FINAL PROJECT REPORT

Project Title: **An Assessment of the Effect of Water Quality on Seagrasses in the Caloosahatchee River/Estuary and Pine Island Sound Using Optical Properties to Predict Light Levels and Thresholds**

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ABSTRACT

Representation of the subsurface light field is a critical component of pelagic ecosystem models and for protection and restoration of SAV (Submerged Aquatic Vegetation) habitats. This study presents four optical models, developed for and specifically tuned to the Caloosahatchee River and Estuary, San Carlos Bay and Pine Island Sound located in Southwest Florida. These tools provide SFWMD and resource managers with a real-time assessment of estuarine conditions relevant to SAV. One model based on i*n situ* optical properties and photosynthetically active radiation (PAR) was used to generate predictions of light attenuation (Kd) and percent light at depth using a real-time dataset from SCCF RECON (http://recon.sccf.org), a coastal observing system established in 2007. This model was also used to estimate maximum concentrations of each of the optical properties, CDOM (colored dissolved organic matter), turbidity, and chlorophyll *a*, and to predict when elevated concentrations of one or more of these properties result in light levels falling below the minimum light requirements of *Thalassia testudinum* at multiple depths. This was accomplished by collecting *in situ* and discrete water column optical properties at SFWMD and SCCF SAV stations, measuring growth rates of *T. testudinum* throughout the study area, and taking advantage of a high quality real-time observing system in the Caloosahatchee. *Thalassia testudinum* growth rates were positively correlated to salinity and temperature and negatively correlated to light attenuation, as expected. The strength of the correlation between growth rate and light attenuation was significant, but since data were only collected in the dry season at the end of a drought, there was little chance to capture responses to light limitation often associated with increased freshwater flows. The performance of four optical models indicated that for the Caloosahatchee, San Carlos Bay, and Pine Island Sound, optical properties more reliably predict blue light attenuation than Kd PAR. This was because the primary optical constituent CDOM explained 64 and 89 percent (PAR, BLUE) of the variability in light attenuation. Given that light absorption by seagrass is not uniform throughout the visible spectrum, blue light attenuation better represents the light conditions that affect seagrass growth. Although good benchmarks for PAR have been established (Dennison et al., 1993), there are currently no good estimates of blue light photosynthetic efficiencies for SAV. Therefore, a commonly applied 20 percent light at depth threshold was used as the minimum light requirements for SAV. Hourly optical data (collected from 10:00 to 14:00) were used to predict Kd PAR for the study period (Dec '07 to May '08). The minimum light requirements were met for all regions of the Caloosahatchee, Pine Island Sound and San Carlos Bay in 1.0 m or less. Minimum light requirements in San Carlos Bay were met at depths less than 1.5 m, while in Pine Island Sound the minimum light requirements were met at all depths less than 1.0 m. These estimates correspond to the depth of the deep edge, with the exception of Pine Island Sound where SAV can be found at depths of up to 3.0 m. This discrepancy in Pine Island Sound was likely caused by localized turbidity spikes registered by sensors at Blind Pass RECON stations that were not observed during monthly SFWMD and SCCF SAV stations. Estimates of the maximum concentrations of optical properties needed to cause light limitation were in good agreement with other estimates for Charlotte Harbor. The PAR and BLUE optical models can be easily coupled to real-time data collected by SCCF's RECON. These models are useful in choosing appropriate SAV restoration sites and the best time to conduct large scale everglades restoration projects, such as C-43 reservoir construction.

INTRODUCTION

Widespread losses, declines in density and changes in the distribution of seagrass communities occur as the result of natural and anthropogenic activities (Short and Wyllie-Echeverria, 1996). Natural stresses include disease (Muehlstein et al., 1988; Durako and Kuss, 1994), herbivory (Zimmerman et al., 2001), and changes in water column clarity due to algal blooms and particulate loading (Hall et al., 1999). Seagrass losses have been documented in the Charlotte Harbor system including the San Carlos Bay and Pine Island regions (see Corbett, 2006). Concerns over such losses have led to the development of water quality-targets (Dixon, 2000, Corbett and Hale, 2006) for natural recovery of seagrass meadows.

Light limitation and salinity are the principle drivers of seagrass distributions in Charlotte Harbor (Kraemer et al., 1999; Greenawalt-Boswell et al., 2006). The primary attenuators of light in the Charlotte Harbor, Pine Island Sound, San Carlos Bay and the Caloosahatchee River and Estuary (CRE) are color (i.e. Colored Dissolved Organic Matter, CDOM), chlorophyll *a* (phytoplankton biomass), and turbidity (an indicator of particulate loading). These optically active water quality constituents have been incorporated in four empirical, multiple regression models to predict the light attenuation coefficient, Kd (McPherson and Miller, 1994). Kd quantifies light attenuation across the entire spectrum of Photosynthetically Active Radiation (PAR). Recently, the effects of light quality (the quantities of light at specific wavelengths of the PAR spectrum) on seagrass growth and management have received attention, especially given the high blue light absorption of CDOM (Milbrandt et al., 2007).

The purpose of this project was to evaluate the relationship between Kds for different regions of the PAR spectrum, and 1) optically active water quality constituents (CDOM, chlorophyll *a*, and turbidity, and 2) growth of the seagrass *Thalassia testudinum* for lower Charlotte Harbor, including the Caloosahatchee River and Estuary (CRE), Pine Island Sound (PIS), and San Carlos Bay (SCB). Data analysis included instrument quality assurance and quality control plus the testing of the following hypotheses; water quality parameters (i.e. CDOM, chlorophyll a, turbidity) can be used to predict light attenuation, light attenuation is correlated *T. testudinum* growth in the CRE and lower PIS, and wavelength-specific light quality (i.e. the quantities of light at specific wavelengths of the PAR spectrum) are better predictors of SAV growth than the full PAR spectrum.

MATERIALS AND METHODS

Study area and types of data collected – The study area was lower Charlotte Harbor which included the Caloosahatchee River and Estuary (CRE), Pine Island Sound (PIS), and San Carlos Bay (SCB) (Figure 1). The study area was divided into three regions based on distributional gradients of SAV species (Chamberlain and Doering, 2000; Bortone and Turpin, 2000). Region 1 was the Caloosahatchee River and Estuary, where *Vallisneria americana* was the dominant macrophyte until recently. Region 2 was San Carlos Bay, where *Thalassia testudinum* and *Halodule wrightii* are co-dominant. Region 3 was Pine Island Sound, and where the dominant species are *Thalassia testudinum* and *Syringodium filiforme*. There were three types of data to be collected; instrument data, water samples and associated laboratory analysis, and *Thalassia testudinum* growth rates. Instrument data were collected monthly at District and SCCF sites using a portable hand-held Hydrolab Quanta (temperature, salinity, sp. conductivity, DO, and turbidity) and *in situ* with a mobile RECON (Satlantic, Wetlabs). *In situ* instruments were also deployed at fixed locations for the duration of this project (Figure 1, blue stars). Each RECON records measurements of CDOM fluorescence, chlorophyll fluorescence, turbidity, conductivity, depth, dissolved oxygen, nitrate, oxygen saturation, salinity, and temperature in real-time (Comeau et al., 2007). Monthly discrete water samples were collected at SFWMD and SCCF SAV stations (Figure 1; Table 1). Water samples were collected, stored, and analyzed at the laboratory for chl *a* (Welschmeyer, 1994) and CDOM analysis (Green and Blough, 1994; Coble, 1996; Coble et al., 1998). In addition, two 2π multi-channel radiometers (Table 2) offset by 100 cm recorded downwelling irradiance and were used to calculate spectral light attenuation (Kd). For calibration and quality control procedures on instrumentation, see Detailed Approach and Methodology section below. Growth rates were collected following Kraemer and Hanisak (2000), where a syringe needle is inserted through the base of a shoot to mark, and after two to three weeks the shoot is harvested. The area from the base of the shoot to the wound created by the needle is measured (see Detailed Approach and Methodology section below).

Detailed Approach and Methodology

Monthly cruises – Monthly cruises were conducted over the course of 2 days each month to collect samples at stations listed in Table 1. Day one was spent visiting stations in the CRE, while day two was spent visiting stations in PIS and SCB. Prior to embarking on a cruise, a benchtop calibration of the Hydrolab Quanta was performed. Calibration standards (Hach) are used to calibrate specific conductivity, pH (2-point), salinity (1-point), dissolved oxygen (1-point) and turbidity (2-point). Dissolved oxygen membranes were changed 24 hours prior to calibration in water saturated air, following manufacturer's instructions. Upon arriving on station, the collection of instrument data commenced along with the collection of a discrete water sample for laboratory analysis. The LOBO (Land Ocean Biogeochemical Observer, Satlantic, Halifax, CA) was deployed in 0.75-1.00 m depth. LOBO instruments are calibrated annually by the manufacturer (Satlantic, WETLabs, Seabird). After initiating the real-time mode, the instrument was allowed to equilibrate for 1 minute prior to logging. Data flow rates were 1 Hz and a file containing 2 minutes of data was stored for each station. For discrete samples, a Nalgene 500 mL plastic bottle was used to collect a surface water sample by rinsing 3X then collecting the sample and avoiding the water air interface. The sample was stored on ice and transported to the laboratory for filtration and subsequent analysis. In addition, Hydrolab Quanta data were recorded from the display and downwelling irradiance was recorded with two 1 m offset radiometers (Biospherical, San Diego, USA). Biospherical radiometers require annual recalibration, the most recent calibration was June 2007. The data were logged internally along with the time of deployment and downloaded at the laboratory following each cruise. Light attenuation coefficients are unitless and are derived from the Lambert-Beer Law expressed in equation (1)

(1) $K_d = \ln(I_1/I_2) / (Z_2-Z_1)$,

where Kd is light attenuation coefficient, I_1 and I_2 are downwelling irradiances at depths at Z_1 and Z_2. Calculations of Kd for four spectral bands, blue, blue-green, green, and PAR were performed with equation 2. Specific wavelengths measured can be found in Table 2.

Discrete Sample Analysis - Upon returning to the laboratory, 150-200 mL of water were filtered through a 25 mm GF/F filter and wrapped in foil for later acetone extraction. Filters were stored at -20C in a desiccator until extraction, but not longer than one month. Filters were analyzed for chlorophyll *a* concentrations (non-acidified) following methods of Welshmeyer (1994); an acetone extraction, homogenization, centrifugation and analysis on an Aminco-Bowman fluorometer. A standard curve (5 point) with standard spinach chl *a* (Sigma, St. Louis, USA) was generated every 3 months to determine final determination of chl *a* in the samples. Blanks consisted of filtered distilled water, following protocols identical to samples, were routinely run. For CDOM estimation from discrete samples, 50 mL of sample was 0.2 µm filtered and stored at room temperature in foil-wrapped 50 mL screwcap tubes. CDOM samples were run on a Cary UV-Vis spectrophotometer (Varian, Palo Alto, USA) that was baseline corrected with distilled water. Spectral scans from 300-700nm were applied to each sample followed by baseline correction. The corrected absorption at 355 nm was used to estimate CDOM concentrations (Green and Blough, 1994; Del Castillo et al. 2000).

SAV Growth Rates - Growth rates of *T. testudinum* were measured using a leaf marking technique (Kraemer and Hanisak, 2000; Short and Coles, 2001). Leaf marking is accomplished by making a pin hole through the sheath to create a scar on the leaf tissue to indicate the tissue present at the time of marking so that the new leaf production can be identified. The pin holes (scars) in the leaves are located in the harvested plants and leaf productivity is calculated by the amount of new leaf added divided by the number of days since the plant was marked. Six shoots are marked with the syringe then attached with a small cable tie to a colored straw for later harvesting. After two to four weeks, stations were re-visited and the marked plants were harvested. Harvested shoots were transported to the lab in ambient water and measured the day of the harvest. For analysis, leaf area added is estimated as the growth parameter for 5 individuals, averaged by station and month. Relationships between growth rates and monthly light attenuation and water quality parameters were determined with Pearson correlations (SAS, 9.12).

Instrument Quality Assurance and Quality Control - Assumptions about *in situ* measurements and methodologies were tested. The first assumption tested was whether pumping water from the surface into an onboard tank was equivalent to suspending the instrument over the side of the boat. The onboard tank was 212L and painted flat black to minimize reflection and focusing of incident light. Water was pumped into the tank at a rate of 1.4×10^3 L per hour. A student's t-test was used to test for differences between tank and open water for each parameter using the combined dataset from all stations visited in December (SAS 9.12, Cary, USA). A second assumption was whether instrument data collected from fixed locations was representative of SFWMD and SCCF stations. Identical instrumentation (LOBO) used in this study are deployed throughout lower Charlotte Harbor and report hourly observations on http://recon.sccf.org. Three stations used for this comparison included Fort Myers, Shell Point, and Blind Pass. Additional details about the location and site history can be found on our website. Since hourly data can only be recorded at a limited number of deep water locations, it was necessary to determine if instrument data from RECON is similar to instrument data collected on or near SAV stations. Box-whisker plots of RECON and SAV stations in each of three regions corresponding to the closest RECON station were generated (SAS 9.12). Hourly RECON data collected on the day of, day before, and day after the monthly cruises were compared to data collected monthly at the SAV stations. Only *in situ* data from identical instruments were considered, data from discrete sampling was not used for this comparison. The plots contain data collected during the entire study period (Dec'07 to May'08). A one-way ANOVA (SAS 9.12) tested whether there were differences between fixed and SAV stations. Finally, *in situ* data are compared to data collected by discrete samples by first standardizing (mean = 0, st.dev. = 1), then plotting *in situ* versus discrete. Pearson correlations were used to determine those parameters which had significant agreement (SAS 9.12).

Optical Model - The first hypothesis in this study is that water quality parameters (i.e. CDOM, chlorophyll *a*, turbidity) can predict light attenuation. To test this hypothesis, a general linear regression model was used to correlate optical properties to spectral Kd. Dependant variables were light attenuation in each channel and the three optical properties were the independent variables. Models for both discrete and *in situ* data were run for all stations during the period of December 2007 through May 2008 (6 cruises, 11 stations). The linear representation of Kd can then be written as equation 2:

$$(2) \quad Kd = Kw + Kc\,(CDOM) + Ka\,(chl\ a) + Kn\,(NTU)$$

where Kd is light attenuation, Kw, Kc, Ka and Kn are the partial attenuation coefficients of water, CDOM, chlorophyll *a*, and turbidity, respectively. For estimating percent contribution of each optical constituent the optical data were first standardized (mean = 0, st. dev.=1) because of the disparity in scales among CDOM, chl *a*, and NTU. The contribution of each property or constituent was expressed as a percent of Kd BLUE and Kd PAR.

Model Predictions – The optical model derived from the *in situ* dataset was used to calculate hourly light attenuation predictions at three fixed RECON sites. Incident irradiation collected from the roof of SCCF Marine Laboratory hourly was used to account for solar angle and other changes in incident irradiance in the study area. Data were collected with a multichannel radiometer calibrated for atmospheric readings (Biospherical, San Diego, USA) and recorded incident light for the channels listed in Table 2. The optical model predicted Kd BLUE and Kd PAR from the optical properties collected at RECON sites. Irradiances and percent light at six depths (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 m) were calculated from the predicted Kds and the measured incident irradiances. A histogram of the percent light at each depth and location was plotted in SAS (v. 9.12). The optical models (*in situ* and discrete) were also used to estimate the maximum concentration of each optical property that would cause percent light at depth to be less than 20 percent of incident light levels. The 20 percent threshold was chosen from minimum light requirements for *T. testudinum* in Florida (Dennison et al., 1993). For each property, the attenuation caused by water (Kw) and the attenuation of the other two constituents was held constant while the equation was solved for the K of interest (CDOM, chl *a*, NTU). Estimated percent light at depth was determined (equation 3) by calculating light at depth from downward irradiance from a sensor mounted atop the SCCF marine laboratory during the study period (Dec 07 to May 08).

(3) % Light at Depth = (Iz / Id)*100,

where Iz is downwelling irradiance at depth and Id is downward irradiance.

RESULTS

During the first cruise of the sampling period (Dec ’07), it was necessary to determine the difference, if any, between optical and water column parameters collected *in situ* versus collected in a reflection-controlled tank with ambient water. Data from all study sites depicted in Figure 1 were averaged by parameter and reported as mean values with standard deviation error bars in Figure 2. There were no differences between data collected *in situ* and data collected in the reflection-controlled tank as means and error bars overlapped between *in situ* and tank. Therefore, all remaining cruises used *in situ* deployments throughout the study area deployed at 0.8 to 1.0 m depth.

In situ water column parameters were plotted by region for each month. Nitrate was highest in region 1 with a decreasing gradient in regions 2 and 3 (Figure 3). Nitrate concentrations ranged from 0 to 15 µM. Conductivity and salinity (Figures 4 and 5) were lowest (15-20 PSU) in region 1 and euryhaline (33-37 PSU) in regions 2 and 3. Variability among stations in salinity was higher in regions 1 and 2 (s.d. 3 PSU). Chlorophyll concentrations were highest in region 1 (up to 6 µg/L) and equivalent in regions 2 and 3 (2 µg/L) during the first half of the study period (Figure 6). During the second half of the sampling period (Mar ’08 to May ’08) chlorophyll concentrations in the lower estuary increased in regions 2 and 3 (3-5 µg/L) and decreased in region 1 (4-6 µg/L). Turbidity (Figure 7) was equivalent in all regions for 5 of 6 months with an increasing gradient from region 1 (2 NTU) downstream (6 NTU) to region 3 during Mar ’08. CDOM (colored dissolved organic matter) concentrations (Figure 8) were highest at upstream stations in region 1 (75-100 Quinine Sulfate Dihydrate Equivalents, QSDE) with a decreasing gradient moving downstream. CDOM in regions 2 and 3 were statistically equivalent and significantly lower than region 1. Dissolved oxygen concentration (Figure 9) showed no consistent pattern and never approached the 2 mg L^{-1} threshold for hypoxia.

Relationships between *in situ* water column parameters were determined with data collected for all stations and all months. The result of the Pearson correlation (SAS 9.12), the sample size, and *p*-value are reported in Table 3. The high sample number (e.g. 5000-9000) was the result of the sampling rate (1 Hz) of the *in situ* instrument used. All parameters were found to be significant because of the high sample sizes which are reflected in degrees of freedom. However, the strength of the correlation can be found in the *R*-square value reported. A high, positive *R*-square value was found between CDOM and nitrate and CDOM and chlorophyll *a*. Negative R-square values were found between salinity and nitrate, salinity and CDOM, and chlorophyll *a* and salinity, indicating that lower salinities were coincident with higher nitrate, CDOM and chlorophyll.

Similar comparisons were made among discrete water column samples and between these samples and growth rates of *T. testudinum* (Table 4). Significant positive relationships were found between growth rates and chlorophyll *a*, salinity, and temperature. A plot of the *T. testudinum* growth rates from region 2 and region 3 indicate that there is slower growth near the source of freshwater (Caloosahatchee) and that there is a strong seasonal component to SAV growth (Figure 10). No growth rates from region 1 were reported because this species does not extend upstream into region 1. There were significant negative (Table 4) relationships found between growth and light attenuation (Kd PAR and Kd BLUE). Other optical properties that were correlated to Kd PAR and Kd BLUE were CDOM, salinity and turbidity (Table 4).

Discrete and *in situ* data collected during the study period were compared by plotting standardized values against each other (Figure 11). Temperature, salinity and CDOM have very good agreement with slopes greater than 0.93 and *R*-squared values greater than 0.87. Turbidity also had good agreement (slope = 0.88; R-squared = 0.80). Dissolved oxygen had moderate agreement between discrete and *in situ* data (slope = 0.79; *R*-squared = 0.62). Chlorophyll *a* showed poor agreement between discrete and *in situ* (slope = 0.55; *R*-squared = 0.30)

In situ and discrete water column optical properties from all stations and all months were aligned with simultaneously collected light attenuation (Kd PAR and Kd BLUE). A general linear model was applied to produce 4 empirical optical models from the data collected; two using discrete samples (4a, 4b) and two using *in situ* samples (5a, 5b). The following equations define the empirical optical models:

(4a) $\text{Kd BLUE} = 1.32 + 3.85\ [\text{CDOM}] - 0.01\ [\text{CHLA}] + 0.72\ [\text{NTU}]$ Discrete

(4b) $\text{Kd PAR} = 0.98 + 0.77\ [\text{CDOM}] - 0.01\ [\text{CHLA}] + 0.05\ [\text{NTU}]$ Discrete

(5a) $\text{Kd BLUE} = 0.090 + 0.045\ [\text{CDOM}] - 0.012\ [\text{CHL}] + 0.165\ [\text{NTU}]$ *In situ*

(5b) $\text{Kd PAR} = 0.448 + 0.015\ [\text{CDOM}] - 0.057\ [\text{CHL}] + 0.112\ [\text{NTU}]$ *In situ*

The empirical optical models were compared in Table 5. The number of stations and months were identical for both models; however, the *in situ* model is ~ 100 times the number of data than the discrete model because of the high frequency (1 Hz) collection rate of the *in situ* instrumentation versus 1 sample (per month) for a discrete sample. The discrete model for Kd BLUE and Kd PAR had significant fit but moderate to poor *R*-squared values (0.46; 0.27). Optical models using *in situ* data were also significant and had 2X greater *R*-squared values (0.84; 0.57). Kd BLUE had greater *R*-squared and F values than Kd PAR and therefore had a better fit to the dataset. The same dataset was used to predict Kd values from optical properties and was plotted against observed values (Figure 12). The percent contribution of optical properties to prediction of Kd in the models is summarized in Table 6. In all models, the contribution by chlorophyll was negative and was therefore ignored. CDOM and turbidity (NTU) were the primary components contributing to light attenuation. Percent contribution (magnitude) of optical constituents to PAR light attenuation differed in the discrete and *in situ* models. In the *in situ* PAR model, the contribution of CDOM was 65% while turbidity (NTU) contributed 35%.

Turbidity was the primary contributor in the discrete model (57%), with CDOM as a secondary component (43%). The primary contributor to BLUE light attenuation, using the *in situ* model, was also CDOM (79%) with turbidity as the secondary contributor (35%).

Water column properties among fixed stations and SAV stations were compared by region. RECON nitrate was more variable than SAV stations, but the median and mean values were within 5 µM in region 1 and within 2 µM in regions 2 and 3 (Figure 13). CDOM variability was equivalent between RECON and SAV stations and mean values were within 10 QSDE in region 1 and within 5 QSDE in regions 2 and 3 (Figure 14). Chlorophyll *a* concentrations were similar in both variability and in central tendency throughout the study area (Figure 15). Median values were within 1 µg L^{-1} for all regions. Dissolved oxygen concentrations were also similar in variability and central tendency (Figure 16), with mean concentrations falling within 1 mg L^{-1} between fixed RECON and SAV stations for all regions. Turbidity had greater variability and central tendency at RECON stations with mean values 2 NTU higher than at SAV stations (Figure 17). Temperature was similar in both variability and central tendency at RECON and SAV stations with mean values often within 2 °C (Figure 18). There were differences in mean salinities between RECON and SAV stations in region 1 (Figure 19). Salinity in regions 2 and 3 were similar with mean values falling within 1 psu.

Light attenuation was predicted from RECON data during the study period. Histograms describing the percent light at depth experienced by SAV during mid day (10:00 to 14:00) were generated for each fixed RECON station (Figures 20-22). For region 1, CRE (Caloosahatchee River and Estuary), the percent PAR at depth during the study period was below 20% for the mid-day period at depths greater than 1.0 m (Figure 20). For region 2, SCB (San Carlos Bay), the percent PAR was below 20% for the entire mid-day period at depths greater than 2.0 m (Figure 21). For region 3, Pine Island Sound, the percent of PAR at depth was less than 20% for the entire mid-day period at depths greater than 2.0 m (Figure 22). From the histograms, the proportions of time (mid day) when light requirements were met or were exceeded are summarized in Table 7.

The maximum concentration of optical constituents that would cause light limitation at various depths is summarized in Tables 8 and 9. Table 8 estimates are calculated using the PAR model; Table 9 estimates are calculated using the BLUE light model. Chlorophyll values in Table 9 are 10X higher than routinely measured and are suspect due to the very low contribution of chlorophyll *a* to the prediction of blue light attenuation (less than 1%).

DISCUSSION

The management and protection of critical habitat is central to discussions of water quality, basin action management plans (BAMP) and to the shared missions of SCCF and SFWMD to protect natural coastal habitats and to improve water quality and natural systems. To be successful, managers must be provided with the high quality information in near real-time and a set of tools that can be applied to protect and restore coastal resources. This research and SCCF RECON provide a data source and a tool for improved management of SAV habitats in the Caloosahatchee River and Estuary, San Carlos Bay, and

Pine Island Sound. Large scale restoration projects can be immediately evaluated if changes to optical constituents are known.

The study period Dec ’07 to May ’08 was a period of little to no rainfall following a significant drought that began in the fall of ’06. Mean salinity near US 41 bridge was 20 psu during the study period; median values are normally less than 15 psu (SFWMD, 2005). Marine water (high salinity, low CDOM) was distributed throughout the study area. However, there was still evidence of the estuarine gradient in the water column from region 1 (CRE) downstream to regions 2 (SCB) and 3 (PIS). There was no *in situ* nitrate data collected in Dec ’07 because of an instrument communication problem. Nitrate, conductivity, salinity, chlorophyll *a*, and CDOM demonstrated evidence of a difference between region 1 (CRE) and regions 2 (SCB) and 3 (PIS) as a result of the estuarine gradient. For most months, turbidity was equivalent throughout the study area, however, in Jan and Mar ’08 there was a gradient of increasing turbidity from upstream to downstream. This was likely caused by wind-driven re-suspension as stations in region 3 have a longer fetch and are not protected by mangrove islands or the constriction of the upper estuary. There was no evidence of the estuarine turbidity maximum (ETM) in region 1 as previously reported. It is likely that the ETM was further upstream and very near the Franklin Lock (Fugate, unpublished data).

When *in situ* data from all months were combined and compared to each other, there was cross correlation between CDOM and nitrate. Given that the instrument calculates nitrate concentrations from portions of the UV spectrum (200 nm to 220 nm), it is possible that there is interference caused by CDOM, as CDOM absorbs in the UV. The peak absorption of CDOM of terrestrial origin is 350 nm (Moran et al., 1991) which is non-overlapping with the nitrate calculations. A strong cross correlation was also found between CDOM and salinity which was expected and recently reviewed by Coble (2007). Chlorophyll was negatively correlated with salinity, but the significance relationship is suspect because of the short duration of the study period that included only a small fraction of the phytoplankton growing season. However, it is reasonable that either grazing pressure by bivalves (Alpine and Cloern, 1992) or low N and P concentrations in the lower estuary caused low phytoplankton biomass. During the same period, concentrations of nitrate in the lower estuary (regions 2 and 3) were often at the detection limits (± 1μM) of the *in situ* instrumentation, suggesting that N-limitation was possible (Howarth and Marino, 2006).

Thalassia testudinum growth was correlated to several optical and other water column properties. A seasonal signal was evident in the positive correlation between growth and temperature. Previous observations indicate that seasonal growth patterns of SAV are expected (Fox et al., 2008; Milbrandt, in prep.). The seasonal increase in chlorophyll *a* in the water column was also expected as day length, solar angle, and temperature increase. A positive correlation between growth and salinity was previously demonstrated by Doering and Chamberlain (2000) and was expected given the salinity gradient present within the study area.

Significant negative correlations were found between growth and Kd PAR (Pearson; R-squared -0.3585; $p<0.05$) and Kd BLUE (Pearson; R-squared -0.32559; $p<0.05$). Given that *T. testudinum* only grows in regions 2 and 3, light attenuation and concentrations of optically active properties were low relative to a wet period. The correlation values were expected to be higher, but because there was zero freshwater discharged to the estuary during the study period, the values were low. Yet, there was some evidence of light limitation as indicated by the significant negative relationships. Additional growth measurements are needed during a period of freshwater discharges to better develop growth response to optically active properties which are associated with freshwater discharges.

Of the four optical models presented, two were created with the discrete dataset (Eq. 4a and 4b) and two with the *in situ* dataset (Eq. 5a and 5b). The two datasets were compared before model development by plotting discrete versus *in situ*. This comparison yielded some new information about *in situ* instrument versus laboratory sampling methods. Perfect agreement was considered to be when slope and R-squared equals 1. Temperature and salinity had excellent agreement, with the exception of an outlier on salinity these two properties would be near perfect. These properties were instrument to instrument comparisons, as a Hydrolab Quanta was used for discrete samples while the Wetlabs WQM was used for *in situ* sampling. Turbidity was also an instrument to instrument comparison and the agreement was good. In another instrument (Hydrolab Quanta) to instrument (Wetlabs WQM) comparison discrete DO and *in situ* DO had fair to good agreement. Good agreement was not expected because *in situ* sensors measure everything suspended in the water column which causes interference. CDOM determination in the laboratory consisted of 0.2 µm filtered samples run on a spectrophotometer and a specific wavelength was used to determine absorption. CDOM determination *in situ* is a fluorometric approach, exciting at 350 nm and measuring emission at 430 nm. Laboratory analyzed samples eliminate particles through filtration; phytoplankton and organic particles absorb UV and emit photons. Despite the differences in methodology there was very good agreement when data were standardized. Good agreement was not found, however, between acetone extracted discrete chlorophyll and *in situ* chlorophyll. Previous comparisons have yielded slopes of 0.5 and R-squared values of 0.80 which was presented at the Charlotte Harbor Summit, but was never published (Milbrandt unpublished data). One of the possible reasons for the failed agreement between acetone extracted and *in situ* chlorophyll may be found in the very low percent contribution by chlorophyll to light attenuation. Possible causes of low percent contribution are that chlorophyll concentrations did not vary as highly as CDOM and turbidity during the study period and therefore was not enough of the possible range of chlorophyll values to develop a predictable relationship. Wet season observations are necessary to adjust this discrepancy.

The performance of the four optical models presented indicated that for the Caloosahatchee, San Carlos Bay, and Pine Island Sound, water column optical properties more reliably predict blue light attenuation than PAR. Also, models derived from the *in situ* dataset predicted Kd more reliably than models using discrete dataset. The *in situ* Kd PAR model differs from previous optical models that encompass the entire Charlotte Harbor (McPherson and Miller, 1994, Corbett and Hale, 2006). While coefficients are difficult to compare directly without first standardizing all models to a mean of 0 and standard deviation of 1, it is possible to compare the magnitude of each optical constituent to light attenuation, a unitless number. Since this is the first report of an optical model which has a spectral component, only PAR can be discussed in relationship to previous efforts. The Caloosahatchee (including Caloosahatchee River and Estuary, San Carlos Bay, and lower Pine Island Sound) optical model (presented in this report) differs from the Charlotte Harbor optical model (McPherson and Miller, 1994) primarily in the influence of chlorophyll. Chlorophyll *a* was responsible for 21% of Kd PAR in Charlotte Harbor, 27% in Tampa Bay, and was a negative constituent in the Caloosahatchee. Chlorophyll was negative because of the very low concentrations during the limited study period. Most of the light attenuation in the Caloosahatchee was from CDOM (65%), where CDOM in Charlotte Harbor, (McPherson and Miller, 1994), was a secondary contributor (18%). Turbidity was the primary contributor to Kd PAR in Charlotte Harbor (55%) and Tampa Bay (54%) (McPherson and Miller, 1994) but was a secondary contributor to Kd PAR in the Caloosahatchee (33%). Given that CDOM absorption was the primary optical constituent, it was expected that blue light would be more reliably predicted than PAR. Previous work also suggests particles absorb and scatter greater in the blue than in the rest of the visible spectrum (Diersson et al., 2006). Previous work suggests that *Thalassia testudinum* does not absorb uniformly throughout the visible spectrum (Zimmerman, 2003). All SAV absorb strongly in the blue and the red and not in the green, orange or yellow. For the Caloosahatchee, absorption of blue light and prediction of water column attenuation of blue light is especially relevant to SAV resource protection because of high concentrations of CDOM.

The criteria used to define light limitation was the 20% light at depth requirement proposed by Dennison et al. (1993) for Florida *T. testudinum*. The principle is that each SAV species has a minimum light requirement to sustain the population. This principle was derived from a series of experiments where the portion of the day that light intensity saturated SAV photosynthesis (Hsat) was manipulated (Dennison and Alberte 1985). Shoot density, growth rates, and reproductive rates depend on the daily light requirement (Hsat) and the minimum light requirement for SAV is expressed as the percent of incident light at the depth of interest (Zimmerman et al., 1995). The proportion of time when percent light at depth was equal to or greater than 20% was provided. The 20% light requirement is adequate to saturate photosynthesis for Florida populations of *Thalassia testudinum* (Dennison et al. 1993). In region 1 (CRE), there is no *T. testudinum*, but was historically *Vallisneria americana*, a species with low light adaptive characteristics which was killed by soaring salinities during the last drought. A 20% light requirement likely overestimates that which is necessary for *V. americana* populations, where 2-9 % light requirement was suggested by Carter et al. (2000) from field observations. Light requirements in region 1 were met 100% of mid-day time in depths of 0.5 m or less. At 1.0 m depth, light requirements were met 21% of time, which is consistent with historical observations of SAV distributions being less than 1 m depth (Doering, personal communication). In region 2, San Carlos Bay, light requirements were met 75% of the time in water less than 1.5 m. These predictions are consistent with the deep edge depth of *T. testudinum* near 1.5 m. In region 3, light requirements were met 75% of time in water less than 1.0 m depth but only 35% of the time at 1.5 m. This is not consistent with deep edge observations which have been observed up to 3 m. This apparent inconsistency may be the result of the contribution of turbidity to light attenuation in the model and the high turbidity readings in this region. For this report, the spiky turbidity data recorded at Blind Pass is questionable and was not cleaned or filtered before the model was used to predict percent light at depth. High turbidity conditions at the

Blind Pass (region 3) RECON station are frequent and an order of magnitude higher than other RECON stations. It is unclear why this occurs, but heavy boat traffic on the ICW (intercoastal waterway) or macrofouling on the fluorometer have been suggested by the manufacturer. A nearby RECON station, Redfish Pass in region 3, does not have a similar history of turbidity spikes and would provide a more realistic prediction of light availability.

The estimated concentrations of optical properties necessary to exceed light requirements of Florida *T. testudinum* at several depths was provided. The estimates assume that all other optical properties are held constant and therefore are overestimates as a combination of optical properties would synergistically cause light limitation. For the first time, the estimates presented in this report provide a benchmark to gauge conditions of the estuary in real-time.

They also further illustrate the differences between the PAR and BLUE optical models. The PAR estimates reflect the relative equally important contributions of CDOM and turbidity in the model. Similarly, the BLUE estimates reflect the primary contribution of CDOM to blue light attenuation. Given that the blue light requirement for *Thalassia testudinum* is unknown, the 20% light at depth rule was applied but should be interpreted cautiously. Given that chlorophyll was a non-contributor either model (0%), estimates that are suspiciously high (up to 627 $\mu g\ L^{-1}$) should be ignored. Generally, the chlorophyll maximum necessary to exceed light requirements agree with previous estimates in Charlotte Harbor (Corbett and Hale, 2006). However, turbidity maximum for each depth was half the maximum reported in the Corbett and Hale (2006) model.

Management Implications

Water column properties are dynamic and therefore more frequent measurements are needed to let managers know when and where light limitation is occurring. While the decision tree for making changes in discharge volumes is often not dictated by threatened estuarine resources, a manager can be aware of conditions that cause light limitation and the possible threats to the resources. If this analysis is not in real-time, the manager is retrospectively assessing conditions and implementing action on those conditions that have probably changed. Therefore, several tools for real-time assessment of light limitation conditions were presented in this report.

The PAR and BLUE models presented can be easily coupled to real-time data collected by SCCF's RECON. As the salinity decreases and light attenuation increases in the lower estuary, the SAV minimum light requirement can be tracked hourly at several stations throughout the Caloosahatchee. In addition, the estimated percent light at depth will be useful in choosing appropriate SAV restoration sites and the best time to conduct such projects. Restoration activities can be timed around episodic discharges because current conditions will be known in real-time.

The work supported the District's mission "to improve water quality and natural systems" by providing data for the evaluation and/or improvement of existing water quality targets in the CRE and lower PIS.

Furthermore, the results of this project support the development of technical documents for Basin Action Management Plans (BMAP) for the Caloosahatchee system as mandated by Florida Senate Bill No. 392 passed in 2007. Specifically the results of this project provide ecologically important information which can be used for 1) identifying the contribution of water quality parameters to light quality and ultimately *T. testudinum* distributions in these estuarine and coastal systems, and 2) to enable future research and monitor efforts to employ methodology appropriate for measuring pertinent water quality parameters for the purpose of assessing the effectiveness/success of Management Measures (e.g. water storage and treatment facilities) to achieve nutrient and organic matter load reduction targets in these estuaries.

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Sources of unpublished data

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Table 1. List of station locations, station type, measurement type, and measurement frequency for optical, chemical, physical and biological data collection for the period of 12/07-6/08.

Monthly Sampling Day	Station	Lat	Lon	Type	Hourly Measurements	Monthly Measurements
1	41 bridge	26.64934 N	-81.88097 W	Fixed/LOBO	CDOM, Chl a, turbidity, conductivity, depth, dissolved oxygen, nitrate, oxygen saturation, salinity, temperature	CDOM, Chl a, turbidity, light attenuation, conductivity, depth, dissolved oxygen, nitrate, oxygen saturation, salinity, temperature
1	Shell Point	26.52332 N	-82.00890 W			
2	Pine Island Sound	26.49550 N	-82.14880 W			
1	SFWMD-1	26.68972 N	-81.83000 W	SFWMD WQ/SAV Monitoring Site	None	
1	SFWMD-2	26.67250 N	-81.86444 W			
1	SFWMD-5	26.52676 N	-81.95605 W			
1	SFWMD-6	26.51322 N	-81.98065 W			
2	SFWMD-7	26.50937 N	-82.04432 W			CDOM, Chl a, turbidity, light attenuation, conductivity, depth, dissolved oxygen, nitrate, oxygen saturation, salinity, temperature and *T. testudinum* growth rates
2	SFWMD-8	26.49810 N	-82.01751 W			
2	SCCF-Toll	26.48357 N	-82.01056 W	SCCF SAV Monitoring Site		
2	SCCF-Causeway	26.47836 N	-82.02574 W			
2	SCCF-WoodPoint	26.46888 N	-82.05756 W			
2	SCCF-TarBay	26.45783 N	-82.08643 W			
2	SCCF-Refuge	26.48415	-82.14769			

Table 2. Spectral channels of radiometers (Biospherical, San Diego, CA)

[redacted]

Table 3. Relationships among *in situ* water column parameters. A Pearson correlation and associated statistics are listed (SAS 9.12). High sample numbers are a result of the 1 Hz output of *in situ* instrumentation.

[redacted]

Table 4. Relationships among discretely sampled water column parameters, including *Thalassia testudinum* growth rates. A Pearson correlation and associated statistics are listed (SAS 9.12); relationships in bold are statistically significant (p<0.05).

Table 5. Optical model performance. Fit statistics and sample sizes of multiple regression models from discrete and *in situ* datasets.

Optical Model		Discrete	*In situ*
K_d BLUE			
	n	63	7623
	R-squared	**0.46**	**0.84**
	F value	17	13402
	p value	<0.001	<0.001
K_d PAR			
	n	62	7509
	R-squared	**0.27**	**0.57**
	F value	7.15	3271
	p value	<0.001	<0.001

Table 6. Contribution of optical properties to the optical models.

Optical Model		Discrete	*In situ*
$K_{d\ BLUE}$			
	CDOM	71%	79%
	chl a	0%	0%
	NTU	27%	21%
$K_{d\ PAR}$			
	CDOM	43%	65%
	chl a	6%	3%
	NTU	57%	35%

Table 7. Percent of time when PAR at depth was adequate for supporting Florida *Thalassia testudinum.* Light requirements are based on 20% minimum light requirements in Dennison et al. (1993). PAR requirements were met when light at depth exceeded 20% of incident light.

	Region 1-CRE PAR requirements		Region 2-SCB PAR requirements		Region 3-PIS PAR requirements	
Depth	met	not met	met	not met	met	not met
0.5 m	100%	0%	100%	0%	98%	2%
1.0 m	78%	22%	97%	3%	84%	16%
1.5 m	0%	100%	73%	27%	24%	76%
2.0 m	0%	100%	8%	92%	0%	100%
2.5 m	0%	100%	0%	100%	0%	100%
3.0 m	0%	100%	0%	100%	0%	100%

Table 8. Estimated maximum concentrations of optical properties necessary to exceed light requirements of Florida *Thalassia testudinum* at the given depth. Exceedance criteria are based on the 20% incident PAR requirement (Dennison, et al., 1993). Estimates are based on the *in situ* empirical optical model for the Caloosahatchee River Estuary, San Carlos Bay, and Pine Island Sound.

	Concentration to exceed light requirements					
	0.5 m	1.0 m	1.5 m	2.0 m	2.5 m	3.0 m
Chl a	46.4	18.2	8.7	3.9	1.1	0
CDOM	196	89	52	34	24	16.7
NTU	25.1	10.8	5.9	3.51	2.1	1.1

Table 9. Estimated concentrations of optical properties hypothesized to cause light limitation of Florida *Thalassia testudinum* at the given depth. Exceedance criteria are based on the 20% blue light requirement proposed in this report. Estimates are based on the *in situ* empirical optical model for the Caloosahatchee River Estuary, San Carlos Bay, and Pine Island Sound.

	Concentration to exceed light requirements BLUE					
	0.5 m	1.0 m	1.5 m	2.0 m	2.5 m	3.0 m
Chl *a* (μg L^{-1})	*627	301	192	138	105	84
CDOM (QSDE)	168	82	53	38	29	24
Turbidity (NTU)	47	23	15	11	9	7

*Chl *a* contributed less than 1% to blue light attenuation (Table 6) and therefore concentrations are suspect.

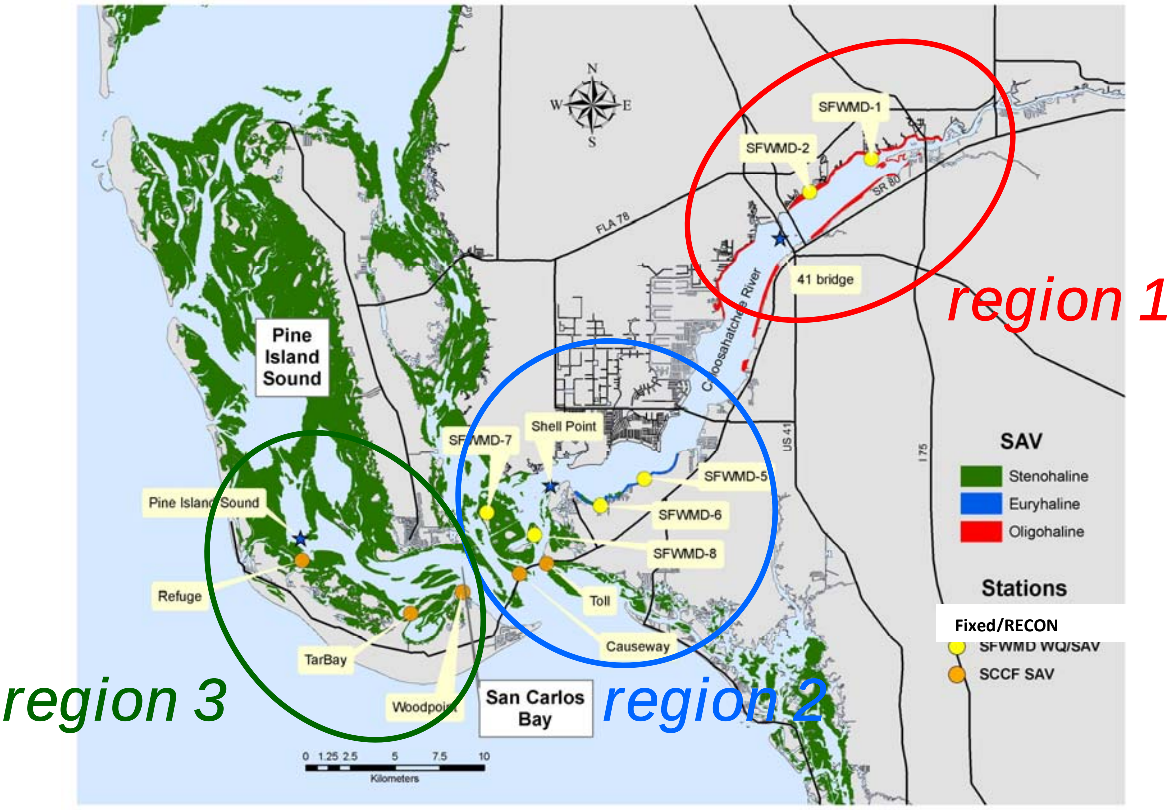

Figure 1. Project map showing station locations for measurement of hourly optical parameters, monthly water quality parameters, and monthly Submerged Aquatic Vegetation (SAV) growth rates. RECON River Estuary Coastal Observing Network (blue stars); South Florida Water Management District water quality stations (yellow circles); Sanibel-Captiva Conservation Foundation SAV stations (orange circles); stenohaline (marine) SAV habitat, from 2006 aerial imagery data; euryhaline (estuarine) SAV habitat, stylized from observational data; and oligohaline (freshwater) SAV habitat, stylized from observational data.

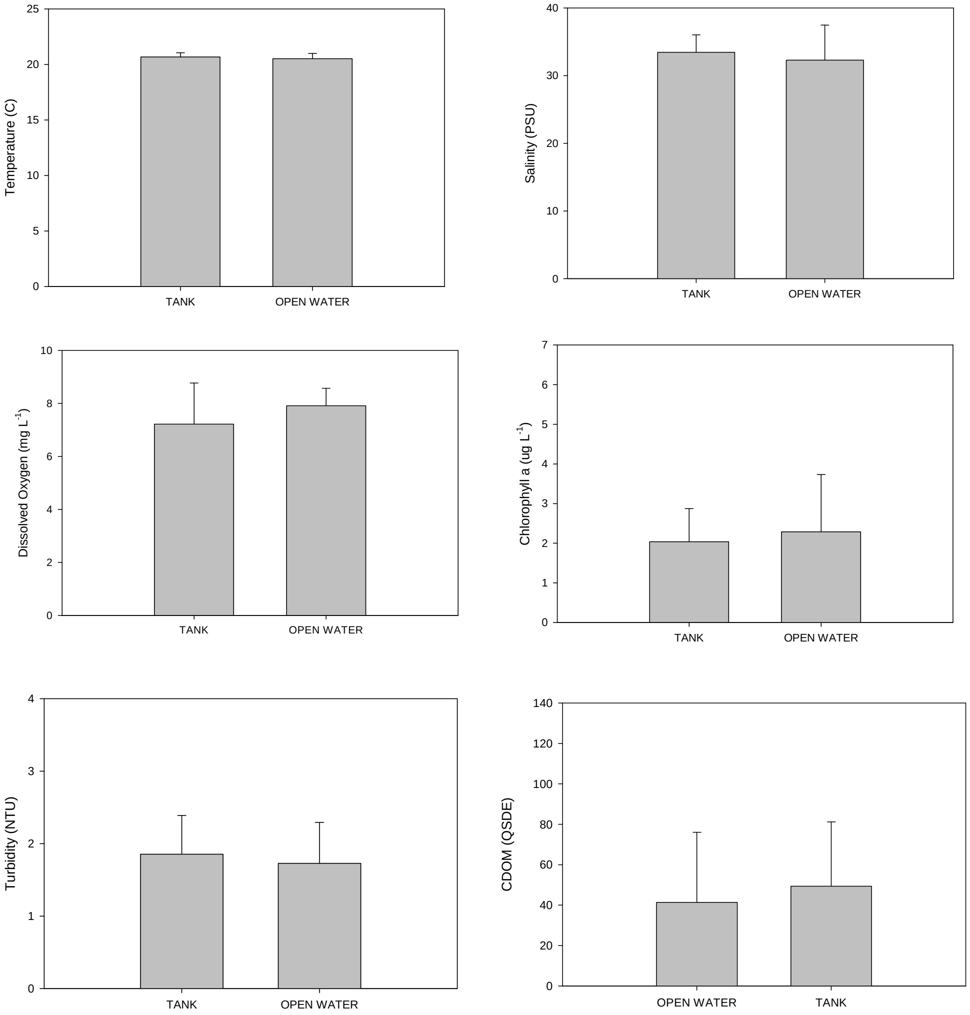

Figure 2. Water column properties measured in an onboard tank versus open water.

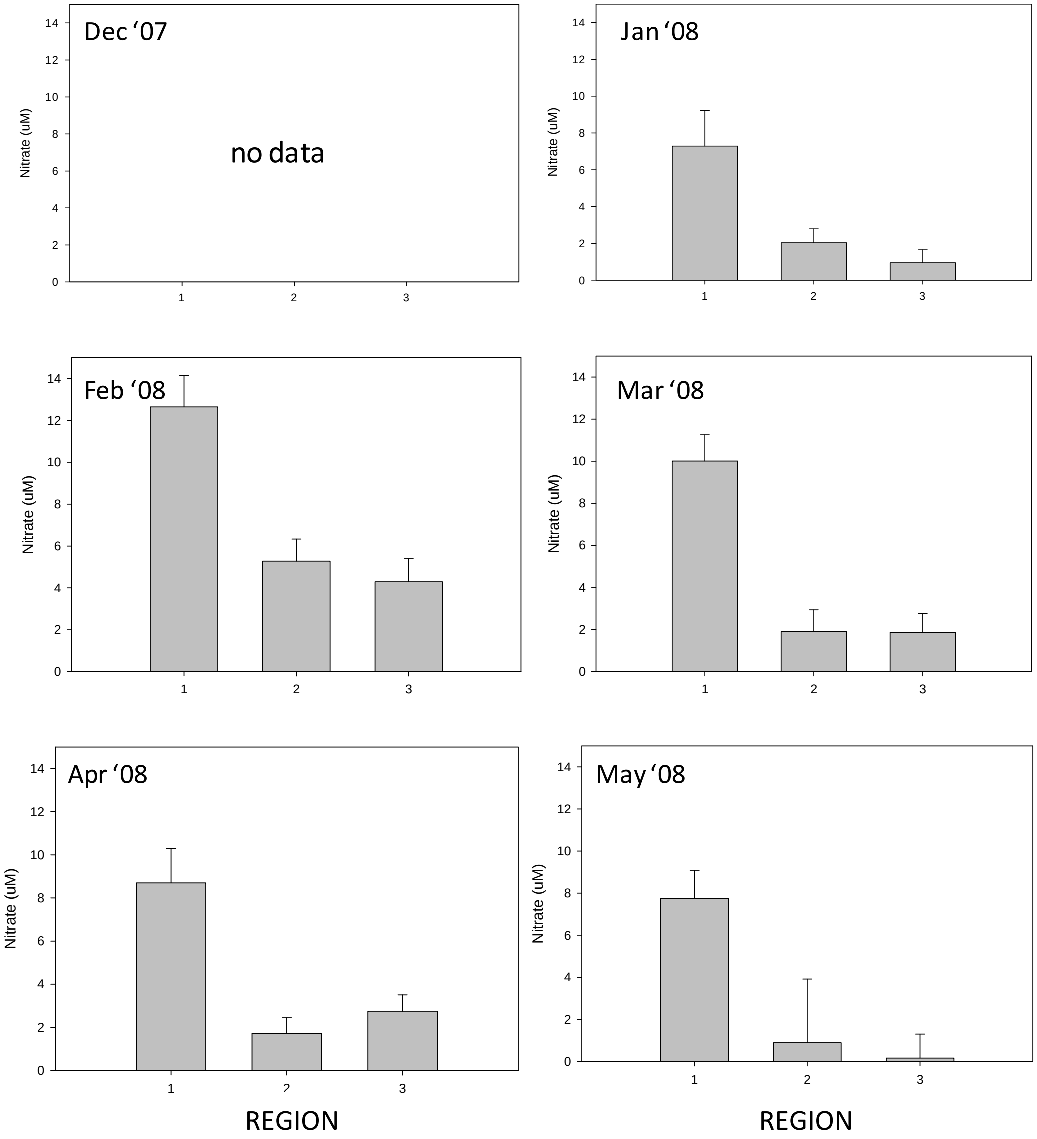

Figure 3. Mean nitrate averaged over the study period (Dec '07 to May '08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

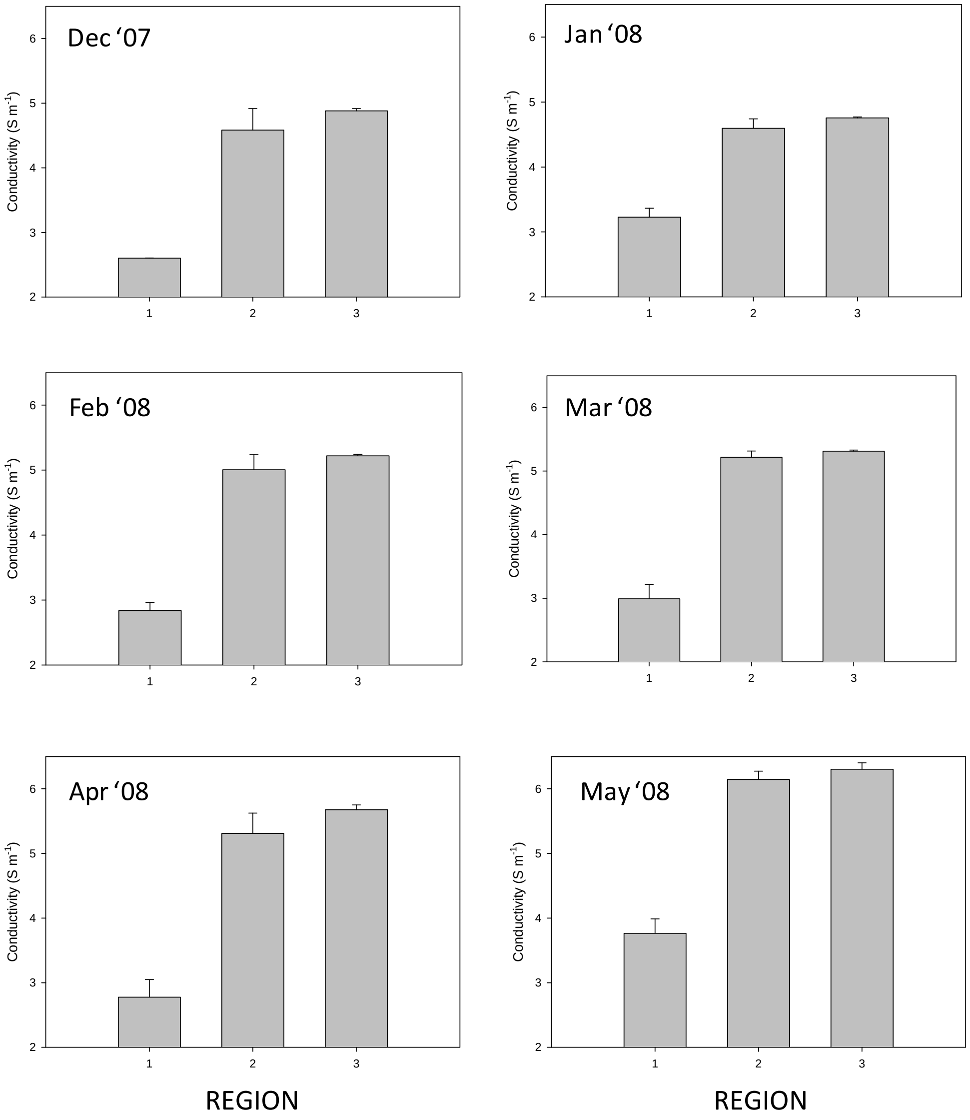

Figure 4. Mean conductivity averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

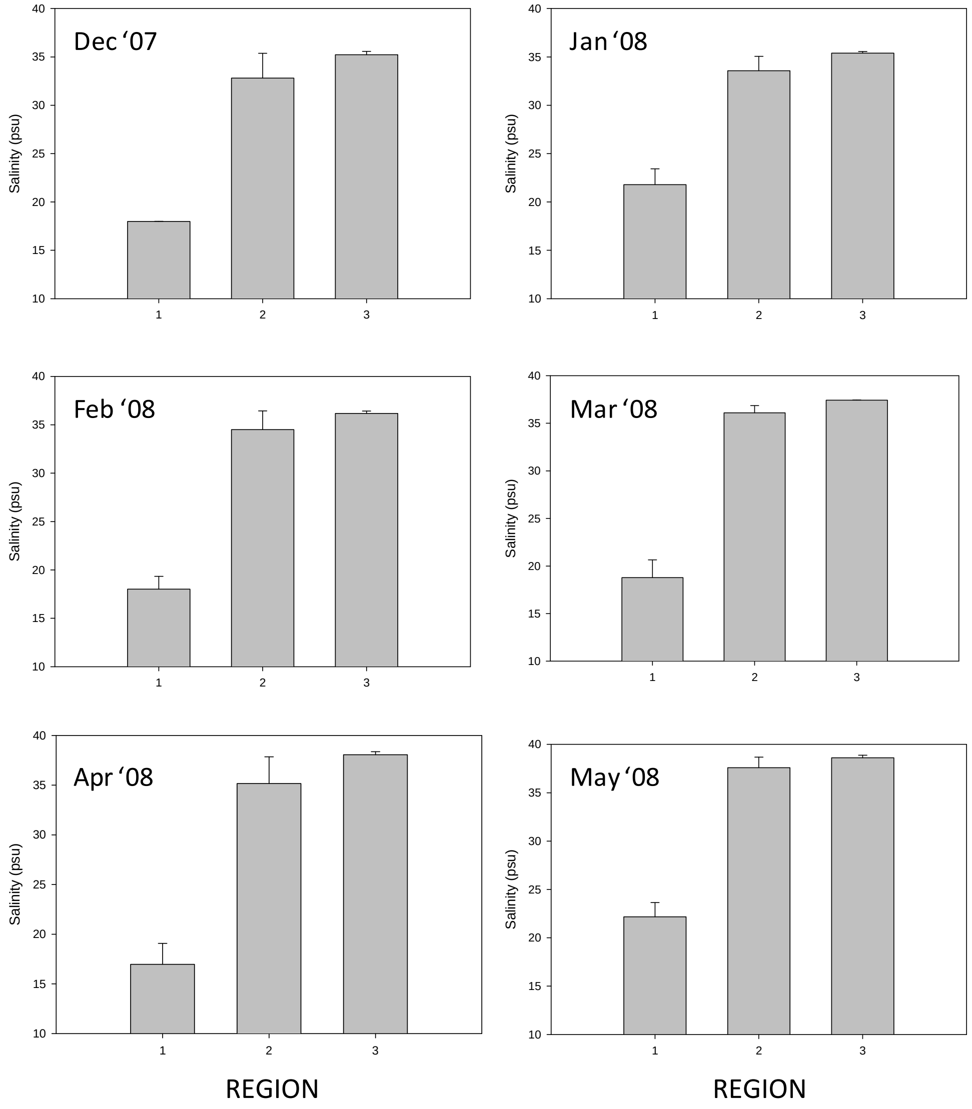

Figure 5. Mean salinity averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

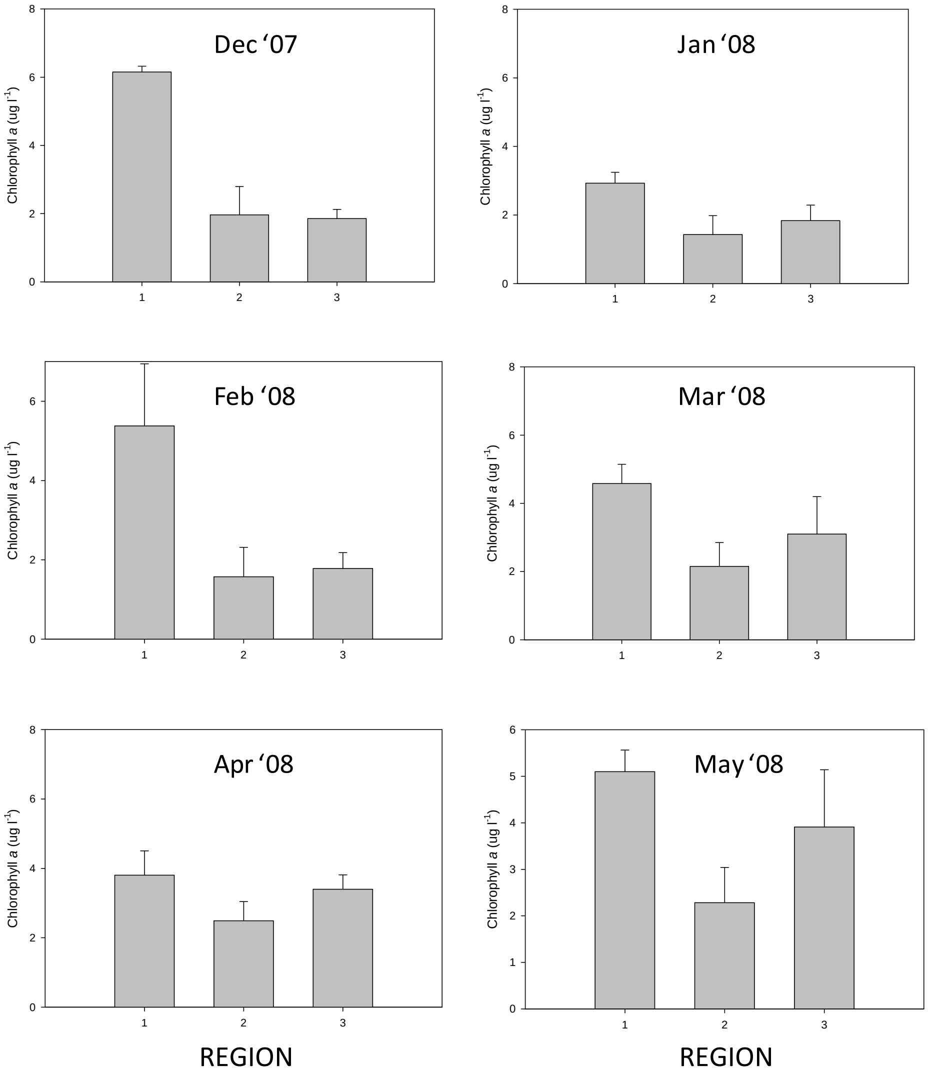

Figure 6. Mean chlorophyll averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

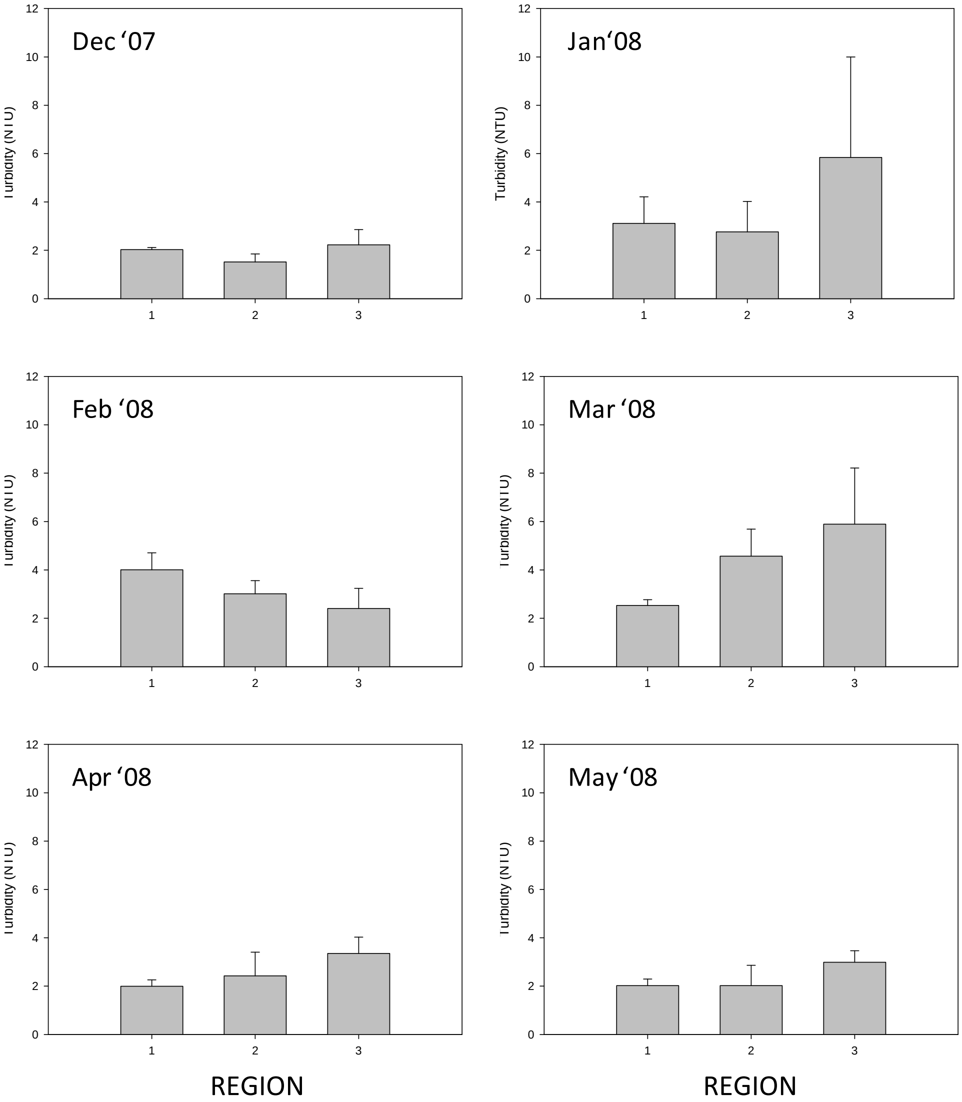

Figure 7. Mean turbidity averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

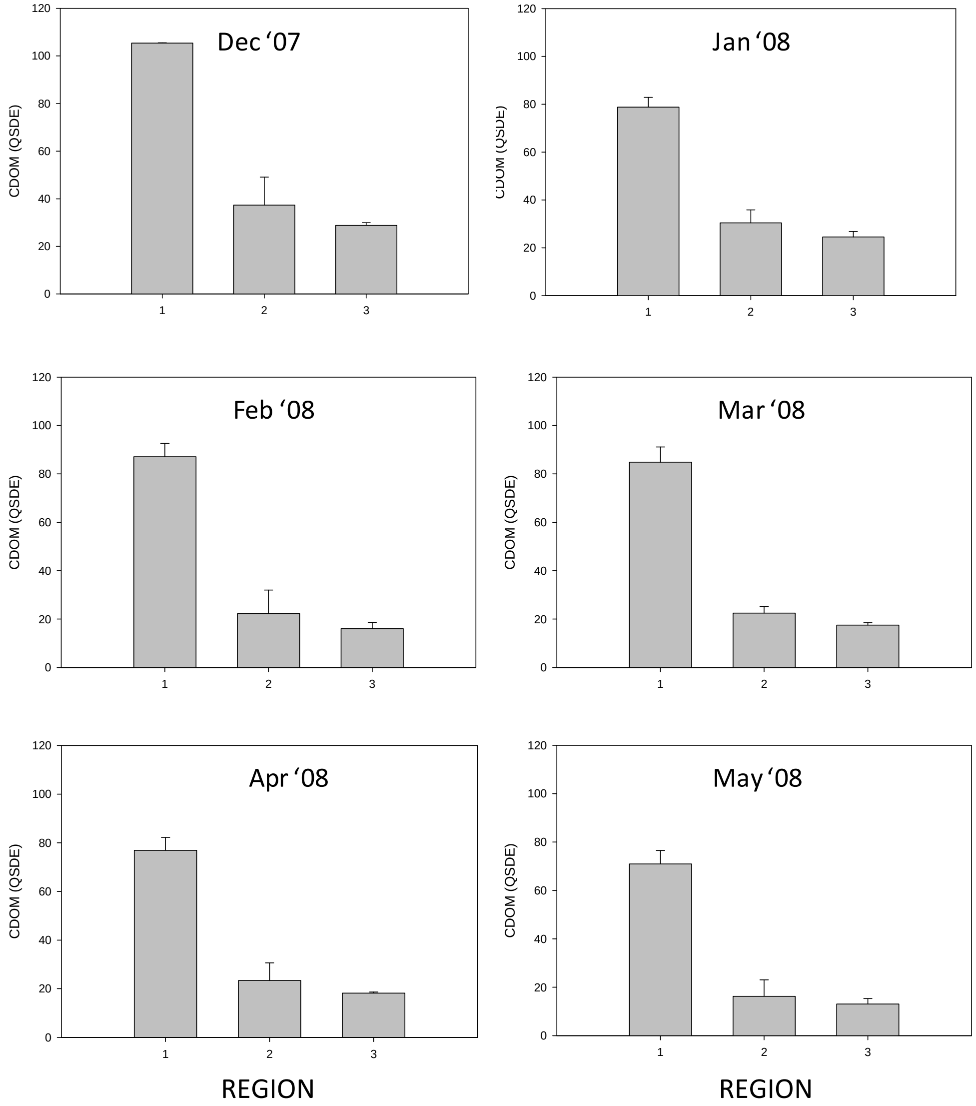

Figure 8. Mean CDOM averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

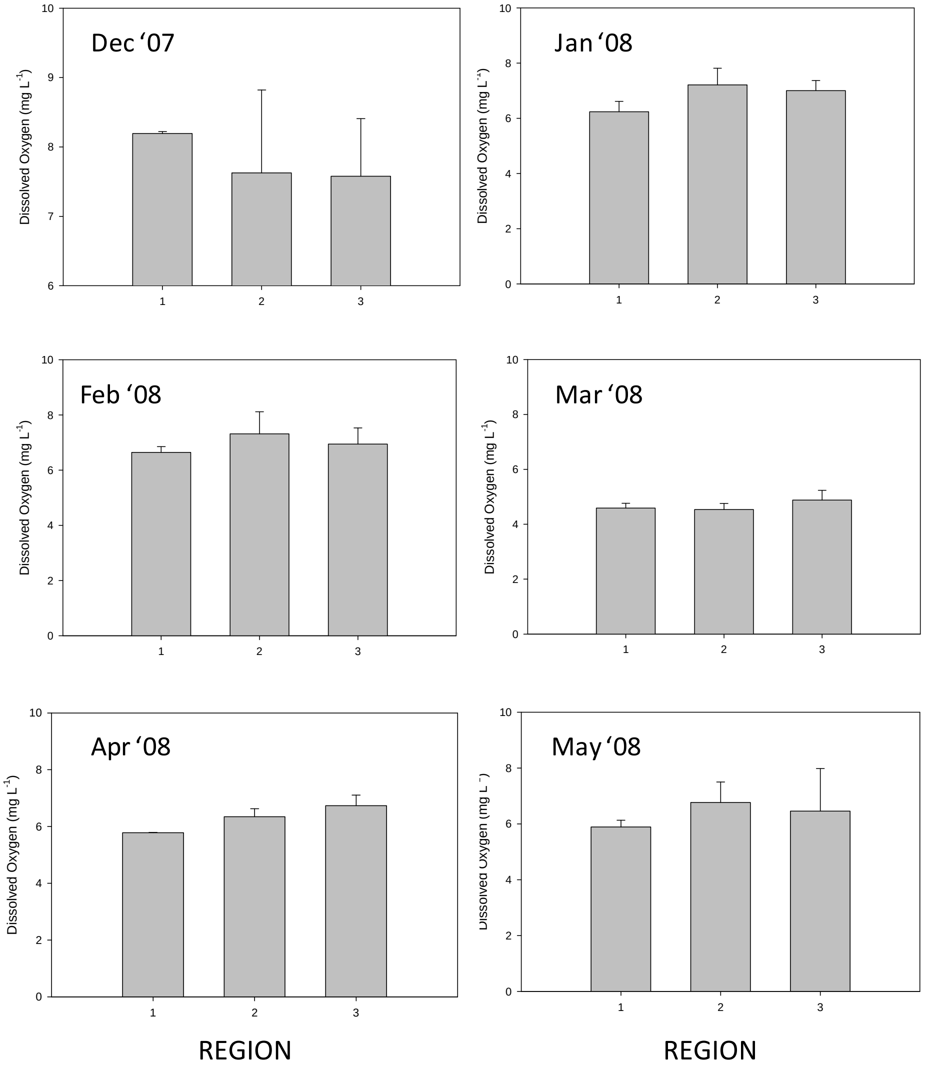

Figure 9. Mean DO (dissolved oxygen) averaged over the study period (Dec ’07 to May ’08). Region 1 is Caloosahatchee River and Estuary (CRE); region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS). Data were collected *in situ* at SCCF and SFWMD stations.

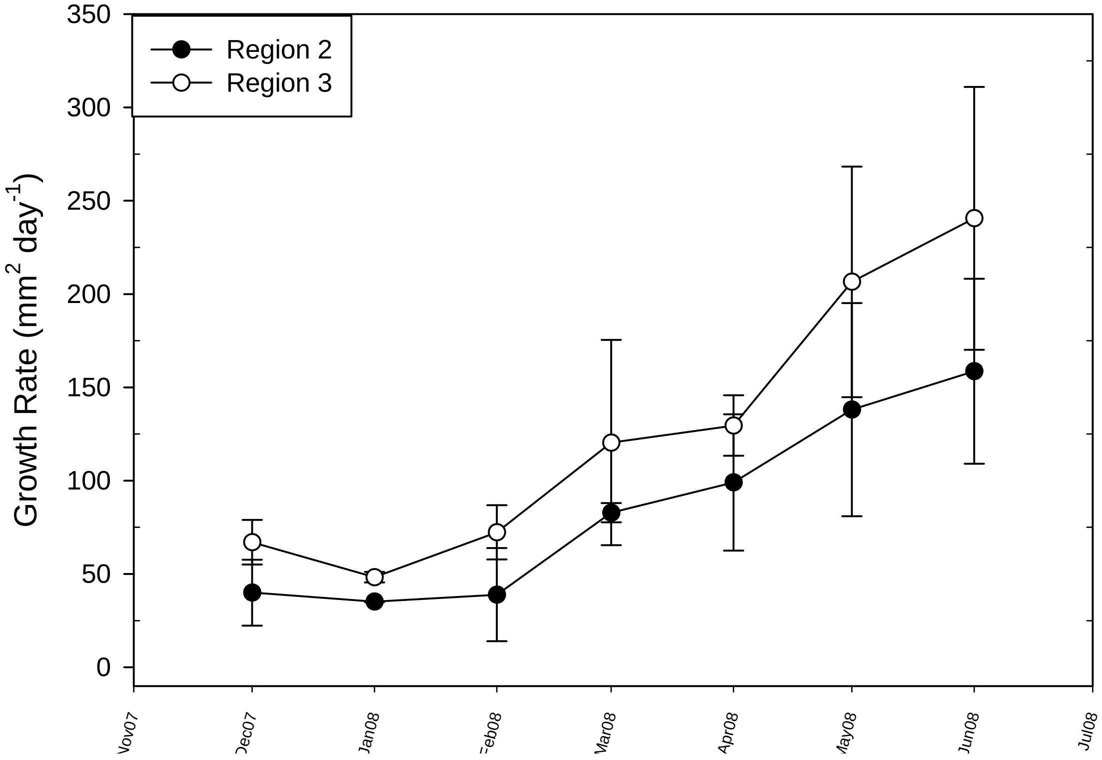

Figure 10. growth averaged by region and month over the study period (Dec’07 to May’08). The distribution of Thalassia does not extend upstream to region 1, therefore it is not included in this plot. Region 2 is San Carlos Bay (SCB) and region 3 is Pine Island Sound (PIS).

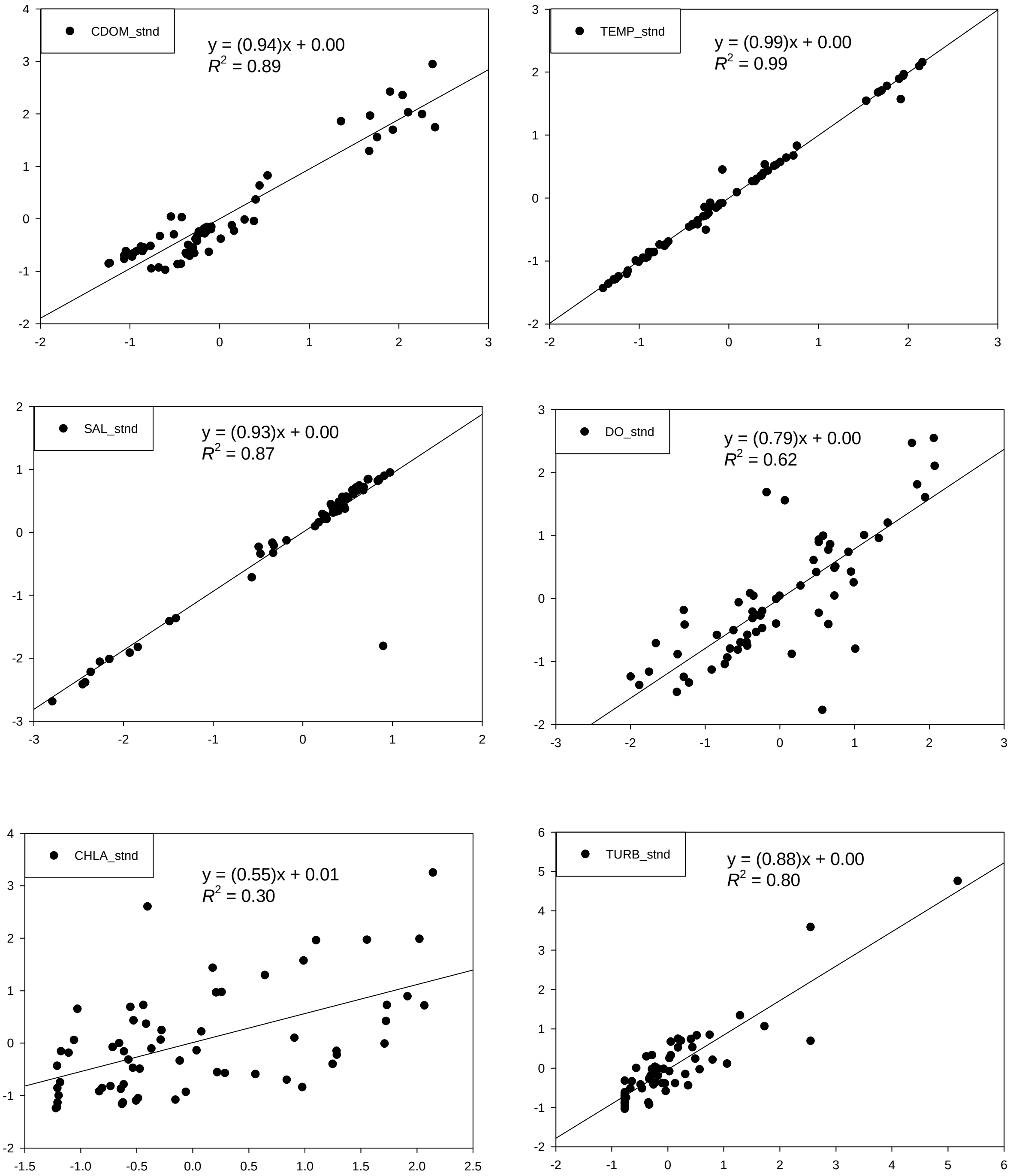

Figure 11. Discrete versus *in situ* water column properties collection at all stations and all months. Discrete and *in situ* data were regressed, axes are dimensionless as data were standardized (mean = 0, standard deviation = 1)Discrete samples required laboratory preparation of CDOM and chlorophyll and used a Hydrolab Quanta for salinity, turbidity, temperature and DO. *In situ* samples were performed by a mobile RECON sensor (Satlantic, Wetlabs).

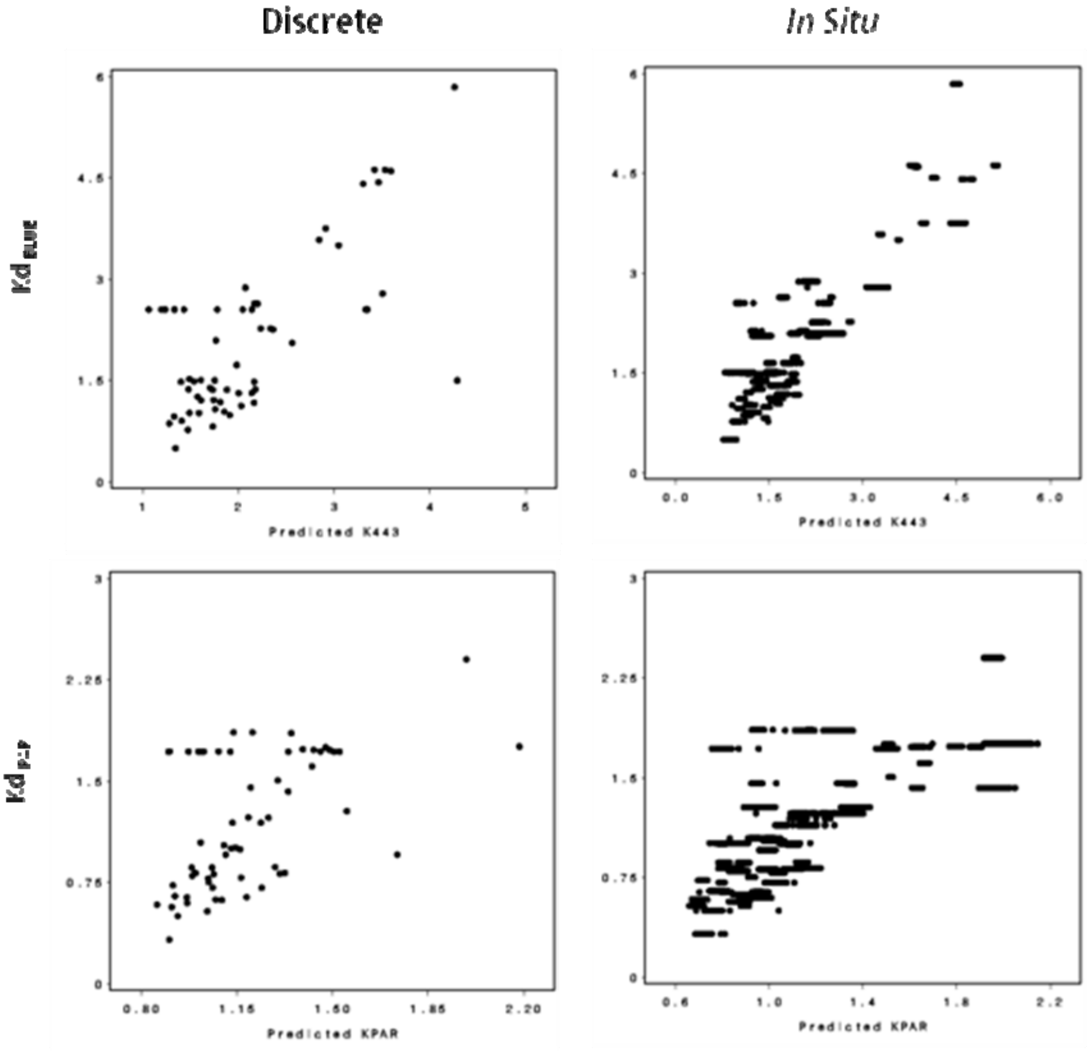

Figure 12. Kd observed versus Kd predicted from empirical optical models, BLUE and PAR. Discrete samples required laboratory preparation of CDOM and chlorophyll and used a Hydrolab Quanta for salinity, turbidity, temperature and DO. *In situ* samples were performed by a mobile RECON sensor (Satlantic, Wetlabs).

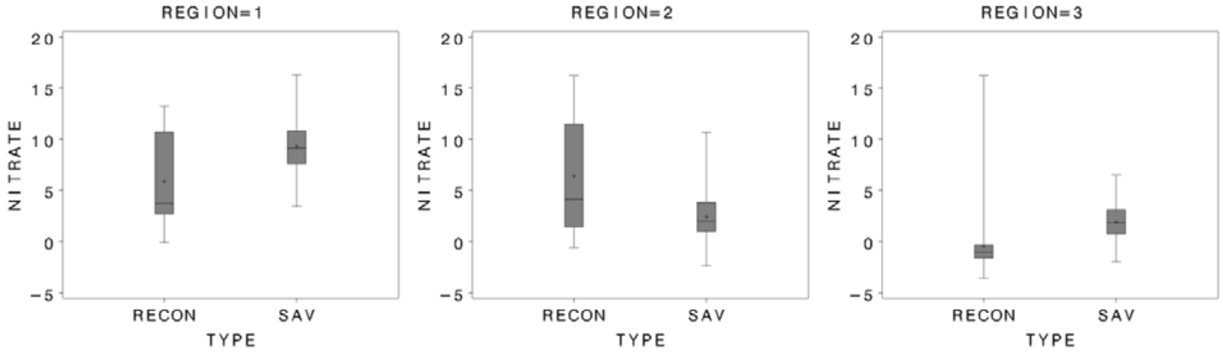

Figure 13. Nitrate (µM) at RECON fixed station versus SAV stations using *in situ* instrumentation.

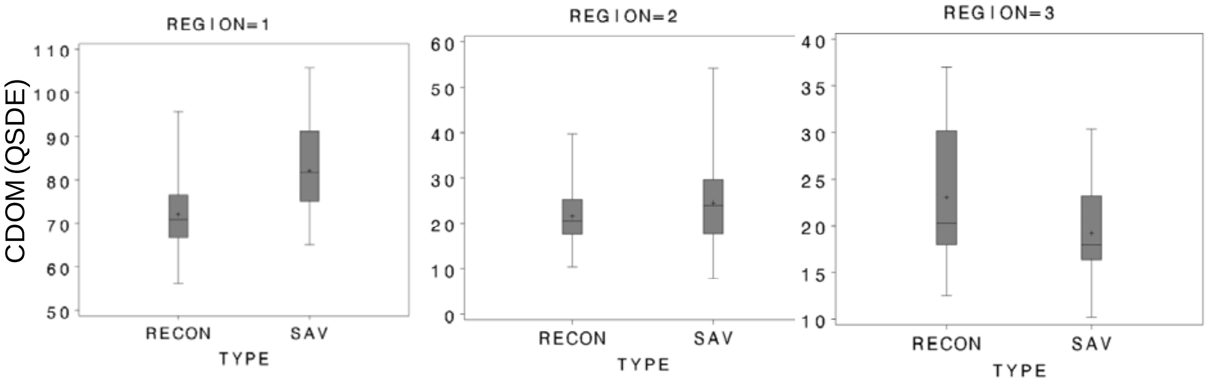

Figure 14. CDOM (quinine sufate dehydrate equivalents [QSDE]) at RECON fixed station versus SAV stations using *in situ* instrumentation.

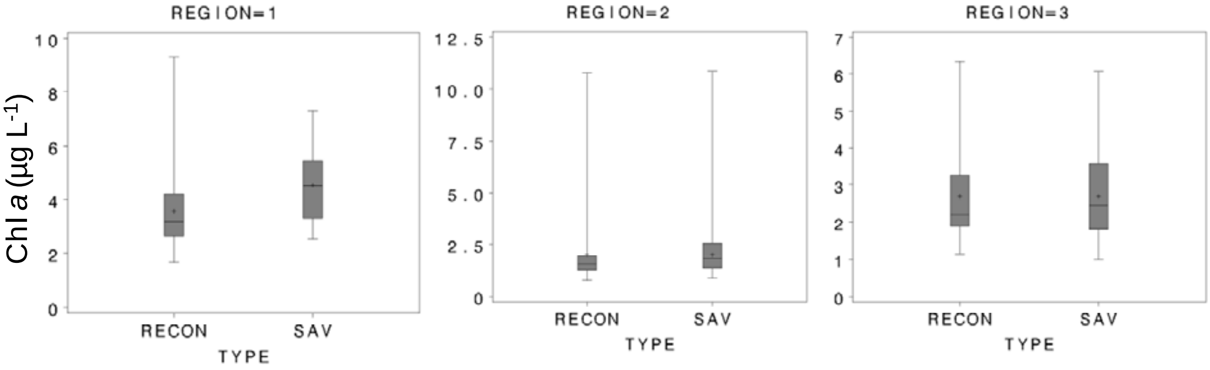

Figure 15. Chlorophyll *a* (µg L^{-1}) at RECON fixed station versus SAV stations using *in situ* instrumentation.

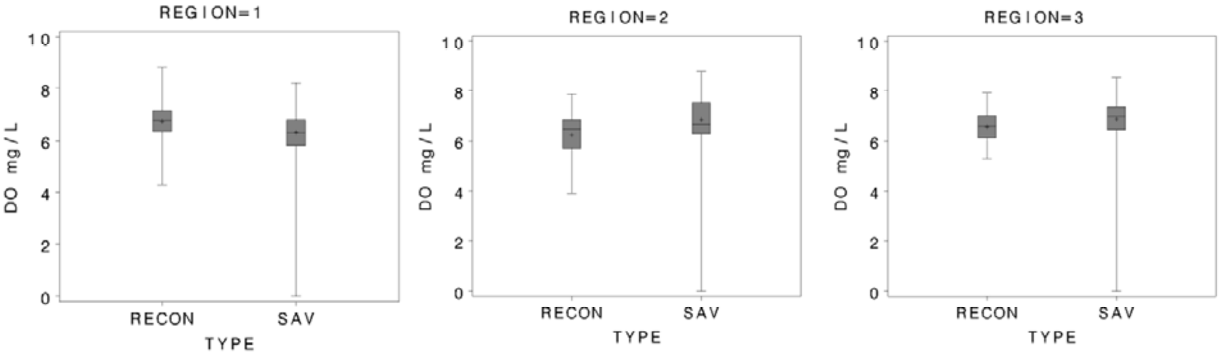

Figure 16. DO (dissolved oxygen in mg L^{-1}) at RECON fixed station versus SAV stations using *in situ* instrumentation.

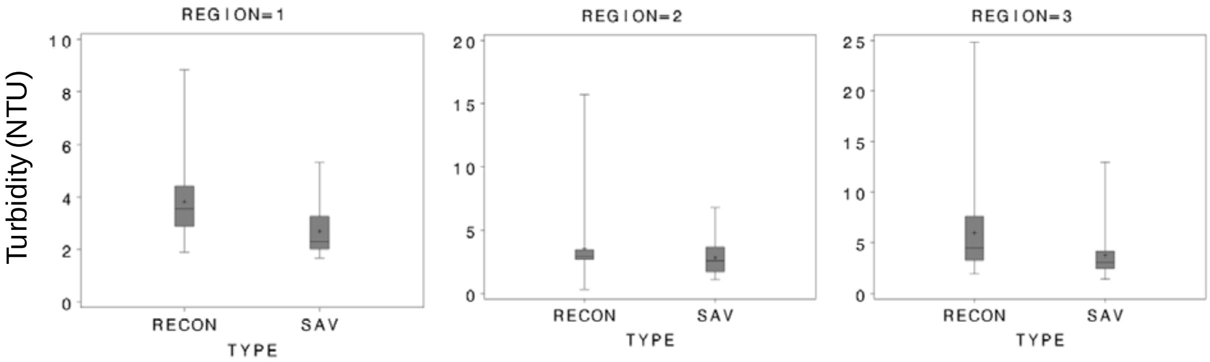

Figure 17. Turbidity (NTU) measured in NTUs at RECON fixed station versus SAV stations using *in situ* instrumentation.

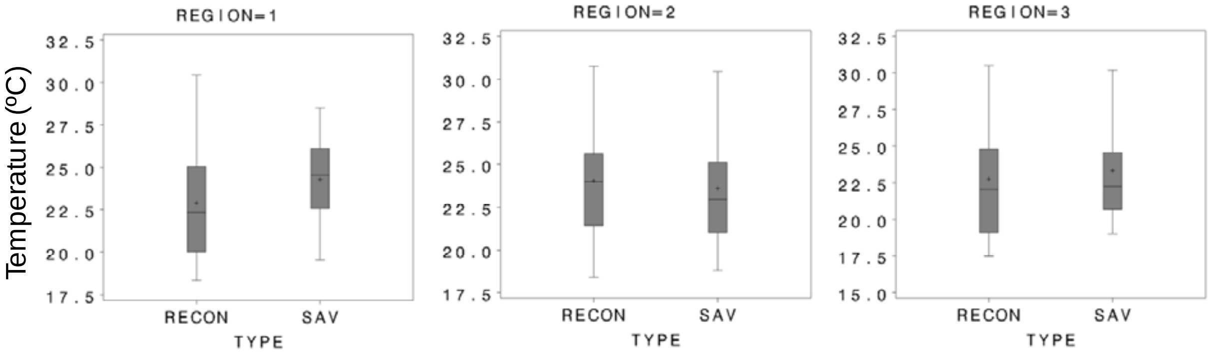

Figure 18. Temperature at RECON fixed station versus SAV stations using *in situ* instrumentation.

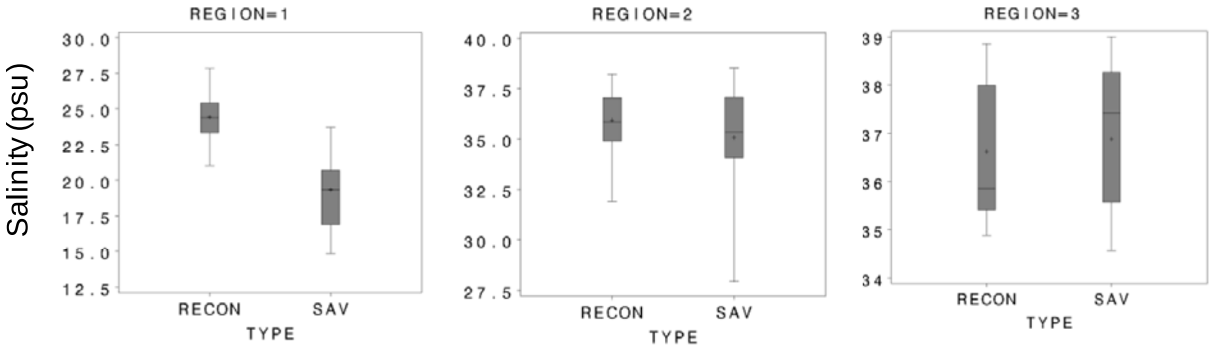

Figure 19. Salinity (psu) at RECON fixed station versus SAV stations using *in situ* instrumentation.

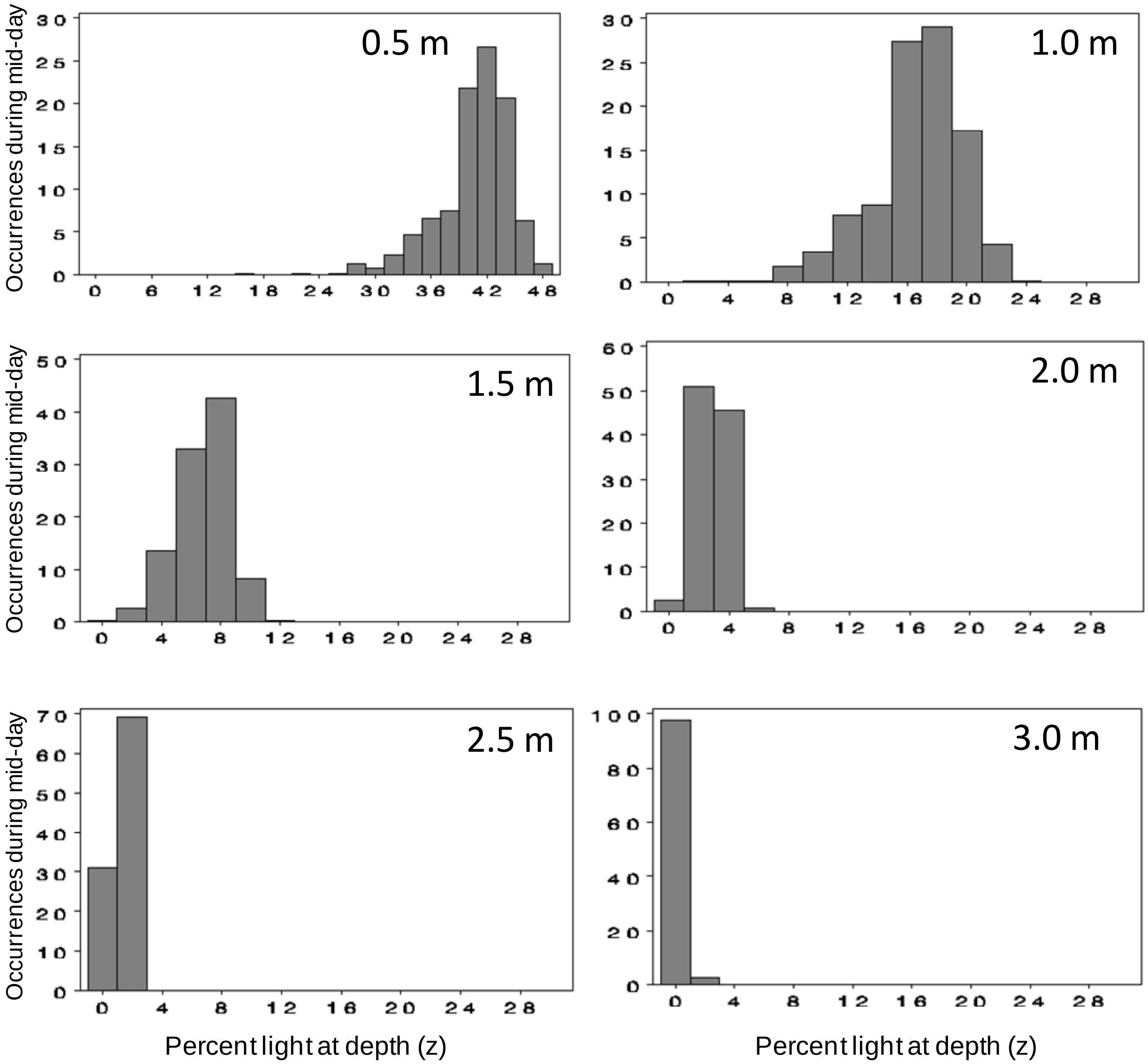

Figure 20. Percent PAR at depth distributions in region 1 from 1000 to 1400 from RECON fixed instruments. Data are recorded every hour and reported depths where SAV are observed; y-axis is the percent of time, x-axis is percent light at depth. Region 1 is Caloosahatchee River and Estuary.

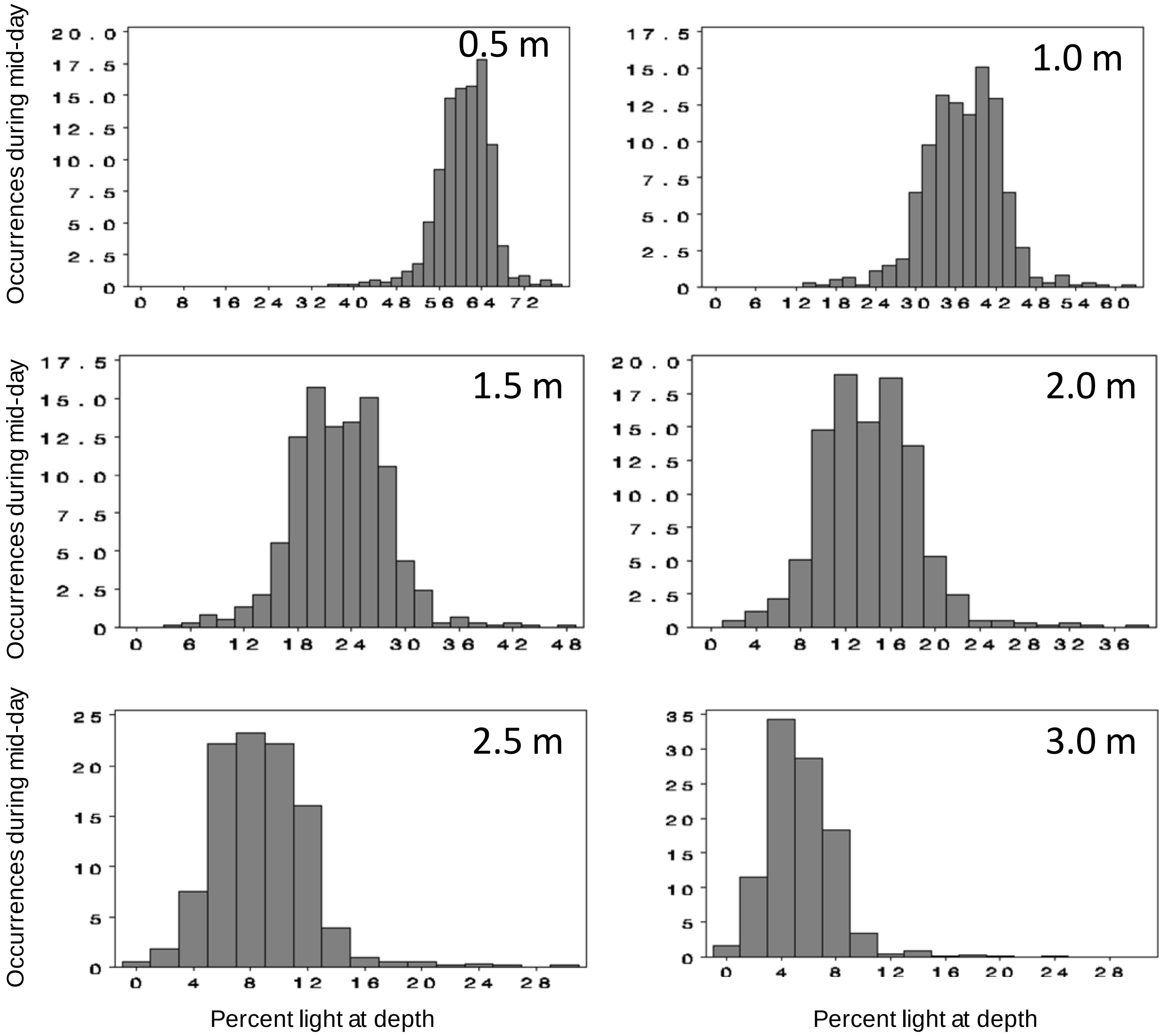

Figure 21. Percent PAR at depth distributions in region 2 from 1000 to 1400 from RECON fixed instruments. Data are recorded every hour and reported depths where SAV are observed; y-axis is the percent of time, x-axis is percent light at depth. Region 2 is San Carlos Bay.

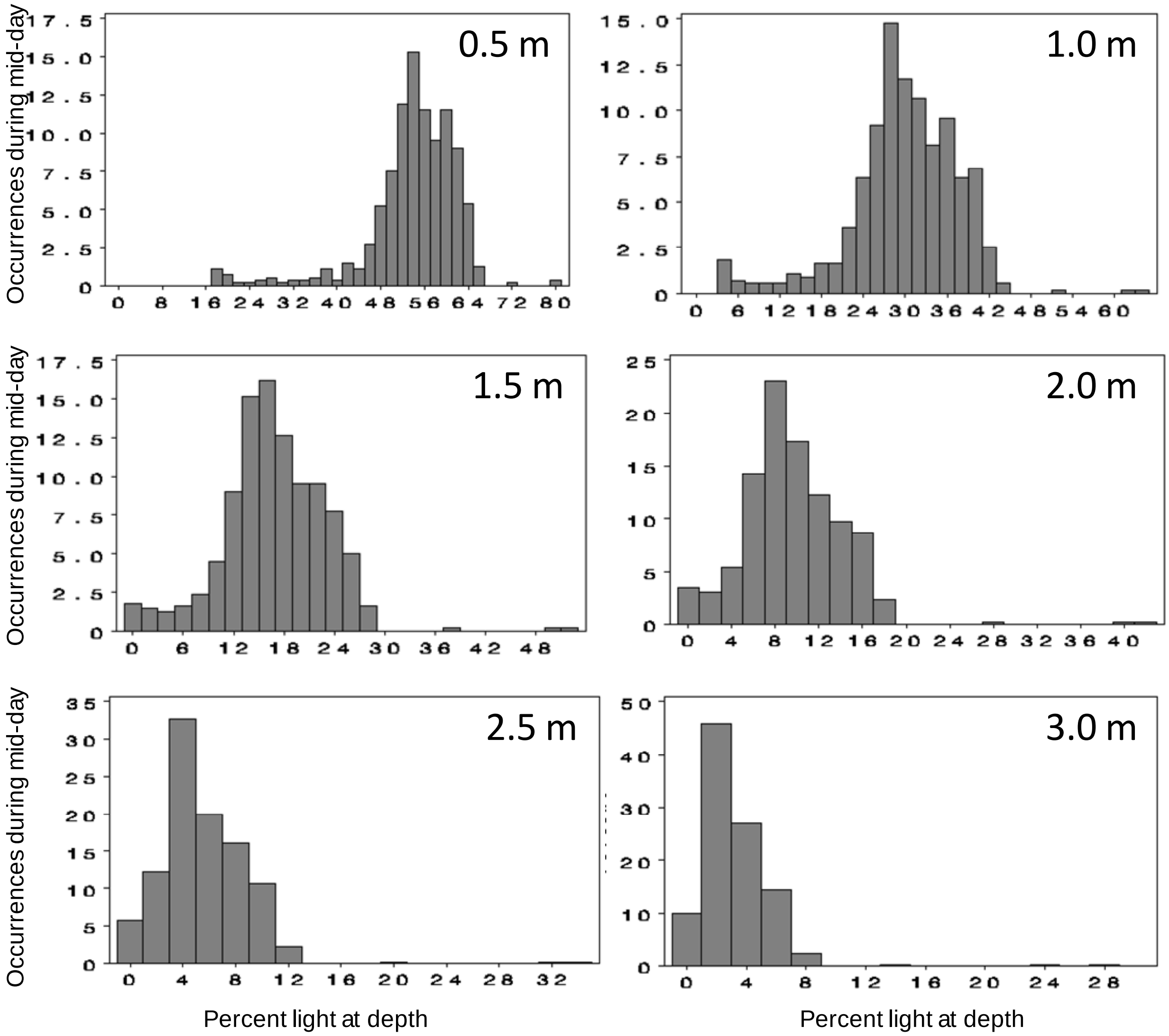

Figure 22. Percent PAR at depth distributions in region 3 from 1000 to 1400 from RECON fixed instruments. Data are recorded every hour and reported depths where SAV are observed; y-axis is the percent of time, x-axis is percent light at depth. Region 3 is Pine Island Sound.

Appendix A – Electronic files used in the report

1) All regions predicted Kd.xls
2) Concentrations to exceed light requirements.xls
3) Optical Model Analysis.doc
4) Plots of LOBO.jnb
5) All DISCRETE dates time series JS.xls