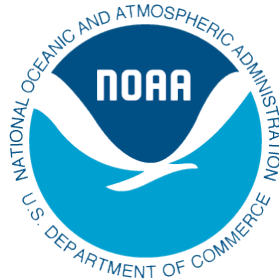


Robust and Powerful Trend Analyses for Continuous Water Quality Monitoring within the Estero Bay Aquatic Preserve (2004-2011)



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Abstract:

Since 2004, the Florida Department of Environmental Protection, Estero Bay Aquatic Preserve staff has monitored water quality within the Estero Bay Aquatic Preserve using continuously deployed datasondes at three locations. The program is intended to continuously track water quality status and trends within the preserve and relate them to influences from the surrounding watershed. Analyses of data from 2004 through 2011 show that dissolved oxygen was declining; and that salinity and pH were increasing for Estero Bay Pass. There were decreasing trends at EB01 for both dissolved oxygen and turbidity; whereas salinity had increased. Site EB02 had a significant decrease in dissolved oxygen, and significant increases in salinity, pH and turbidity. Site EB03 was the most stable of all three sites. Overall, site EB02 was more dissimilar from sites EB01 and EB03 than they were of each other. Continued monitoring is necessary in order to relate water quality trends region-wide to seagrass trends and to aid in the management of submerged resources within the region.

Introduction:

The Charlotte Harbor watershed, located in southwest Florida, drains into the vast estuarine complex of Charlotte Harbor which is one of the most pristine and socio-economically important ecosystems in the state. Located within this complex are many sub-estuaries, from Lemon Bay in the north to Estero Bay in the south, comprising nearly 250,000 acres of submerged habitat (e.g., Duffey et al, 2007; Leary, 2009; and Leary 2010). Contained within the estuarine complex are six aquatic preserves. Aquatic preserves are areas of exceptional submerged resources that were set aside by the Florida legislature to be maintained in their essentially natural condition for future generations (Chapter 18-20, FAC). The aquatic preserves are managed by the FDEP Office of Coastal and Aquatic Managed Areas (CAMA). Five of the local aquatic preserves are managed by the Charlotte Harbor Aquatic Preserves' office in Punta Gorda, and include: Lemon Bay, Gasparilla Sound-Charlotte Harbor, Cape Haze, Pine Island Sound, and Matlacha Pass Aquatic Preserves. The sixth local aquatic preserve is the Estero Bay Aquatic Preserve, which is managed from the Estero Bay office located in Fort Myers Beach (Leary 2012b).

The purpose of this report is to describe trends in water quality within the Estero Bay Aquatic Preserve for more than seven years of continuous water quality monitoring and monthly sampling (July 2004 – August 2011). The parameters of interest for trend analyses are data collected by datasondes including: dissolved oxygen (as a percentage of total saturation; DO), temperature, pH, turbidity, and salinity. These parameters are important in assessing the health of the estuary. This report summarizes water quality and relates it to location for the Estero Bay Aquatic Preserve.

Methods:

Study Area:

Situated in southwest Florida, the Estero Bay Aquatic Preserve is located within Lee County. The bay is adjacent to the cities of Fort Myers, Fort Myers Beach, Estero, and Bonita Springs. Although Estero Bay receives freshwater indirectly from the Caloosahatchee River, its main tributaries are: Hendry, Mullock and Spring Creeks, and the Estero and Imperial Rivers. Estero

Bay is approximately 14 km long and 3 km wide, and encompasses approximately 11,000 acres of submerged habitat and mangrove islands. It is a shallow, micro-tidal, bar-built sub-estuary of the greater Charlotte Harbor estuarine system. The Estero Bay Aquatic Preserve was the first aquatic preserve established in the state Florida in 1966.

Field Methods:

Two datasondes were deployed in north and central Estero Bay during July 2004, and a third in November 2004, in order to depict general open water conditions in real time. Station EB01 is in the northern part of Estero Bay, and is influenced by waters from the Caloosahatchee River (e.g., Byrne 2003, Byrne and Flanigin 2005, Byrne and Gabaldon 2007, SFWMD 2008, etc.); EB02 in the central portion of Estero Bay is located near the mouth of Spring Creek; and EB03 in the southern section which is primarily affected by waters from the Imperial River. The datasondes are encased in PVC housing with a lock top to prevent theft. Every two weeks to one month, the sondes are swapped out in order to control for drifting of data and probe fouling. The datasondes are located 0.5 m off the bottom and collect data every 15 minutes. Seven parameters were measured with the sondes, including: temperature, salinity, conductivity, pH, DO, turbidity and depth (e.g., Brown 2011). For additional information on field methods and quality control standards, refer to the NOAA National Estuarine Research Reserves CDMO manual (Small 2008).

Statistical Methods (adapted from Leary, 2012b):

Statistics were run using multiple methods on the following computer programs: SPSS 19.0 (IBM), Kendall.exe (USGS), KTRLLine 1.0 (USGS), G*Power 3 (Faul et al 2007), and PAST 2.14 (Hammer et al 2001).

A priori power analyses were run based on Cohen's D and adjusted to R^2 in order to determine the minimum number of results (samples) required for statistical analyses ($\alpha=0.05$; $\beta=0.05$; and two tailed). It was determined that a minimum of 28 samples are required for regression analyses in order to detect a large effect size ($R^2 \geq 0.26$; e.g., Faul et al 2007, Cohen 1992, Lenth 2001, Lenth 2007 and Leech et al 2005). In addition, trend tests require a minimum of four to five years of data, depending on the specific analysis. Afterwards, *post hoc* power analyses were run to measure effect size. Exploratory/summary statistics were also run. Multiple tests were then run in order to test the assumption of normality, including: Johnson's SU transformation for skew; Anscombe & Glynn's transformation for kurtosis; and Jarque & Bera LM test (Jarque and Bera 1980, Jarque and Bera 1987 and DeCarlo 1997). Outliers were identified using Mahalanobis D^2 and by SARIMA (Seasonal Auto-Regressive Integrated Moving Average). The assumption of homoscedasticity was tested using the Breusch-Pagan test (Breusch and Pagan 1979).

From these statistics, assumptions of normality and homoscedasticity can be assessed; however since both parametric and non-parametric tests were run, the data were not transformed. In addition, assumptions of linearity were examined using plots of observed versus predicted values and residuals versus predicted values (e.g., Park 2008). Assumptions of independence were assessed using autocorrelation function (ACF) combined with the Durbin-Watson d test (e.g., Durbin and Watson 1950, Durbin and Watson 1951, Watson and Durbin 1951 and Dufour

and Dagenais 1985). For time series analyses the assumption of stationarity (data are statistically in equilibrium, with constant mean and variance) was tested using ACF, Partial Auto-Correlation Function (PACF) and Ljung-Box Q; and is adjusted for by differencing in the model of the raw data, and is removed in the seasonally adjusted data (Box and Pierce 1970, Ljung and Box 1978, and Hamza 2009). In addition, using SARIMA, rather than ARIMA, allows for analyzing data multiplicatively by incorporating both a non-seasonal and a seasonal component (e.g., Durdu 2010).

Outliers that arise from obvious equipment malfunctions, transcription errors, or do not meet laboratory quality control criteria were excluded from analyses; or in the case of transcription errors corrected when identified (i.e., CDMO 2007, Small 2008 and YSI 2009). Outliers related to specific events (e.g., hurricanes, etc.) will be retained in the analyses. Laboratory data reported as below the method detection limit (MDL) will be analyzed as the maximum MDL for that parameter, as well as all values reported below the maximum MDL. Although this substitution method overestimates the data, it was used due to its ease and speed (e.g., Helsel 1990 and Helsel 2010). In order to better handle such a large dataset with multiple cyclical components (daily, tidal, annual, etc.), monthly means and medians were used for analyses (e.g., Burkholder et al 2006). Missing data were re-coded for time series analyses as the mean or median for that parameter for that particular month. Raw data was adjusted using Seasonal Decomposition, which results in three data sets: the original “raw” data; seasonally adjusted data (data that have annual cyclical patterns removed; SAS); and trend-cycle smoothed data (a smoothed version of the seasonally adjusted data; STC). All data were assumed to have at least an annual periodicity for Seasonal Decomposition.

Multiple tests (parametric, robust, and non-parametric) were used to detect trends, including: Seasonal Kendall Trend Test (SKTT; Hirsch et al 1982, Hirsch and Slack 1984, Hirsch et al 1991, Helsel and Hirsch 2002, and Helsel et al 2006); Theil-Kendall robust line regression (KTRL; Theil 1950a, Theil 1950b, Theil 1950c, Granato 2006 and Sen and Saleh 2010); SARIMA models; exponential smoothing models (these and SARIMA are collectively called time series models); Robust Regression (based on M-estimators, rather than mean or median; RR; e.g., Nevitt and Tam 1998); and Ordinary Least Squares regression (OLS; e.g., Burkholder et al 2006, and Weber and Perry 2006). All models, with the exception of SKTT and KTRL, were run on the raw data; SAS data; and STC data. However, only results for those models where assumptions were met are presented. **Note:** SPSS Time Series Modeler includes a procedure called Expert Modeler, which identifies the best fitting SARIMA or exponential smoothing model, was used rather than modeling the parameters by trial and error; in addition this procedure automatically transforms (either square root or natural log) data when necessary (SPSS 2010). Trends in time series models were obtained by using the Least Squares method on the predicted (modeled) values (Burkholder et al 2006). Measures of goodness of fit include: stationary R^2 , R^2 , root mean square error (RMSE), mean absolute percent error (MAPE), maximum absolute percent error (MaxAPE), mean absolute error (MAE), normalized Bayesian information criterion (BIC), median deviation, median absolute deviation (MAD), non-parametric prediction error sum of squares (NPPRESS), and bias correction factor (BCF). Both t-tests and F-tests were performed on the observed versus the predicted values in the time series analyses in order to determine if they were statistically significantly different.

When statistical assumptions are met, parametric statistics (based on metrics; for example mean, median, standard deviation, etc.) offer the best power and accuracy, but if they are violated the error components of the analyses become overly inflated and the statistics become unreliable (e.g., Yaffee 2002). When this occurs, it becomes necessary to employ either robust statistics (based on estimators) or nonparametric statistics (based on ranks; Erceg-Hum and Miroseвич 2008). Although robust statistics can improve power and accuracy when assumptions are violated, there are no set standards for when the data are too non-normal, heteroscedastic, or when outliers are too influential. Therefore nonparametric statistics were employed, as well. These multiple methods allow for better interpretation of results (Wilcox 2001).

When data do not meet specified parametric assumptions of normality or homoscedasticity, transformations can be applied to the data in order to run parametric statistics. Typical transformations include: logarithmic, square root, exponential and reciprocal. For reciprocal, Box-Cox and log transformations, the data must not equal zero (e.g., Yamamura 1999). Although transformations can help to stabilize variance, and in the process aid in producing a normal distribution, there are instances where transformations will not satisfy assumptions. These include: heavy tailed distributions; bimodal or multimodal distributions; distributions with a large number of equal values; and where transformations lead to variation in the p-value. In addition, transformations can only be used on discrete or continuous data (e.g., Yaffee 2002 and Helal 2008). When assumptions are not met and transformations do not help or cannot be used, non-parametric statistics or robust statistics must be used. In addition, transformations can change the underlying nature of the data (Osborne 2002). Therefore, for this paper, transformations were not used, with the exception of SARIMA models where SPSS Expert Modeler did so.

Where data were normal ANOVA, Levene's, Welch F, and Gabriel's tests were used for site comparisons, and the mean was reported. However, if data were non-normal, Kruskal-Wallis H and Man-Whitney U were used for site comparisons, and the median was reported.

Results are given for the Estero Bay Aquatic Preserve as a whole and by site. For detailed statistics, see the appendices contained within Leary, 2012b.

Results:

Field Parameters

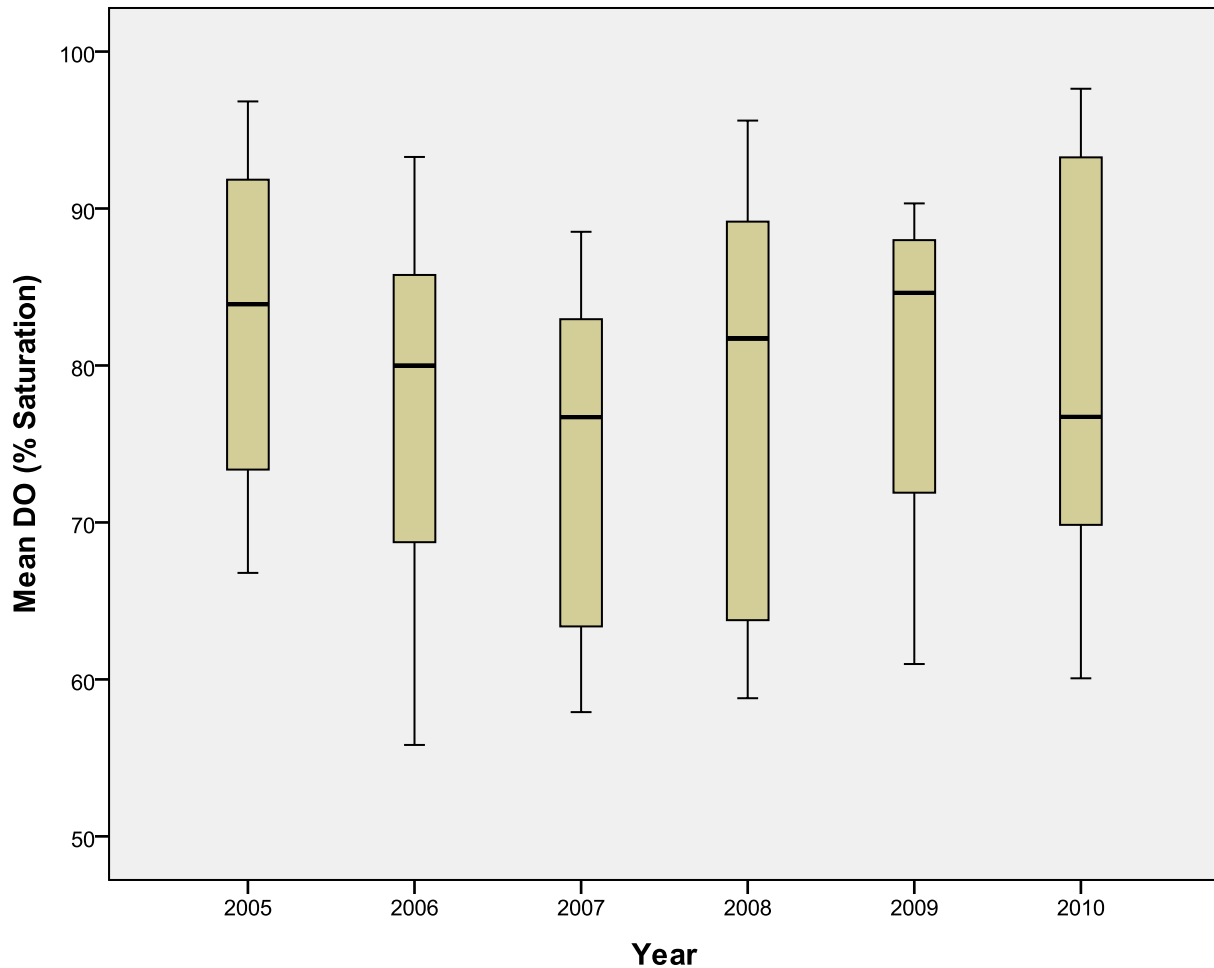
Dissolved Oxygen

Statistical assumptions of independence were violated for all DO data within the Estero Bay Aquatic Preserve, therefore nonparametric results were used. Both the SKTT and KTRL results showed significant ($p < 0.05$ for both) declining trends in DO at rates between -1.5% and -1.6% per year, respectively. In addition, Estero Bay Aquatic Preserve's all three SARIMA models of DO showed significant decreasing trends in DO; as did the OLS and RR regressions of the DO data ($p < 0.05$ for all trends and models). The same general pattern was observed for sites EB01 and EB02, as well; with significant declining trends in all models and trend tests. Both the SKTT and KTRL were decreasing significantly ($p < 0.05$ for both) at rates of approximately -2.1% per year for EB01. Site EB02 had significant decreasing trends for SKTT and KTRL, with rates

falling at between -1.1% and -1.6% per year, respectively. There were no statistically significant trends or models at site EB03, however all showed declining trends in DO. There were no other significant trends in DO. There was no statistical difference between sites with regard to DO (mean DO: EB03=79%, EB01=81%, EB02=81%; p=0.480). The mean DO for Estero Bay Aquatic Preserve was 80% (95% CI=79 – 82%).

DO (% Saturation)											
Estero Bay Aquatic Preserve			EB01			EB02			EB03		
Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P
Simple Seasonal	-1.88	0.000	Simple Seasonal	-2.13	0.000	SARIMA (1,0,0)(0,1,1) ₁₂	-1.57	0.002	SARIMA (1,0,0)(0,1,0) ₁₂	-0.45	0.533
SAS_SARIMA (0,1,1)(0,0,0) ₁₂	-2.27	0.000	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	-1.15	0.000	SAS_SARIMA (0,1,1)(0,0,0) ₁₂	-2.92	0.000	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	-0.05	0.799
STC_SARIMA (3,1,5)(0,0,0) ₁₂	-2.23	0.000	STC_SARIMA (0,1,3)(0,0,0) ₁₂	-2.50	0.000	STC_SARIMA (3,1,0)(0,0,0) ₁₂	-3.20	0.000	STC_Damped Trend	-0.40	0.792
OLS	-1.13	0.067	OLS	-1.54	0.029	OLS	-1.27	0.036	OLS	-0.25	0.743
SAS_OLS	-1.80	0.000	SAS_OLS	-2.43	0.000	SAS_OLS	-1.79	0.000	SAS_OLS	-0.18	0.659
STC_OLS	-1.83	0.000	STC_OLS	-2.44	0.000	STC_OLS	-1.84	0.000	STC_OLS	-0.20	0.524
RR	-1.18	0.077	RR	-1.64	0.029	RR	-1.29	0.045	RR	-0.30	0.717
SAS_RR	-1.25	0.000	SAS_RR	-2.00	0.000	SAS_RR	-1.32	0.002	SAS_RR	-0.29	0.451
STC_RR	-1.14	0.000	STC_RR	-2.27	0.000	STC_RR	-0.87	0.019	STC_RR	-0.23	0.479
SKTT	-1.50	0.002	SKTT	-2.12	0.000	SKTT	-1.13	0.027	SKTT	-0.10	0.858
KTRL	-1.58	S	KTRL	-2.11	S	KTRL	-1.60	S	KTRL	-1.01	NS

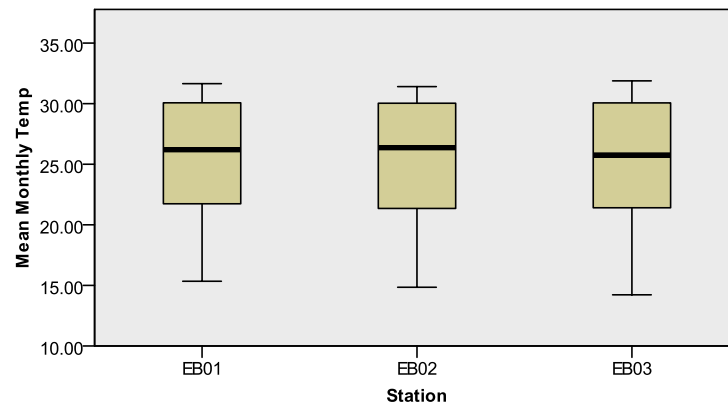
Note: KTRL does not report P-values, therefore if the 95% CI did not contain zero it was considered significant (S).



Temperature

Statistical assumptions of normality and independence were violated for all temperature data within the Estero Bay Aquatic Preserve, therefore nonparametric results were used. In addition, the data appeared to have a bimodal distribution. A *post hoc* two-step cluster analysis was performed on the data and showed two clusters, one centered around a mean of 29.51°C for the months of May through October (summer); and the other centered around a mean of 21.26°C for November through April (winter). The datasets were then split based upon membership within these clusters, and were found to be normal and homoscedastic with the exception of the “winter” data for the individual sites (all were heteroscedastic). Regressions (KTRL, OLS, and RR) of these seasonal data all showed a general increasing trend for “summer;” and a general decreasing trend for “winter.” However, none of these analyses were statistically significant. There was no significant differences between sites with regard to temperature (median temperature: EB03=25.75, EB01=26.20, and EB02=26.36°C; $p=0.940$). The median temperature for the aquatic preserve was 26.19°C. There were no other significant trends in temperature.

Independent-Samples Kruskal-Wallis Test



Total N	254
Test Statistic	.123
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.940

1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Clusters

Input (Predictor) Importance

■ 1.0 ■ 0.8 ■ 0.6 ■ 0.4 ■ 0.2 ■ 0.0

Cluster	2	1
Label		
Description		
Size	 50.4% (128)	 49.6% (126)
Inputs	Mean Monthly Temp 29.51	Mean Monthly Temp 21.26
	Month 8 (18.0%)	Month 11 (16.7%)

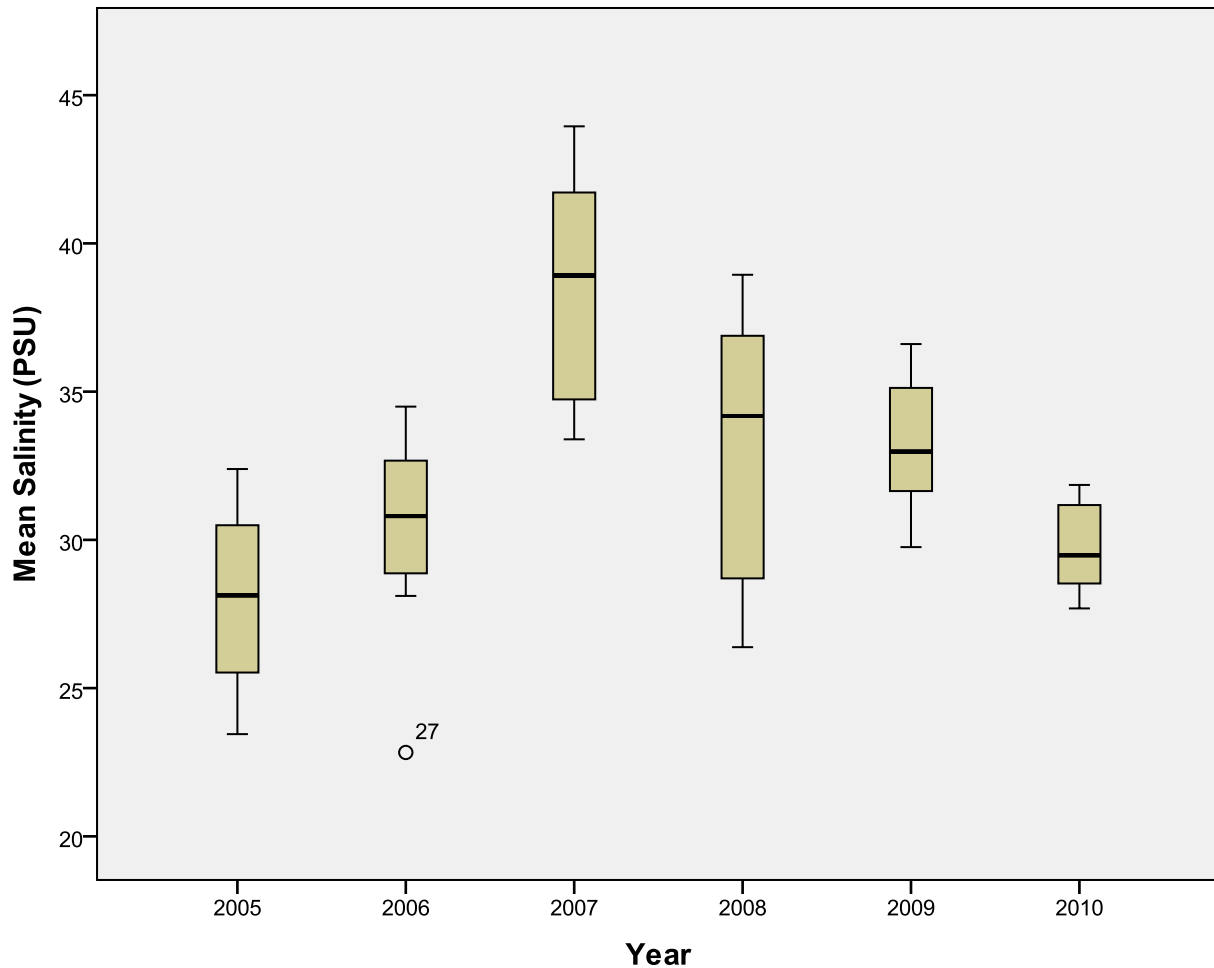
Salinity

Statistical assumptions of independence were violated for all salinity data within the Estero Bay Aquatic Preserve, therefore nonparametric results were used. Salinity increased for all models and trend analyses for Estero Bay Aquatic Preserve as a whole, and at all three individual sites. However, not all analyses or models were statistically significant, with one exception: all trend analyses and models for EB01 increased significantly. Nonparametric trend results for SKTT and KTRL were increasing at rates of 0.47 and 0.64 PSU per year, respectively for EB01 ($p < 0.05$ for both). For Estero Bay Aquatic Preserve as a whole, salinity increased significantly for both the OLS and RR regressions of the raw data, as well as for the SARIMA model of the raw data. In addition, for the aquatic preserve as a whole, the RR regression of the STC data and the SARIMA model of these data were increasing significantly, however assumptions were violated. All OLS regressions and RR regressions of the raw and STC data were significantly increasing at EB02, as was the SARIMA model of the STC data, however assumptions were violated. Only RR regression of the raw and STC data were increasing significantly at EB03. It should be noted that salinity data for EB03 appear to be bimodal, two-step cluster analysis revealed that January through July of 2007 to be in a separate cluster; the driest year within the

study period. Site EB02 had a mean salinity that was significantly greater than EB01, but not EB03 (mean salinity: EB01=30.63; EB03=32.08; and EB02=33.52 PSU; $p < 0.001$). The mean salinity for Estero Bay Aquatic Preserve was 32.08 PSU. There were no other significant trends in salinity.

Salinity (PSU)											
Estero Bay Aquatic Preserve			EB01			EB02			EB03		
Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P
Simple Seasonal	0.36	0.032	SARIMA (1,0,0)(1,0,0) ₁₂	0.35	0.006	SARIMA (1,0,0)(0,0,0) ₁₂	0.11	0.118	SARIMA (1,0,0)(1,0,0) ₁₂	0.09	0.732
SAS_SARIMA (1,0,0)(0,0,0) ₁₂	0.21	0.086	SAS_SARIMA (0,1,0)(1,0,0) ₁₂	0.36	0.006	SAS_SARIMA (1,0,0)(0,0,1) ₁₂	0.11	0.078	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	0.11	0.562
STC_Damped Trend	0.62	0.000	STC_Damped Trend	0.57	0.000	STC_Damped Trend	0.32	0.000	STC_SARIMA (0,1,1)(0,0,0) ₁₂	0.43	0.064
OLS	0.47	0.034	OLS	0.77	0.001	OLS	0.30	0.013	OLS	0.18	0.650
SAS_OLS	0.30	0.116	SAS_OLS	0.62	0.002	SAS_OLS	0.23	0.033	SAS_OLS	0.05	0.880
STC_OLS	0.32	0.059	STC_OLS	0.65	0.000	STC_OLS	0.25	0.007	STC_OLS	0.04	0.888
RR	0.48	0.029	RR	0.70	0.005	RR	0.26	0.039	RR	0.50	0.046
SAS_RR	0.33	0.073	SAS_RR	0.56	0.004	SAS_RR	0.21	0.055	SAS_RR	0.36	0.117
STC_RR	0.42	0.009	STC_RR	0.67	0.000	STC_RR	0.27	0.006	STC_RR	0.27	0.044
SKTT	0.35	0.095	SKTT	0.47	0.030	SKTT	0.09	0.226	SKTT	0.10	0.502
KTRL	0.39	NS	KTRL	0.64	S	KTRL	0.25	NS	KTRL	0.22	NS

Note: KTRLLine does not report P-values, therefore if the 95% CI did not contain zero it was considered significant (S).



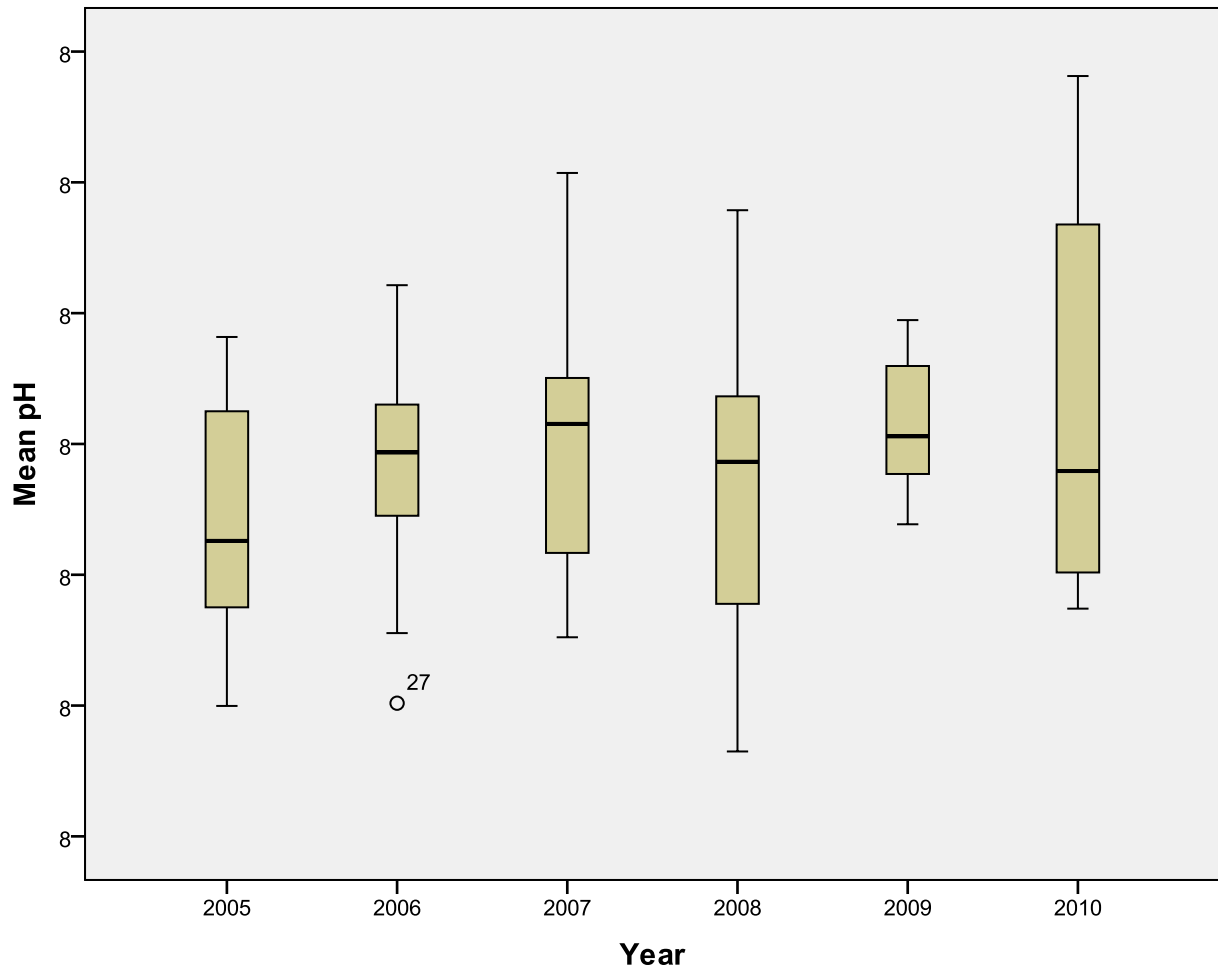
pH

Statistical assumptions of independence and homoscedasticity were violated for all pH data within the Estero Bay Aquatic Preserve, therefore nonparametric results were used. All trend analyses and models of pH for the Estero Bay Aquatic Preserve showed increasing trends, with the exception of SKTT which was constant. The KTRL results showed pH to be increasing at approximately 0.003 SU per year, however they were not significant. At EB01, most analyses of pH showed increases, but some showed decreases, and only an increasing trend in the modeled STC value was significant but violated assumptions of stationarity. All models and analyses showed increasing pH at EB02; and all OLS regressions, RR regressions of the raw and STC data, and the STC SARIMA were significant. In addition, nonparametric SKTT showed pH to be increasing at 0.01 SU per year at EB02 ($p=0.036$). All models and trend analyses at EB03 were increasing, with the exception of the SARIMA STC model, but none were significant. However, site EB01 only had significant decreasing trends in the regressions (both OLS and RR) of the SAS and STC pH data; and a significant decreasing trend in the SARIMA of the STC data, however assumptions were violated. Site EB03 had significant decreasing trend for pH for all parametric regressions and SARIMA models of the SAS and STC data, however only the SAS data did not violate assumptions. There were no other significant trends for pH. Site EB02 was significantly ($p<0.001$) greater than the other sites, whereas EB01 was significantly ($p<0.001$) less

than the other sites (mean pH: EB01=7.94, EB03=7.97, and EB02=8.05). The mean pH for the aquatic preserve was 7.99.

pH											
Estero Bay Aquatic Preserve			EB01			EB02			EB03		
Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P
Simple Seasonal	0.003	0.332	SARIMA (0,0,1)(1,1,0) ₁₂	0.001	0.444	SARIMA (1,0,0)(0,0,0) ₁₂	0.005	0.203	Simple Seasonal	0.001	0.850
SAS_SARIMA (1,0,0)(1,0,0) ₁₂	0.002	0.290	SAS_SARIMA (1,0,0)(0,0,1) ₁₂	0.001	0.542	SAS_SARIMA (1,0,0)(0,0,1) ₁₂	0.004	0.235	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	0.001	0.688
STC_SARIMA (0,1,1)(1,0,0) ₁₂	0.007	0.000	STC_SARIMA (0,1,3)(0,0,0) ₁₂	0.007	0.005	STC_SARIMA (3,1,0)(0,0,1) ₁₂	0.009	0.001	STC_SARIMA (0,1,1)(0,0,0) ₁₂	-0.002	0.555
OLS	0.008	0.104	OLS	0.009	0.240	OLS	0.014	0.019	OLS	0.004	0.526
SAS_OLS	0.004	0.285	SAS_OLS	0.003	0.623	SAS_OLS	0.012	0.036	SAS_OLS	0.003	0.600
STC_OLS	0.004	0.067	STC_OLS	0.003	0.307	STC_OLS	0.012	0.001	STC_OLS	0.002	0.536
RR	0.006	0.247	RR	0.006	0.463	RR	0.008	0.118	RR	0.007	0.286
SAS_RR	0.002	0.495	SAS_RR	-0.001	0.789	SAS_RR	0.007	0.092	SAS_RR	0.004	0.470
STC_RR	0.004	0.096	STC_RR	0.002	0.474	STC_RR	0.009	0.004	STC_RR	0.004	0.359
SKTT	0.000	1.000	SKTT	0.000	0.900	SKTT	0.010	0.036	SKTT	0.004	0.686
KTRL	0.003	NS	KTRL	0.002	NS	KTRL	0.010	NS	KTRL	0.005	NS

Note: KTRLLine does not report P-values, therefore if the 95% CI did not contain zero it was considered significant (S).



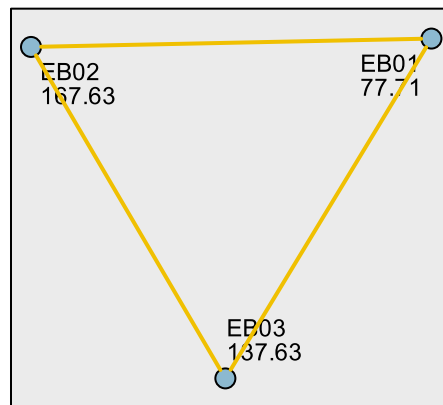
Turbidity

Statistical assumptions of normality were violated for all turbidity data within the Estero Bay Aquatic Preserve, therefore nonparametric results were used. All models and trend tests for the Estero Bay Aquatic Preserve of turbidity data were increasing, however none were significant. Site EB01 had significant decreasing trends in turbidity for all RR regressions and for SKTT, with turbidity declining at a rate of between -0.3 and -0.5 NTU per year (p ranged from 0.005 to 0.010). All models and trend tests at EB02 were increasing for turbidity. The KTRL results showed an average annual increase of 1.6 NTU per year ($p < 0.05$). The raw data at EB02 were transformed and re-run for turbidity; all of these data showed statistically significant increases in turbidity. At site EB03, all trend tests showed significant increases in turbidity, as did the SARIMA of the STC data, however these were not significant. There were no other significant turbidity trends. The median turbidity for all sites was significantly different (median turbidity: EB01=4.5, EB03=11.5, and EB02=17.8 NTU; $p < 0.001$). The median turbidity for the aquatic preserve was 10.7 NTU.

Turbidity											
Estero Bay Aquatic Preserve			EB01			EB02			EB03		
Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P	Model or Trend Test	Slope	P
Simple Seasonal	0.28	0.344	SARIMA (1,0,0)(0,1,1) ₁₂	-0.11	0.850	SARIMA (1,0,0)(0,0,0) ₁₂	0.33	0.696	SARIMA (1,0,0)(0,0,0) ₁₂	-0.62	0.478
SAS_Simple Seasonal	0.32	0.092	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	-0.16	0.445	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	0.87	0.282	SAS_SARIMA (1,0,0)(0,0,0) ₁₂	-0.34	0.608
STC_SARIMA (1,1,1)(0,0,0) ₁₂	0.03	0.892	STC_SARIMA (3,1,0)(0,0,0) ₁₂	0.41	0.079	STC_SARIMA (1,1,3)(0,0,0) ₁₂	1.22	0.026	STC_SARIMA (0,0,1)(1,0,0) ₁₂	0.05	0.748
OLS	0.28	0.650	OLS	-0.01	0.844	OLS	1.34	0.293	OLS	0.05	0.949
SAS_OLS	0.46	0.340	SAS_OLS	-0.22	0.657	SAS_OLS	2.14	0.049	SAS_OLS	0.29	0.668
STC_OLS	0.39	0.201	STC_OLS	-0.29	0.368	STC_OLS	2.06	0.003	STC_OLS	0.24	0.520
RR	0.52	0.200	RR	-0.48	0.010	RR	1.40	0.022	RR	0.49	0.244
SAS_RR	0.59	0.143	SAS_RR	-0.51	0.005	SAS_RR	1.50	0.016	SAS_RR	0.60	0.147
STC_RR	0.38	0.247	STC_RR	-0.49	0.005	STC_RR	1.68	0.012	STC_RR	0.47	0.167
SKTT	0.10	0.646	SKTT	-0.30	0.008	SKTT	0.07	0.676	SKTT	0.43	0.098
KTRL	0.55	NS	KTRL	-0.34	NS	KTRL	1.61	S	KTRL	0.63	NS

Note: KTRLLine does not report P-values, therefore if the 95% CI did not contain zero it was considered significant (S).

Pairwise Comparisons of Station



Each node shows the sample average rank of Station.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
0-2	-59.925	11.340	-5.285	.000	.000
0-1	-89.919	11.204	-8.026	.000	.000
2-1	29.994	11.340	2.645	.008	.025

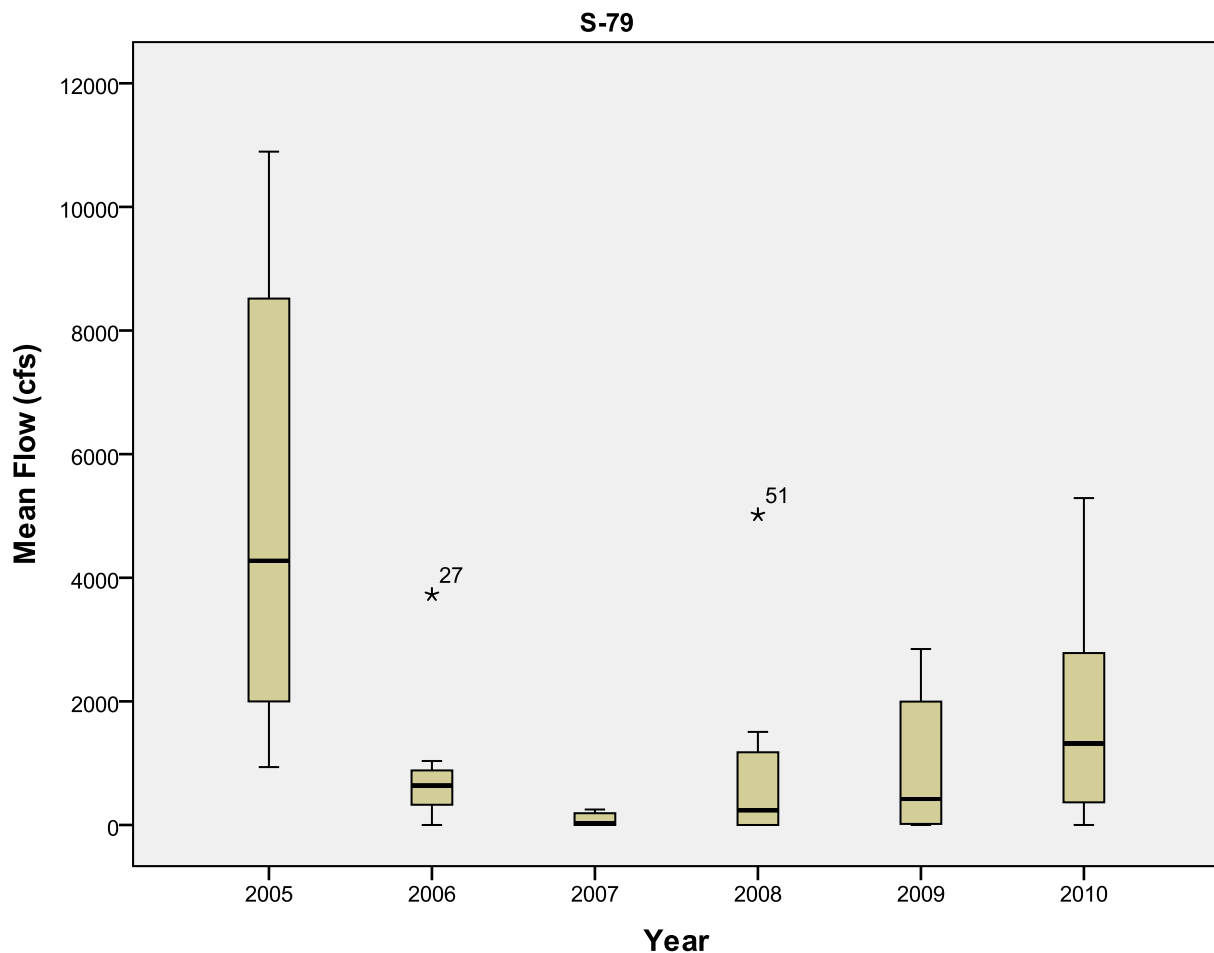
Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

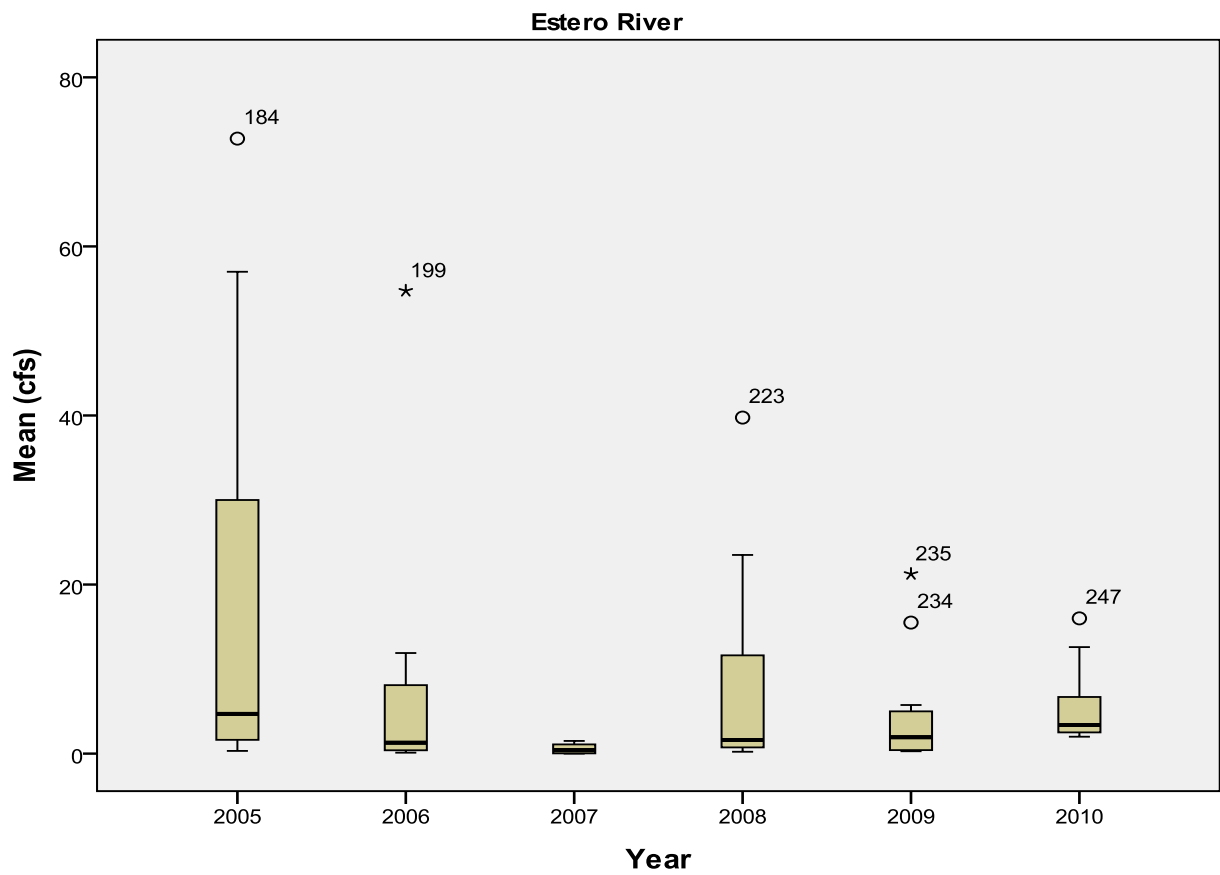
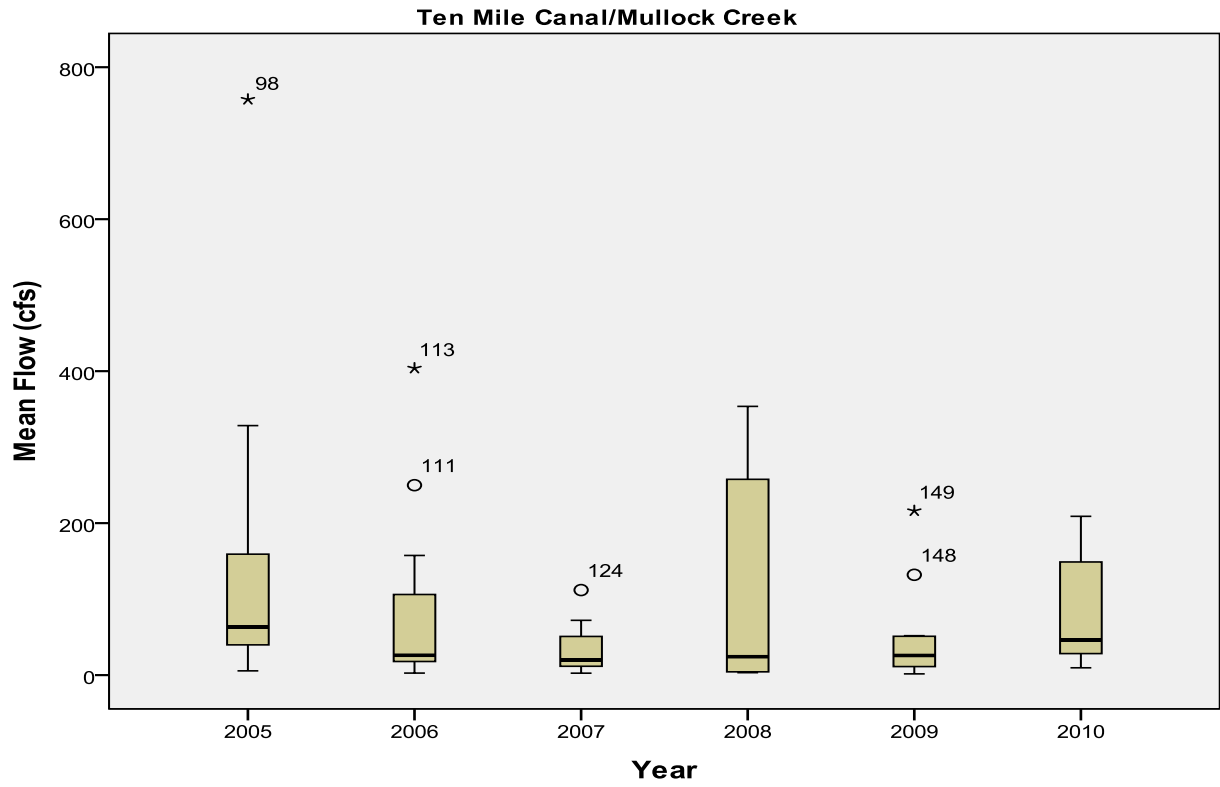
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

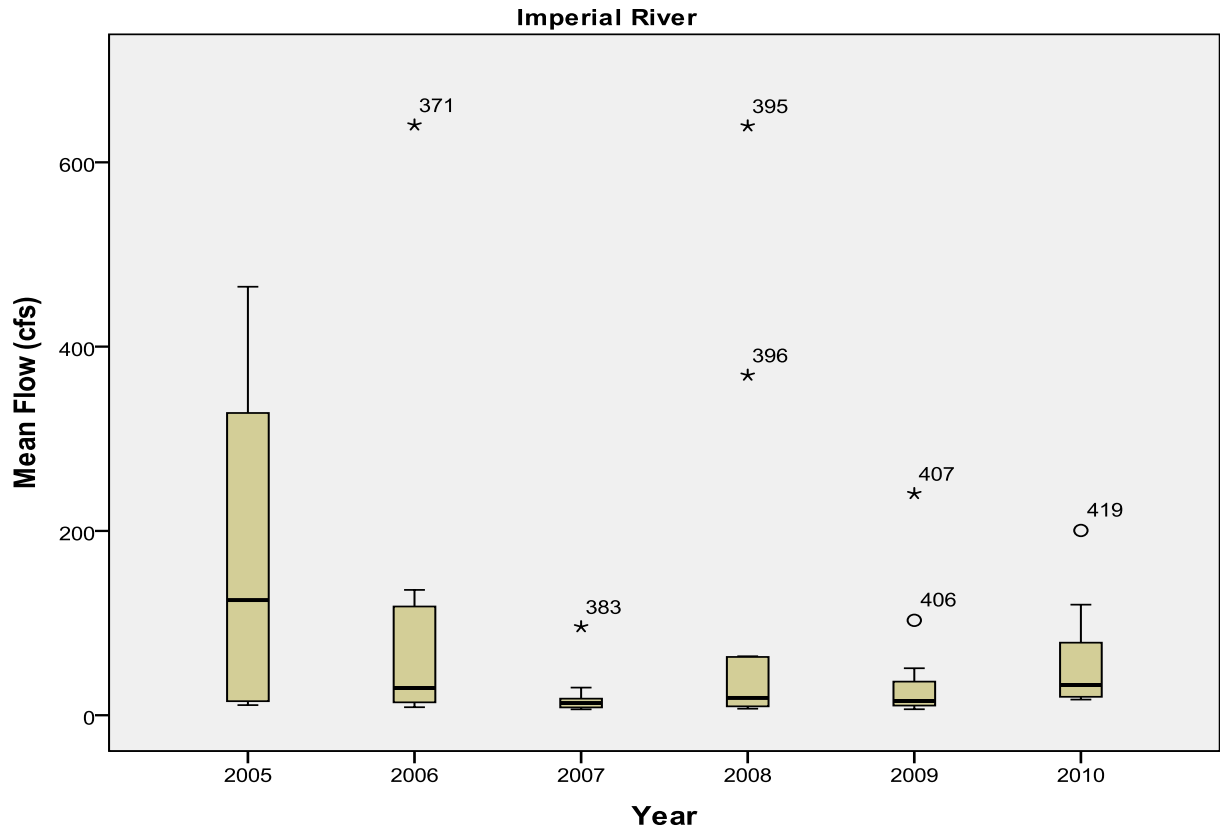
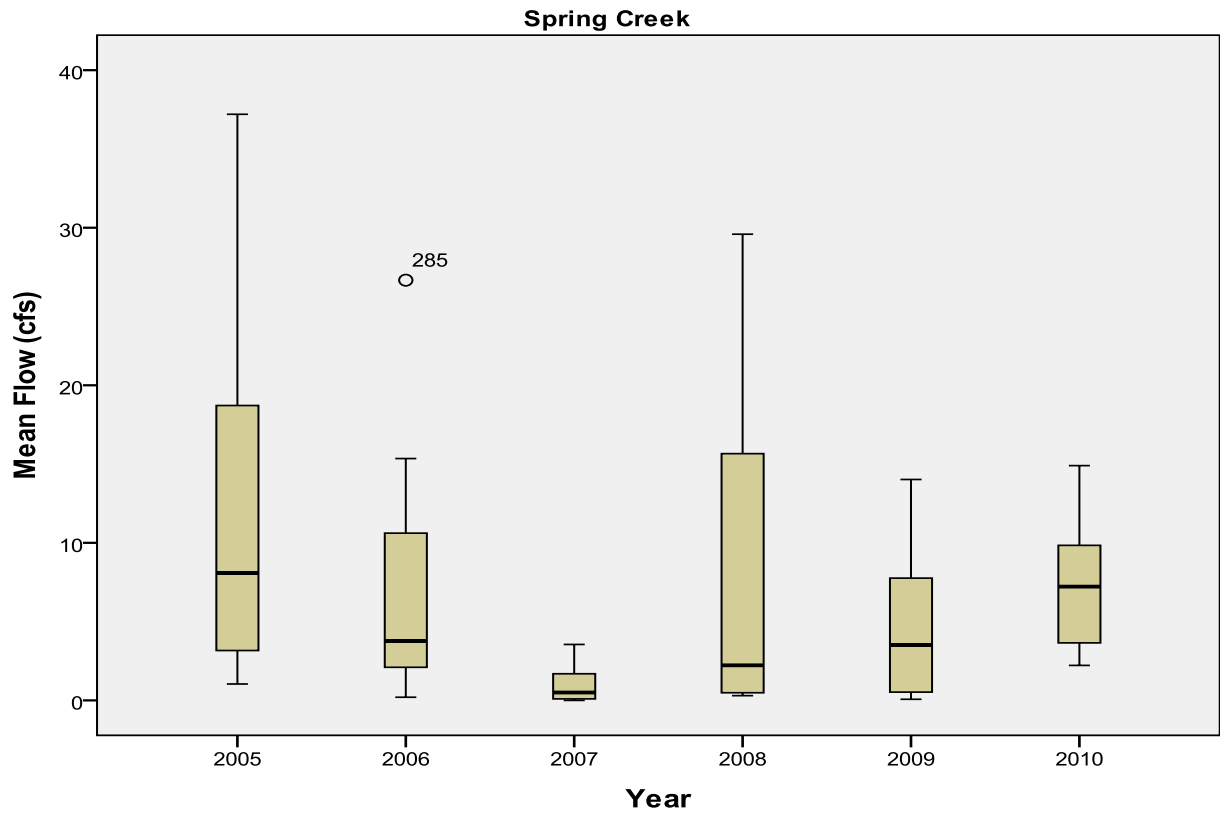
Rainfall, Flow and Freshwater Releases

Spearman's (rank based, nonparametric) correlation, followed by forward stepwise regression of water quality parameters against flow from the S-79 Franklin Lock and Dam, Ten Mile Canal/Mullock Creek, Estero River, Spring Creek, and the Imperial River (mean monthly flow, cfs) and rainfall (average monthly rainfall, m) were conducted *post hoc* in order to quantify their impact on water quality within the Estero Bay Aquatic Preserve. The average flows from July 2004 through August 2011 were: S-79=1635.6 cfs, Ten Mile Canal/Mullock Creek=86.4 cfs, Estero River=8.1 cfs, Spring Creek=6.3cfs, Imperial River=85.7 cfs, and total average flow=53.4 cfs. For the aquatic preserve as a whole, regression analyses showed rainfall to be the primary factor negatively affecting DO ($R^2=0.31$, $p<0.001$); temperature was positively influenced by rainfall followed by flow from S-79 ($R^2=0.45$, $p=0.048$); salinity was primarily negatively influenced by Spring Creek and S-79 flows and rainfall ($R^2=0.62$, $p=0.012$); and pH was negatively influenced by flow from the Imperial River ($R^2=0.36$, $p<0.001$). There were no significant correlations or regressions for turbidity for the aquatic preserve as a whole. At EB01, DO was negatively influenced by rainfall, followed by flows from the Imperial and Estero

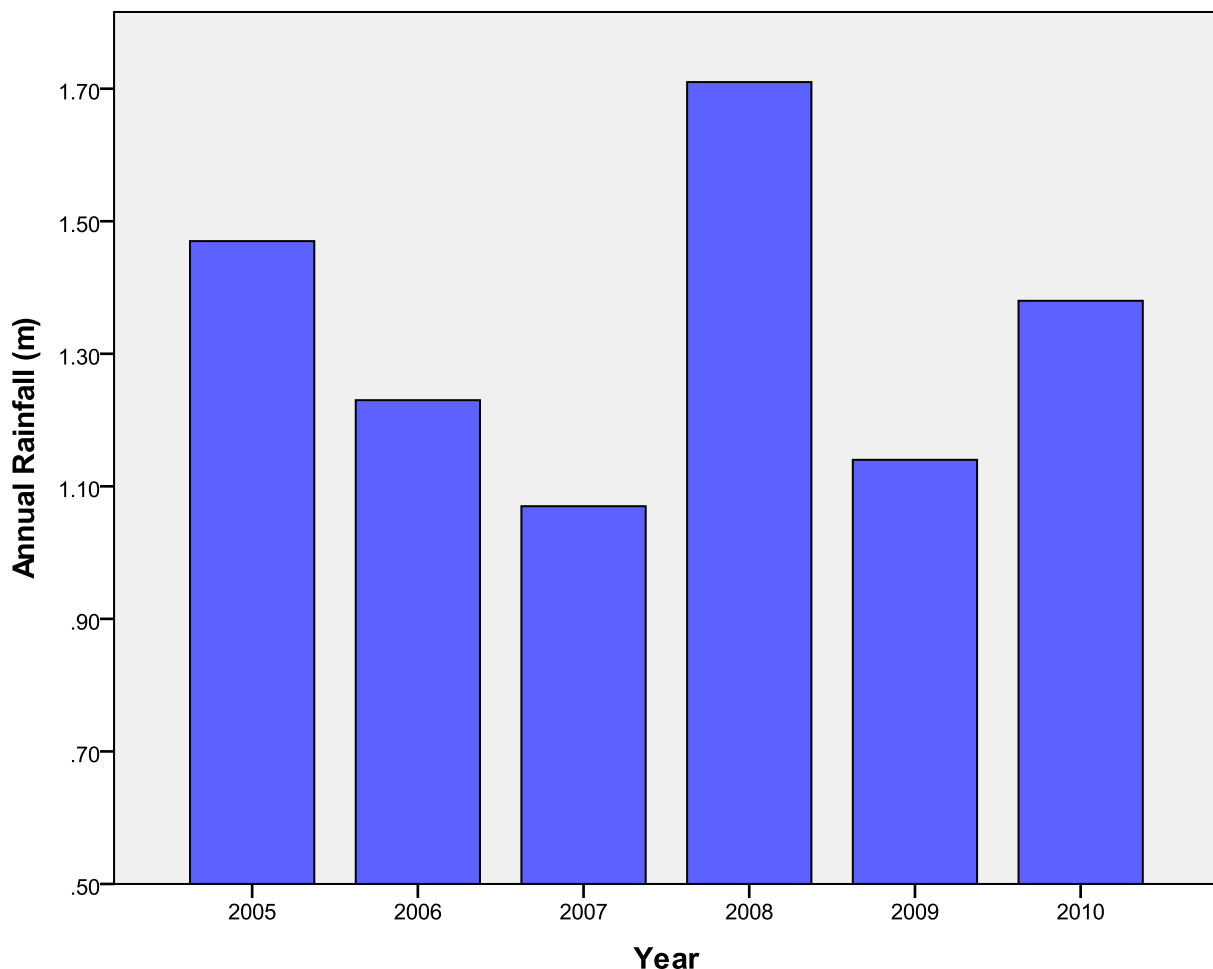
Rivers and S-79 ($R^2=0.38$, $p=0.048$); temperature was positively influenced by rainfall ($R^2=0.41$, $p<0.001$); salinity was negatively affected by flow from both the Imperial River and S-79 ($R^2=0.75$, $p<0.001$); pH was negatively influenced by Imperial and Estero River flows and rainfall ($R^2=0.43$, $p=0.017$); and turbidity was positively influenced by Estero River, Spring Creek and S-79 flows ($R^2=0.37$, $p=0.020$). The DO levels at EB02 were negatively influenced by rainfall ($R^2=0.16$, $p<0.001$); temperature was positively influenced by rainfall ($R^2=0.42$, $p<0.001$); salinity was negatively influenced by flows from Spring Creek and S-79 and by rainfall ($R^2=0.67$, $p=0.004$); and pH was negatively influenced by flow from S-79 ($R^2=0.19$, $p<0.001$). No flow patterns or rainfall significantly influenced turbidity at EB02. Site EB03 DO was negatively affected by flow from the Estero River and by rainfall ($R^2=0.35$, $p=0.004$); temperature was negatively influenced by rainfall and flow from Spring Creek and negatively influenced by flow from the Estero River ($R^2=0.24$, $p=0.007$); salinity was negatively influenced by flow from the Imperial River ($R^2=0.31$, $p<0.001$); pH was negatively influenced by flows from both the Estero River and Spring Creek ($R^2=0.24$, $p=0.005$); and turbidity was negatively influenced by rainfall ($R^2=0.09$, $p=0.006$).







Stepwise regression results of significant water quality parameters as predicted by flow and rainfall.							
	Parameter	Model Order	Predictor	F	ρ	R ²	SE
Estero Bay Aquatic Preserve	DO	1	Rainfall	38.032	-0.539	0.312	10.067
	Temperature	1	Rainfall	61.596	0.672	0.423	3.584
		2	S-79	4.034	0.360	0.450	3.521
	Salinity	1	Spring	90.803	-0.882	0.519	3.052
		2	S-79	14.839	-0.748	0.592	2.828
		3	Rainfall	6.547	-0.294	0.622	2.738
	pH	1	Imperial	47.415	-0.521	0.361	0.078
EB01	DO	1	Rainfall	27.530	-0.503	0.247	12.040
		2	Imperial	4.448	-0.259	0.285	11.800
		3	Estero	7.896	-0.311	0.348	11.339
		4	S-79	4.035	-0.066	0.379	11.134
	Temperature	1	Rainfall	57.565	0.659	0.407	3.583
	Salinity	1	Spring	131.864	-0.852	0.606	2.928
		2	S-79	46.303	-0.813	0.744	2.360
	pH	1	Imperial	46.099	-0.540	0.354	0.125
		2	Rainfall	5.344	-0.390	0.393	0.122
		3	Estero	5.895	-0.479	0.434	0.118
	Turbidity	1	Estero	24.640	0.076	0.218	2.327
		2	Spring	12.052	0.126	0.309	2.187
		3	S-79	5.654	0.333	0.345	2.128
EB02	DO	1	Rainfall	15.777	-0.405	0.158	10.938
	Temperature	1	Rainfall	59.871	0.685	0.416	3.607
	Salinity	1	Spring	101.899	-0.863	0.543	1.624
		2	S-79	20.346	-0.715	0.628	1.464
		3	Rainfall	8.854	-0.259	0.660	1.399
	pH	1	S-79	19.588	-0.420	0.189	0.104
EB03	DO	1	Estero	32.384	-0.353	0.278	18.328
		2	Rainfall	8.662	-0.578	0.346	17.545
	Temperature	1	Rainfall	7.158	0.552	0.079	6.815
		2	Estero	8.683	0.178	0.166	6.524
		3	Spring	7.588	0.268	0.236	6.279
	Salinity	1	Imperial	37.764	-0.843	0.310	8.174
	pH	1	Estero	16.910	-0.326	0.168	1.552
		2	Spring	8.397	-0.328	0.244	1.488
	Turbidity	1	Rainfall	8.001	-0.263	0.087	3.371



Discussion:

Estero Bay Aquatic Preserve:

There was good agreement among the various trend analyses for DO in the Estero Bay Aquatic Preserve; all of which showed a declining trend between -1.1 and -2.3% per year. This suggests that the significant increasing trend in salinity (between 0.21 and 0.62 PSU per year) was driving down DO saturations. A declining trend in DO concentrations (-0.8 mg/L per year) within Estero Bay was also noted for the Coastal Charlotte Harbor Monitoring Network (CCHMN) from 2001 through 2007 (Leary 2009). In addition, the overall average DO saturation (80%) suggests that the estuary was becoming hypoxic (Cary Institute of Ecosystem Studies 2009). Trends in chlorophyll *a* within Estero Bay, as well as in nutrients, need to be further examined as they can lead to hypoxic conditions as well (e.g., Lowery 1998). In waterbodies, DO is produced primarily by dissolution from the atmosphere (Stickney 1984), through wind and wave action, and to a lesser extent by photosynthesis; and may serve as an indicator of biotic productivity and estuarine health (e.g., Stickney 1984 and Colling 2006). Super saturation of DO can occur due to high rates of photosynthesis and surface mixing caused by wind and wave activity (e.g., Garcia and Gordon 1992). In the Charlotte Harbor estuarine complex, low DO values may be due to the combined effects of high water temperature, high levels of colored

dissolved organic matter (CDOM) and anthropogenic sources of nutrients and other materials (Morrison et al 1998, Camp, Dresser & McKee, Inc. 1998 and Leary 2009). Therefore, declines in DO should be investigated.

Temperature was bimodal and, as noted earlier, summer temperatures increasing, whereas winter temperatures were trending down; suggesting increasing environmental variability in the region. The same general trends were found for the nearby Matlacha Pass Aquatic Preserve (Leary 2012c). Increasing variability in temperature may prove to be detrimental to certain organisms in the estuary (e.g., Roessig 2004, Folguera et al 2009, Florida Oceans and Coastal Council 2009, Nye 2010, Estay et al 2011, Folguera et al 2011). Temperature is one of the most important properties of water (e.g., Stickney 1984 and Colling 2006), and is a controlling factor in a many chemical and biological processes within estuaries and may serve as a biological predictor (Duffey et al 2007). For example, temperature plays a key role in seasonal primary productivity and triggers life stage changes in many finfish and shellfish species. In general, temperature varies with: depth, mixing due to wind and tides, season, time of day, and anthropogenic influences (EPA 1993). Further analyses of temperature with respect to individual seasonal trends are required.

There was good agreement between all models and trend analyses for salinity, indicating an increase of between 0.21 and 0.62 PSU per year. Changes in salinity regimes are important to estuarine processes, as they can influence DO concentrations (Stickney 1984). In a previous study, Leary (2010) found salinity to be the primary factor influencing DO concentrations within Estero Bay. In addition, variations in salinity may affect many biological, chemical and physical processes within the estuary. Furthermore, many estuarine species are adapted to particular salinity ranges including seagrass (e.g., Greenawalt et al 2006; Corbett et al 2005; Chamberlain and Doering 1998), fish (e.g., Bortone et al 2006), and shellfish (e.g., Baker et al 2002, Duffey et al 2007).

There was good agreement between all models and trend analyses for pH, indicating an increase of less than 0.01 SU per year; however, results were not significant. The pH of the waterbody affects a variety of biological and chemical processes (Stickney 1984). The pH of the estuary can be influenced by the local geology and hydrology of the surrounding watershed (e.g., Stickney 1984 and Boggs 2006). Many aquatic organisms are adapted to narrow pH ranges, with optimal pH ranges for many fish being about 6.5-8.5 (e.g., Chamberlain et al 1980). Furthermore, changes in pH in aquatic systems are positively linked with changes in salinity and photosynthesis (e.g., Stickney 1984, Day et al 1989, etc.).

Turbidity within Estero Bay Aquatic Preserve, as a whole, appears to be increasing, however statistical assumptions were typically violated and nonparametric tests were not significant. Leary (2009) noted a significant increasing trend in turbidity (0.34 NTU per year) within Estero Bay from 2001 through 2007 based on data collected by the Charlotte Harbor Estuaries Volunteer Water Quality Monitoring Network (CHEVWQMN). Excessive turbidity can have a variety of effects on physical and biological processes, including: increased light attenuation (Kirk 1983, McPherson and Miller 1994, Ott et al 2006), and potentially decreasing the productivity, growth and survival of shellfish (Volety and Encomio 2006). Freshwater inflows from rivers are primary sources of sediment loading into the Charlotte Harbor estuarine complex (Hammett 1990), with nonpoint sources contributing almost 97% of the total

suspended solids (Squires et al 1998). However, resuspension of sediments due to wind and wave action may be the primary cause for increased turbidity in shallow estuaries, such as Estero Bay (de Jorge and Beusekom 1995). Turbidity results should continue to be monitored and compared with other programs within the Estero Bay Aquatic Preserve.

Overall, rainfall appeared to be a more important factor influencing the physical parameters within Estero Bay than flow. This is not surprising, as the southwest Florida rainy season falls within the warmest months of the year. Also, as rainfall increases, flow increases as well. Therefore, as rainfall increases, so does temperature, which was observed in all the models (for individual sites and for Estero Bay Aquatic Preserve as a whole). In addition, the combined influence of temperature and rainfall cause DO levels to decline. This was evident in all the models, as well. However, for turbidity neither flow nor rainfall was statistically significant, suggesting that turbidity patterns within Estero Bay may be due to wind-driven resuspension of sediments. Salinity and pH patterns appeared to be most influenced by Spring Creek and the Imperial River, respectively. However, this is unlikely to be the case and may be due to flow increasing when rainfall increases. These general trends appear similar at the individual locations, as well. Further research is needed to quantify how flow and rainfall effect the physical processes within Estero Bay and how the influence the biology of the region.

EB01

For DO at site EB01, there was good agreement among all models and trend tests, all of which showed declining trends between -1.15 and -2.50% per year. This site also had the lowest mean salinity; however, all models and trend tests should significant increases between 0.35 and 0.77 PSU per year. This may be due to a reduction in releases from the S-79 Franklin locks and dam to the tidal portion of the Caloosahatchee River (e.g., Brown et al 2012). In addition, EB01 had the lowest mean pH of all the sites. Flow from the Imperial River appeared to have the greatest influence on pH; however, this is unlikely to be the case, as it is the farthest tributary from the site, and is more likely due to rainfall. This site was the only one in Estero Bay that showed significant declines in turbidity, with all robust and nonparametric trend tests showing declines of -0.30 to -0.51 NTU per year. Station EB01 had the lowest median turbidity (4.5 NTU), and was significantly lower than the other two sites. This site is located approximately halfway between two seagrass transects, EB01 and EB02. Leary (2012a) documented that seagrass transect EB02 was the most improved of the seagrass transects within the Estero Bay Aquatic Preserve, and that transect EB02 appeared to be improving although bed lengths were declining from 2002 – 2009; results from both studies should be examined in order to aid in management decisions for the aquatic preserve.

EB02

There was a declining trend in DO at EB02. This trend was true for all modeled results and all trend tests. In addition, all models and trend analyses showed increasing trends in salinity, pH, and turbidity. Results for temperature were inconclusive due to violations of assumptions and low statistical significance. This site had the highest mean salinity and pH, and the highest median temperature and turbidity. Site EB02 appears to be the most dynamic of the three locations in Estero Bay. As with the other sites and the aquatic preserve as a whole, rainfall and its association with temperature patterns is the most likely driver of the physical parameters.

Results from this site need to be compared with results from the nearby seagrass transect EB03, which had declining trends in total abundance from 2002 through 2009 (Leary 2012a).

EB03

For DO at site EB03, there was good agreement among all models and trend tests, all of which showed declining trends. However, none of the models or trend tests were significant. In addition, all models and trend analyses showed an increasing trend in salinity; this was true for pH as well, with the exception of one model. Results for salinity, temperature and turbidity were inconclusive due to violations of assumptions and low statistical significance. This site appears to be the most stable within Estero Bay. As with the other sites and the aquatic preserve as a whole, rainfall and its association with temperature patterns is the most likely driver of the physical parameters. Results from this site need to be compared with results from the nearby seagrass transect EB04, which was the most stable seagrass transect within Estero Bay from 2002 through 2009 (Leary 2012a).

Conclusions:

Declining trends for DO within the Estero Bay Aquatic Preserve and at all three sites is of concern. However, these results were tied to rainfall during this time frame, and were likely impacting DO concentrations. In addition, these trends in DO had been documented from an earlier study. Temperature was found to be bimodal, and was increasing during summer months while decreasing during winter months. This means that there was increasing variability in temperature, and may stress organisms within the aquatic preserve. Increasing trends in salinity and pH need to be further examined, as they can impact the biota within the aquatic preserve, as well. The findings in this study need to be compared with nutrients within the region and their relationship with seagrasses in Estero Bay.

Comparisons between this program and other monitoring programs in the area need to be examined, and site specific trend analyses should be conducted for the CHEVWQMN. In addition, the program needs to be continued and expanded to cover the entire Charlotte Harbor estuarine complex in order to quantify region-wide trends and to determine if DO levels are declining throughout the estuary. Further research and continued protection of the waters and submerged resources are required in order to maintain the socio-economic and ecological value of the Estero Bay Aquatic Preserve.

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