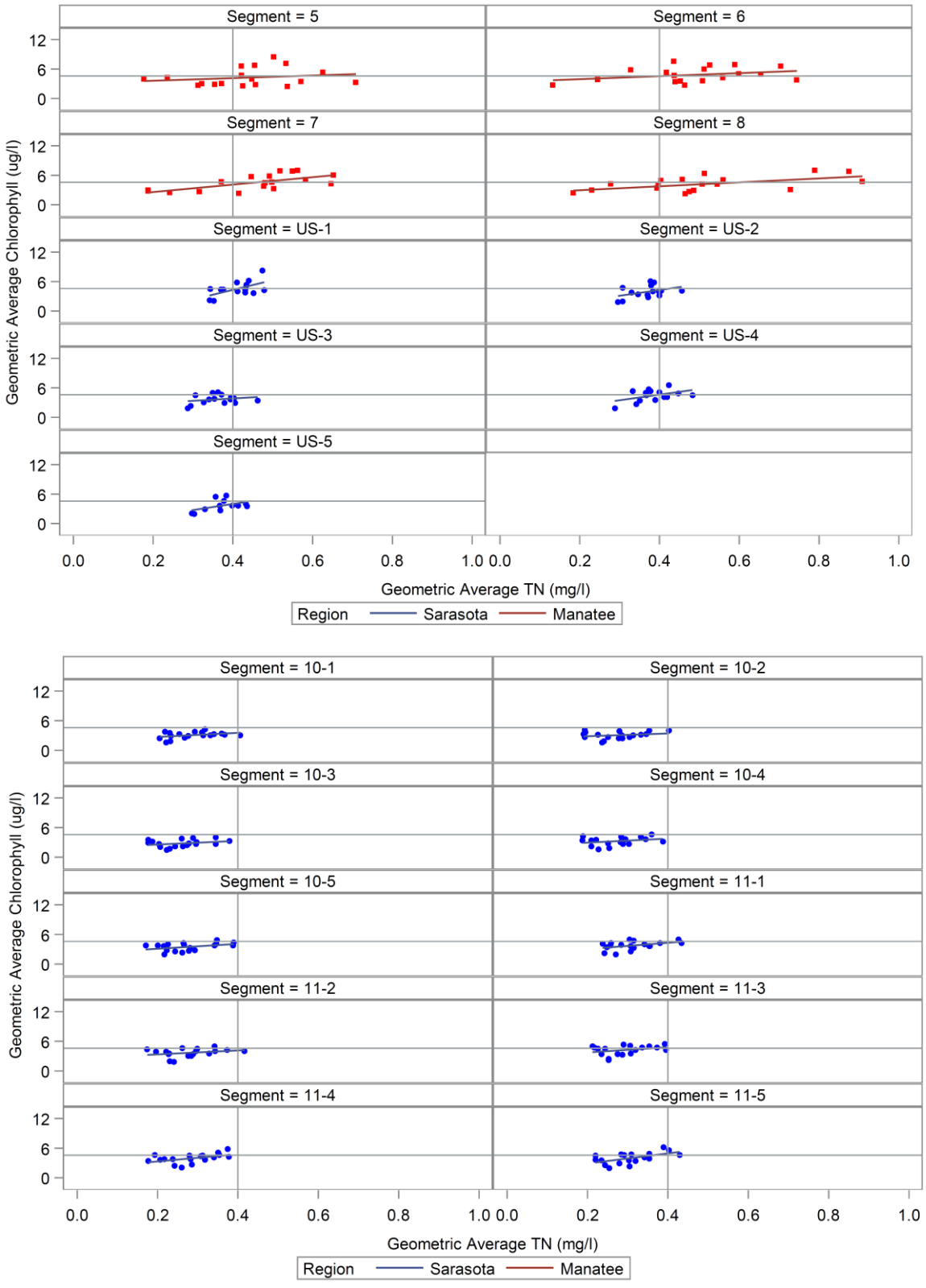


Figure 8. Segment specific relationship between annual geometric mean total nitrogen (TN) and chlorophyll in the Sarasota Bay ENR.

$$Y_{ijk} = \alpha + \alpha_j + B_1 * TN_{ijk} + e_{ijk} + e_k$$

Where:

Y_{ijk} = AGM chlorophyll for each year (i), strata (j), and segment (k)

TN_{ijk} = AGM total nitrogen for each year, strata, and segment

α = overall intercept

α_j = random intercept for strata

B_1 = Deterministic effect of TN on chlorophyll

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

e_k = residual covariance among segments

The model allows for the expression of the relationship between TN and chlorophyll to be defined as a deterministic (Fixed Effect) component while including variance components (Random Effects) that allow for the stratum intercepts to vary and for the correlation that exists among segments to be accounted for when evaluating the statistical significance of the regression. This final aspect of the mixed effects model is critical to account for correlation that exists among segments to obtain appropriate estimates of the standard errors for hypothesis testing. The resulting model was highly statistically significant (Figure 9). The likelihood ratio test comparing the mixed effects parametrization relative to the null model and the solution for fixed effects (Pr>|t| for geo_tn) were both highly statistically significant ($p<0.0001$). Residual diagnostics were also evaluated to assess model fit and assumptions associated with the regression. The model residuals fit the quantile plot of the normal distributed and were approximately normal with a mean near zero and standard deviation near 1. The residual were also randomly distributed as function of the predicted mean (Figure 10) indicating the model assumptions were met.

The regression was then used to predict the AGM TN concentration that would result in an AGM chlorophyll value of 4.58 ug/l (i.e. the reference period threshold) by rearranging the fixed effects component of the regression. That is (4.58 -2.04)/5.23 = 0.49 mg/l . The resulting regression lines for each stratum are plotted in Figure 11 along with the data used in the regression. The predicted TN value associated with the chlorophyll threshold is represented by the solid blue reference lines in the plot.

Fixed Effects

The Mixed Procedure

Null Model Likelihood Ratio Test		
DF	Chi-Square	Pr > ChiSq
18	79.97	<.0001

Solution for Fixed Effects					
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	2.0402	0.2501	30.4	8.16	<.0001
geo_tn	5.2277	0.5823	84.7	8.98	<.0001

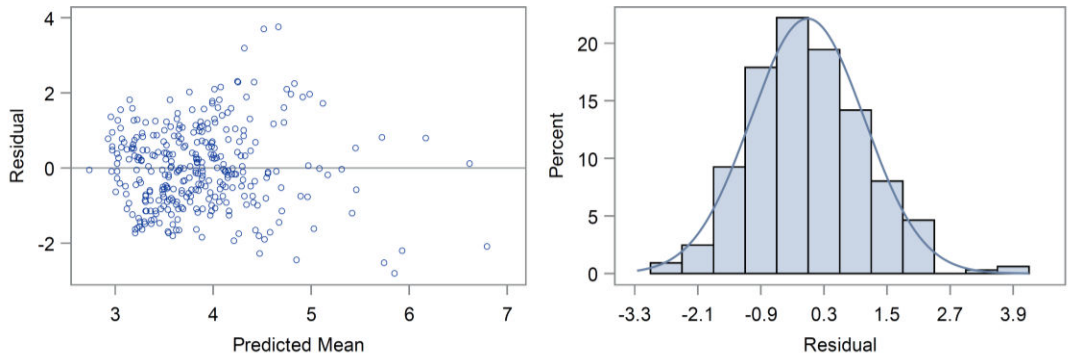
Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
geo_tn	1	84.7	80.60	<.0001

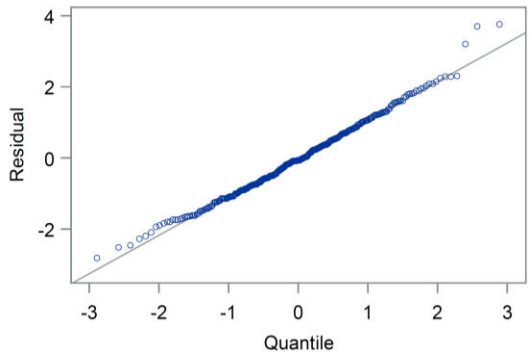
Fit Statistics	
-2 Log Likelihood	889.1
AIC (Smaller is Better)	931.1
AICC (Smaller is Better)	934.2
BIC (Smaller is Better)	889.1

Random Effects

Covariance Parameter Estimates						
Cov Parm	Subject	Ratio	Estimate	Standard Error	Z Value	Pr Z
Variance	strata	0.04751	0.05327	0.04417	1.21	0.1139
TOEP(2)	segment	0.2913	0.3266	0.07606	4.29	<.0001
TOEP(3)	segment	-0.1065	-0.1194	0.08365	-1.43	0.1535
TOEP(4)	segment	-0.1289	-0.1445	0.07740	-1.87	0.0619
TOEP(5)	segment	0.08950	0.1003	0.08123	1.24	0.2167
TOEP(6)	segment	-0.04440	-0.04978	0.09859	-0.50	0.6137
TOEP(7)	segment	-0.07361	-0.08253	0.07960	-1.04	0.2998
TOEP(8)	segment	-0.09759	-0.1094	0.1031	-1.06	0.2887
TOEP(9)	segment	-0.00583	-0.00653	0.09955	-0.07	0.9477
TOEP(10)	segment	0.1556	0.1744	0.1160	1.50	0.1327
TOEP(11)	segment	0.2871	0.3219	0.1575	2.04	0.0409
TOEP(12)	segment	-0.03943	-0.04420	0.1401	-0.32	0.7523
TOEP(13)	segment	-0.2583	-0.2896	0.1412	-2.05	0.0403
TOEP(14)	segment	-0.2246	-0.2519	0.1703	-1.48	0.1392
TOEP(15)	segment	0.002558	0.002868	0.1862	0.02	0.9877
TOEP(16)	segment	0.06788	0.07610	0.2138	0.36	0.7219
TOEP(17)	segment	-0.09762	-0.1095	0.2230	-0.49	0.6236
TOEP(18)	segment	0.1339	0.1501	0.1984	0.76	0.4491
Residual		1.0000	1.1212	0.1080	10.38	<.0001

Figure 9. Results for mixed effects model showing the model fit statistics for the fixed and random effects components of the model.





Residual Statistics	
Observations	324
Minimum	-2.806
Mean	-0.011
Maximum	3.7538
Std Dev	1.0813

Fit Statistics	
Objective	889.12
AIC	931.12
AICC	934.18
BIC	929.98

Figure 10. Residual diagnostics for the mixed effects model.

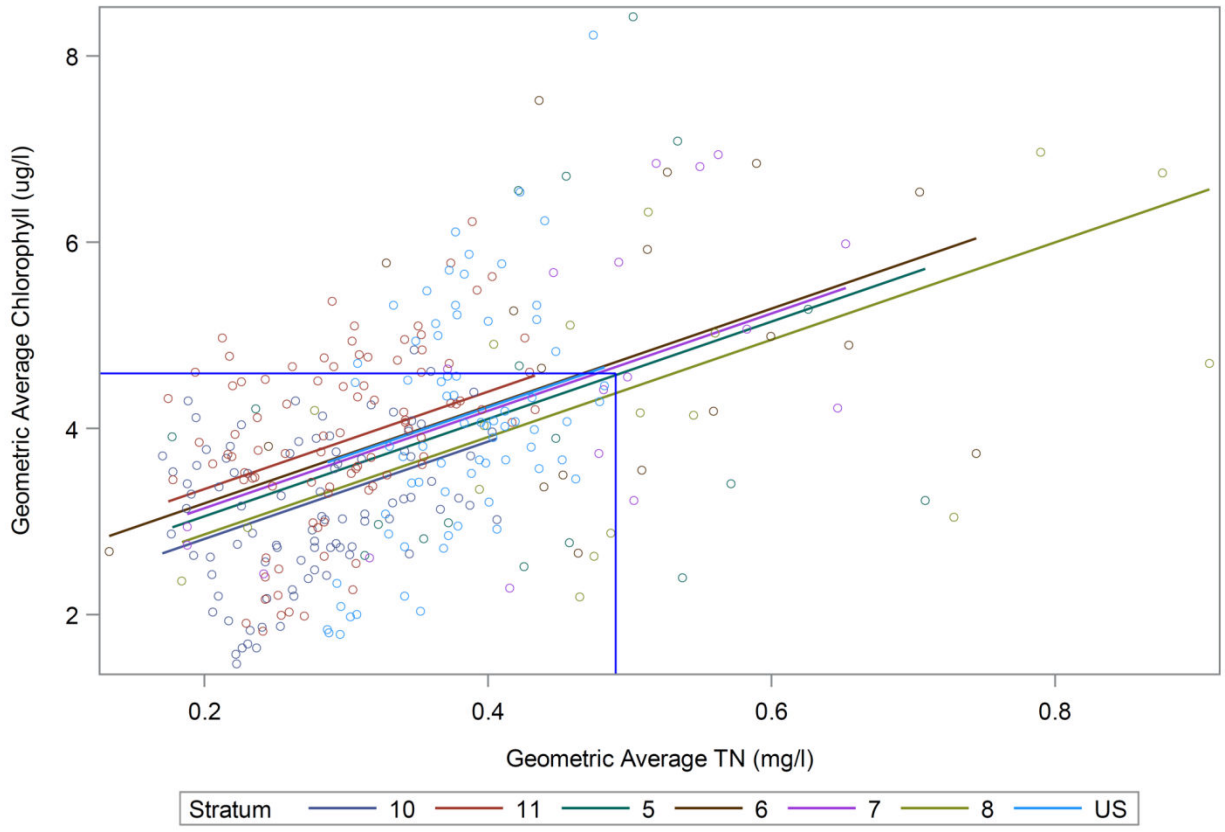


Figure 11. Observed (open circles) and predicted (solid lines) strata specific relationship between AGM TN concentrations and AGM chlorophyll concentrations. Solid blue reference lines indicate the TN criterion value (x axis) at the threshold reference period chlorophyll value (y axis).

The regression predicts that when the AGM TN value is 0.49, the chlorophyll AGM will, on average, be at the threshold level for 50% of the strata in Sarasota Bay. This interpretation conforms to the manner in which the chlorophyll threshold was determined in that the threshold was defined as a annual average chlorophyll over the entire ENR meaning that approximately 50% of the values were above and 50% were below the overall average (assuming a normal distribution). Therefore an AGM TN of 0.49 mg/l is the proposed criterion value based on this analysis. It is important to understand that the chlorophyll criterion value, and therefore the TN criterion value, represents a reference condition without direct evidence of an adverse effect. A similar but alternative reference approach would be to use DEPs Healthy Conditions approach which had not been adopted by DEP when the original work was proposed. This approach is discussed in the next section.

FDEP's Healthy Conditions Approach:

The FDEP identified an approach for developing NNC in lieu of a stressor- response relationship for marine water bodies that were deemed to be in healthy condition. For a "healthy existing conditions" dataset, the Department's proposed criterion expression is to set the magnitude expression as an

annual geometric established at the upper 80 percent prediction limit of the spatially averaged annual geometric means, with a frequency and duration of no more than 1 annual geometric mean exceeding the limit in a 3-year period (FDEP 2012 equation 3: page 29). The equation is reproduced below for convenience and relies on calculation of annual geometric averages and the standard deviation of those AGMs.

$$P.I. = e^{\left(\bar{y}+t_{(\alpha,n-1)}*\sqrt{\sigma_{\bar{y}}^2+\sigma_y^2/n}\right)}$$

where,

$\bar{y}$ = the mean of the log-transformed data
σ^2_y = the variance of the log-transformed data
n=sample size (number of years)

To apply this approach to the Sarasota Bay ENR, the same approach used to establish the original targets was used. That is:

- data were averaged by region and month,
- the values were log transformed,
- the average over the 2001-2005 time period was calculated as the reference mean,
- the variance was calculated based on the annual log transformed means between 1998 – 2009
- The reference mean and variance estimates were applied to the FDEP equation above

The values generated for TN included a reference natural log mean of -0.9695780 and a standard deviation of the natural log means of 0.16282 with 12 years of annual means used to calculate the standard deviation. The resulting healthy conditions criterion value for the Sarasota Bay ENR resulting from these values would be an AGM TN of 0.44 mg/l.

Discussion:

The regression analysis described here followed the same logic employed in the original work with respect to the establishment of the reference period approach targets and thresholds. The chlorophyll threshold was defined based on a standard deviation above the reference period average but was re-expressed as an AGM threshold value. Results of the revised regression analysis suggest that a TN AGM of 0.49 would limit chlorophyll concentration to the threshold value re-expressed as an AGM. The difference in results between the regression approach and DEPs healthy conditions approach is 0.05 mg/l, the minimum detection limit for TN. The healthy conditions approach simply relies on the reference condition distribution of TN, not the relationship between TN and chlorophyll which is thought to limit light availability to seagrasses. Therefore, the results of the regression approach may be more indicative of a response endpoint than the healthy conditions approach if applied in rule as the revised Sarasota Bay ENR NNC criterion value not to be exceeded more than 1 year in 3 in accordance with application of the

Impaired Water Rule evaluation. However, it is important to note that this process does remain a reference approach without observation of an adverse effect. Therefore, it is not guaranteed that exceedence of the threshold value will result in adverse effects to the system. The reference period approach is more aligned with DEP's anti degradation policy for healthy waterbodies then with an explicit estimate to the assimilative capacity of the system.

The fact that the ENR is so large and incorporates two Counties with two different sampling schemes complicates the derivation and inference associated with development of protective criteria for the entire Sarasota Bay ENR as a single unit. It is therefore important that evaluation of either reference approach, if adopted, be applied consistently with how the original work was conducted. That is, the data should be averaged monthly by region, log transformed, and then the annual average of the regional values should be computed and exponentiated to generate the AGM values. Because Sarasota Bay samples with greater intensity than Manatee County, simply calculating geometric averages based on all data would weight the mean towards the Sarasota County data. The revised regression approach used the strata effect to retain a higher number of observations but more evenly weight the estimation of the criterion value. Including covariates in this relationship was considered but excluded from consideration based on the fact that salinity and color were both significantly correlated with TN concentrations and therefore including them as covariates would violate a principal assumption associated with multiple regression analysis. An alternative method of estimation might be to develop Region specific regressions and average the results but this would require redefining the chlorophyll thresholds based on the original work and was beyond the scope of this effort. An optical model recently completed for the SBEP may also inform the derivation of the NNC as it explicitly links chlorophyll concentrations to the light requirements of seagrass at target depths throughout the ENR. This approach would fill in the stressor response link absent from the current reference period approach in that the reference period approach is not directly linked to the light attenuation requirements of the seagrass communities. However, under the constraint of the reference condition approach, the two derivations described above represent the best currently available options from which to incorporate a revised criterion value for the Sarasota Bay NNC into rule making.

References:

Florida Department of Environmental Protection 2012. Overview of approaches for numeric nutrient criteria development in marine waters. Division of Environmental Assessment and Restoration. Standards and Assessment Section. Florida Department of Environmental Protection. Tallahassee, FL 32399

Janicki Environmental, Inc. 2011. Proposed numeric nutrient criteria for Sarasota Bay. Report submitted to the Sarasota Bay Estuary Program. Sarasota, FL.

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