Final Report

Seagrass Response to Wastewater Inputs: Implementation of a Seagrass Monitoring Program in Two Texas Estuaries

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Acronyms

Acronym	Definition
% SI	percent surface irradiance
AOI	area of interest
BPE	B-type phycoerythrin
CBOD	carbonaceous biochemical oxygen demand (5-day)
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
EF	East Flats
Epi	epiphytes
EY	eosin Y
F	fluorescence
F.U.	fluorescence units, arbitrary
GIS	geographic information systems
GLO	General Land Office
Green F	green-excited fluorescence
GTLT	greater than or less than
Hal	*Halodule wrightii*
Halodule	*Halodule wrightii*
IHS	intensity-hue-saturation
K_d	light attenuation coefficient
lbs/day	pounds per day
MANERR	Mission Aransas National Estuarine Research Reserve
MGD	million gallons per day
mM	millimoles per liter
µM	micromoles per liter
NELAC	National Environmental Laboratory Accreditation Conference
NOAA	National Oceanic and Atmospheric Administration
Norm	normalized
NTU	nephelometric turbidity units
PAR	photosynthetically-active radiation
PB	Port Bay
ppt	parts per thousand
QAPP	quality assurance project plan
R/G	ratio of red-excited fluorescence intensity to green-excited fluorescence intensity (unitless)
r^2	determination coefficient (square of correlation coefficient r)

Red F	red-excited fluorescence
RGB	red-green-blue
ROI	region of interest
RSR	root:shoot ratio
Ruppia	*Ruppia maritima*
SD	standard deviation
SE	standard error of the mean
SI	surface irradiance
S-M-D	depth: shallow-medium-deep
Sp-Su-Fa	season: spring-summer-fall
SWQM	Surface Water Quality Monitoring
SWQMIS	Surface Water Quality Monitoring Information System
Syringodium	*Syringodium filiforme*
T	transect
T1, T2, T3	Transect 1, 2, 3 respectively
TAMU-CC	Texas A & M University Corpus Christi
TCEQ	Texas Commission on Environmental Quality
TCOON	Texas Coastal Ocean Observation Network
Thal	*Thalassia testudinum*
Thalassia	*Thalassia testudinum*
TOC	total organic carbon
TPWD	Texas Park and Wildlife Department
TSS	total suspended solids
UTMSI	University of Texas Marine Science Institute
VSS	volatile suspended solids
WCID	Water Control and Improvement District

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Executive Summary

The Coastal Management Program of the General Land Office (GLO) funded Texas Parks and Wildlife Department (TPWD) to conduct a seagrass monitoring project in two central coast Texas estuaries. Monitoring was done in Port Bay, a secondary bay of the Copano Bay system, and East Flats in Corpus Christi Bay, following recommendations outlined in Dunton *et al.* (2007). The project had two main purposes: to assess the impact of a domestic wastewater discharge on the seagrass beds, and to test recent recommendations for coastwide seagrass monitoring. Identical procedures were used in East Flats and Port Bay and included three major components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) epiphyte fluorescence analysis. Methods and quality assurance protocols are detailed in the Quality Assurance Project Plan (QAPP) for the project. Work was conducted in collaboration with Dr. Kenneth Dunton of the University of Texas Marine Science Institute (UTMSI), Dr. Warren Pulich of Texas State University and Dr. Kirk Cammarata of Texas A&M University - Corpus Christi (TAMU-CC).

Port Bay and East Flats 2009 aerial photography was studied using imagery classification and transect analysis. In 2010 transect analysis was employed for both sites and imagery classification was done for Port Bay. Deep edge analysis was also performed both years. 2009 aerial imagery for East Flats showed 137 acres of bare area and 243 acres of largely continuous vegetated area. Macroalgae deposits covered 41 acres. Transect analysis found 25 - 30% bare length per transect. The deep edge occurred at a tide-adjusted depth of 1.42 m. 2009 aerial imagery for Port Bay indicated 92 acres of bare area and 131 acres of largely continuous vegetated area. Vegetated area increased in 2010 with a concommitant decrease in bare area. Algae were not present at Port Bay. Transect analysis found 29 - 74% bare length per transect. The average deep edge occurred at a tide-adjusted depth of 1.04 m. In 2010 bare patches ranged from 7 – 41% of the transect length for East Flats and 3 – 42% for Port Bay. The 2010 average deep edge occurred at a tide-adjusted depth of 1.32 m for East Flats and 1.19 m for Port Bay.

Water temperature in both bays showed typical seasonal trends. Average salinity at East Flats, 31.5 ppt, was much higher than at Port Bay, 7.5 ppt. East Flats had higher percent surface irradiance at the seagrass canopy. Overall, turbidity was low in both systems. Secchi depth measurements were lower at Port Bay than East Flats and Port Bay tended to have higher total suspended solids concentrations than East Flats. Water column nutrient and chlorophyll-*a* concentrations in Port Bay and East Flats were low throughout study, with Port Bay having consistently higher concentrations of ortho-phosphate, nitrate+nitrite, and chlorophyll-a than East Flats. Ortho-phosphate concentrations increased in both bays from spring to fall. Sediments in both bays were dominated by sand (≥ 90%). Seasonally averaged sediment porewater ammonia concentrations ranged from 0.95 – 2.2 mg L^{-1} for East Flats and 1.1 – 1.4 mg L^{-1} for Port Bay. In East Flats porewater ammonia-N concentrations were highest in summer and were lowest in fall, while Port Bay showed no seasonal trend.

Throughout the study, Port Bay was dominated by *Halodule wrightii* with some *Ruppia maritima* present. Total seagrass coverage varied across transects with almost 100% coverage at T3 each season. East Flats displayed a multi-species assemblage of seagrass, with *Halodule wrightii* and *Thalassia testudinum* dominant. Transects 1 and 2 had >90% mean seagrass cover each season,

but T3 had lower percent coverage in summer and fall. *Syringodium filiforme* was also noted at T2 and T3 each season and *Ruppia maritima* was observed occasionally. For both sites, macroalgae biomass was variable across transects and seasons. *Gracilaria* was the only genus identified in Port Bay, while 10 genera were identified in East Flats.

Halodule shoot density in Port Bay was highly variable and declined from spring through fall at T3, but remained consistent at T1 and T2. Biomass estimates were also highly variable, with values at T3 higher than T1 or T2. There was no clear seasonal trend. In East Flats shoot density and biomass were also highly variable. For *Thalassia*, above-ground, below-ground, and total biomass tended to be higher in summer.

Root:shoot ratios above 1.0 are generally thought to represent healthy plants (Appendix E). Root:shoot ratio for *Halodule* ranged from 0.52 to 3.4 at Port Bay, and from 0.54 to 7.4 at East Flats. For *Thalassia* in East Flats, root:shoot ratio ranged from 1.6 to 6.5. Root:shoot ratio did not show a strong seasonal trend at Port Bay, but it did appear elevated during summer and fall at East Flats for *Halodule* and *Thalassia*.

Normalized epiphyte load was determined four different ways by three different laboratories. Conventional scraping techniques were used by TPWD, UTMSI and TAMU-CC to obtain measures of dried epiphyte weight per unit area and per unit seagrass dry weight. Fluorescence techniques were employed by TAMU-CC to determine epiphyte load normalized to the areal distribution of the red-excited fluorescence signal and to seagrass dry weight. For *Halodule* in Port Bay, normalized epiphyte loads by all measurement techniques were consistently smaller than those measured for East Flats *Halodule* and *Thalassia* and show a slight decrease from spring to fall. Independent determinations of epiphyte load per unit seagrass dry weight by TPWD and TAMU-CC agreed fairly well across the seasons, as did determinations of epiphyte load per unit area by TPWD and UTMSI in the fall. Using scraping techniques, *Halodule* and *Thalassia* in East Flats showed an increase in normalized epiphyte loading from spring to fall for both species. Fluorescence techniques for scanned seagrass blades tracked scraping methods for *Halodule* in Port Bay. In East Flats fluorescence signal of scanned seagrass blades normalized to seagrass dry weight tracked the seasonal increase observed for *Halodule*, but not for *Thalassia*. However, when epiphytes were scraped from the blades, normalized green-excited fluorescence tracked the scraping methods for both sites. Fluorescence signal normalized to the areal distribution of the red-excited fluorescence signal did not track the scraping methods for either species.

Because the wastewater plant was not built on Port Bay during the study, it is not possible to evaluate the impacts of wastewater on the seagrass community there. TPWD intends to resample Port Bay following completion of the wastewater discharge plant. The three seasons of data that were collected will serve as baseline, pre-operational data for that site. As originally proposed, East Flats was to serve as a reference site for evaluation of wastewater impacts to Port Bay. It turns out that the sites differ in salinity, seagrass species and epiphyte communities. Future work will focus on the use of baseline data and internal references to evaluate wastewater impacts.

Developing a statewide seagrass monitoring program is the keystone of seagrass management in Texas. Resource managers must have accurate information of the status and trends of seagrass

beds along the Texas coast and regulatory decisions must be science-based. An ideal monitoring program would focus the state's limited resources on collecting the data that best describe seagrass condition and give sufficient information to understand the environmental stressors affecting seagrasses. Some components of a statewide monitoring program that must be considered include: best time of year to conduct monitoring (that is, to define an "index period"), essential parameters to sample, level of effort, cost, laboratory capability and applicability of the program to all parts of the coast where seagrasses grow. This work demonstrated that state staff can accurately and efficiently conduct monitoring and analyze seagrass samples. This effort has already borne fruit in the form of a pilot statewide seagrass monitoring project initiated by the Texas Commission on Environmental Quality (TCEQ) in conjunction with TPWD.

Introduction

The Coastal Management Program of the General Land Office (GLO) funded Texas Parks and Wildlife Department (TPWD) to conduct a seagrass monitoring project in two Texas estuaries. The project had two main purposes: to assess the impact of a domestic wastewater discharge on the seagrass beds, and to test recent recommendations for coastwide seagrass monitoring.

Seagrass (submerged aquatic vegetation) has been identified as a critical habitat under the Coastal Coordination Act. Seagrass beds serve as critical nursery habitat for estuarine fisheries and wildlife. Seagrasses provide food for fish, waterfowl and sea turtles, contribute organic material to estuarine and marine food webs, cycle nutrients, and stabilize sediments. They are economically important based on their function in maintaining Gulf fisheries by serving as nursery habitat for juvenile fish and invertebrates. Growing coastal populations and increasing coastal development threaten seagrass habitat.

Three state agencies with primary responsibility for conserving coastal natural resources, Texas General Land Office, TCEQ, and the Texas Parks and Wildlife Department, signed the Seagrass Conservation Plan for Texas in 1999 (TPWD 1999). Currently, TPWD facilitates quarterly meetings of a Seagrass Monitoring Work Group comprised of experts from academics, government and non-governmental organizations. The group's main purpose is to develop a statewide seagrass monitoring plan. Some participants from the group did work under previous grants (Dunton *et al*. 2005, Dunton and Pulich 2007), resulting in recommendations for a seagrass monitoring program incorporating landscape analysis and field-based indicators of environmental quality and seagrass condition.

This project evaluated seagrass condition in two areas of the central Texas coast following recommendations outlined in Dunton and Pulich (2007). One area is more or less "pristine" (East Flats in Corpus Christi Bay) and the other area has a proposed domestic wastewater treatment discharge into a seagrass bed (Port Bay). Identical procedures were used in East Flats and Port Bay and included three major components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) epiphyte fluorescence analysis.

The landscape monitoring portion included aerial photography obtained twice during the study at each site. Each site was photographed at a 1:9,600 scale using color imagery. The imagery was analyzed to determine vegetative cover, and to help assess potential indicators of seagrass stress, including patchiness, depth limit of seagrass coverage, and macroalgae abundance.

A suite of seagrass condition and water quality indicators were evaluated at each site, based on the recommendations of Dunton *et al.* (2007), which identified several potential indicators of stress on seagrasses that might work in Texas coastal waters. Water quality data collected included dissolved nutrients, chlorophyll-*a*, suspended solids, light attenuation, salinity, dissolved oxygen, and temperature. Sediment was analyzed for porewater ammonia, total organic carbon (TOC), and grain size. Seagrass condition indicators evaluated include total biomass, root:shoot biomass ratio, shoot density, leaf length and width, leaf area index, percent

cover, carbon and nitrogen isotope ratios (to measure human influence), and ratios of carbon-to-nitrogen in seagrass tissue. TPWD staff worked with Dr. Kenneth Dunton of the University of Texas Marine Science Institute (UTMSI) to collect the seagrass condition and water quality indicators.

The final component of this study was exploration of a novel technique developed by Dr. Kirk Cammarata of Texas A&M University - Corpus Christi (TAMU-CC) to quantify and analyze epiphytic algae growth on seagrass leaves. In nutrient-enriched waters, epiphytic algae growth may increase; at some point interfering with photosynthesis and causing seagrass loss. Measurements of epiphytic algal density are a sensitive way to measure impacts of increased nutrient loadings. This study compared traditional measurements of epiphytic algal biomass (obtained from leaf scrapings) with fluorescence measurements made in Dr. Cammarata's laboratory (Cammarata *et al.* 2009).

Data collected at East Flats and Port Bay was used to test the Dunton *et al.* (2007) seagrass monitoring protocol as well as help determine whether the effluent limitations that TCEQ has permitted are effective in protecting seagrasses in the vicinity of the planned discharge in Port Bay. Seagrass condition, water quality indicators and epiphytic algae analysis were conducted along three transects each at Port Bay and East Flats during the spring, summer, and fall of 2010. At this time, the wastewater facility on Port Bay has not begun discharging. The Port Bay data is considered baseline which can be compared with data obtained after the plant is operational. Once the wastewater plant has a steady discharge, we can compare the environmental and biological condition of the area to the data collected before the wastewater treatment plant was built.

Study Area

East Flats and Port Bay are in the Coastal Bend of the Texas coast. East Flats is a small shallow embayment within Corpus Christi Bay, TCEQ Segment 2481, approximately 5 km west of the City of Port Aransas (Figure 1). Port Bay lies within the Copano Bay system, TCEQ Segment 2472, of the Mission and Aransas Rivers. It is a narrow bay with a small watershed that enters the southernmost corner of Copano Bay approximately 9 km west of the City of Rockport (Figure 2).

Areas of interest (AOIs) have been defined for both East Flats and Port Bay. The AOIs represent a subsample of the bays containing seagrass (Figure 3 and Figure 4). They were the focus for landscape analysis of aerial imagery and where water quality and seagrass condition measures were sampled at three transects. The AOIs for East Flats and Port Bay are approximately 154 ha and 109 ha, respectively. Transects in both study areas were placed to contain the deep edge of the seagrass bed. In Port Bay, transects were also placed in relationship to the planned domestic discharge in order to measure any potential impacts to the AOI.

Figure 1. East Flats study area.

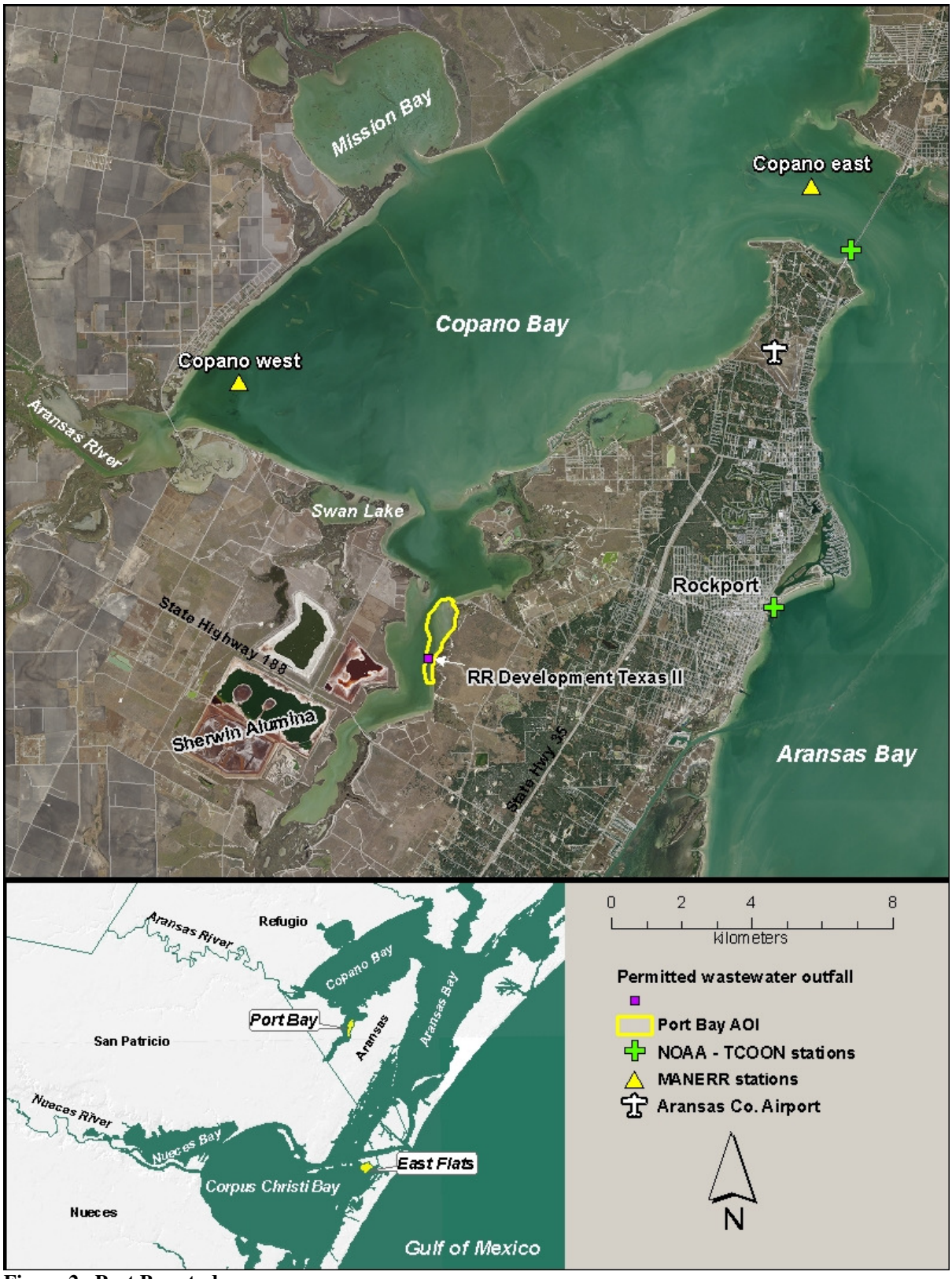


Figure 2. Port Bay study area.

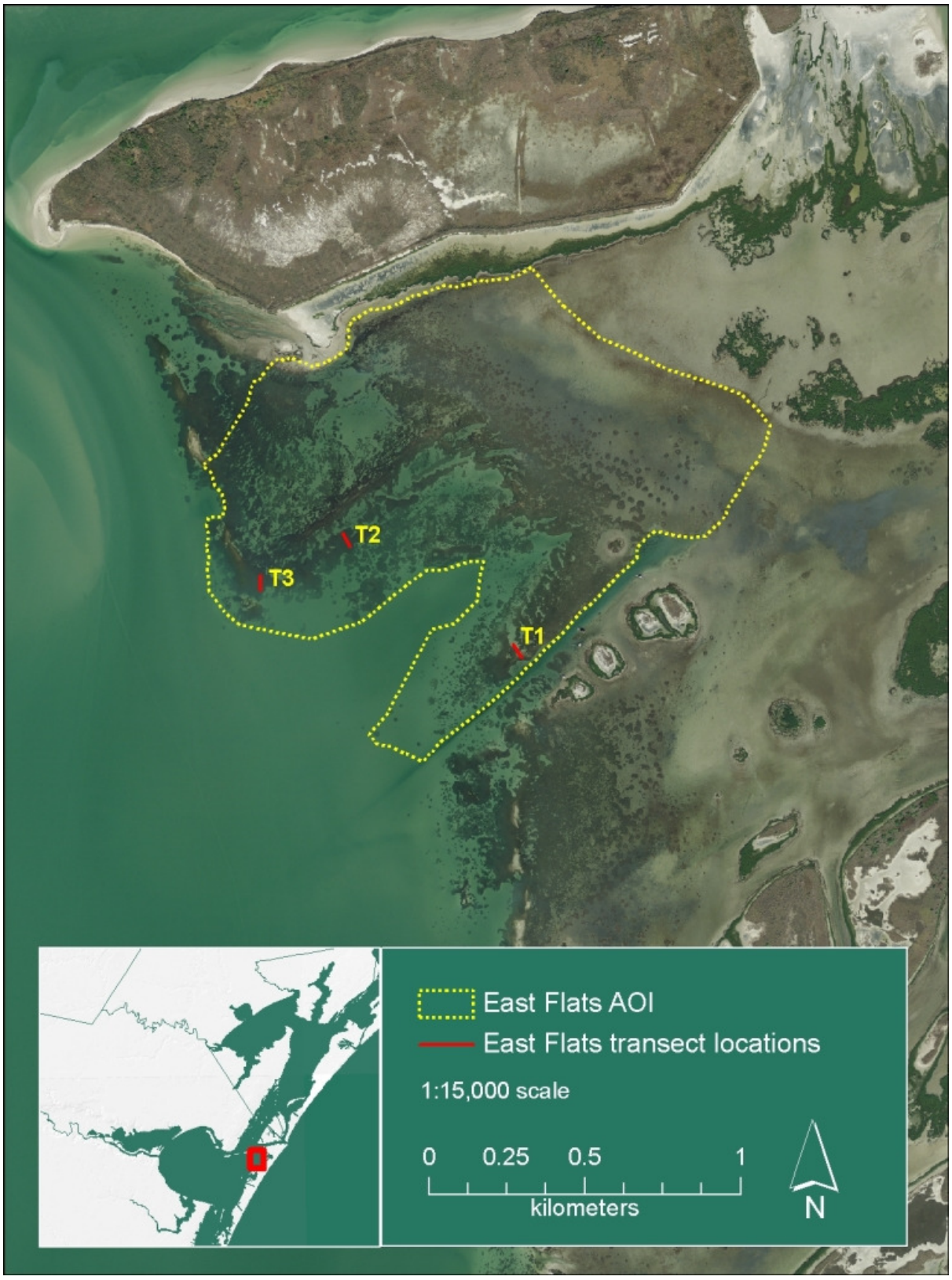


Figure 3. East Flats study area, area of interest (AOI), and transects.

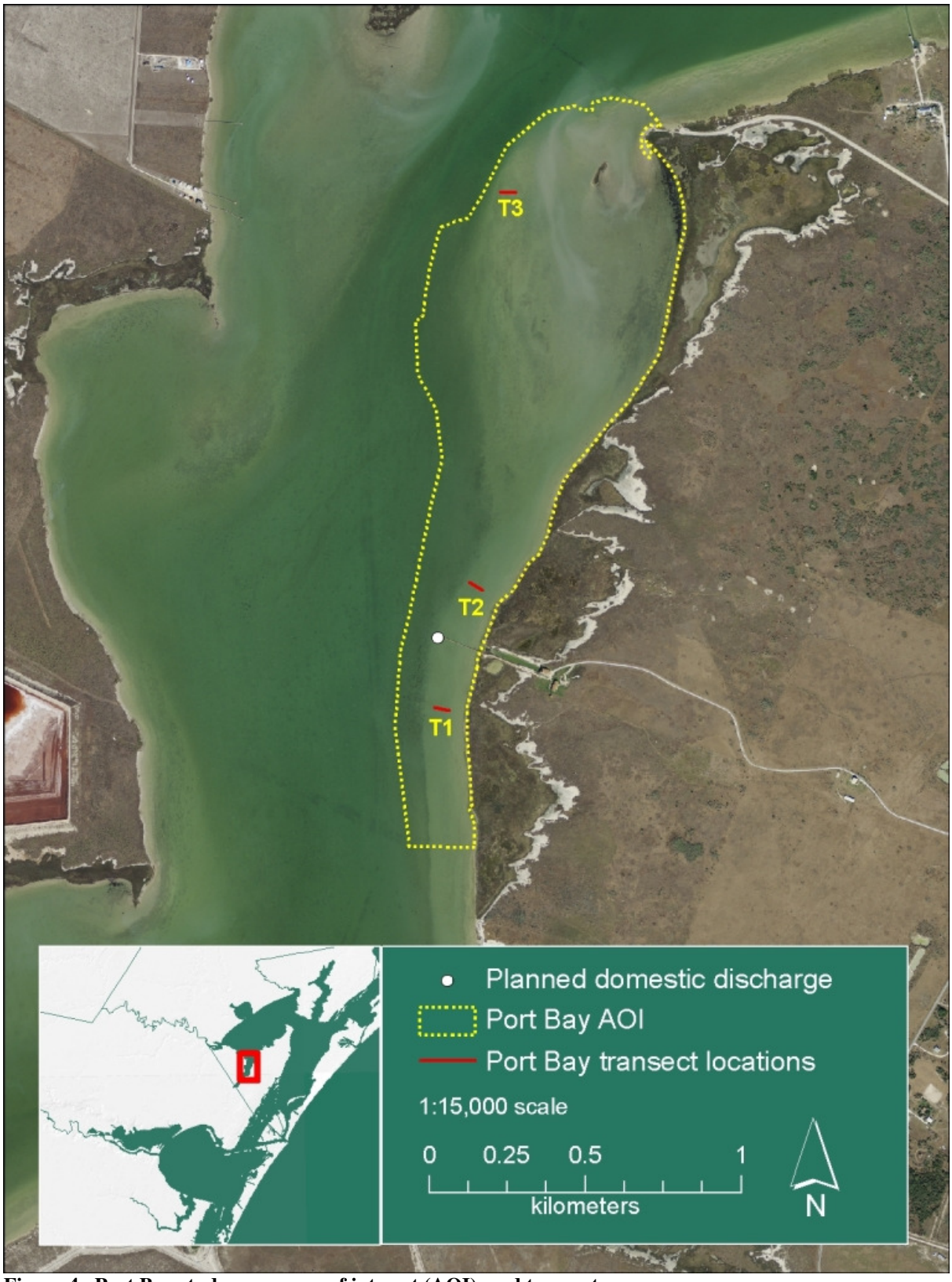


Figure 4. Port Bay study area, area of interest (AOI), and transects.

Methods

Detailed descriptions of sampling methods and quality assurance protocols used in this project are described in the quality assurance project plan (QAPP) (Radloff 2010).

Seagrass Basics

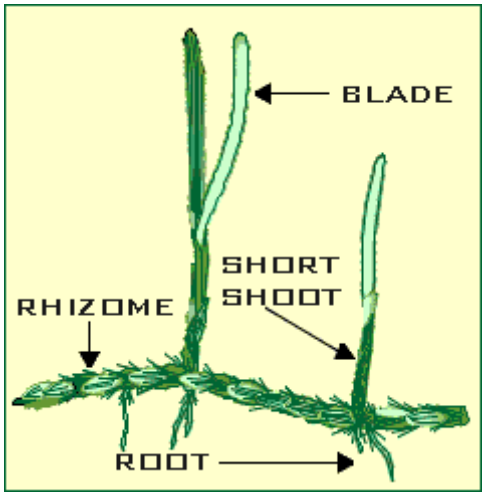


Figure 5. Seagrass parts (University of Florida 2011).

Seagrass parts and major functions have been described by the University of Florida (University of Florida 2011), "*The major function of the blades is photosynthesis, but they also function in nutrient absorption and in elimination of waste products. The short shoot can be thought of as the "stem" of the plant, where the blades originate. Rhizomes are subterranean organs that function in propagation of the clone, in anchoring the plants to the substrate, in translocation of materials throughout the clone, and are also involved in nutrient absorption and gas exchange. Short shoots and roots emanate from the rhizomes. Roots are much thinner than rhizomes and function primarily in nutrient absorption. They also contribute to anchorage of the plant and to the elimination of waste products*." The terms "leaf" and "blade" will be used interchangeably in this report.

Aerial Imagery

Acquisition

High resolution (1:9,600 scale) true color aerial photography was acquired on clear, calm days: 20 Dec 2009 and 19 Nov 2010. Accuracy of one meter or better was obtained. For each event, the vendor provided a set of 9-inch by 9-inch color film diapositives, a set of scanned, digitized, georeferenced, full frame images, the actual flight plan, and metadata that met Federal Geographic Data Committee standards. The film diapositives were scanned at 12.5 micron resolution using a Leica DSW500 photogrammetric scanner. The resulting cellsize was 0.15 m by 0.15 m. Details of the imagery requirements are given in the QAPP (Radloff 2010).

Analysis

Analysis of aerial imagery in this study included image classification, generation of landscape metrics and depth limit analysis. For 2009 imagery, image classification was used to create a thematic map of discrete habitats. The classified image was further analyzed to determine patchy and continuous seagrass and bare habitat. The number of patches and the total area were recorded for each habitat type, as well as average shape index, average perimeter-to-area ratio and average size.

In 2009 and 2010, virtual transects were used to obtain landscape metrics. Transitions between vegetation and bare area were identified along each transect by interpreting the original photography. The number of transitions, the transect length and the overall length of bare patches were obtained.

Depth limit analysis was used to determine the deepest water at which the seagrass grows. Through photo-interpretation, the estimated edge between vegetation and open water was delineated. The deep edge was validated in the field and the depth was recorded at the site. Results are displayed as a graphic showing the locations of the tide-adjusted depths, as well as with descriptive statistics (mean, standard deviation, and range).

Details of methods are given in Appendix C.

Field Data Collection

General Overview

Study design for field data collection included three identical sampling events in 2010, one each in spring, summer, and fall, to attempt to capture seasonal variations in measurements at each site. Field data collection was carried out by TPWD staff at Port Bay, and UTMSI staff at East Flats, both teams using the same procedures.

At each site three transects were selected roughly perpendicular to shore, which encompassed the deep edge of the seagrass bed. In Port Bay, transect selection took into account the location of a proposed wastewater plant discharge. One transect (T1) was located approximately 0.24 km south of the wastewater discharge and another transect (T2) was approximately 0.19 km north of the discharge. A third transect (T3) was located considerably further away (approximately 1.5 km), with the expectation that at this distance the wastewater discharge would not impact this site and it could later be considered a reference transect. In East Flats, replicate transects were selected to encompass the available seagrass habitat.

Sampling at each of the three transects was identical (Figure 6). Transects were permanent for the duration of the study, and were 50 m long. Transect ends were located using GPS during each event, and were marked with white PVC poles for convenience when sampling. Sampling for percent coverage and macroalgae occurred along each transect at 10 points that were randomly selected before each trip. Seagrass cores, shoots and sediment cores for other analyses were collected near the transect, but far enough away to keep the transect undisturbed. Transects were digitally videographed using an underwater camera (950 Sea-Drop color camera, SeaViewer, Inc.).

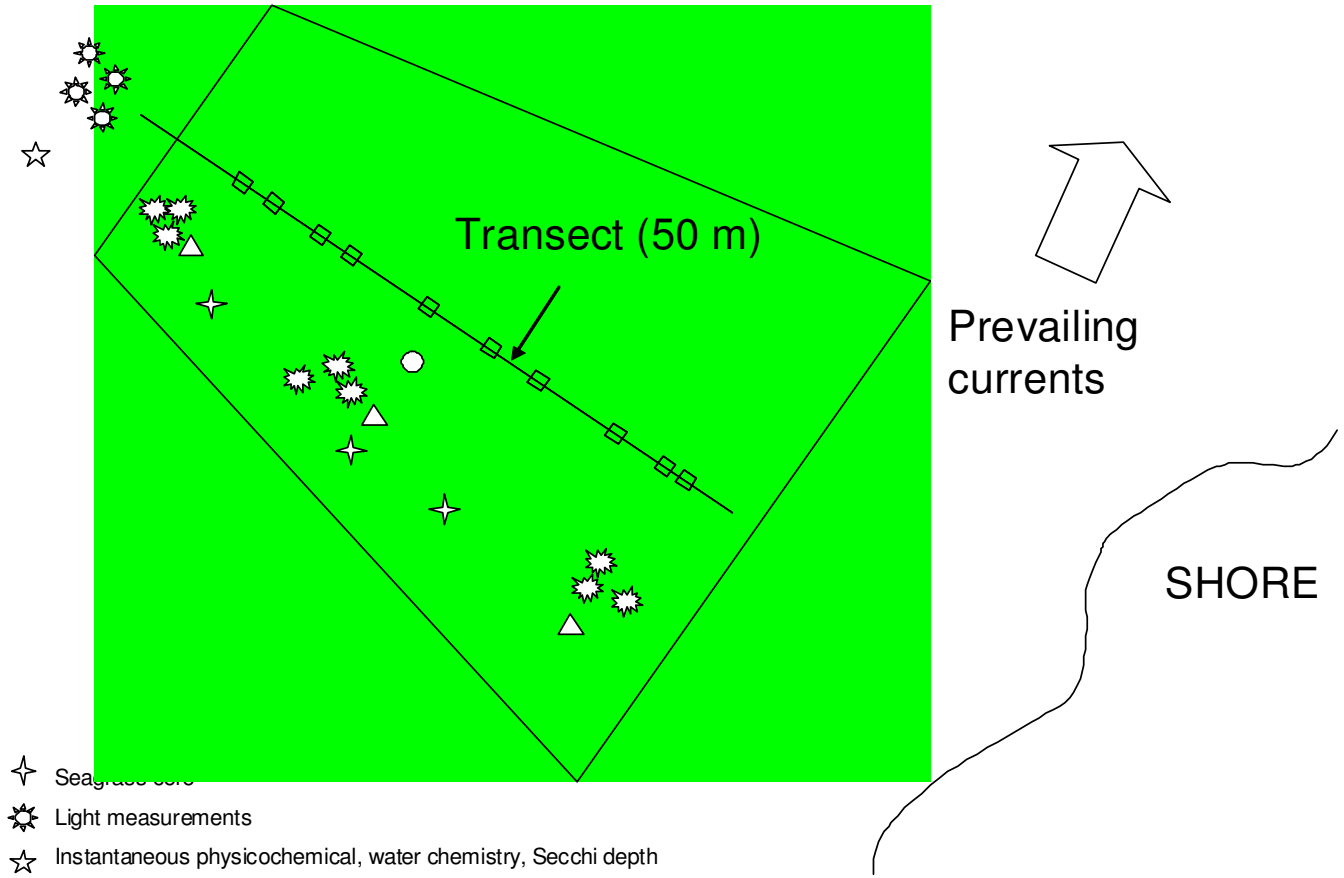


Figure 6. Schematic of field sampling design.

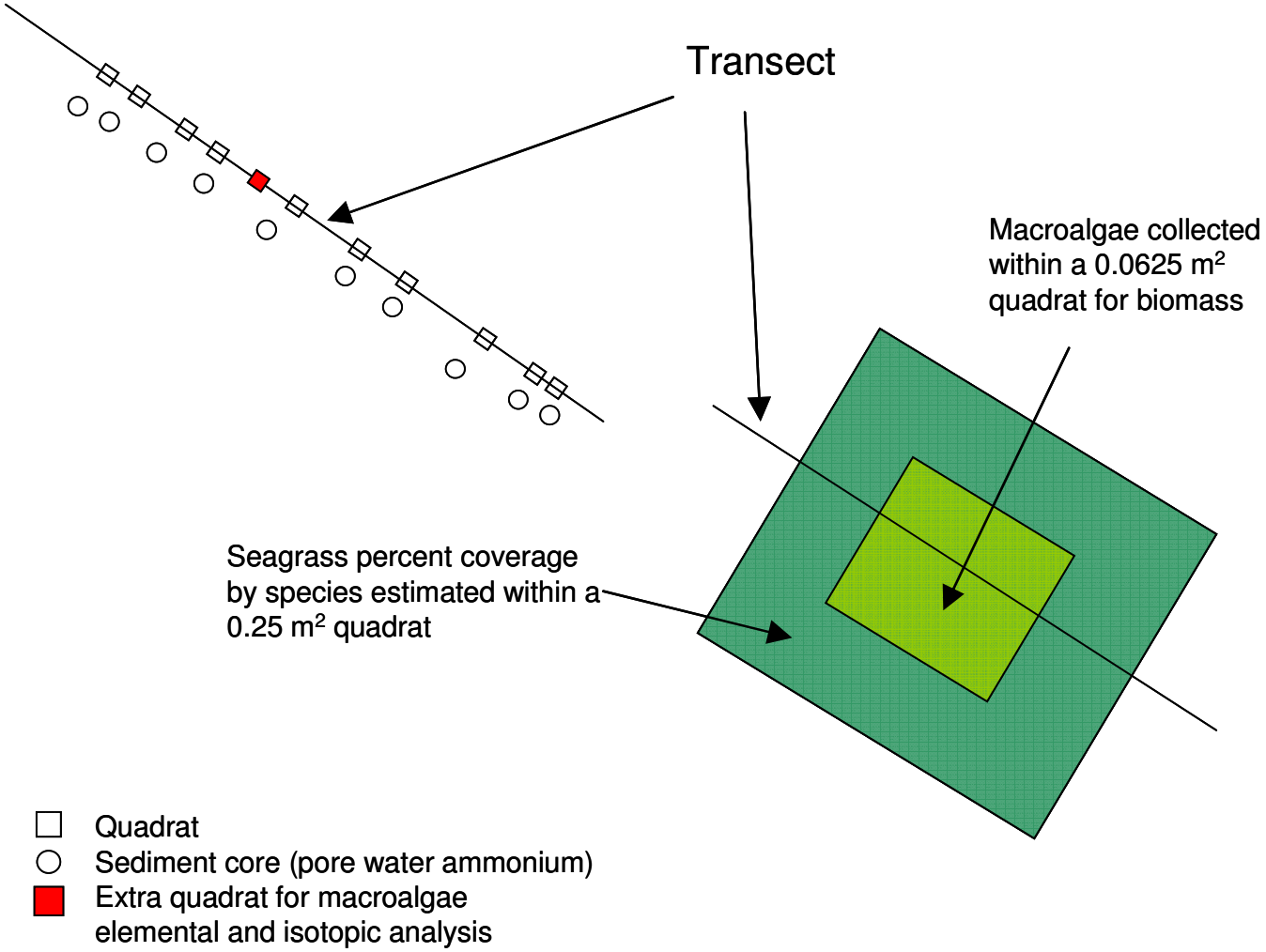


Figure 7. Close-up of field sampling design showing quadrat.

Instantaneous Physicochemical Measurements

For each transect, dissolved oxygen (DO), specific conductivity, pH, salinity, and temperature were measured using a multiprobe instrument (YSI 600XLM or equivalent). Secchi depth and total depth were measured. Four replicate light measurements were made using a Li-Cor quantum sensor at the water surface and the top of the seagrass canopy, with a corresponding measurement made on the boat. These measurements were made before field staff entered the water, to prevent disturbing the sediments and influencing water quality measurements.

Long-term Water Quality Measurements

Datasondes were deployed at each site to provide a long-term record of water quality during the study. A YSI OMS datasonde was deployed within each AOI near the deep edge of a seagrass bed, recording turbidity, temperature, specific conductivity, and water level. A YSI LS datasonde was also deployed in Port Bay at the State Highway 188 bridge to obtain additional temperature, specific conductivity, and water level measurements. Deployments lasted approximately six weeks before sondes were retrieved, cleaned, post-calibrated, and re-deployed.

Long-term PAR measurements were also collected at East Flats using a Li-COR continuously recording light meter at the deep edge of a seagrass bed.

Water Chemistry

Replicate water samples were collected during each event at each transect for ammonia (NH_3, NH_4^+)[1], nitrate (NO_3^-), nitrite (NO_2^-), ortho-phosphate (OP, PO_4^{-3}), chlorophyll-*a*, and total suspended solids (TSS) analysis. Sample containers, syringe and filter heads used to collect nutrient samples were acid-washed. Samples were collected at the surface near the deep water end of a transect using a hand syringe and filtered on site with a ~0.7 µm glass fiber filter. Samples were immediately preserved on ice for transport to the laboratory. Porewater ammonia, ammonia, nitrate, nitrite and ortho-phosphate samples were analyzed using marine methods at a UTMSI laboratory. Chlorophyll-*a* and TSS samples from East Flats were analyzed at UTMSI, while chlorophyll-*a* and TSS samples from Port Bay were analyzed at the Lower Colorado River Authority's (LCRA's) Environmental Services Laboratory.

Sediment Chemistry

Sediment samples were collected at each site once during the study at each transect for total organic carbon (TOC) and grain size (texture) analysis. Sediment samples were collected along each transect near each quadrat (see Figure 7) at each site and event, and analyzed for porewater ammonia. Samples were obtained using a plastic 60 cc syringe barrel (2.5 cm diameter, 10 cm long, with plunger removed). The syringe barrel was pushed straight down into the sediment to a depth of approximately 10 cm. The open end of the syringe barrel was covered to retain a vacuum while the syringe was pulled up out of the sediment. Any water retained on top of the core was poured off, and the sediment core was shaken or extruded into a pre-labeled Whirl-Pak bag, and immediately placed on ice for transport to the laboratory. Additional cores were collected for TOC and grain size analysis to provide enough sediment (at least 100 g) for analysis. Sediment samples from East Flats were analyzed at UTMSI, while those from Port Bay

[1] The term "ammonia" will be used throughout this report to refer to both ammonia (NH_3) and ammonium (NH_4^+).

were analyzed at the Lower Colorado River Authority's (LCRA's) Environmental Services Laboratory.

Quality Control for Water and Sediment Samples

Field quality control requirements as outlined in the *TCEQ Surface Water Quality Monitoring Procedures Manual* (SWQM)*, vol. 1* (TCEQ 2003) were met. Multi-parameter datasondes were calibrated against known standards, following specified procedures, within 24 hours prior to sampling. Expired standards were not used. Only instruments passing calibration were used to collect data. Within 24 hours following sampling, instruments were checked against calibration standards to ensure that measurements were within required limits as specified in the SWQM Manual. Any data collected by instruments which did not meet the post-calibration check requirements was flagged and not used in analysis. Calibration records were kept on file.

For water samples, field splits were collected on a 10% basis at both sites, except for the first sampling trip, when field splits were not obtained. Field splits consisted of a single water sample subdivided by field staff immediately following collection and submitted to the laboratory as two separately identified samples according to procedures specified in the SWQM Manual. Split samples were preserved, handled, shipped, and analyzed identically and used to assess variability in all of these processes. Field split analysis results were evaluated by calculating relative percent difference (RPD) using the following equation:

$$RPD = (X_1-X_2)/((X_1+X_2)/2))*100$$

Where X_1 and X_2 are the analysis results for the split samples. A 30% RPD criteria was used to screen field split results as a possible indicator of excessive variability in the collection and analytical system. Professional judgment during data validation was relied upon to interpret any high RPD values and take appropriate action.

Methods Comparison for Water and Sediment Samples

Seagrass researchers measuring water quality often use marine methods to avoid saltwater interference and maintain a low method detection limit for samples. Due to lack of information about comparability of marine methods with standard methods used by TCEQ for its statewide Surface Water Quality Monitoring Program, some water and sediment samples were split between labs to allow comparison of results.

At Port Bay on the July sampling trip, field split samples were collected as described above at each transect for nitrate, nitrite, ortho-phosphate, and ammonia in water. One set of samples was analyzed by the LCRA laboratory and one by the UTMSI laboratory. One sediment sample for porewater ammonia at each transect was also split between the two labs. At East Flats on the July sampling trip, split samples were collected at each transect for chlorophyll-a and TSS. At East Flats on the November sampling trip, one sediment sample per transect was split for grain size and TOC.

Macroalgae Collection

Macroalgal biomass was determined from the collection of all algal material within ten - 0.0625 m^2 quadrats placed along each transect at pre-determined random distances from the zero meter

mark (Figure 6). Material from each replicate was placed in sealed plastic bags and transported to the laboratory in cooled containers. Additional algal samples were collected for analysis for elemental composition (C:N) and stable isotope ratio ($\delta^{13}C$ and $\delta^{15}N$).

Percent Vegetative Cover

After macroalgae had been collected, vegetative percent cover was estimated by examining ten quadrats (0.25 m^2) placed along each transect at the same pre-determined random distances from the zero meter mark that were used to collect the macroalgae. A new set of random sampling positions was chosen before each sampling event. Each quadrat was examined underwater by a diver (Figure 8). The quadrat area was first cleared of all macroalgae, and then all seagrass species occurring in the quadrat were identified (Figure 9). Cover, defined as the percentage of the total quadrat area that was obscured by a particular species when viewed from directly above, was determined for each species. A Braun-Blanquet score based on the cover of the species within that quadrat was assigned (Table 1).

Figure 8. Diver estimating seagrass coverage at Port Bay T2.

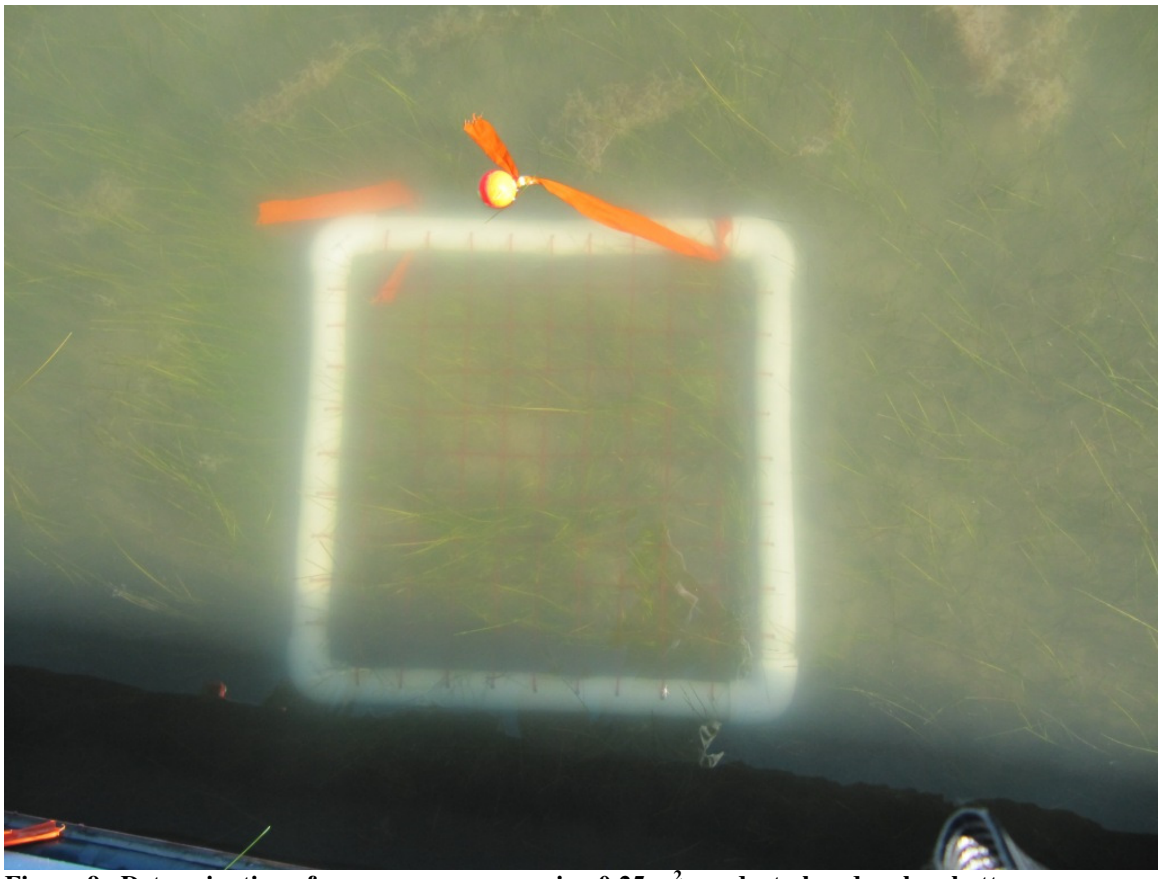


Figure 9. Determination of seagrass coverage using 0.25 m^2 quadrat placed on bay bottom.

Table 1. Braun-Blanquet abundance scores.

S	Interpretation
0	Absent from quadrat
1	< 5% cover
2	5%-25% cover
3	25%-50% cover
4	50%-75% cover
5	75%-100% cover

Seagrass Cores

Three replicate cores at each transect were used for estimates of above- and below-ground biomass. In Port Bay, three replicate cores were collected. In East Flats, one core was collected near the shallow end, middle, and deep end of the transect. A 15 cm (ID) diameter corer was used to sample *Thalassia,* and a 9 cm (ID) diameter corer was used to sample *Halodule*, *Syringodium*, and *Ruppia*.

The 15 cm corer was made of PVC, with a cap on top that had a small hole in the center which could be plugged with a rubber stopper (Figure 10). The 9 cm corer was a hollow cylinder made

of PVC or polycarbonate (Figure 11). Prior to placing the 15 cm core on the seabed, the rubber stopper was removed from the top of the core. For both 9 cm and 15 cm cores, before pressing the corer into the sediment, the diver felt carefully around the bottom of the core and removed any seagrass that had been caught under the edge of the coring device. The diver then pressed and twisted the core down into the sediment 10 to 15 cm. The stopper was re-installed in the 15 cm core, and the corer rocked back and forth. The diver then worked a hand under the core and removed it from the seagrass bed, keeping a hand under the bottom of the core to prevent loss of sample. The diver emptied the core into the sieve and removed any broken shoots since these were likely exterior shoots cut by the core tube (Figure 12). Samples were placed in pre-labeled Ziploc bags and immediately placed on ice.

Seagrass Shoot Collection

For epiphyte fluorescence studies, three replicate samples of seagrass shoots were collected near the shallow end, middle and deep end of each transect. Additional samples were obtained if a second seagrass species was present at a significant level of abundance (estimated to exceed 20% of the total seagrass coverage at the site). Thus, if two species were present, each at >20% of the total seagrass coverage, separate samples were obtained for each seagrass species. Single-species seagrass shoot samples (up to 50) were obtained by gently pulling up seagrass rhizomes with shoots attached, handling only at the base or rhizome to avoid disturbing attached epiphytes and transferring to widemouth sample bottles. For each sample, the number of shoots collected depended on the average blade length of the shoots (Table 2). Care was taken during sampling events to avoid trampling the area where shoots would be collected later.

Figure 10. 15 cm PVC corer with cap.

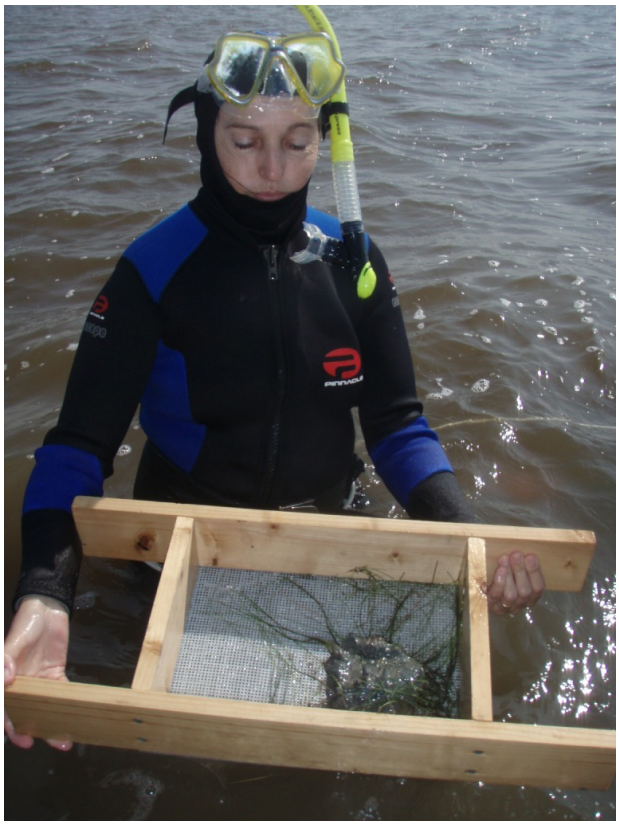


Figure 11. Left - A 9 cm corer used to collect *Halodule and Syringodium* samples.
Figure 12. Right - Diver washing seagrass core after collection using 9 cm corer.

Table 2. Shoot collection guidance for epiphyte fluorescence; minimum number of shoots to collect by species.

Blade Length (cm)	Halodule	Thalassia
<10	45	20
10 – 20	35	15
> 20	25	10

Shoots were also collected for estimating epiphyte load using the traditional scraping and weighing method, and for stable isotope and C:N analysis. One sample, consisting of several shoots, was collected at the shallow end, middle and deep end of each transect for epiphyte load. Likewise, one sample, consisting of several shoots, was collected at the shallow, middle and deep end of each transect for stable isotope and C:N analysis. Shoots were collected by gently pulling up seagrass rhizomes with shoots attached, handling only at the base or rhizome to avoid disturbing attached epiphytes and transferring to Whirl-Pak bags. At least 10 shoots per sample were needed for the epiphyte load analysis. At least 20 shoots per sample were needed for the stable isotope and nutrient content analysis.

Processing and Analysis of Biological Samples

Macroalgae

Samples from each quadrat were cleaned of debris and non-algal material. Samples were placed in a plastic "salad spinner" and spun to force water off the vegetation. Samples were then placed in pre-labeled and pre-weighed aluminum foil packets and weighed. Samples were dried to a constant weight at 60°C in the foil packets, and the dry weight recorded.

Seagrass Cores

Each seagrass core was removed from its sample bag and placed in a white sorting tray (Figure 13). Cores were rinsed and cleaned to remove sediment, dead plant material and other debris, which were discarded. Number of shoots was counted using a tally counter for accuracy and recorded individually for each species present. Above-ground and below-ground tissue was separated by cutting with a scalpel at the achlorophyllous (whitish) base of the shoot. Above-ground tissue included leaves (including sheath material) and floral parts, while below-ground tissues included root and rhizome material. Leaves were carefully cleaned of attached biota by scraping with a wet cloth, scalpel, forceps or razor blade. Leaf morphometrics were obtained for each sample by randomly selecting five shoots to measure. For each shoot, the number of blades and the length and width of the longest blade were recorded. Above-ground and below-ground material for each sample was placed in separate pre-labeled, pre-weighed foil packets for drying. Above-ground and below-ground tissue were dried to a constant weight (60 °C) and weighed to the nearest milligram. Drying typically took 2-5 days. The biomass values for above- and below-ground biomass are used to calculate a root:shoot ratio.

Figure 13. Processing of *Halodule* seagrass core sample.

Seagrass Shoots for Epiphyte Load

Algal epiphytes were harvested from blades using traditional scraping techniques for determination of epiphyte biomass. Leaf samples for epiphytic biomass were processed within three days of collection. In the laboratory epiphytes were separated from the leaf surface by scraping with a scalpel and measurements made of the length and width of the area scraped. Scraped material was collected and retained on pre-weighed glass fiber filters. The collected epiphytic biomass was dried to a constant weight at 60 °C for determination of epiphyte biomass per area seagrass scraped. In Port Bay, scraped seagrass leaves were also retained and dried to a constant weight at 60 °C. This allowed epiphyte biomass to also be expressed as a fraction of scraped seagrass biomass. This additional measurement was made since estimates of epiphyte biomass made on an areal basis are strictly accurate only for *Thalassia*, since *Halodule* and *Syringodium* leaf shapes are terete in cross-section instead of flat like *Thalassia* (Appendix A).

Data Analysis

Replicate measurements were averaged for each species for each transect at a site. Descriptive statistics were calculated for parameters with replication at each transect. Measured parameters from each transect were compared to the other two transects. For parameters where no significant differences were found, data from transects at each site was averaged to summarize results for the site.

Results

Aerial Imagery

East Flats 2009

In East Flats, three AOIs including shallow water, deep water and problem areas, were required to accurately classify the image into a thematic map (Figure 14). Classification was a relatively simple process, based on prior experience with aerial imagery from this site (Pulich and Summers 2010). Shallow flats occur on the northeast side of the image with deeper areas towards the southwest. Problem areas exist in deep areas where dark water can be confused with vegetation. All AOIs were processed using the intensity band.

The classified image of East Flats showed 137 acres of bare area and 243 acres of vegetated area (Table 3 and Figure 15), with an overall classification accuracy of 88.2% (Table 4). Dense algae deposits covered 41 acres of the study area.

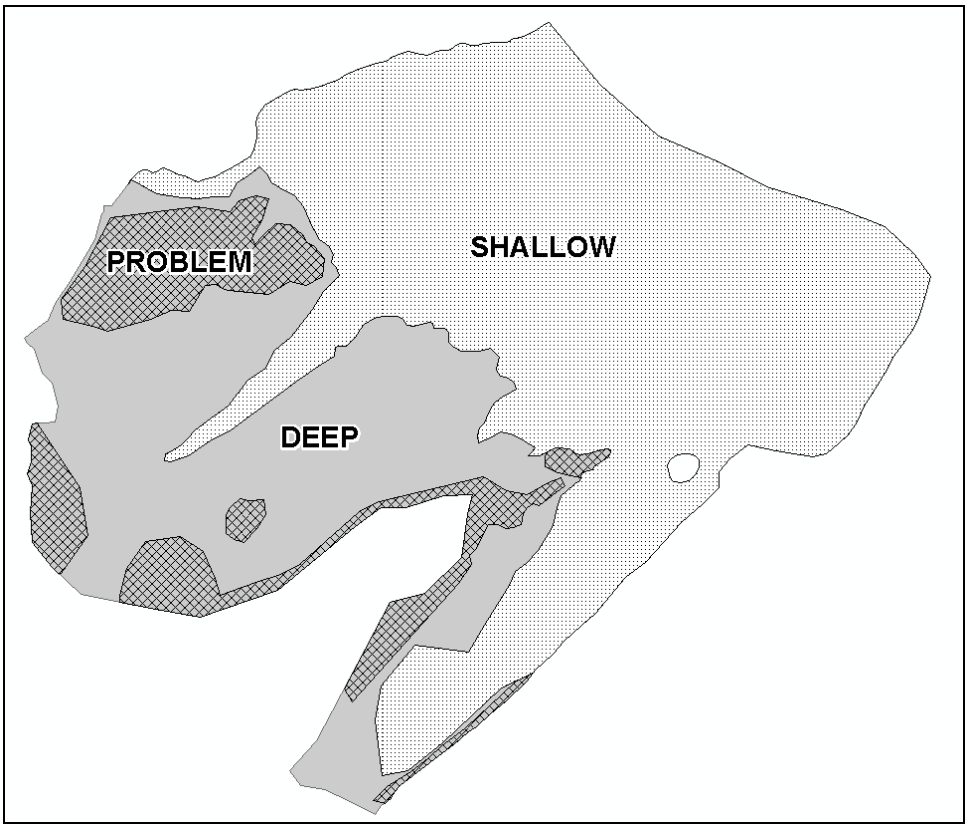


Figure 14. East Flats 2009 areas of interest (AOIs).

Table 3. East Flats 2009 habitat areas.

Habitat	Cell size	Pixel count	Total acreage
Bare	0.15 m x 0.15 m	24,652,130	137
Vegetation	0.15 m x 0.15 m	43,760,605	243
Total		68,412,735	380

Table 4. East Flats 2009 classification accuracy assessment.

		Reference data			
		Vegetation	Bare	Row total	User's accuracy
Classified data	Vegetation	20	0	20	100.0%
	Bare	4	10	14	71.4%
	Column total	24	10	34	
	Producer's accuracy	83.3%	100.0%		
	Overall accuracy				88.2%

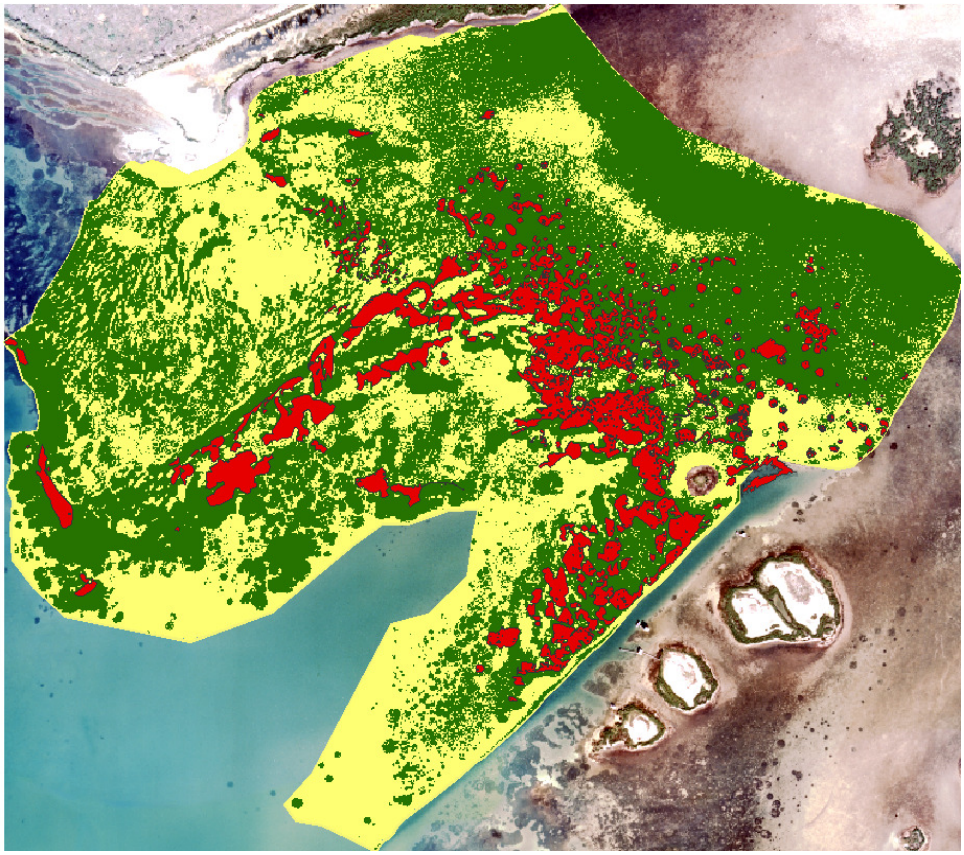


Figure 15. East Flats 2009 classified image.
Bare areas are shown in yellow; vegetated areas in green; algae deposits in red.

Patch analysis of habitat polygons generated from the classified image showed the majority of the vegetated area to be continuous (Table 5 and Figure 16). The area threshold for classing data as either continuous or patchy was set at 400 m^2. (Greater than 400 m^2 is continuous; less than or equal to 400 m^2 is patchy.) Shape index and perimeter-to-area ratio differentiated continuous vegetation from other habitat types, with shape index being higher and perimeter-to-area ratio being lower for continuous areas than for both patchy and bare polygons (Table 5). Patchy and bare polygons appeared to have the same level of shape complexity according to the shape index and perimeter-to-area ratio metrics.

Table 5. East Flats 2009 landscape metrics.

	Average shape index	Average perimeter-to-area ratio	Average size ± SD (m^2)	Polygon count	Total size (m^2)	Total size (acres)
Continuous	8.8	0.7	37,592 ± 179,724	25	939,814	232
Patchy	2.0	2.7	18 ± 41	2247	40,490	10
Bare	2.0	2.7	101 ± 2,609	5362	544,129	134

Polygons less than two square meters were excluded from the patch analysis, based on limits of the vectorization technique. This eliminated 9,665 vegetated polygons and 20,023 bare polygons from the study area (0.3% and 0.7% of the total area, respectively).

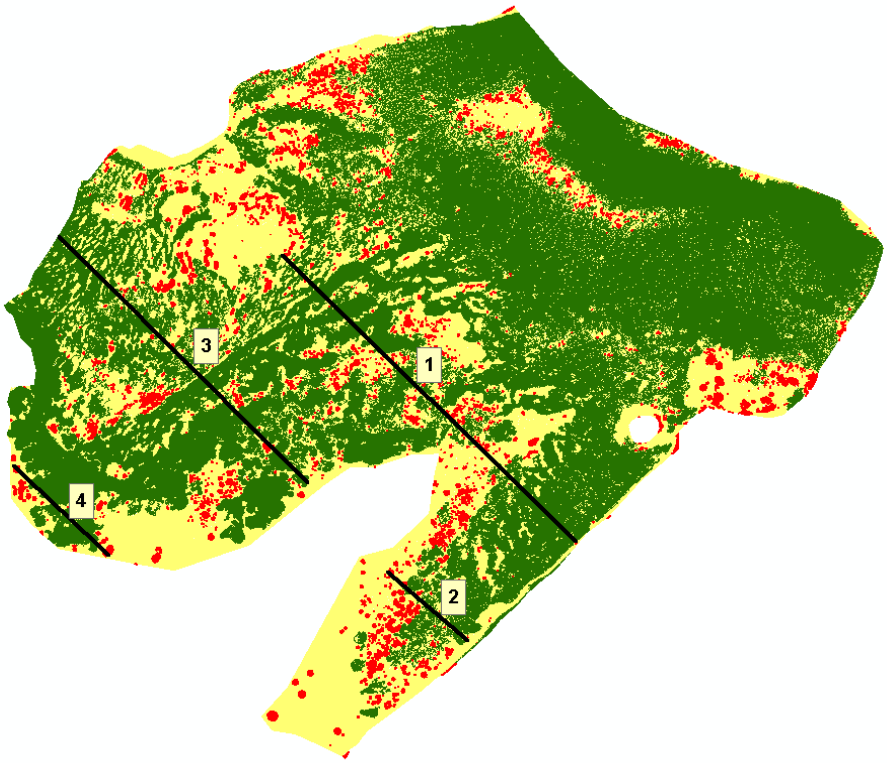


Figure 16. East Flats 2009 landscape graphic.
Patchy areas are shown in red; continuous areas in green; bare areas in yellow; virtual transects in black.

Additional landscape metrics were calculated in East Flats by evaluating four virtual transects following Dowty (2005). The number of transitions per transect between bare and vegetation ranged between 15 and 108 with transect length ranging from 219 m to 855 m (Table 6). Bare patches on all transects made up 25-30% of the transect length. Transects 1, 2 and 3 appeared to have similar average patch sizes and number of transitions per 100 m. From the landscape graphic, these three transects also appear to run through similar habitat (Figure 16). Transect 4, in the southwestern corner of the study area, had the least number of normalized transitions and the largest average bare patches.

Table 6. East Flats 2009 virtual transects.

Transect number	Number of transitions between vegetated and bare areas	Transect length (m)	Total length of bare patches (m)	Average length of bare patches (m)	Bare length/ transect length	Normalized transitions/100 m transect length
1	90	855	259	6	0.30	11
2	26	219	62	5	0.28	12
3	108	728	203	4	0.28	15
4	15	271	67	10	0.25	6

The average deep edge for seagrass at East Flats occurred at a tide-adjusted depth of 1.42 m ± 0.09 m. Depths at the deep edge ranged from 1.3 m to 1.55 m (Figure 17).

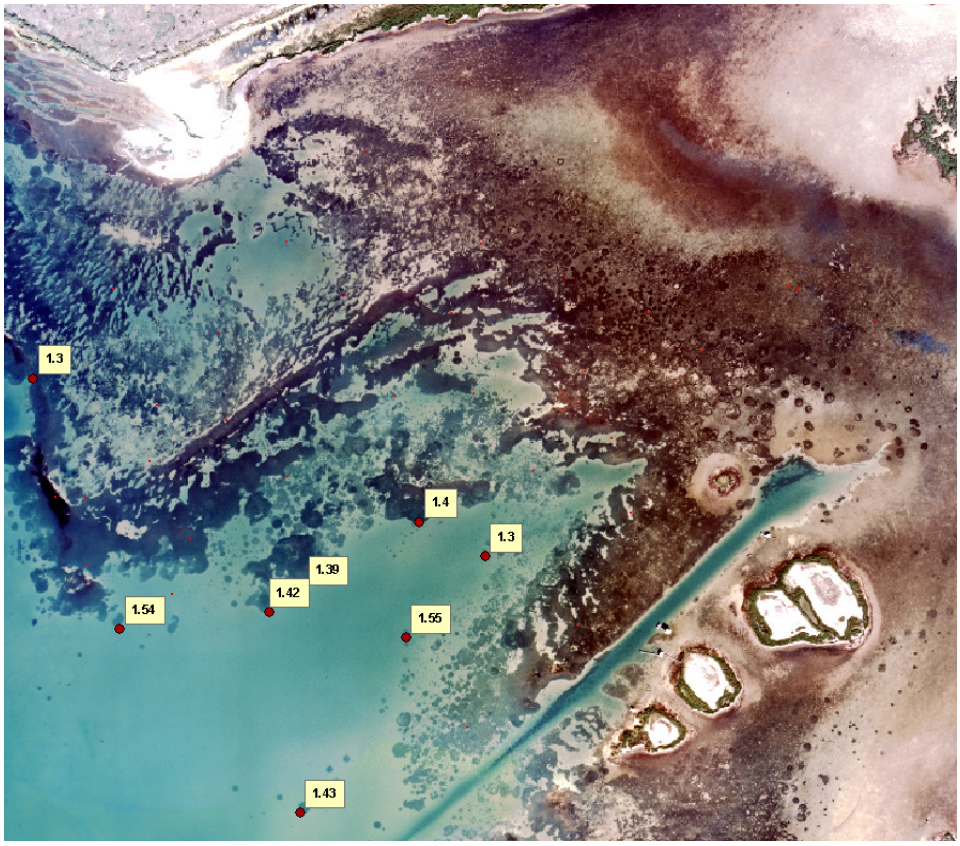
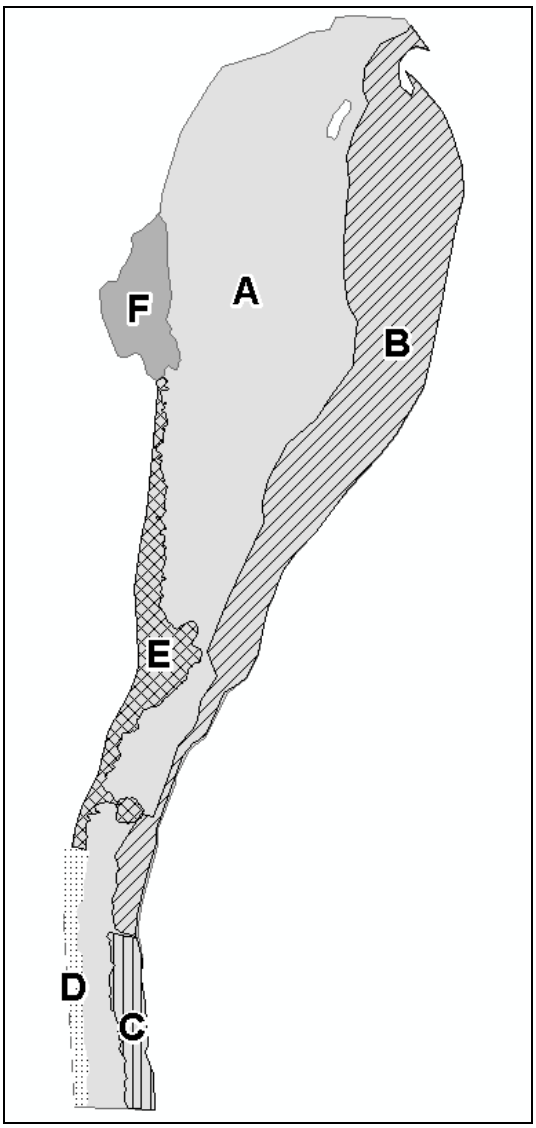


Figure 17. East Flats 2009 deep-edge graphic.
Depth at edge of seagrass at eight field-collected points. Labeled depths have been adjusted for tide.

Port Bay 2009

Analysis of Port Bay required six AOIs (A – F) to address differences in intensity and saturation values by location (Figure 18). For example, the value of a bare pixel may have one pixel threshold in the northeastern portion of the study area, but may require a completely different threshold in the southwestern portion. In East Flats, this was noticed to a limited extent, but the AOI boundaries were easily drawn around obviously shallow or deep habitat. Defining the AOIs in Port Bay was more difficult because the values for bare and vegetated pixels fluctuated considerably over relatively small areas and AOIs were not correlated with specific landscape differences, such as "shallow" or "deep." Presumably, these inconsistencies in pixel values were due to varying densities of vegetation and variation in water color. Determining the best possible outlines and thresholds for the AOIs was an iterative and, in this case, time-consuming task. AOI creation is also subjective, so the time required to attain the "best fit" is variable and changes with the analyst.

Although the intensity and saturation bands inconsistently differentiated vegetation from bare across the image, analysis revealed that these bands were useful for eliminating plume effects throughout the entire study area. A large plume was visible on the original photography in the northern portion of the study area but was not visible in either the intensity or saturation bands. This kept the turbid area from showing up in the classified image and thus improved the accuracy of the classification. Four of the AOIs were processed with the saturation band and two used the intensity band.



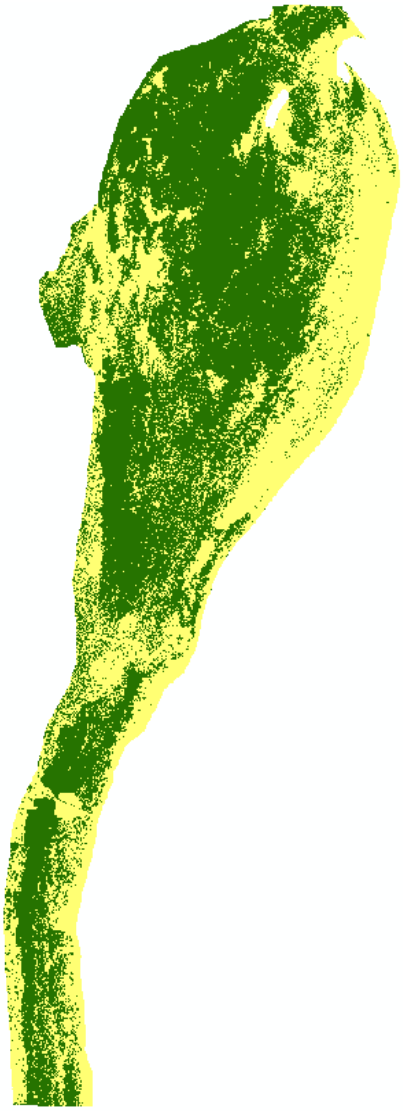


Figure 18. Left - Port Bay 2009 areas of interest (AOIs).
Figure 19. Right - Port Bay 2009 classified image.
Bare areas shown in yellow; vegetated areas in green.

The classified image of Port Bay showed 92 acres of bare area and 131 acres of vegetated area (Table 7 and Figure 19), with an overall classification accuracy of 82.1% (Table 8). Algae was not observed at Port Bay.

Table 7. Port Bay 2009 habitat areas.

Habitat	Cell size	Pixel count	Total acreage
Bare	0.15 m x 0.15 m	16,626,782	92
Vegetation	0.15 m x 0.15 m	23,488,862	131
Total		40,115,644	223

Table 8. Port Bay 2009 classification accuracy assessment.

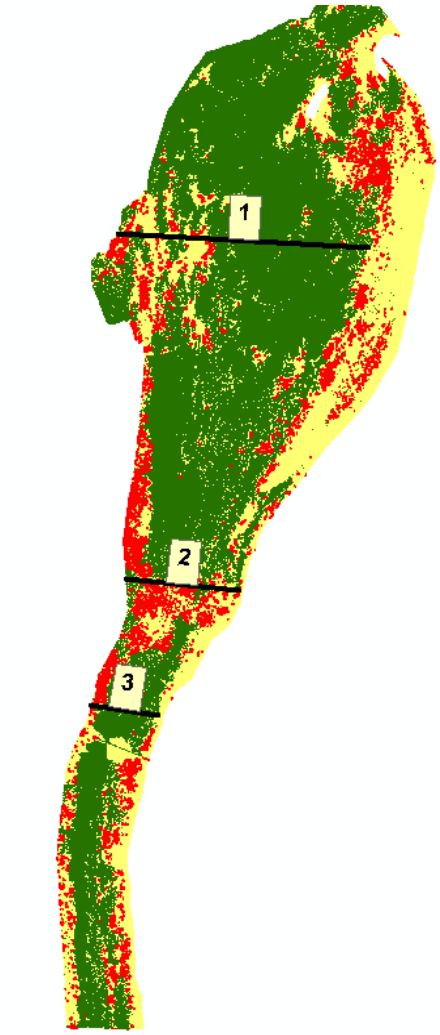
		Reference data			
		Vegetation	Bare	Row total	User's accuracy
Classified data	Vegetation	24	2	26	92.3%
	Bare	5	8	13	61.5%
	Column total	29	10	39	
	Producer's accuracy	82.8%	80.0%		
	Overall accuracy				82.1%

Patch analysis of habitat polygons generated from the classified image showed the majority of the vegetated area to be continuous, just as in East Flats (Table 9 and Figure 20). The area threshold for classing data as either continuous or patchy was set at 400 m^2. (Greater than 400 m^2 is continuous; less than or equal to 400 m^2 is patchy.) Similar to East Flats results, shape index and perimeter-to-area ratio differentiated continuous vegetation from other habitat types, with shape being higher and perimeter-to-area ratio being lower for continuous polygons than both patchy and bare polygons (Table 9). Patchy and bare appeared to have the same level of shape complexity according to the shape index and perimeter-to-area ratio metrics.

Table 9. Port Bay 2009 landscape metrics.

	Average shape index	Average perimeter-to-area ratio	Average size ± SD (m^2)	Polygon count	Total size (m^2)	Total size (acres)
Continuous	20.0	1.4	27,939 ± 86,451	17	474,966	117
Patchy	2.3	3.3	11 ± 25	2895	34,154	8
Bare	2.2	3.1	97 ± 3,859	3721	362,430	90

Polygons less than two square meters were excluded from the patch analysis, based on limits of the vectorization technique. This eliminated 21,168 vegetated polygons and 24,494 bare polygons from the study area (0.8% and 1.1% of the total area, respectively).



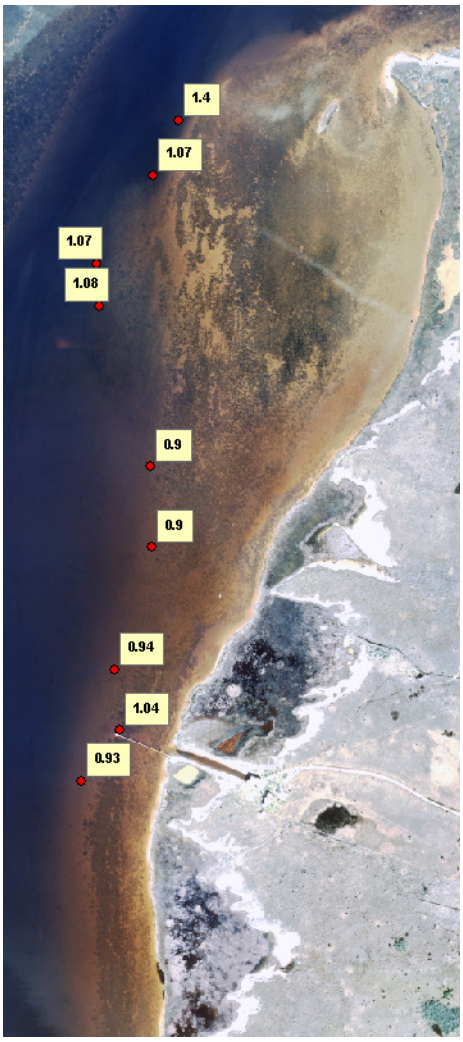


Figure 20. Left - Port Bay 2009 landscape graphic.
Patchy areas shown in red; continuous areas in green; bare in yellow; virtual transects in black.
Figure 21. Right - Port Bay 2009 deep-edge graphic.
Depth at edge of seagrass at nine field-collected points. Labeled depths have been adjusted for tide.

Additional landscape metrics were calculated in Port Bay by evaluating three virtual transects. The number of transitions per transect between bare and vegetation ranged between 22 and 27 with a transect length ranging from 157 m to 577 m (Table 10). Transect 3 had more bare patches (when standardized to transect length) and smaller average bare patches when comparing to Transect 1 and 2. Transect 2 showed the most bare area along the transect (74%) and ran through an area primarily made up of patchy polygons on the landscape graphic (Table 10 and Figure 20). Transect 1 was the longest transect but had the least transitions when standardized to transect length. In the landscape graphic, Transect 1 traversed an area primarily covered in continuous vegetation (Figure 20).

The average deep edge for seagrass at Port Bay occurred at a tide-adjusted depth of 1.04 m ± 0.16 m. Depths at the deep edge ranged from 0.9 m to 1.4 m (Figure 21).

Table 10. Port Bay 2009 virtual transects.

Transect number	Number of transitions between vegetated and bare areas	Transect length (m)	Total length of bare patches (m)	Average length of bare patches (m)	Bare length/ transect length	Normalized transitions/100 m transect length
1	27	577	168	12	0.29	5
2	28	262	194	14	0.74	11
3	22	157	46	4	0.29	14

East Flats 2010

During the second year of analysis in the East Flats study area, imagery classifications and automated landscape metrics were not used. Instead, 15 virtual transects were evaluated to provide landscape metrics (Figure 22). Transects were stratified based on primary species type, with Transects 1-4 extending through *Halodule* habitat and Transects 5-15 extending through *Thalassia* habitat.

Figure 22. East Flats 2010 virtual transects.

The number of transitions per transect between bare and vegetated ranged between 7 and 85 with a transect length ranging from 216 m to 1124 m (Table 11). Bare patches ranged between 7% and 41% of the transect length. Transects 1, 9, and 12 had the highest number of normalized transitions between vegetated and bare habitat. The average length of bare patches was generally smaller in the *Halodule* habitat (Transects 1-4) than in the *Thalassia* habitat (Transects 5-15).

The average deep edge for seagrass at East Flats occurred at a tide-adjusted depth of 1.32 m ± 0.15 m. Depths at the deep edge ranged from 1.11 m to 1.48 m (Figure 23).

Table 11. East Flats 2010 virtual transects

Transect number	Number of transitions between vegetated and bare areas	Transect length (m)	Total length of bare patches (m)	Average length of bare patches (m)	Bare length/ transect length	Normalized transitions/100 m transect length
1	56	361	59	2	0.16	16
2	46	533	88	4	0.17	9
3	16	622	59	7	0.09	3
4	23	668	47	4	0.07	3
5	62	1124	357	12	0.32	6
6	71	855	271	8	0.32	8
7	23	295	92	8	0.31	8
8	15	233	62	8	0.27	6
9	34	219	67	4	0.31	16
10	12	216	65	11	0.30	6
11	70	898	371	11	0.41	8
12	85	809	241	6	0.30	11
13	48	728	153	6	0.21	7
14	44	717	128	6	0.18	6
15	7	272	58	15	0.21	3

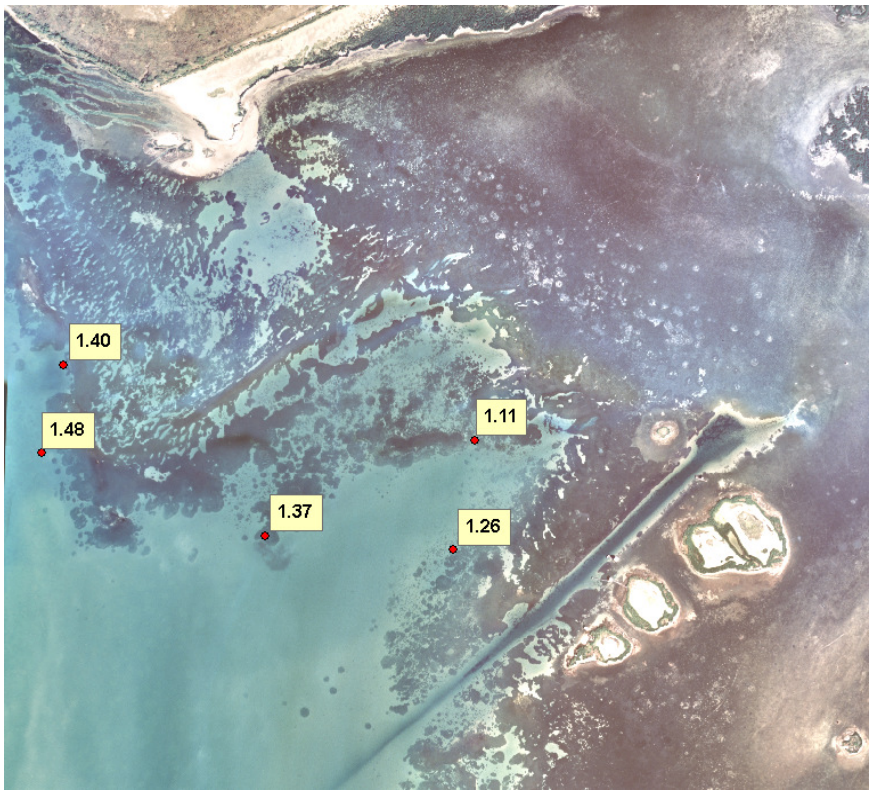


Figure 23. East Flats 2010 deep edge graphic.
Depth at edge of seagrass at five field-collected points. Labeled depths have been adjusted for tide.

Port Bay 2010

Both imagery classification and transect analysis were used to evaluate the 2010 Port Bay imagery. Classification analysis was performed using ENVI™ image processing software.[2] One Region of Interest (ROI) was required to accurately classify the image into a thematic map (Figure 24).

The classified image of Port Bay showed 59 acres of bare area and 158 acres of vegetation (Table 12 and Figure 25), with an overall classification accuracy of 85.1% based on 23 ground-truth points taken by differential GPS (Table 13). Very little macroalgae was observed at Port Bay.

Table 12. Port Bay 2010 habitat areas.

Habitat	Cell size	Pixel count	Total acreage
Bare	0.25 m x 0.25 m	3,841,845	59
Vegetation	0.25 m x 0.25 m	10,255,869	158
Total		14,097,714	217

Table 13. Port Bay 2010 classification accuracy assessment.

		Reference data			
		Vegetation	Bare	Row total	User's accuracy
Classified data	Vegetation	19	0	19	100.0%
	Bare	2	2	4	50.0%
	Column total	21	2	23	
	Producer's accuracy	90.5%	100.0%		
	Overall accuracy				85.1%

Patch analysis of habitat polygons generated from the classified image was not conducted for the 2010 imagery.

[2] ENVI™, like ERDAS™, also performs RGB color space transformations, resulting in conversion of the pixel color bands to hue, saturation, and intensity color space values. The intensity band in ENVI is referred to as the value band, and this band was used for subsequent separation of bare from vegetated areas. Unlike the 2009 Port Bay image, only one ROI (= region of interest, analogous to the AOI in ERDAS) was used rather than six AOIs. This ROI was developed from the shape file ('pb_ALL.shp') produced previously for the 2009 photoimage. Density slicing was then performed on the value band image (= intensity image) to differentiate vegetation from bare areas. It was determined that a threshold DN (= digital pixel number) value of 180 in the value band adequately separated the bare from vegetated areas throughout the entire ROI. Pixels less than DN 180 corresponded to seagrass/submerged vegetation, while pixels >180 DN were identified as bare areas. Presumably, the inconsistencies in pixel values previously encountered in a single AOI of the 2009 image were not present here in 2010 due to lack of variation in overlying water color. The density-sliced overlay image was then filtered with a 3x3 median convolution filter, and the image was permanently classified using an unsupervised isodata algorithm to produced a bare and vegetated image. The classified image was then displayed as an 8-bit thematic image with the two classes.

To facilitate comparison of the 2009 and 2010 images, the classified file prepared for the 2009 image was reanalyzed by adjusting the pixel size from 0.15 m per pixel to 0.25 m per pixel (Table 14).

Table 14. Port Bay 2009 habitat areas using 0.25 m pixel size.

Habitat	Cell size	Pixel count	Total acreage
Bare	0.25 m x 0.25 m	5,958,689	92
Vegetation	0.25 m x 0.25 m	8,265,054	128
Total		14,223,743	220

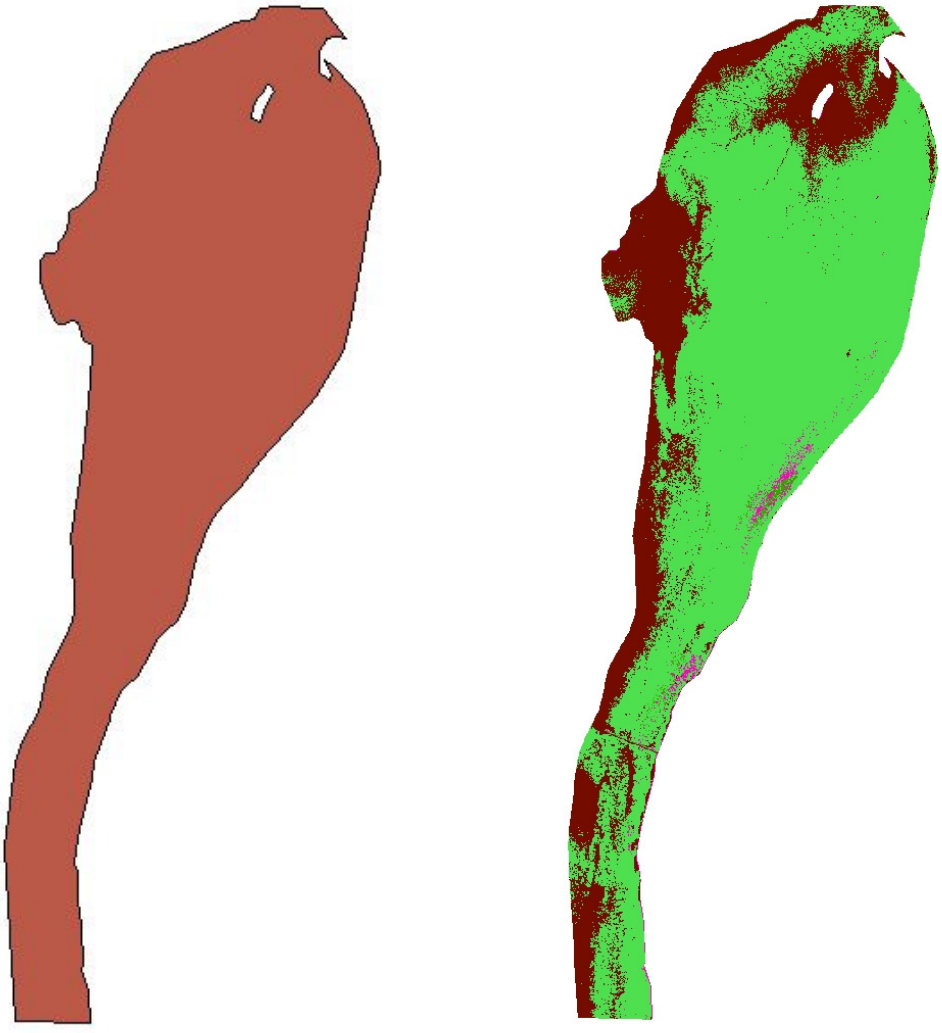
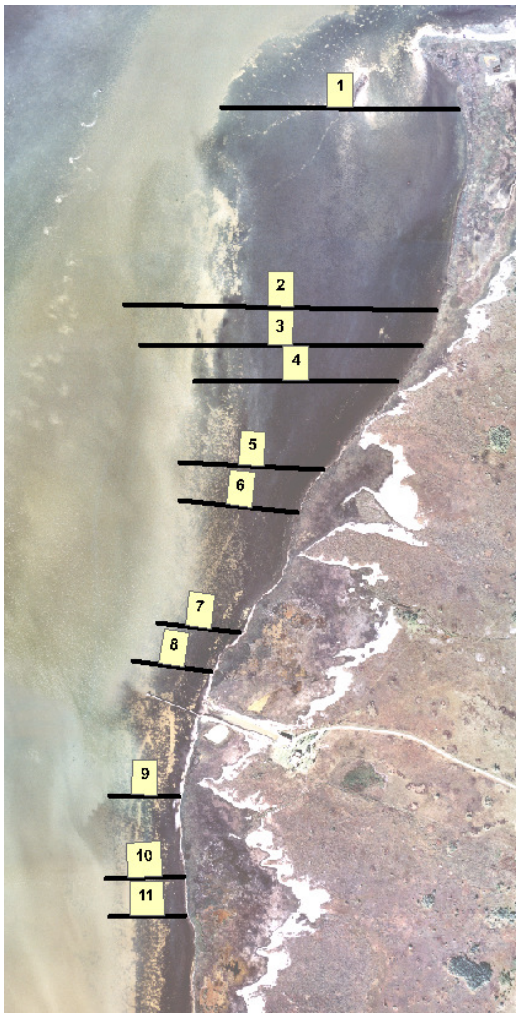


Figure 24. Left - Port Bay 2010 area of interest (ROI).
Figure 25. Right - Port Bay 2010 classified image.
Bare areas are shown in brown; vegetated areas in green and pink.

In Port Bay, 11 virtual transects were randomly selected perpendicular to shore according to Dowty (2005) and evaluated to provide landscape metrics (Figure 26).

The number of transitions per transect between bare and vegetation ranged between 5 and 53 with a transect length ranging from 170 m to 762 m (Table 15). Bare patches ranged between 3% and 42% of the transect length. The southernmost transects (9, 10, and 11) had the most bare area overall. Transects 2, 3 and 10 contained the longest patches on average. Transects 1 and 9 were the patchiest, as indicated by their high normalized transition count.

The average deep edge for seagrass at Port Bay occurred at a tide-adjusted depth of 1.19 m ± 0.11 m. Depths at the deep edge ranged from 1.10 m to 1.32 m (Figure 27).



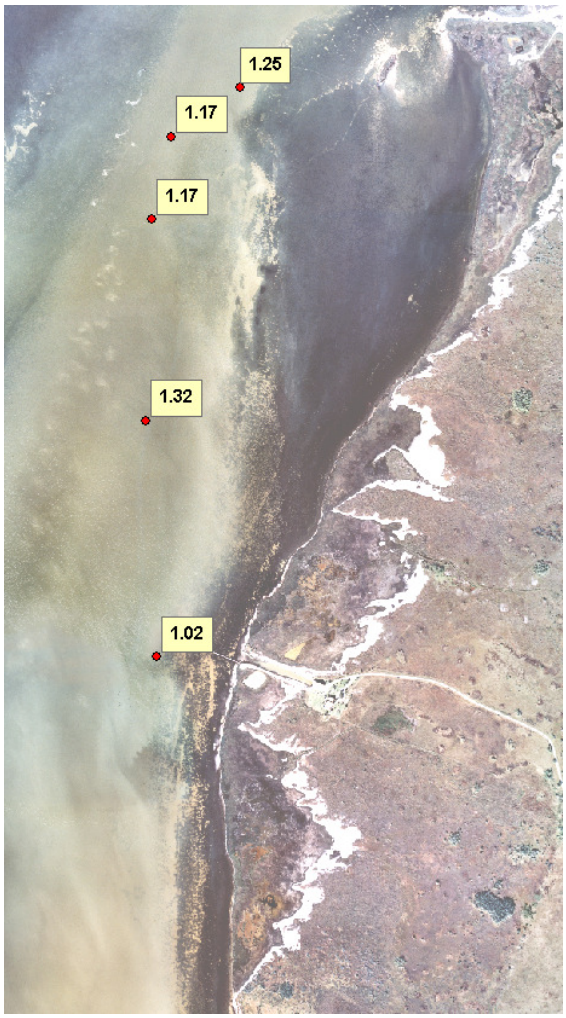


Figure 26. Left - Port Bay 2010 virtual transects.
Figure 27. Right - Port Bay 2010 deep edge graphic.
Depth at edge of seagrass at nine field-collected points. Labeled depths have been adjusted for tide.

Table 15. Port Bay 2010 virtual transects.

Transect number	Number of transitions between vegetated and bare areas	Transect length (m)	Total length of bare patches (m)	Average length of bare patches (m)	Bare length/ transect length	Normalized transitions/100 m transect length
1	53	581	75	3	0.13	9
2	8	762	59	15	0.08	1
3	10	689	50	10	0.07	1
4	10	498	17	3	0.03	2
5	13	350	57	8	0.16	4
6	15	290	29	4	0.10	5
7	5	205	20	7	0.10	2
8	8	195	20	5	0.10	4
9	24	170	51	4	0.30	14
10	12	191	81	12	0.42	6
11	12	185	56	9	0.30	6

Site Characterization

Instantaneous Physicochemical Measurements

Water temperature showed typical seasonal trends, with warmest temperatures at both sites in the summer, followed by spring and fall (Table 16). Salinity at East Flats was much higher than at Port Bay, which is not surprising since Port Bay is a secondary bay off Copano Bay, and thus more influenced by runoff from the mainland. Salinity at Port Bay decreased over 2010 in response to higher than average rainfall in the spring, early summer and fall. Dissolved oxygen was higher at East Flats than at Port Bay, and was supersaturated during the spring sampling period.

Measures of Water Clarity

Since light availability may limit seagrass growth, several measures of water clarity were obtained in this study, including instantaneous measurements of Secchi depth and photosynthetically-active radiation (PAR), long-term measurements of turbidity (both sites) and PAR (East Flats), and instantaneous measurement of total suspended solids (presented in the water chemistry section below).

Secchi depth measurements were lower at Port Bay than East Flats (Table 16). The deepest Secchi measurement in Port Bay occurred at T3 in the summer (0.9 m), while the shallowest was also at T3 in the fall (0.4 m). East Flats had Secchi depths greater than 1.0 m and at times the bay bottom was visible through the water column at the deep edge of T1 and T2.

Table 16. Instantaneous physicochemical measurements for Port Bay and East Flats, May – Nov 2010.

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Port Bay									
Temperature (ºC)	27.1	27.1	27.1	29.2	29.4	29.7	22.7	22.6	22.5
Salinity (ppt)	11.3	11.2	11.2	8	8.8	9.6	2.4	2.4	2.7
Specific conductance (mS cm^{-1})	19.1	18.95	19	13.84	15.25	16.44	4.4	4.5	4.9
pH	8.2	8.2	8.3	8.11	8.2	8.3	8	7.8	8.2
Dissolved oxygen (mg L^{-1})	7.4	6.4	6.4	6.6	6.5	6.7	7	7	6.9
Dissolved oxygen (%)	99	85.5	84.9	93.4	91.3	93.7	82.6	82.4	81.3
Secchi depth (m)	0.5	0.6	0.8	0.6	0.8	0.9	0.5	0.6	0.4
Percent surface irradiance	12.6	18.0	42.6	11.5	22.8	34.9	16.1	14.8	16.2
Light attenuation coefficient (m^{-1})	1.88	1.56	1.42	2.89	1.85	1.32	1.83	1.91	1.65
East Flats									
Temperature (ºC)	30.5	30.4	30.0	31.9	31.0	30.1	19.4	19.8	20.0
Salinity (ppt)	32.1	32.0	32.1	-	-	-	31.1	31.0	30.8
Specific conductance (mS cm^{-1})	49.4	49.2	49.3	-	-	-	47.7	47.6	47.2
pH	7.3	7.6	7.5	7.4	7.4	8.2	8.0	8.0	7.9
Dissolved oxygen (mg L^{-1})	9.9	9.8	7.2	5.1	5.3	6.0	5.7	6.5	6.8
Dissolved oxygen (%)	153.9	152.8	113.4	84.7	85.9	96.7	75.4	85.5	89.1
Secchi depth (m)	>0.5	1.0	0.7	>0.45	>1.0	0.8	>0.4	>1.0	1.1
Percent surface irradiance	83.9	69.0	26.9	98.8	49.0	52.0	82.8	78.0	51.9
Light attenuation coefficient (m^{-1})	0.55	0.37	1.01	0.03	0.60	0.59	0.63	0.33	0.60

PAR data may be used to calculate percent surface irradiance (% SI) and a diffuse light attenuation coefficient (K_d). Percent surface irradiance available at the seagrass canopy was calculated as:

$$\% \text{ SI} = (I_z/I_0) \times 100$$

where I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively.

A diffuse light attenuation coefficient was calculated using the transformed Beer Lambert equation:

$$K_d = -[\ln(I_z/I_0)]/z$$

where K_d is the attenuation coefficient (m^{-1}) and I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively. Large values of K_d indicate rapid attenuation as light penetrates the water column.

Instantaneous PAR measurements were highly variable, even for measurements collected on the same day at a site (Table 16). The majority of the surface irradiance values were above the minimum light requirements for seagrass, 14 to 16% (Czerny and Dunton 1995). Exceptions were in Port Bay at T1 in spring and summer. East Flats had the highest surface irradiance

values (26.9% - 98.8%). Port Bay light attenuation coefficient values were all within the range observed historically at East Flats, 0.3 – 2.5 m^{-1} (Mutchler and Dunton 2007). All but one light attenuation coefficient value at East Flats was within the range previously found in East Flats. The exception was at Transect 3 in the summer (0.03 m^{-1}).

Long-Term Physicochemical Measurements

Long-term physicochemical measurements of temperature, specific conductance and turbidity were monitored at each site to capture the range of conditions that are likely to affect seagrass. In addition, long-term PAR data was available for East Flats. Wind, tide and rainfall data from nearby weather and Texas Coastal Ocean Observation Network (TCOON) stations was also obtained to help understand the changes in water quality.

Long-term water quality measurements in Port Bay and East Flats showed seasonal changes that influence seagrass growth. As expected, for both bays water temperatures gradually increased through the summer followed by several periods of temperature declines from September through November (Figure 28). The two systems differed in that Port Bay was much fresher than East Flats (Figure 28). Specific conductance in Port Bay had two significant declines in concentration, July and at the end of September. Both of these declines coincided with rain events. Specific conductance did not recover to levels measured prior to each rain event. Drops in specific conductance also occurred in East Flats, but in July the specific conductance rebounded within two weeks.

Overall turbidity was low in both Port Bay and East Flats. Both sites had occasional spikes in turbidity which were closely related to increases in wind, decreases in water level, and rainfall events (Figure 29). In both sites, water levels were the highest in July and September, when monthly tide fluctuations were large. The highest observed water levels appear to correlate with high wind speeds. Water levels at both sites were the lowest in late October through November. In these months the wind changed from a prevailing southeasterly direction to a variable combination of winds from all directions (Figure 29). The highest turbidities were observed at the end of October and into November. These higher turbidities are attributed to the variable wind directions, which include winds from the north and west, and decreasing water levels. Port Bay has a roughly north-south orientation along the coast and the study site was on the eastern shoreline. As such, winds from the north and west have a greater likelihood of disturbing the site. When water levels decrease, the bay bottom is closer to the water surface, allowing more disturbances of fine sediments. East Flats had one occasion where turbidity gradually increased in July. It is not clear what may have caused this increase.

In Port Bay temperature and specific conductance were collected in two locations, within the AOI and at State Highway 188. Temperature measurements were close to identical between the two locations (Figure 30). Specific conductance values were similar. After the rain event in July, specific conductance concentrations at State Highway 188 did not recover to concentrations within the AOI until the end of August.

Long-term PAR was also measured at East Flats. UTMSI reports that average daily maximum percent surface irradiance was above the minimum light requirements for seagrass growth (Appendix E).

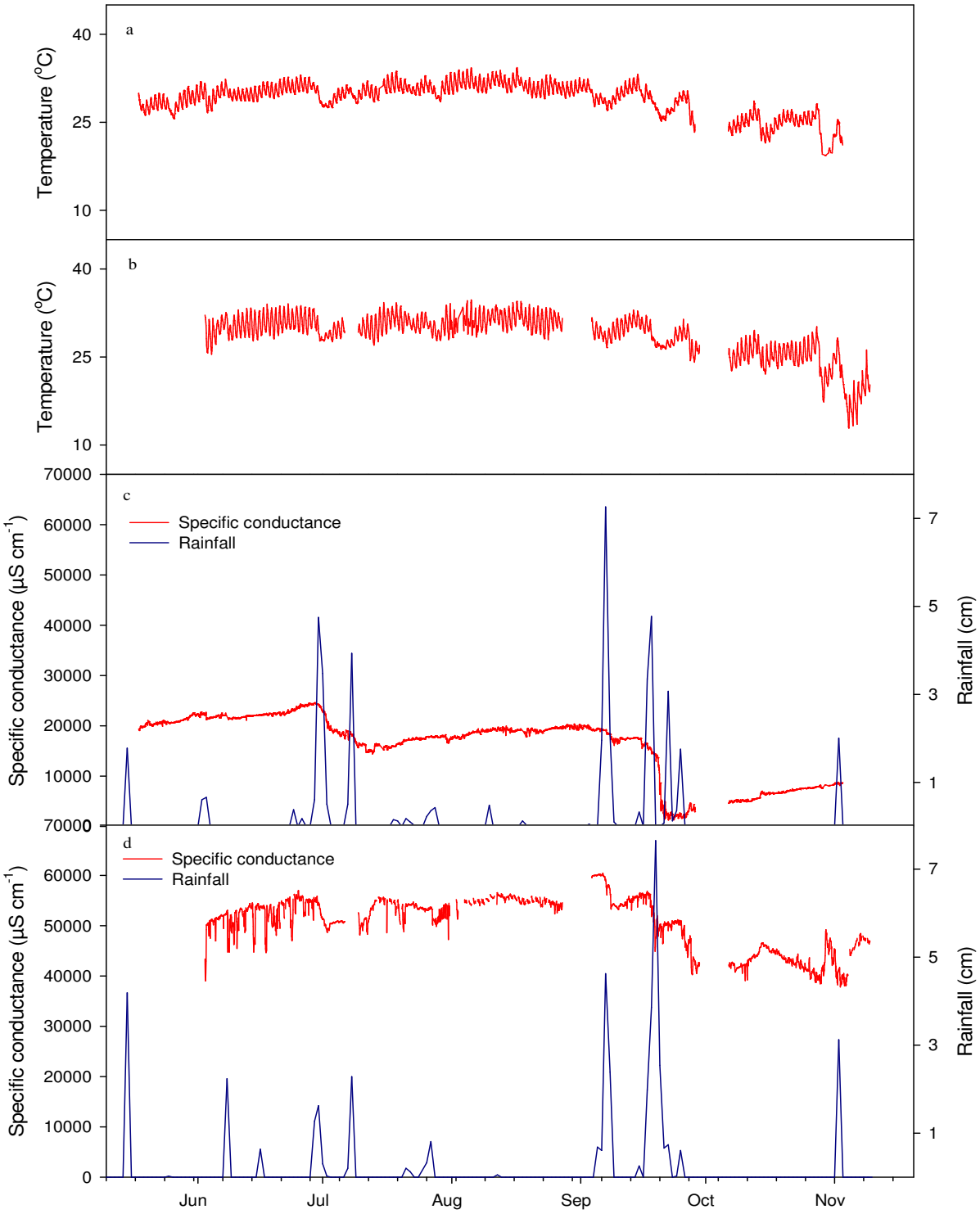


Figure 28. Port Bay temperature (a), East Flats temperature (b), Port Bay specific conductance and daily rainfall (c), East Flats specific conductance and daily rainfall (d) measurements, May – Nov 2010.

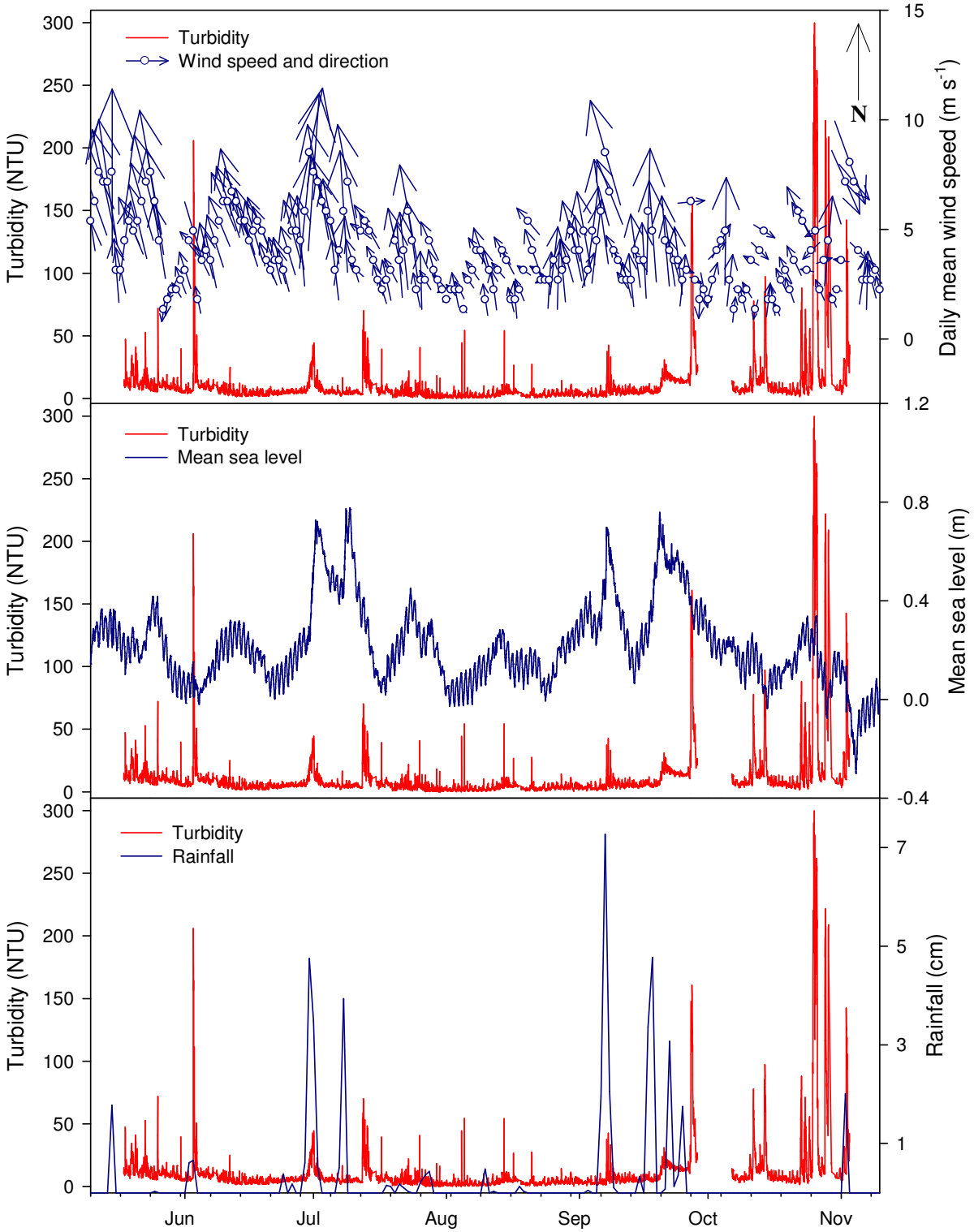


Figure 29. Turbidity, daily mean wind direction and speed, mean sea level, and daily rainfall measurements for Port Bay, May – Nov 2010.

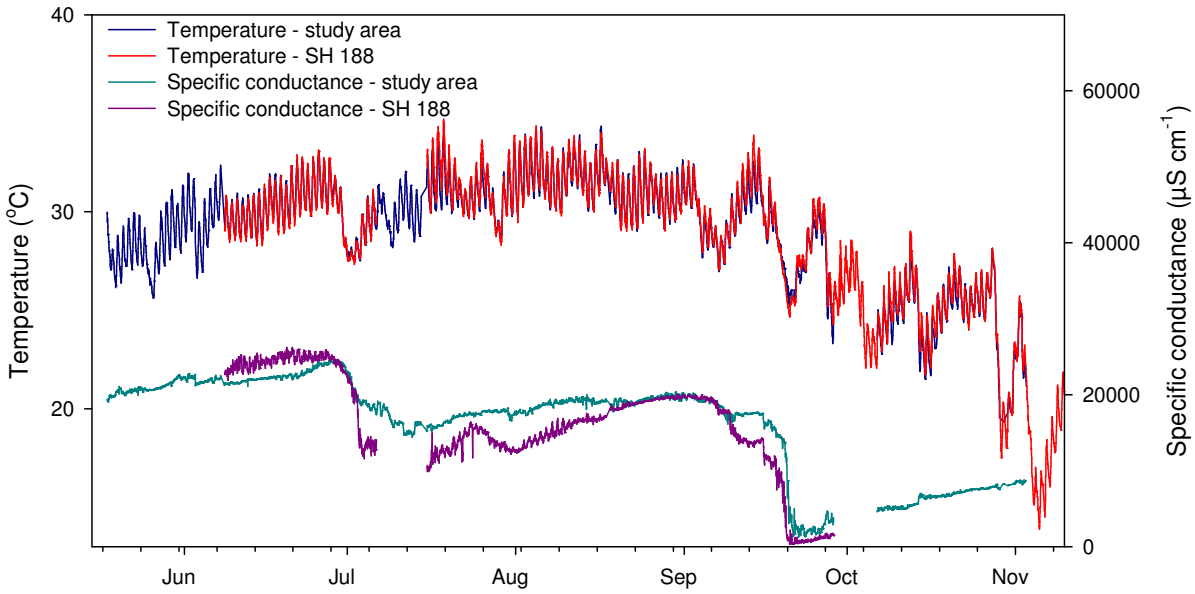


Figure 30. Temperature and specific conductance measurements for Port Bay, within AOI and at State Highway 188.

Water Chemistry

Water column nutrient and chlorophyll-*a* concentrations in Port Bay and East Flats were low throughout the study (Table 17). Ortho-phosphate-P concentrations were greater in Port Bay (4.1 µg L^{-1} – 50.5 µg L^{-1}) than in East Flats (0.4 µg L^{-1} – 3.7 µg L^{-1}). Each site had an increase in ortho-phosphate-P from spring to fall. Concentrations among transects in Port Bay were similar within a season. Chlorophyll-*a* concentrations were also greater in Port Bay (3.0 µg L^{-1} – 14.7 µg L^{-1}) than in East Flats (1.4 µg L^{-1} – 4.7 µg L^{-1}). They increased from spring to fall in Port Bay, and were similar among transects within a season, while in East Flats concentrations remained low and did not appear to vary with season. Nitrate-N + Nitrite-N concentrations were generally lower in East Flats (0.2 µg L^{-1}- 5.7 µg L^{-1}) than Port Bay (0.2 µg L^{-1} – 17.7 µg L^{-1}) and were variable by season, transect, and study site. Ammonia-N concentrations were near or below the analytical detection limit.

Total suspended solids (TSS) were also measured at each site. Port Bay tended to have higher concentrations (13.1 mg L^{-1} – 35.6 mg L^{-1}) than East Flats (7.1 mg L^{-1}– 22.5 mg L^{-1}). For all sampling events except East Flats in the fall, transect concentrations were consistent within a season.

Sediment Characteristics

Characterization of sediments is important since seagrasses are rooted in sediment and take up nutrients through below-ground tissues, as well as through their leaves. While data is available from both LCRA and UTMSI laboratories, since the laboratories used somewhat different analytical methods the results are not necessarily comparable. This section only summarizes

grain size and total organic carbon (TOC) data from the LCRA laboratory and porewater ammonia-N data from the UTMSI laboratory.

Porewater ammonia-N reflects an important source of nutrients to the seagrass plants in the bed, but can be toxic to seagrasses at high levels. Port Bay porewater ammonia-N concentrations were variable between transects with no distinct seasonal trend (Figure 31 and Table 17). In East Flats however, porewater ammonia-N concentrations were highest in summer (1.9 mg L^{-1} – 2.4 mg L^{-1}) at all three transects and lowest in fall (0.5 mg L^{-1} – 1.5 mg L^{-1}) (Figure 32).

Total organic carbon reflects the amount of organic material present in the sediments. Total organic carbon concentrations in sediment were similar in Port Bay (1230 mg kg^{-1} – 1660 mg kg^{-1}) between transects (Table 17). Total organic carbon concentrations in East Flats (250 mg kg^{-1} – 3080 mg kg^{-1}) were more variable.

Grain size analysis provides a general categorization of the type of sediment present in a seagrass bed. Sediment texture samples from Port Bay and East Flats were dominated by sand, 90% and 96%, respectively (Figure 33). The remainder of the sediment was made up of silts and clays, and only a fraction (0.1%) was gravel.

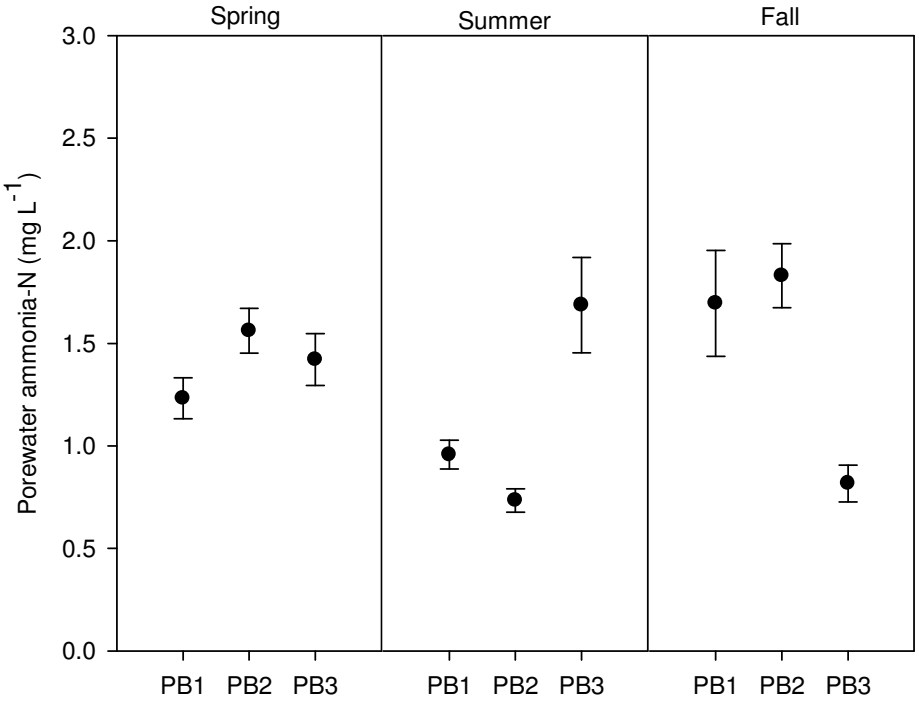


Figure 31. Port Bay porewater ammonia-N by transect and season, May – Oct 2010. Sample size is 10 except for PB2 spring (N=9). Mean ± SE.

Table 17. Sediment and water chemistry for Port Bay and East Flats, May – Nov 2010. Mean ± SE (N).
All values reported as greater than the method detection limit were included in averages. Values reported as non-detects were included in the averages at half the method detection limit.

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Port Bay									
Sediment									
Porewater ammonia-N ($mg\ L^{-1}$)	1.23 ± 0.10 (10)	1.56 ± 0.11 (10)	1.42 ± 0.13 (10)	0.96 ± 0.07 (10)	0.73 ± 0.06 (10)	1.69 ± 0.23 (9)	1.70 ± 0.26 (10)	1.83 ± 0.16 (10)	0.82 ± 0.09 (10)
Total organic carbon ($mg\ kg^{-1}$)[a]				1230 (1)	1660 (1)	1270 (1)			
Water									
Ammonia-N ($\mu g\ L^{-1}$)	1.4 ± 0.0 (2)	13.4 ± 10.7 (2)	0.9 ± 0.1 (2)	0.7 ± 0.0 (3)	0.7 ± 0.0 (2)	0.7 ± 0.0 (2)	3.8 ± 0.5 (3)	4.5 ± 1.1 (2)	4.4 ± 0.6 (2)
Chlorophyll-*a* ($\mu g\ L^{-1}$)	4.35 ± 0.65 (2)	4.17 ± 0.28 (3)	3.00 ± 0.10 (2)	8.87 ± 0.35 (3)	8.00 ± 1.10 (2)	6.20 ± 0.20 (2)	11.13 ± 1.04 (3)	14.70 ± 0.60 (2)	7.00 ± 0.20 (2)
Pheophytin-*a* ($\mu g\ L^{-1}$)	2.0 ± 0.2 (2)	1.2 ± 0.1 (3)	0.8 ± 0.1 (2)	1.8 ± 0.1 (3)	1.7 ± 0.2 (2)	1.0 ± 0.1 (2)	4.8 ± 0.5 (3)	4.4 ± 0.2 (2)	3.8 ± 0.1 (2)
Nitrate-N + nitrite-N ($\mu g\ L^{-1}$)	5.6 ± 0.3 (2)	6.3 ± 0.6 (2)	5.9 ± 0.1 (2)	17.7 ± 0.2 (3)	3.8 ± 0.7 (2)	0.2 ± 0.0 (2)	10.5 ± 0.2 (3)	0.5 ± 0.3 (2)	9.4 ± 0.5 (2)
Ortho-phosphate-P ($\mu g\ L^{-}$)	4.1 ± 0.4 (2)	4.2 ± 0.3 (2)	4.3 ± 0.6 (2)	38.5 ± 0.5 (3)	23.2 ± 0.5 (2)	9.9 ± 0.2 (2)	50.1 ± 0.5 (3)	43.3 ± 0.4 (2)	50.5 ± 0.2 (2)
Total suspended solids ($mg\ L^{-1}$)	35.6 ± 4.0 (2)	30.2 ± 0.2 (2)	23.2 ± 1.5 (2)	15.8 ± 0.6 (2)	15.2 ± 0.3 (3)	13.1 ± 0.3 (2)	18.7 ± 1.1 (2)	13.9 ± 0.4 (3)	16.4 ± 0.4 (2)
East Flats									
Sediment									
Porewater ammonia-N ($mg\ L^{-1}$)	1.72 ± 0.11 (10)	1.61 ± 0.13 (10)	1.36 ± 0.06 (10)	1.87 ± 0.21 (10)	2.34 ± 0.27 (10)	2.44 ± 0.25 (8)	0.51 ± 0.06 (10)	0.95 ± 0.11 (10)	1.52 ± 0.24 (8)
Total organic carbon ($mg\ kg^{-1}$) [a]							3080 (1)	1380 (1)	250 (1)
Water									
Ammonia-N ($\mu g\ L^{-1}$)	2.0 ± 0.6 (2)	1.4 ± 0.7 (2)	0.3 ± 0.0 (2)	0.7 ± 0.0 (3)	14.6 ± 13.9 (2)	86.6 ± 13.9 (2)	9.0 ± 2.7 (2)	11.1 ± 3.5 (2)	8.5 ± 0.0 (1)
Chlorophyll-*a* ($\mu g\ L^{-1}$)	2.54 ± 0.43 (2)	2.54 ± 0.43 (2)	2.47 ± 0.50 (2)	4.69 ± 0.05 (3)	2.97 ± 0.59 (2)	3.56 ± 0.00 (2)	3.45 ± 0.43 (3)	2.46 ± 0.20 (2)	1.36 ± 0.22 (2)
Nitrate-N + nitrite-N ($\mu g\ L^{-1}$)	4.5 ± 0.1 (2)	5.7 ± 0.5 (2)	4.2 ± 1.1 (2)	0.4 ± 0.1 (3)	0.2 ± 0.0 (2)	0.2 ± 0.0 (2)	0.7 ± 0.4 (2)	0.4 ± 0.0 (2)	1.1 ± 0.0 (1)
Ortho-phosphate-P ($\mu g\ L^{-}$)	0.5 ± 0.0 (2)	1.7 ± 1.2 (2)	0.5 ± 0.0 (2)	0.5 ± 0.0 (3)	0.5 ± 0.0 (2)	0.5 ± 0.0 (2)	1.3 ± 0.5 (2)	1.8 ± 1.1 (2)	3.7 ± 0.0 (1)
Total suspended solids ($mg\ L^{-1}$)	9.5 ± 1.1 (2)	17.1 ± 1.8 (2)	18.0 ± 1.1 (2)	22.5 ± 1.4 (3)	18.6 ± 0.2 (2)	20.1 ± 1.7 (2)	18.8 ± 1.1 (3)	12.1 ± 1.1 (2)	7.1 ± 0.5 (2)

a – Only LCRA values reported.

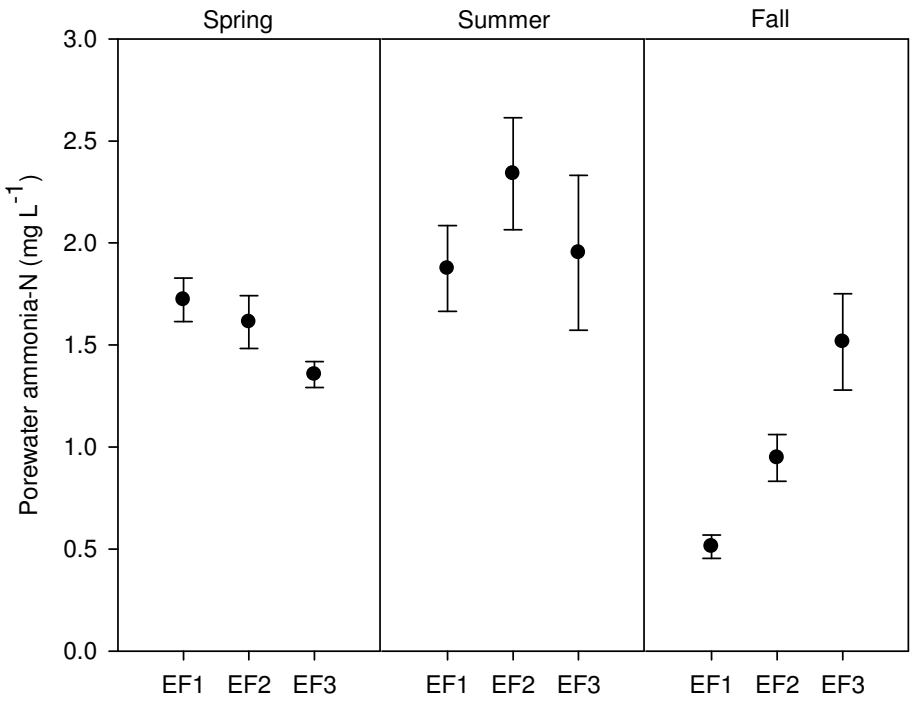


Figure 32. East Flats porewater ammonia-N by transect and season, Jun – Nov 2010. Sample size is 10 except for EF3 fall (N=8). Mean ± SE.

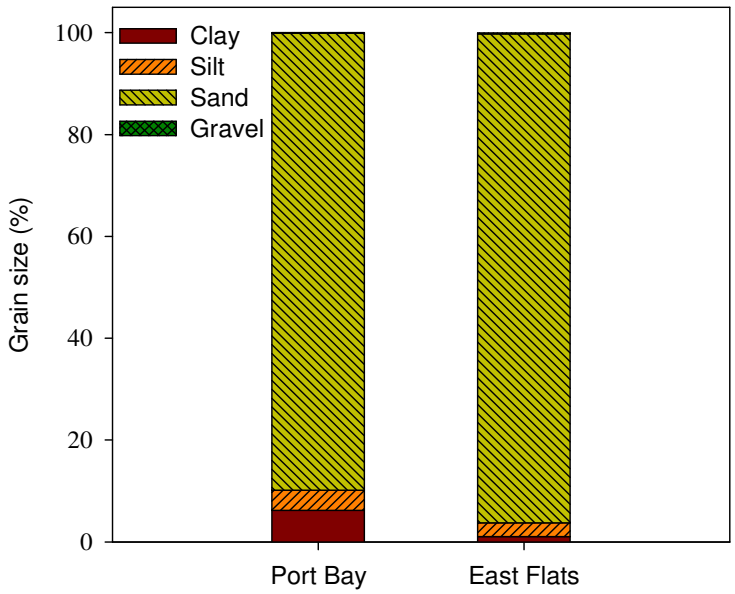


Figure 33. Average sediment texture for Port Bay and East Flats, 2010.

Inter-laboratory Comparisons

Relative percent difference (RPD) was calculated for each field and laboratory split sample (Appendix D. Table 24). For many of the parameters, one or more of the samples was noted as non-detect ("ND") or between the PQL and MDL ("J" flagged). In these instances, the calculated RPD represents a small change in small values that do not meet specified reliability

criteria. As such, large RPDs associated with ND or J flagged values are not considered significant. With this understanding, agreement among field split samples is generally good.

UTMSI and LCRA used different methods to measure most water and sediment quantities (Radloff 2010), making differences in reported values potentially difficult to interpret. Nonetheless, RPDs were calculated for samples split between laboratories. Inter-laboratory comparisons for chlorophyll-*a*, total suspended solids and porewater ammonia exceed 30% and cannot be explained by small sample values.

Sediment total organic carbon RPD values are large, but probably not significant. LCRA used method SW9060, while UTMSI used a method generally known as "loss on ignition" (Radloff 2010). Similarly, large RPDs observed for sediment grain size are probably not significant, as LCRA and UTMSI used somewhat different methods for sediment grain size determination (Radloff 2010). Both methods showed sand as the dominant substrate.

Six replicate porewater ammonia samples were obtained at Port Bay on the fall sampling trip (Figure 34). While mean values of the three samples analyzed at each laboratory differ by only 21%, the three samples analyzed by UTMSI have a larger spread than those analyzed by LCRA.

Various factors in addition to differing methods may contribute to the differences. The laboratories have differing quality assurance protocols (the LCRA Environmental Services Laboratory is NELAC-certified, while the UTMSI laboratory is not). The LCRA laboratory methods are used primarily for freshwater, and may require dilution for certain parameters (particularly nutrients), while the marine methods used by the UTMSI laboratory do not typically require dilution and thus often achieve lower detection limits.

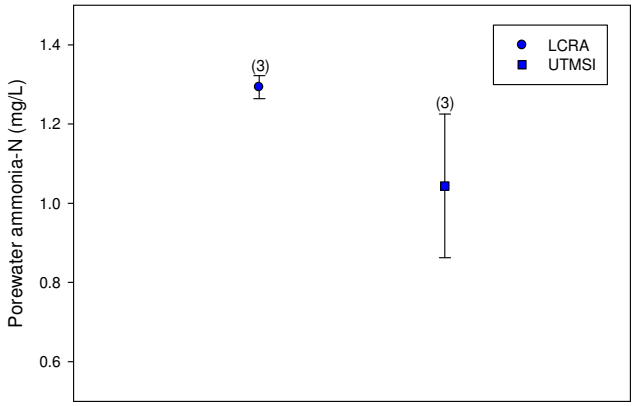


Figure 34. Comparison of replicate porewater ammonia samples analyzed by LCRA and UTMSI laboratories. Mean ± SE (N).

Biological Parameters

Seagrass Percent Coverage

Throughout the study, Port Bay was dominated by *Halodule* (Table 18, Figure 35). Total seagrass percent coverage increased across transects (T1<T2<T3), with almost 100% coverage at

T3 each season. *Ruppia* was present at T1 and T2 in summer and fall. Although the percent coverage methodology did not document *Ruppia* at T3, *Ruppia* was present in seagrass cores collected there in fall (see Seagrass Shoot Density and Biomass section below), and field notes document the presence of *Ruppia* near T3 in both summer and fall.

East Flats displayed a multi-species assemblage of seagrass, with *Halodule* and *Thalassia* dominant. Transects 1 and 2 had >90% mean seagrass cover each season, but T3 had lower percent coverage in summer and fall. *Syringodium* was also noted at T2 and T3 each season. Total seagrass coverage and species composition was consistent across seasons at Port Bay and East Flats (Figure 36).

Seagrass coverage was not only determined by measurements in the field, but also by analysis of aerial imagery, as discussed above. The average value of the fall 2010 quadrat analysis for each site can be compared with 2009 and 2010 aerial imagery classification and transect analyses (Table 19). Considering only the imagery analysis techniques, vegetated area coverage appears to have increased from 2009 to 2010. This may be due to differences in imagery acquisition dates. Images were acquired 20 Dec 2009 and 19 Nov 2010. The average percent coverage obtained from the quadrat technique for each site falls within the range of coverages observed from the 2010 image analysis. For Port Bay, the quadrat average is lower than the transect analysis average, while for East Flats it is higher. This may again be due at least in part to differences in data acquisition dates, with the Port Bay quadrat analysis having been conducted in early October and the East Flats analysis in early November. Another possible explanation is that the very low salinities in Port Bay in 2010 stimulated the growth of *Ruppia,* which is reflected in greater seagrass coverage in Port Bay in 2010. Since the landscape cover measurement (at a broad, bed-scale) corresponds reasonably to the seagrass cover measurement from quadrats (at the field or transect scale), it suggests that a more rigorous examination of this relationship may be warranted.

Table 18. Seagrass percent coverage by species for each season and transect. Mean ± SE, N = 10.

Season	Transect	*Halodule*	*Syringodium*	*Ruppia*	*Thalassia*	Bare substrate	Total seagrass
				Port Bay			
Spring	T1	28 ± 10	0 ± 0	0 ± 0	0 ± 0	72 ± 10	28 ± 10
Spring	T2	52 ± 11	0 ± 0	0 ± 0	0 ± 0	48 ± 11	52 ± 11
Spring	T3	94 ± 2	0 ± 0	0 ± 0	0 ± 0	6 ± 2	94 ± 2
Summer	T1	46 ± 14	0 ± 0	2 ± 1	0 ± 0	52 ± 14	48 ± 14
Summer	T2	36 ± 11	0 ± 0	27 ± 6	0 ± 0	38 ± 11	63 ± 11
Summer	T3	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	100 ± 0
Fall	T1	19 ± 11	0 ± 0	8 ± 6	0 ± 0	72 ± 13	28 ± 13
Fall	T2	43 ± 8	0 ± 0	15 ± 8	0 ± 0	43 ± 6	57 ± 6
Fall	T3	96 ± 3	0 ± 0	0 ± 0	0 ± 0	4 ± 3	96 ± 3
				East Flats			
Spring	T1	63 ± 15	0 ± 0	0 ± 0	36 ± 15	1 ± 1	99 ± 1
Spring	T2	10 ± 8	12 ± 10	0 ± 0	79 ± 13	0 ± 0	100 ± 0
Spring	T3	57 ± 16	36 ± 15	0 ± 0	3 ± 2	4 ± 3	96 ± 3
Summer	T1	58 ± 15	0 ± 0	0 ± 0	42 ± 15	1 ± 1	100 ± 1
Summer	T2	21 ± 13	30 ± 15	0 ± 0	50 ± 17	0 ± 0	100 ± 0
Summer	T3	55 ± 16	24 ± 10	0 ± 0	0 ± 0	21 ± 10	79 ± 10
Fall	T1	51 ± 16	0 ± 0	0 ± 0	48 ± 16	0 ± 0	100 ± 0
Fall	T2	16 ± 11	15 ± 10	0 ± 0	60 ± 13	9 ± 3	91 ± 3
Fall	T3	51 ± 14	10 ± 10	0 ± 0	0 ± 0	40 ± 14	60 ± 14

Table 19. Vegetated area, percent vegetated length and seagrass coverage for Port Bay and East Flats, 2009 – 2010.

Parameter	Method	Sample date	Port Bay	East Flats
Percent vegetated area	Aerial imagery classification	Fall 2009	59%	64%
Percent vegetated length range	Image transect analysis	Fall 2009	26% - 71%	70% - 75%
Average percent vegetated length	Image transect analysis	Fall 2009	59%	71%
Average percent seagrass coverage	Quadrat method (N=30)	Fall 2010	60%	84%
Percent vegetated length range	Image transect analysis	Fall 2010	58% - 97%	59% - 93%
Average percent vegetated length	Image transect analysis	Fall 2010	87%	75%

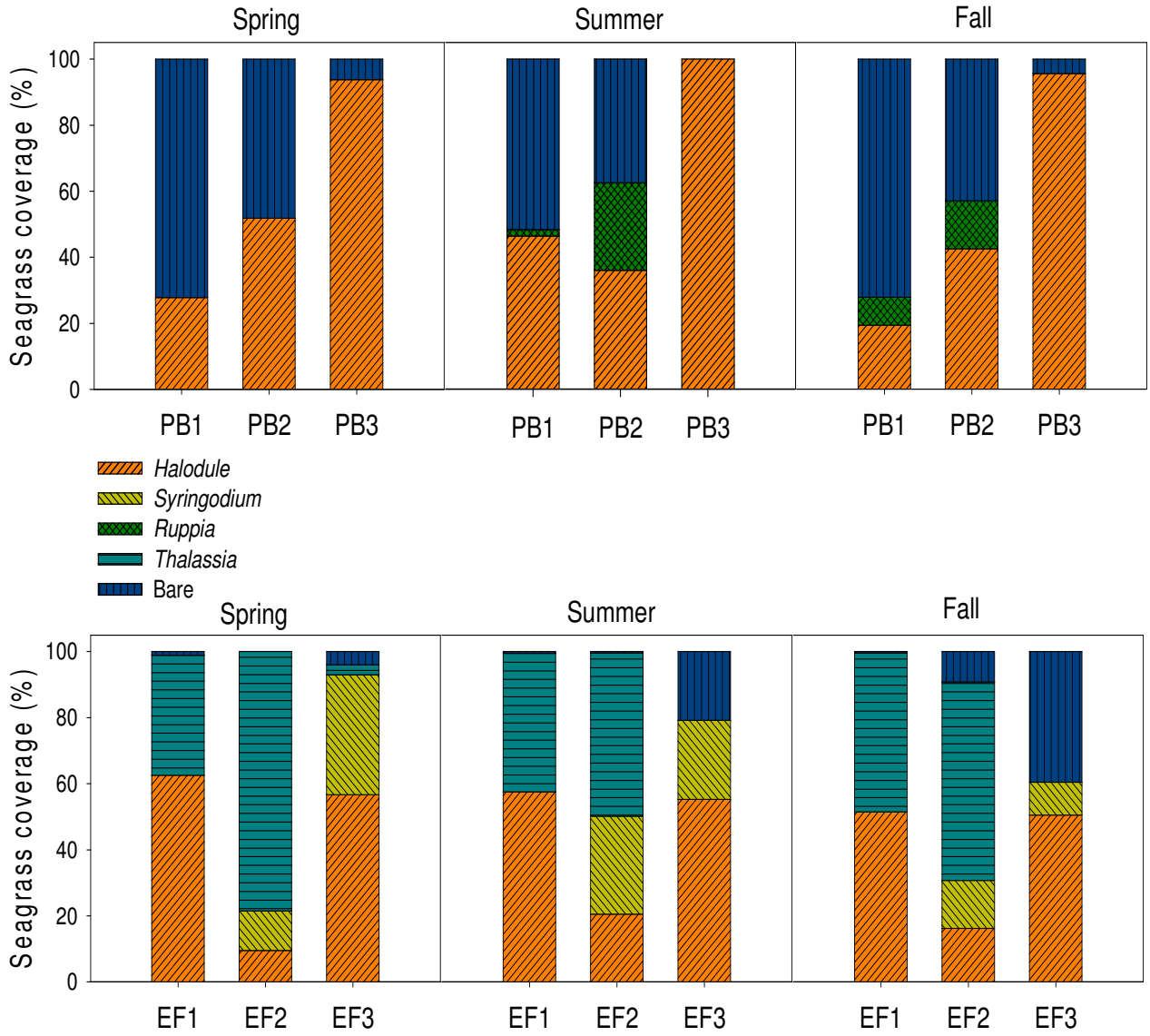


Figure 35. Seagrass coverage for Port Bay and East Flats by transect and season, May – Nov 2010. Mean ± SE, N=10.

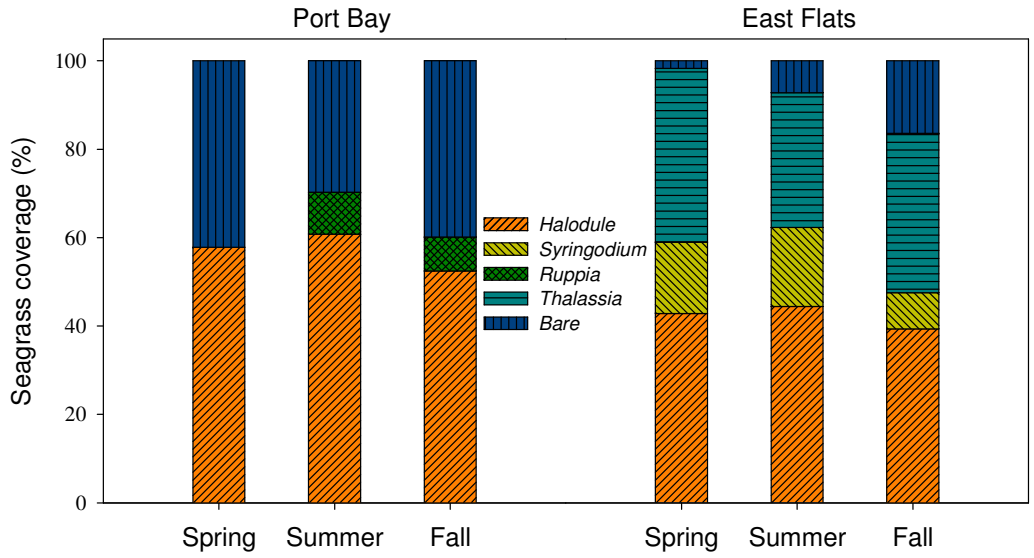


Figure 36. Seagrass coverage for Port Bay and East Flats by season, May – Nov 2010. Mean ± SE, N=30.

Macroalgae Biomass

Macroalgae biomass was variable across transects and seasons (Table 20). Average macroalgae biomass did not differ between Port Bay and East Flats. *Gracilaria* was the only genus identified in Port Bay, while 10 genera were identified in East Flats (see UTMSI report in Appendix E).

Table 20. Macroalgae biomass for Port Bay and East Flats, May – Nov 2010. Mean ± SE, N=10.

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
				Port Bay					
Macroalgae biomass ($g\ m^{-2}$)	28.8 ± 10.5	13.6 ± 6.1	2.6 ± 2.1	25.5 ± 12.3	34.1 ± 17.9	0.0 ± 0.0	89.3 ± 24.1	12.6 ± 6.1	0.6 ± 0.4
				East Flats					
Macroalgae biomass ($g\ m^{-2}$)	5.7 ± 2.0	10.1 ± 6.9	9.3 ± 3.6	23.3 ± 11.1	47.9 ± 23.0	12.1 ± 5.2	24.4 ± 12.6	1.0 ± 0.8	3.9 ± 2.3

Seagrass Shoot Density and Biomass

Shoot density of *Halodule* in Port Bay was highly variable, ranging from 1,100 to 16,700 shoots m^{-2} (Table 21). Shoot density tended to be higher at T3 than at T1 or T2 (Figure 37). Over the duration of the study, shoot density declined from spring through fall at T3, but remained consistent at T1 and T2. Biomass estimates were also highly variable (total biomass ranged from 24.5 to 291 $g\ m^{-2}$), and values at T3 were higher than T1 or T2, especially below-ground biomass and total biomass. There was no clear seasonal trend in biomass.

In East Flats shoot density and biomass were estimated for four species of seagrass, which reduced the number of replicates for a given species in a given season (Table 22). Shoot density and biomass were highly variable. For example, *Halodule* shoot density ranged from 57 to

7,640 m^{-2} and total biomass ranged from 0.28 to 433 g m^{-2}. For *Thalassia*, above-ground, below-ground, and total biomass tended to be higher in summer and fall (Figure 38). Lack of replication made it difficult to spot other trends among transects or seasons.

Root:shoot ratio represents the relative allocation that the plant makes between below-ground biomass and above-ground (photosynthetic) biomass. Root:shoot ratio for *Halodule* ranged from 0.52 to 3.4 at Port Bay, and from 0.54 to 7.4 at East Flats (Figure 39). For *Thalassia* in East Flats, root:shoot ratio ranged from 1.6 to 6.5. Values above 1.0 are generally thought to represent healthy plants. Root:shoot ratio did not show a strong seasonal trend at Port Bay, but it did appear elevated during summer and fall at East Flats for *Halodule* and *Thalassia* (Figure 40). This would be consistent with plants growing and storing energy in below-ground structures for the coming winter.

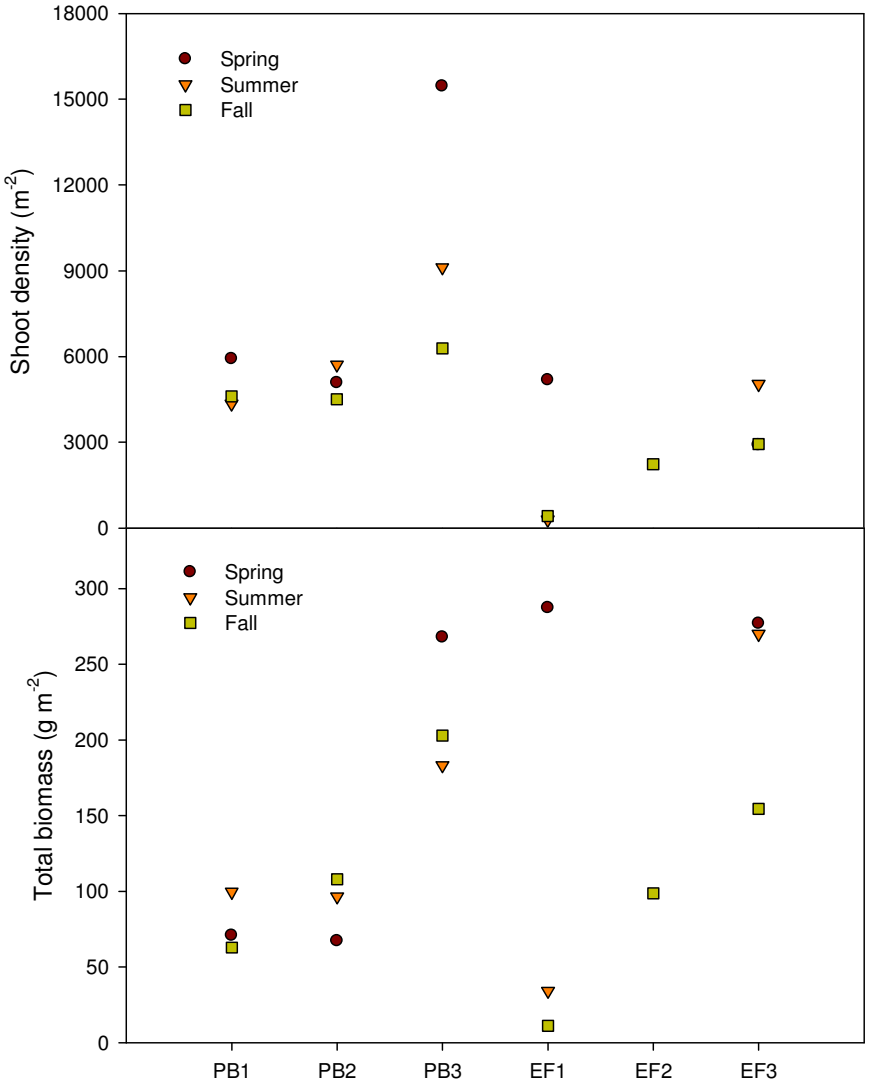


Figure 37. Mean shoot density and mean total biomass for each season by transect for *Halodule* at Port Bay and East Flats, May - Nov 2010.

Table 21. Seagrass condition indicators for Port Bay, May – Oct 2010.
Mean ± SE (N) for each season by transect.

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Halodule									
Shoot density (number m^{-2})	5,921 ± 1,291 3	5,082 ± 1,520 3	15,457 ± 979 3	4,349 ± 2,634 3	5,711 ± 2,353 3	9,117 ± 1,494 3	4,611 ± 2,905 3	4,506 ± 823 3	6,288 ± 1,921 3
Above-ground biomass (g m^{-2})	38.2 ± 14.1 3	25.7 ± 10.1 3	109.6 ± 17.9 3	60.0 ± 46.8 3	52.4 ± 14.1 3	54.9 ± 5.2 3	23.4 ± 8.6 3	42.3 ± 5.7 3	80.7 ± 20.6 3
Below-ground biomass (g m^{-2})	32.7 ± 5.1 3	41.5 ± 12.5 3	158.2 ± 15.9 3	39.5 ± 25.1 3	44.1 ± 21.3 3	128.3 ± 26.9 3	39.5 ± 21.3 3	65.6 ± 13.8 3	122.1 ± 28.3 3
Total biomass (g m^{-2})	70.9 ± 18.7 3	67.2 ± 22.1 3	267.9 ± 17.7 3	99.5 ± 71.8 3	96.5 ± 34.4 3	183.2 ± 27.9 3	62.8 ± 23.8 3	107.8 ± 15.3 3	202.8 ± 48.3 3
Root:shoot ratio	0.99 ± 0.19 3	1.74 ± 0.22 3	1.53 ± 0.29 3	1.00 ± 0.35 3	0.77 ± 0.19 3	2.38 ± 0.56 3	1.98 ± 0.82 3	1.59 ± 0.38 3	1.57 ± 0.15 3
LAI	0.87 ± 0.27 3	0.58 ± 0.17 3	2.04 ± 0.18 3	0.93 ± 0.67 3	1.14 ± 0.53 3	1.91 ± 0.33 3	0.87 ± 0.61 3	0.78 ± 0.23 3	1.48 ± 0.55 3
Number of blades (shoot^{-1})	2.7 ± 0.2 3	2.6 ± 0.1 3	2.6 ± 0.2 3	2.1 ± 0.3 3	2.3 ± 0.2 3	2.7 ± 0.1 3	2.3 ± 0.1 3	2.1 ± 0.1 3	2.5 ± 0.2 3
Leaf length (cm)	14.1 ± 1.3 3	11.3 ± 0.2 3	13.2 ± 0.9 3	18.2 ± 2.8 3	18.8 ± 1.6 3	21.5 ± 3.2 3	17.5 ± 2.2 3	17.4 ± 4.7 3	21.9 ± 2.6 3
Leaf width (mm)	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3	1 ± 0 3
Ruppia									
Shoot density (number m^{-2})				1,179 ± 550 2	472 1		472 1	472 1	
Above-ground biomass (g m^{-2})				3.8 ± 0.7 2	8.4 1		25.4 1	3.5 1	
Below-ground biomass (g m^{-2})				3.2 ± 0.8 2	2.0 1		18.6 1	1.1 1	
Total biomass (g m^{-2})				6.9 ± 0.1 2	10.4 1		44.0 1	4.6 1	
Root:shoot ratio				0.92 ± 0.40 2	0.24 1		0.73 1	0.33 1	
LAI				0.09 ± 0.05 2	0.05 1		0.05 1	0.03 1	
Number of blades (shoot^{-1})				5.3 ± 0.5 2	3.6 1		16.0 1	8.0 1	
Leaf length (cm)				7.8 ± 0.3 2	9.8 1		10.5 1	7.0 1	
Leaf width (mm)				1 ± 0 2	1 1		1 1	1 1	

Table 22. Seagrass condition indicators for East Flats, Jun – Nov 2010.
Mean ± SE (N) for each season by transect.

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Halodule									
Shoot density (number m^{-2})	5,178 ± 2,462 2		2,908 ± 1,179 2	283 1		5,030 ± 1,271 3	415 ± 197 3	2,229 ± 2,172 2	2,934 ± 1,424 3
Above-ground biomass (g m^{-2})	93.6 ± 63.2 2		152.3 ± 17.4 2	4.1 1		107.5 ± 43.8 3	3.6 ± 2.0 3	25.1 ± 24.6 2	48.0 ± 26.9 3
Below-ground biomass (g m^{-2})	193.6 ± 82.6 2		124.6 ± 33.7 2	30.0 1		162.4 ± 38.2 3	7.5 ± 3.7 3	73.6 ± 73.2 2	106.3 ± 52.5 3
Total biomass (g m^{-2})	287.2 ± 145.7 2		276.9 ± 16.3 2	34.1 1		269.9 ± 66.2 3	11.1 ± 5.4 3	98.7 ± 97.8 2	154.4 ± 77.5 3
Root:shoot ratio	2.70 ± 0.94 2		0.85 ± 0.32 2	7.38 1		1.95 ± 0.57 3	2.09 2	1.92 ± 1.03 2	2.21 2
LAI	1.23 ± 0.75 2		0.81 ± 0.22 2			1.59 ± 0.67 2	0.07 1	0.48 ± 0.47 2	0.66 ± 0.04 2
Number of blades ($shoot^{-1}$)	2.4 ± 0.0 2		2.5 ± 0.1 2			2.8 ± 0.4 2	2.4 1	2.7 ± 0.1 2	3.0 ± 0.4 2
Leaf length (cm)	21.8 ± 4.2 2		29.6 ± 4.4 2			26.2 ± 3.7 2	16.5 1	14.6 ± 4.8 2	14.8 ± 2.2 2
Leaf width (mm)	1 ± 0 2		1 ± 0 2			1 ± 0 2	1 1	1 ± 0 2	1 ± 0 2
Thalassia									
Shoot density (number m^{-2})	396 ± 170 2	1,867 ± 340 2		1075 ± 204 3	1,103 ± 85 2		1,188 ± 142 3	934 ± 368 2	
Above-ground biomass (g m^{-2})	95.5 ± 73.3 2	187.8 ± 39.6 2		188.2 ± 32.2 3	213.5 ± 63.0 2		193.6 ± 30.1 3	76.4 ± 11.9 2	
Below-ground biomass (g m^{-2})	218.9 ± 169.5 2	345.7 ± 58.0 2		464.7 ± 152.1 3	797.8 ± 51.3 2		502.3 ± 53.7 3	465.5 ± 45.9 2	
Total biomass (g m^{-2})	314.4 ± 242.8 2	533.5 ± 97.7 2		652.9 ± 159.1 3	1011.2 ± 114.3 2		695.8 ± 51.4 3	541.9 ± 57.8 2	
Root:shoot ratio	2.27 ± 0.03 2	1.86 ± 0.08 2		2.54 ± 0.89 3	4.02 ± 0.94 2		2.76 ± 0.56 3	6.15 ± 0.36 2	
LAI	0.54 ± 0.40 2	1.76 ± 0.17 2		3.25 ± 0.58 2	3.39 ± 0.56 2		2.71 ± 0.57 2	1.80 ± 0.64 2	
Number of blades ($shoot^{-1}$)	3.1 ± 0.3 2	3.3 ± 0.3 2		3.4 ± 0.2 2	2.6 ± 0.0 2		2.4 ± 0.4 2	2.7 ± 0.1 2	
Leaf length (cm)	17.9 ± 5.7 2	18.4 ± 4.7 2		40.3 ± 7.8 2	57.3 ± 6.7 2		31.6 ± 0.1 2	30.2 ± 1.1 2	
Leaf width (mm)	6.0 ± 1.0 2	5.5 ± 1.0 2		7.5 ± 0.5 2	5.4 ± 0.2 2		6.7 ± 0.4 2	6.5 ± 0.0 2	
Syringodium									
Shoot density (number m^{-2})		5,659 1	2,436 ± 1,022 2		3,144 1			2,043 1	
Above-ground biomass (g m^{-2})		376.9 1	106.9 ± 26.9 2		101.9 1			151.4 1	
Below-ground biomass (g m^{-2})		247.4 1	93.7 ± 28.1 2		258.9 1			167.1 1	
Total biomass (g m^{-2})		624.4 1	200.6 ± 55.0 2		360.8 1			318.5 1	
Root:shoot ratio		0.66 1	0.86 ± 0.05 2		2.54 1			1.10 1	

	Spring						Summer				Fall			
	T1		T2		T3		T1	T2	T3		T1	T2	T3	
LAI			2.24	1	0.56 ± 0.18	2								
Number of blades (shoot^{-1})			1.0	1	1.3 ± 0.1	2								
Leaf length (cm)			36.0	1	24.5 ± 3.0	2								
Leaf width (mm)			1	1	1 ± 0	2								
								Ruppia						
Shoot density (number m^{-2})	57	1												
Above-ground biomass (g m^{-2})	7.1	1												
Below-ground biomass (g m^{-2})	1.2	1												
Total biomass (g m^{-2})	8.4	1												
Root:shoot ratio	0.17	1												
LAI	0.01	1												
Number of blades (shoot^{-1})	1.0	1												
Leaf length (cm)	25.0	1												
Leaf width (mm)	1	1												

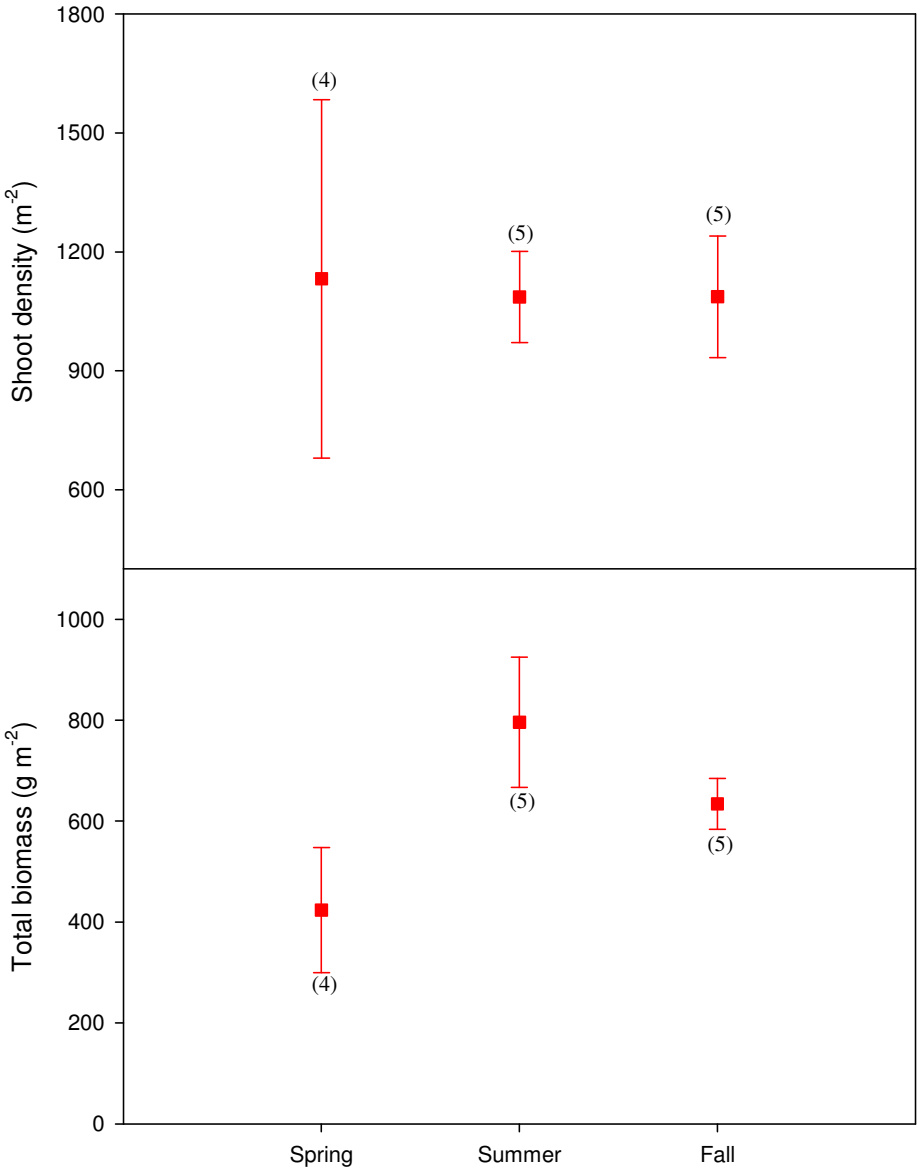


Figure 38. Mean shoot density and mean total biomass for *Thalassia* at East Flats, Jun - Nov 2010, (N).

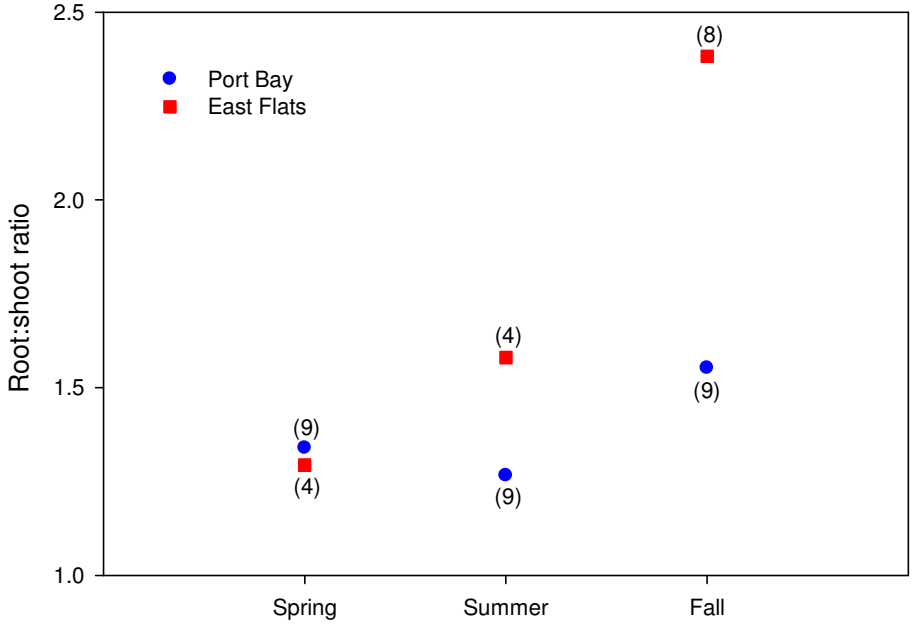


Figure 39. *Halodule* root:shoot ratio for Port Bay and East Flats, May - Nov 2010. Ratio based on mean above- and below-ground biomass obtained from (N) observations.

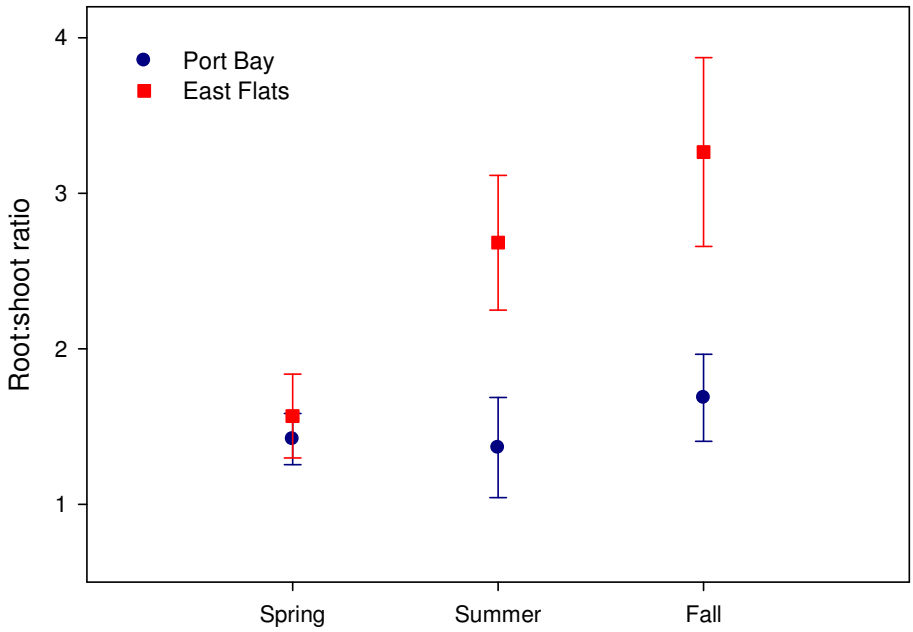


Figure 40. Seagrass root:shoot ratio for Port Bay and East Flats, May – Nov 2010. Mean ± SE, averaged by core, N=9. *Halodule* and *Ruppia* for Port Bay and *Halodule, Thalassia, Syringodium* and *Ruppia* for East Flats.

Seagrass Leaf Area Index

Leaf area index was variable over transects and seasons at both Port Bay and East Flats (Table 21, Table 22 and Figure 41). Leaf area index did not show a strong seasonal trend, with the possible exception of *Thalassia* at East Flats, which appeared elevated in summer. *Halodule* in Port Bay had longer leaves in summer and fall than in spring. In East Flats, *Thalassia* had the shortest average leaf length in spring, longest leaves in summer, and intermediate leaf length in fall.

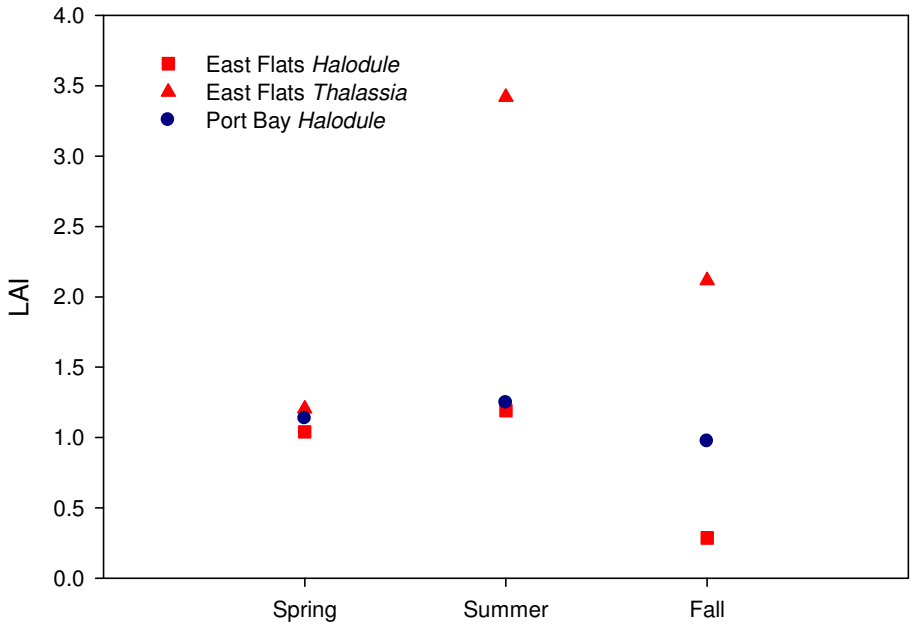


Figure 41. Leaf area index (LAI) for Port Bay and East Flats, May – Nov 2010. *Halodule* only for Port Bay and *Halodule* and *Thalassia* for East Flats.

Epiphyte Loading

Among the parameters sampled, epiphyte biomass is unique in that normalized epiphyte load could be determined four different ways by three different laboratories. Conventional scraping and weighing techniques were used to obtain measures of dried epiphyte weight per unit area and per unit seagrass dry weight. Fluorescence techniques were used to determine epiphyte load normalized to the areal distribution of the red-excited fluorescence signal and to seagrass dry weight. The basis of the fluorescence techniques is the postulated relationships between the magnitude of the green-excited fluorescence signal and epiphyte load and between the areal distribution of the red-excited fluorescence signal and scraped seagrass leaf area (which is itself related to seagrass dry weight). The fluorescence techniques are discussed in detail in Appendix F. Note that what is characterized here as epiphyte biomass is sometimes referred to as epiphyte cover in Appendix E.

For *Halodule* in Port Bay, normalized epiphyte loads by all measurement techniques were consistently smaller than those measured for East Flats *Halodule* and *Thalassia* (Figure 43 and Figure 44) and did not vary by transect (Figure 42). For Port Bay, seasonally averaged values determined by all methods show a slight decrease from spring to fall. Individual values for scraping techniques ranged from 0.21 – 0.32 mg cm^{-2} in the spring (205 – 280 mg g^{-1}), from 0 – 0.18 mg cm^{-2} in the summer (0 - 244 mg g^{-1}) and from 0.02 – 0.09 mg cm^{-2} in the fall (15 – 84 mg g^{-1}). Independent determinations of epiphyte load per unit seagrass dry weight by TPWD and TAMU-CC agreed fairly well across the seasons, as did determinations of epiphyte load per unit area by TPWD and UTMSI in the fall. Values of normalized epiphyte load for Port Bay determined as weight per unit area are well-correlated with values determined as weight percent dry seagrass ($R^2 = 0.85$).

The seasonal decrease in normalized epiphyte load observed in Port Bay differs from the seasonal pattern observed using scraping techniques for both *Halodule* and *Thalassia* in East Flats, which showed an increase in loading from spring to fall for both species. Individual *Halodule* values ranged from 0.11 – 2.17 mg cm^{-2} in the spring, from 0.18 – 1.78 mg cm^{-2} in the summer and from 2.03 – 3.25 mg cm^{-2} in the fall. Individual *Thalassia* values ranged from 0.09 – 2.17 mg cm^{-2} in the spring, from 0.12 – 1.60 mg cm^{-2} in the summer and from 2.47 – 6.06 mg cm^{-2} in the fall. Epiphyte loading for *Syringodium* was measured only in the summer, when values ranged from 0.16 – 2.74 mg cm^{-2}.

Fluorescence scans of seagrass blades track scraping methods for *Halodule* in Port Bay (Figure 44) in showing a slight decrease from spring to fall. In East Flats, however, the ability of the fluorescence techniques to track the scraping results was mixed. For scanned seagrass leaves, green-excited fluorescence signal normalized to seagrass dry weight did track the seasonal increase observed for *Halodule*, but not for *Thalassia*. Fluorescence signal normalized to the areal distribution of the red-excited fluorescence signal did not track the scraping methods for either species. This means that a normalized measure of epiphyte loading based solely on fluorescence is not currently feasible. However, when epiphytes were scraped from the blades, the green-excited fluorescence techniques tracked the scraping methods for all species at both sites (Figure 45). Red-excited fluorescence of removed epiphytes tracked scraping methods for *Halodule* and *Thalassia* at East Flats, but not for *Halodule* at Port Bay.

The fluorescence methodology applied to scraped epiphytes revealed additional insight into epiphyte colonization (Figure 45). The ratio of red- to green-excited fluorescence for *Halodule* at Port Bay was much larger than for either *Halodule* or *Thalassia* at East Flats, implying different epiphyte community compositions in the two bays. The results suggest that Port Bay may be dominated by green algal epiphyte colonization and that other epiphytes, such as red algae, brown algae or diatoms, predominate at East Flats. The ratio of red- to green-excited fluorescence varied seasonally at both sites, decreasing from spring to fall at East Flats for both species, while peaking in the summer in Port Bay. Overall, conditions appear to be more conducive to epiphyte colonization and growth, relative to growth of the seagrass blade, at East Flats.

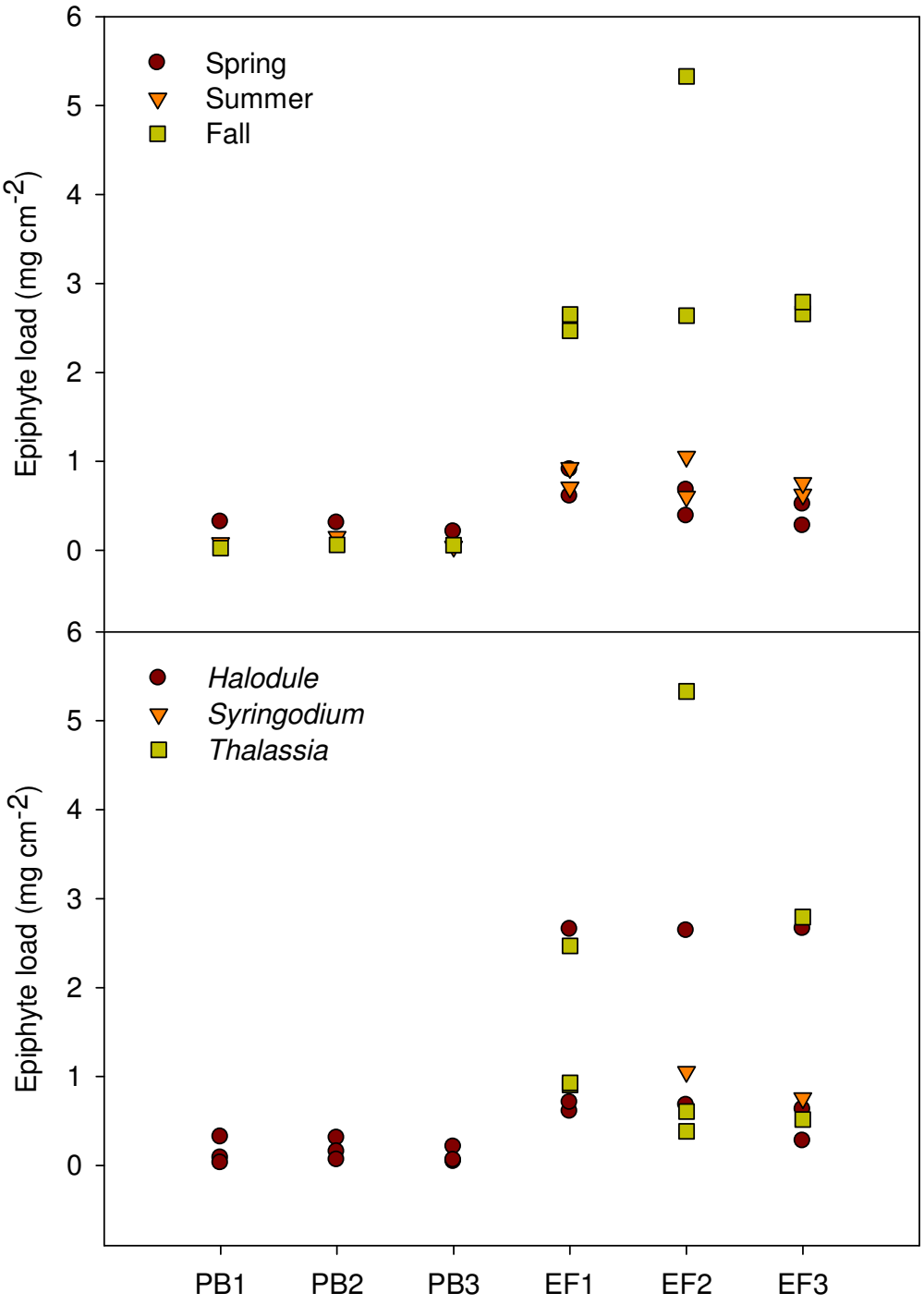


Figure 42. Normalized epiphyte load for Port Bay and East Flats by transect, May - Nov 2010. Upper panel by season and lower by seagrass species.

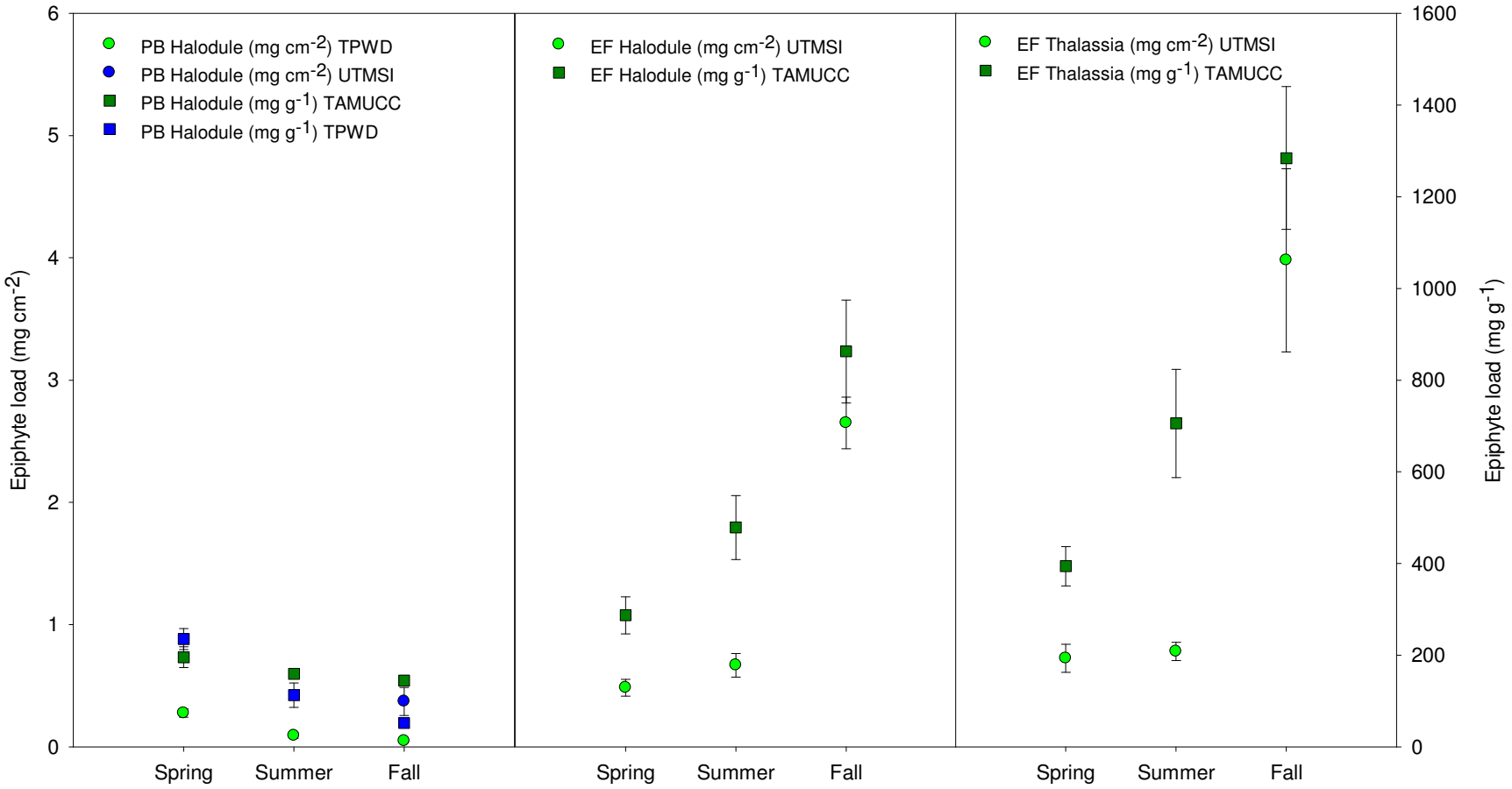


Figure 43. Normalized epiphyte loading obtained from scraping methods as dried epiphyte weight per unit area and per unit seagrass dry weight for Port Bay (PB) and East Flats (EF), May - Nov 2010. Mean ± SE.
Sample sizes for PB are: TPWD Spring - 3, Summer and Fall - 9; TAMU-CC Spring , Summer and Fall - 27; UTMSI Fall – 9. Sample sizes for EF *Halodule* are: UTMSI Spring - 35, Summer - 20, Fall - 5; TAMU-CC Spring - 19, Summer - 12, Fall - 15; for *Thalassia* are: UTMSI Spring - 25, Summer - 22, Fall - 5; TAMU-CC Spring - 15, Summer and Fall - 11.

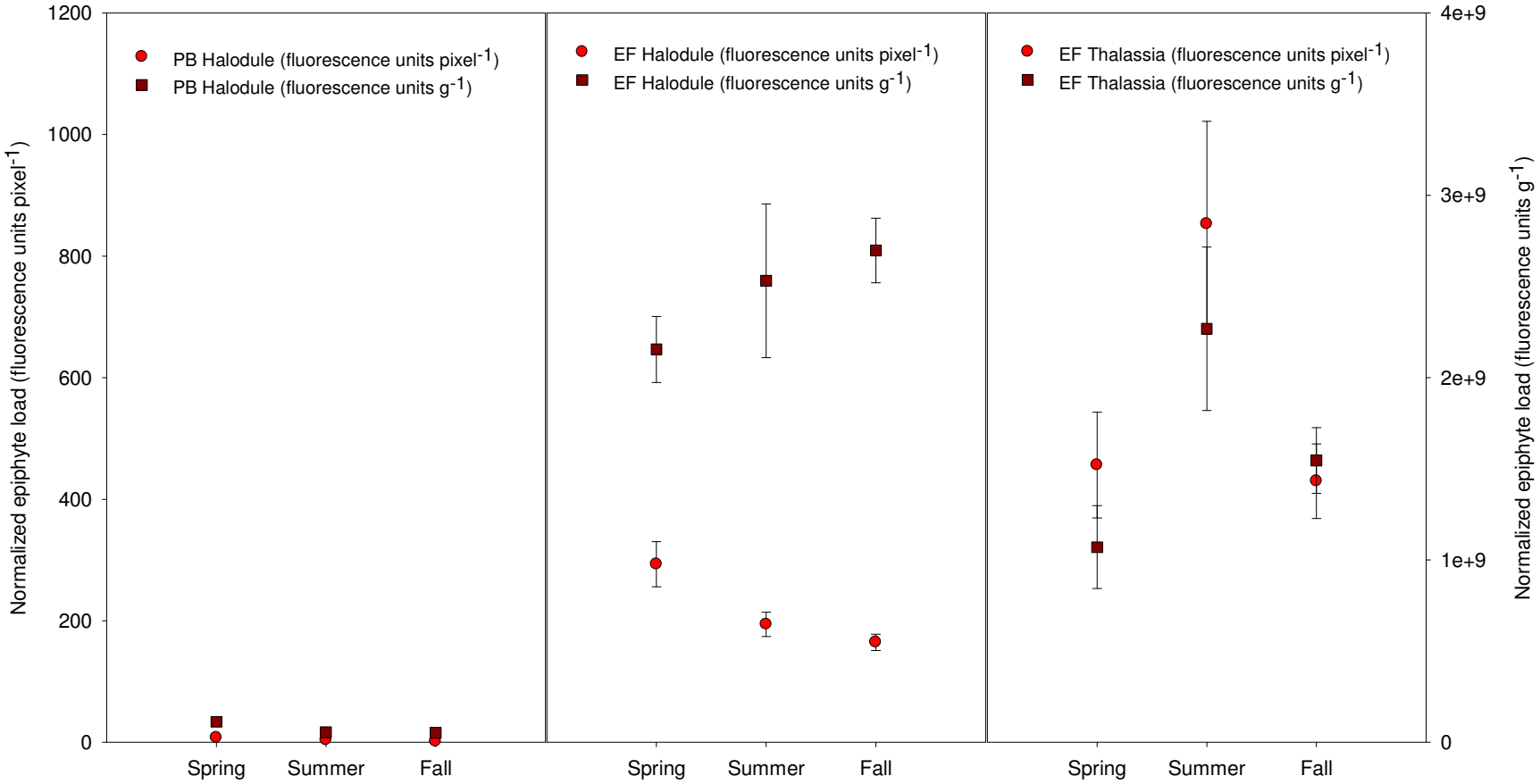


Figure 44. Normalized epiphyte loading obtained from fluorescence techniques applied to seagrass blades, normalized to the areal distribution of the red-excited fluorescence signal and to seagrass dry weight for Port Bay (PB) and East Flats (EF), May – Nov 2010. Mean ± SE.
Sample sizes for PB are: Spring , Summer and Fall – 27. Sample sizes for EF *Halodule* are: Spring - 19, Summer - 12, Fall - 15; for *Thalassia* are: Spring - 15, Summer and Fall - 11.

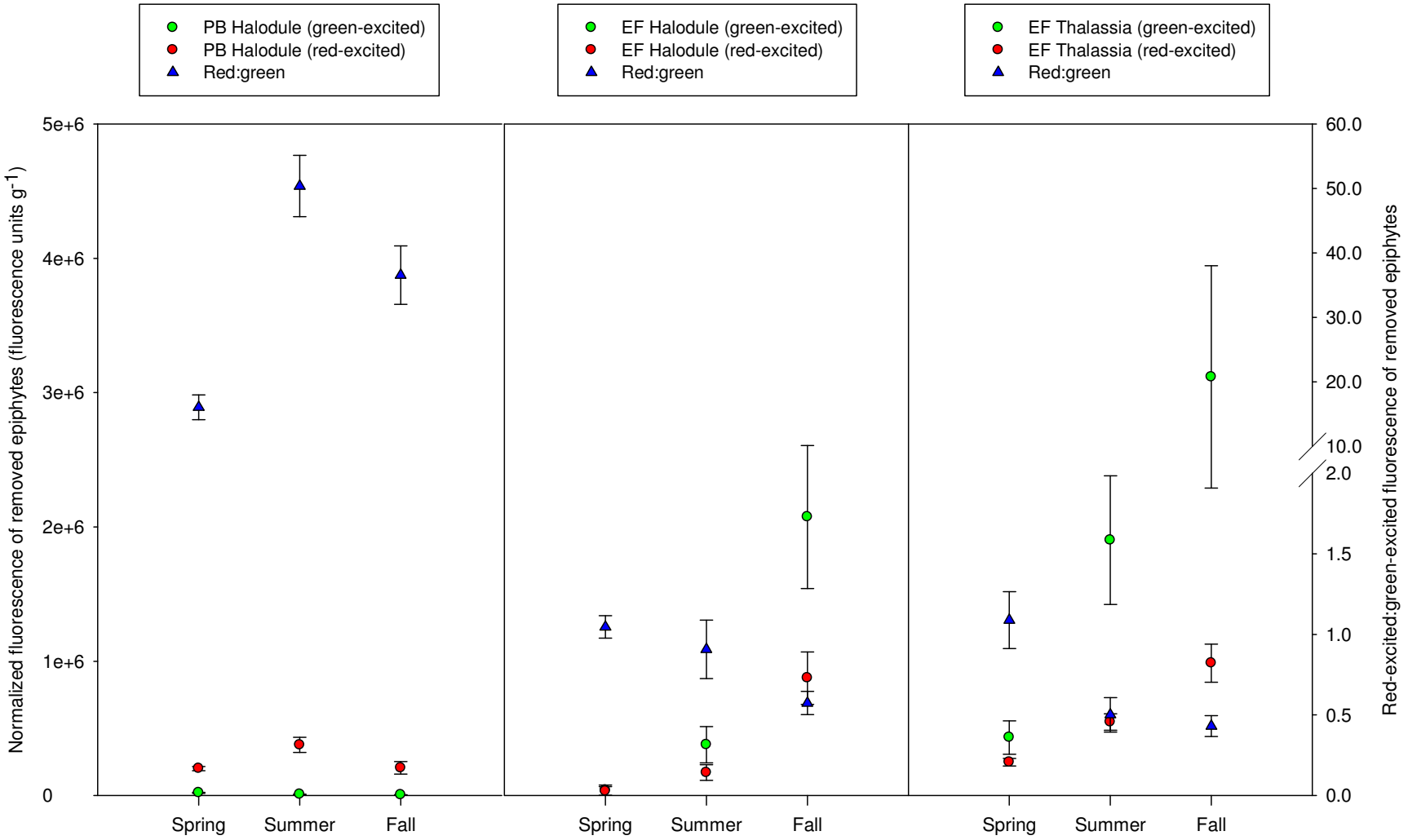


Figure 45. Normalized epiphyte loadings and ratios of red-excited to green-excited fluorescence obtained from fluorescence techniques applied to removed epiphytes, normalized to seagrass dry weight for Port Bay (PB) and East Flats (EF), May – Nov 2010. Mean ± SE.
Sample sizes for PB are: Spring , Summer and Fall – 27. Sample sizes for EF *Halodule* are: Spring - 19, Summer - 12, Fall - 15; for *Thalassia* are: Spring - 15, Summer and Fall - 11.

Table 23. Normalized epiphyte load by scraping methods for Port Bay and East Flats, May - Nov 2010. Mean ± SE (N).

	Spring			Summer			Fall		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Port Bay									
Halodule epiphyte load (mg cm^{-2})	0.32 (1)	0.31 (1)	0.21 (1)	0.08 ± 0.02 (3)	0.15 ± 0.02 (3)	0.04 ± 0.04 (3)	0.02 ± 0.01 (3)	0.06 ± 0.01 (3)	0.06 ± 0.01 (3)
Halodule epiphyte load (mg g^{-1} dry seagrass)	280 (1)	221 (1)	205 (1)	138 ± 56 (3)	160 ± 10 (3)	40 ± 35 (3)	33 ± 9 (3)	58 ± 13 (3)	65 ± 10 (3)
East Flats									
Halodule epiphyte load (mg cm^{-2})	0.61 ± 0.19 (10)	0.68 ± 0.08 (10)	0.28 ± 0.04 (15)	0.71 ± 0.15 (10)	-	0.63 ± 0.13 (10)	2.65 (1)	2.64 ± 0.61 (2)	2.66 ± 0.26 (2)
Thalassia epiphyte load (mg cm^{-2})	0.91 ± 0.17 (15)	0.38 ± 0.13 (5)	0.52 ± 0.14 (5)	0.93 ± 0.09 (12)	0.61 ± 0.10 (10)	-	2.47 (2)	5.33 ± 0.73 (2)	2.79 (1)
Syringodium epiphyte load (mg cm^{-2})		1.05 ± 0.49 (5)	0.76 ± 0.37 (5)						

Table 24. Epiphyte fluorescence and scraping data for *Halodule* in Port Bay, May - Oct 2010. Mean ± SE (N).

Seagrass species	Season	Parameter	T1			T2			T3		
Halodule	Spring	Scanned leaf area (number of pixels of red-excited fluorescence)	1.03E+06	± 7.22E+04	(9)	7.73E+05	± 8.94E+04	(9)	9.76E+05	± 6.80E+04	(9)
		Leaf dry weight (g)	0.066	± 0.007	(9)	0.050	± 0.006	(9)	0.070	± 0.006	(9)
		Green-excited fluorescence (fluorescence units)	4.13E+06	± 5.51E+05	(9)	4.14E+06	± 5.28E+05	(9)	1.27E+07	± 9.60E+05	(9)
		Scraped epiphyte dry weight (mg)	13.6	± 1.9	(9)	13.5	± 3.1	(9)	7.3	± 1.0	(9)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	3.9	± 0.3	(9)	5.5	± 0.6	(9)	13.2	± 0.8	(9)
		Normalized epiphyte load (fluorescence units g^{-1})	6.48E+07	± 6.55E+06	(9)	8.50E+07	± 9.16E+06	(9)	1.83E+08	± 5.86E+06	(9)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	219	± 33	(9)	263	± 45	(9)	107	± 13	(9)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	4.12E+02	± 6.52E+01	(9)	5.51E+02	± 5.24E+01	(9)	2.78E+03	± 2.41E+02	(9)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	6.67E+03	± 8.20E+02	(9)	1.30E+04	± 1.07E+03	(9)	1.47E+04	± 1.16E+03	(9)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	18.0	± 2.6	(9)	24.7	± 2.2	(9)	5.5	± 0.5	(9)
	Summer	Scanned leaf area (number of pixels of red-excited fluorescence)	1.39E+06	± 1.07E+05	(9)	9.94E+05	± 1.20E+05	(9)	1.11E+06	± 1.23E+05	(9)
		Leaf dry weight (g)	0.081	± 0.012	(9)	0.070	± 0.004	(9)	0.078	± 0.003	(9)
		Green-excited fluorescence (fluorescence units)	3.00E+06	± 3.02E+05	(9)	2.51E+06	± 1.34E+05	(9)	6.97E+06	± 5.14E+05	(9)
		Scraped epiphyte dry weight (mg)	13.5	± 2.0	(9)	11.7	± 1.1	(9)	10.4	± 1.1	(9)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	2.1	± 0.1	(9)	2.9	± 0.5	(9)	6.7	± 0.7	(9)
		Normalized epiphyte load (fluorescence units g^{-1})	4.00E+07	± 4.31E+06	(9)	3.70E+07	± 2.57E+06	(9)	8.94E+07	± 4.52E+06	(9)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	177	± 27	(9)	169	± 14	(9)	132	± 10	(9)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	2.74E+02	± 2.58E+01	(9)	2.59E+02	± 2.75E+01	(9)	1.40E+03	± 1.46E+02	(9)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	9.38E+03	± 1.57E+03	(9)	1.95E+04	± 2.46E+03	(9)	5.81E+04	± 5.65E+03	(9)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	32.2	± 3.6	(9)	77.0	± 8.3	(9)	41.9	± 0.7	(9)
	Fall	Scanned leaf area (number of pixels of red-excited fluorescence)	1.71E+06	± 2.39E+05	(9)	2.20E+06	± 6.76E+04	(9)	2.28E+06	± 9.02E+04	(9)

Seagrass species	Season	Parameter	T1		T2		T3	
		Leaf dry weight (g)	0.056 ± 0.005	(9)	0.073 ± 0.004	(9)	0.070 ± 0.003	(9)
		Green-excited fluorescence (fluorescence units)	2.85E+06 ± 5.74E+05	(9)	3.53E+06 ± 2.59E+05	(9)	4.07E+06 ± 3.97E+05	(9)
		Scraped epiphyte dry weight (mg)	6.3 ± 0.4	(9)	12.1 ± 1.8	(9)	10.4 ± 1.5	(9)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	1.6 ± 0.1	(9)	1.6 ± 0.1	(9)	1.8 ± 0.1	(9)
		Normalized epiphyte load (fluorescence units g^{-1})	5.06E+07 ± 7.51E+06	(9)	4.88E+07 ± 3.49E+06	(9)	5.79E+07 ± 4.06E+06	(9)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	118 ± 12	(9)	168 ± 24	(9)	147 ± 19	(9)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	1.83E+02 ± 8.53E+00	(9)	2.22E+02 ± 1.15E+01	(9)	5.35E+02 ± 8.84E+01	(9)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	2.33E+03 ± 2.83E+02	(9)	9.25E+03 ± 1.37E+03	(9)	3.22E+04 ± 7.82E+03	(9)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	13.3 ± 2.0	(9)	41.0 ± 5.4	(9)	55.3 ± 7.2	(9)

Table 25. Epiphyte fluorescence and scraping data for *Halodule*, *Thalassia* and *Syringodium* in East Flats, Jun - Nov 2010. Mean ± SE (N).

Seagrass species	Season	Parameter	T1		T2		T3	
Halodule	Spring	Scanned leaf area (number of pixels of red-excited fluorescence)	1.03E+06 ± 1.45E+05	(6)	9.75E+05 ± 1.73E+05	(4)	1.06E+06 ± 8.93E+04	(9)
		Leaf dry weight (g)	0.082 ± 0.011	(6)	0.138 ± 0.042	(4)	0.161 ± 0.015	(9)
		Green-excited fluorescence (fluorescence units)	1.69E+08 ± 4.75E+07	(6)	4.26E+08 ± 2.30E+08	(4)	3.79E+08 ± 4.64E+07	(9)
		Scraped epiphyte dry weight (mg)	40.1 ± 9.7	(6)	33.4 ± 13.4	(4)	39.6 ± 5.4	(9)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	147.3 ± 24.5	(6)	380.4 ± 137.2	(4)	351.1 ± 23.5	(9)
		Normalized epiphyte load (fluorescence units g^{-1})	1.48E+09 ± 2.35E+08	(6)	2.64E+09 ± 6.18E+08	(4)	2.31E+09 ± 1.27E+08	(9)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	421 ± 120	(6)	218 ± 36	(4)	243 ± 25	(9)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	4.38E+04 ± 1.57E+04	(6)	4.11E+04 ± 1.86E+04	(4)	4.67E+04 ± 9.04E+03	(9)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	4.45E+04 ± 1.36E+04	(6)	3.87E+04 ± 1.64E+04	(4)	4.12E+04 ± 6.54E+03	(9)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	1.2 ± 0.2	(6)	1.1 ± 0.2	(4)	0.9 ± 0.1	(9)
	Summer	Scanned leaf area (number of pixels of red-excited fluorescence)	1.35E+06 ± 3.21E+05	(4)	1.59E+06 ± 3.46E+05	(2)	1.84E+06 ± 1.62E+05	(6)
		Leaf dry weight (g)	0.143 ± 0.034	(4)	0.125 ± 0.051	(2)	0.281 ± 0.181	(6)
		Green-excited fluorescence (fluorescence units)	2.25E+08 ± 3.25E+07	(4)	3.64E+08 ± 4.26E+07	(2)	3.39E+08 ± 5.25E+07	(6)
		Scraped epiphyte dry weight (mg)	72.2 ± 21.5	(4)	72.5 ± 37.1	(2)	52.2 ± 9.4	(6)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	194.5 ± 54.7	(4)	233.6 ± 24.0	(2)	180.8 ± 19.9	(6)
		Normalized epiphyte load (fluorescence units g^{-1})	1.68E+09 ± 2.30E+08	(4)	3.31E+09 ± 1.01E+09	(2)	2.84E+09 ± 7.33E+08	(6)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	490 ± 27	(4)	548 ± 72	(2)	448 ± 142	(6)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	4.51E+04 ± 1.08E+04	(4)	8.12E+04 ± 1.73E+04	(2)	8.73E+04 ± 1.15E+04	(6)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	7.35E+04 ± 2.09E+04	(4)	5.28E+04 ± 2.96E+04	(2)	4.24E+04 ± 5.13E+03	(6)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	1.7 ± 0.2	(4)	0.6 ± 0.2	(2)	0.5 ± 0.0	(6)
	Fall	Scanned leaf area (number of pixels of red-excited fluorescence)	1.32E+06 ± 1.76E+05	(5)	1.79E+06 ± 1.20E+05	(4)	1.89E+06 ± 1.35E+05	(6)

Seagrass species	Season	Parameter	T1		T2		T3	
		Leaf dry weight (g)	0.072 ± 0.010	(5)	0.106 ± 0.018	(4)	0.129 ± 0.013	(6)
		Green-excited fluorescence (fluorescence units)	2.02E+08 ± 1.06E+07	(5)	2.53E+08 ± 2.87E+07	(4)	3.33E+08 ± 4.17E+07	(6)
		Scraped epiphyte dry weight (mg)	61.8 ± 11.4	(5)	131.2 ± 18.6	(4)	67.9 ± 7.9	(6)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	169.9 ± 34.5	(5)	141.6 ± 14.2	(4)	175.4 ± 17.5	(6)
		Normalized epiphyte load (fluorescence units g^{-1})	3.09E+09 ± 4.91E+08	(5)	2.44E+09 ± 1.62E+08	(4)	2.54E+09 ± 9.90E+07	(6)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	888 ± 135	(5)	1325 ± 240	(4)	532 ± 51	(6)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	1.36E+05 ± 5.18E+04	(5)	5.43E+05 ± 2.08E+05	(4)	1.29E+05 ± 1.82E+04	(6)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	7.72E+04 ± 1.66E+04	(5)	2.14E+05 ± 2.65E+04	(4)	5.67E+04 ± 7.46E+03	(6)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	0.8 ± 0.2	(5)	0.5 ± 0.1	(4)	0.4 ± 0.0	(6)
Thalassia	Spring	Scanned leaf area (number of pixels of red-excited fluorescence)	1.41E+06 ± 1.30E+05	(9)	1.57E+06 ± 4.75E+05	(6)	-	
		Leaf dry weight (g)	0.677 ± 0.091	(9)	0.818 ± 0.215	(6)	-	
		Green-excited fluorescence (fluorescence units)	3.56E+08 ± 5.33E+07	(9)	1.37E+09 ± 5.06E+08	(6)	-	
		Scraped epiphyte dry weight (mg)	261.1 ± 35.0	(9)	215.6 ± 73.9	(6)	-	
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	267.3 ± 39.9	(9)	739.1 ± 149.5	(6)	-	
		Normalized epiphyte load (fluorescence units g^{-1})	4.54E+08 ± 7.73E+07	(9)	2.06E+09 ± 2.08E+08	(6)	-	
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	420 ± 63	(9)	352 ± 53	(6)	-	
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	9.13E+04 ± 1.24E+04	(9)	4.60E+05 ± 1.64E+05	(6)	-	
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	1.26E+05 ± 1.07E+04	(9)	2.09E+05 ± 8.79E+04	(6)	-	
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	1.5 ± 0.2	(9)	0.5 ± 0.1	(6)	-	
	Summer	Scanned leaf area (number of pixels of red-excited fluorescence)	2.31E+06 ± 1.64E+05	(6)	1.90E+06 ± 2.95E+05	(5)	-	
		Leaf dry weight (g)	0.896 ± 0.124	(6)	0.768 ± 0.035	(5)	-	
		Green-excited fluorescence (fluorescence units)	1.07E+09 ± 1.41E+08	(6)	2.54E+09 ± 5.09E+08	(5)	-	
		Scraped epiphyte dry weight (mg)	434.1 ± 49.7	(6)	710.1 ± 141.8	(5)	-	

Seagrass species	Season	Parameter	T1			T2			T3		
		Normalized epiphyte load (fluorescence units pixel^{-1})	482.9	± 72.3	(6)	1407.5	± 201.8	(5)	-		
		Normalized epiphyte load (fluorescence units g^{-1})	1.41E+09	± 3.41E+08	(6)	3.30E+09	± 6.66E+08	(5)	-		
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	505	± 43	(6)	946	± 218	(5)	-		
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	5.54E+05	± 8.16E+04	(6)	2.46E+06	± 3.46E+05	(5)	-		
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	3.69E+05	± 2.72E+04	(6)	4.99E+05	± 4.53E+04	(5)	-		
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	0.7	± 0.1	(6)	0.2	± 0.0	(5)	-		
	Fall	Scanned leaf area (number of pixels of red-excited fluorescence)	2.34E+06	± 2.40E+05	(4)	2.67E+06	± 1.54E+05	(5)	2.48E+06	± 4.00E+05	(2)
		Leaf dry weight (g)	0.704	± 0.197	(4)	0.763	± 0.087	(5)	0.584	± 0.045	(2)
		Green-excited fluorescence (fluorescence units)	9.07E+08	± 1.61E+08	(4)	1.56E+09	± 3.33E+08	(5)	4.58E+08	± 1.08E+08	(2)
		Scraped epiphyte dry weight (mg)	887.7	± 190.0	(4)	1248.8	± 307.0	(5)	323.1	± 2.6	(2)
		Normalized epiphyte load (fluorescence units pixel^{-1})	381.4	± 27.4	(4)	567.4	± 94.5	(5)	182.5	± 14.1	(2)
		Normalized epiphyte load (fluorescence units g^{-1})	1.38E+09	± 1.19E+08	(4)	1.99E+09	± 2.35E+08	(5)	7.74E+08	± 1.25E+08	(2)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	1314	± 68	(4)	1551	± 244	(5)	556	± 39	(2)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	1.04E+06	± 9.70E+04	(4)	3.72E+06	± 4.19E+05	(5)	3.03E+05	± 5.55E+03	(2)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	7.11E+05	± 8.95E+04	(4)	8.62E+05	± 6.38E+04	(5)	1.25E+05	± 1.73E+03	(2)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	0.7	± 0.0	(4)	0.2	± 0.0	(5)	0.4	± 0.0	(2)
Syringodium	Summer	Scanned leaf area (number of pixels of red-excited fluorescence)	-			1.48E+06	± 4.86E+05	(2)	2.50E+06	± 2.98E+05	(3)
		Leaf dry weight (g)	-			0.501	± 0.254	(2)	0.252	± 0.012	(3)
		Green-excited fluorescence (fluorescence units)	-			5.95E+08	± 2.61E+07	(2)	1.20E+08	± 1.75E+07	(3)
		Scraped epiphyte dry weight (mg)	-			117.6	± 12.4	(2)	42.6	± 4.8	(3)
		Normalized epiphyte load (fluorescence units pixel^{-1})	-			444.0	± 128.3	(2)	50.8	± 12.3	(3)
		Normalized epiphyte load (fluorescence units g^{-1})	-			1.63E+09	± 8.79E+08	(2)	4.85E+08	± 8.84E+07	(3)

Seagrass species	Season	Parameter	T1	T2		T3	
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	-	299 ± 127	(2)	168 ± 11	(3)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	-	2.65E+05 ± 1.39E+05	(2)	3.46E+04 ± 6.44E+03	(3)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	-	1.31E+05 ± 2.79E+04	(2)	3.84E+04 ± 2.96E+03	(3)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	-	0.6 ± 0.2	(2)	1.2 ± 0.1	(3)
	Fall	Scanned leaf area (number of pixels of red-excited fluorescence)	-	-		2.92E+06 ±	(1)
		Leaf dry weight (g)	-	-		0.200 ±	(1)
		Green-excited fluorescence (fluorescence units)	-	-		1.80E+08 ±	(1)
		Scraped epiphyte dry weight (mg)	-	-		78.7 ±	(1)
		Normalized epiphyte load (fluorescence units $pixel^{-1}$)	-	-		61.5 ±	(1)
		Normalized epiphyte load (fluorescence units g^{-1})	-	-		8.99E+08 ±	(1)
		Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1})	-	-		394 ±	(1)
		Green-excited fluorescence of scraped epiphytes (fluorescence units)	-	-		1.45E+05 ±	(1)
		Red-excited fluorescence of scraped epiphytes (fluorescence units)	-	-		7.36E+04 ±	(1)
		Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes	-	-		0.5 ±	(1)

Seagrass Stable Isotope

Stable isotopes have long been used in environmental studies as tracers of source material to an ecosystem. Naturally occurring isotopes of carbon and nitrogen are of special interest when looking at nutrient and organic material cycling in aquatic ecosystems, since sources (such as domestic wastewater) often have distinctive ratios of the heavier isotope to the more common isotope of the element. For example, domestic wastewater often shows an enriched $\delta^{15}N$ signature relative to environmental sources of nitrogen, due to a larger proportion of ^{15}N in wastewater. For carbon the isotope of interest is ^{13}C, the heavier isotope of carbon. Likewise for nitrogen the isotope of interest is ^{15}N. In tracer studies the parameter of interest is the deviation of the isotope ratio of a sample from that of a standard, and is expressed in parts per thousand:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times (1{,}000)$$

where $R = {}^{13}C/{}^{12}C$ (or $^{15}N/^{14}N$).

Port Bay *Halodule* had lower $\delta^{13}C$ than East Flats *Halodule* (Table 26, Table 27 and Figure 46). Port Bay and East Flats *Halodule* had similar $\delta^{15}N$ content. Macroalgae at Port Bay had higher $\delta^{13}C$ and $\delta^{15}N$ than macroalgae at East Flats (Figure 47 and Figure 48).

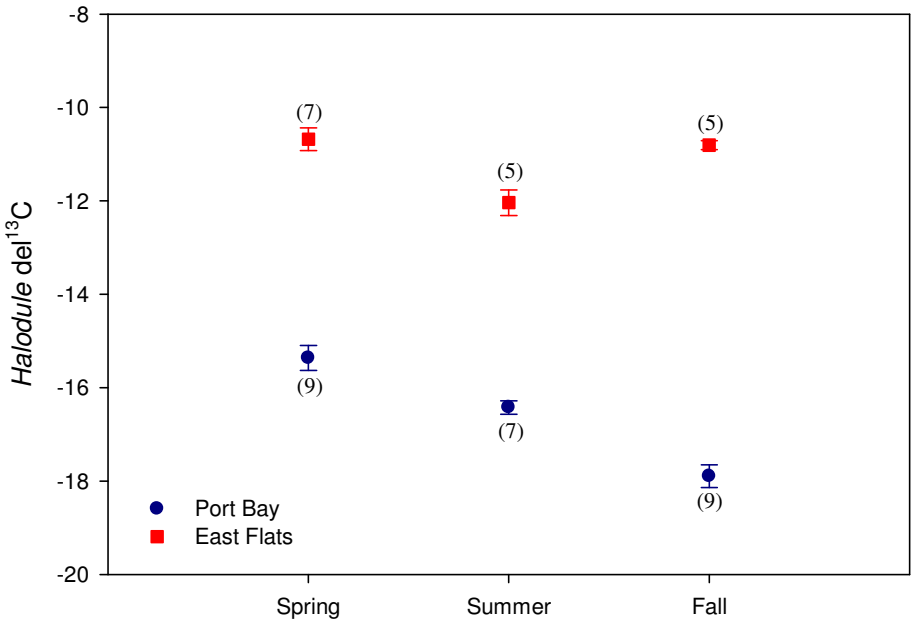


Figure 46. Stable isotope ratio, $\delta^{13}C$, for *Halodule* from Port Bay and East Flats, May – Nov 2010. Mean ± SE (N).

Table 26. Isotopic composition by season of seagrasses, epiphytes and macroalgae in Port Bay and East Flats, May – Nov 2011. Mean ± SE.

	$\delta^{13}C$	$\delta^{15}N$	C:N	N
		Port Bay		
		Seagrasses		
Halodule				
Spring	-15.4 ± 0.3	5.0 ± 0.5	14.6 ± 0.6	9
Summer	-16.4 ± 0.1	4.7 ± 0.3	15.4 ± 0.5	7
Fall	-17.9 ± 0.2	2.6 ± 0.4	10.9 ± 0.3	9
Ruppia				
Summer	-17.3 ± 0.2	5.1 ± 0.6	11.7 ± 0.1	2
		Epiphytes		
on *Halodule*				
Spring	-15.2 ± 0.5	6.7 ± 0.5	6.8 ± 0.2	6
Fall	-19.8 ± 0.7	2.5 ± 0.2	7.9 ± 0.4	3
		Macroalgae		
Spring	-18.8 ± 1.3	8.3 ± 0.3	15.3 ± 1.2	3
Summer	-22.5 ± 1.3	7.8 ± 1.1	19.9 ± 4.2	2
Fall	-23.5 ± 0.4	6.8 ± 0.2	12.7 ± 0.8	6
		East Flats		
		Seagrasses		
Halodule				
Spring	-10.7 ± 0.2	4.1 ± 0.5	20.2 ± 0.4	7
Summer	-12.0 ± 0.3	3.6 ± 0.3	15.4 ± 0.1	5
Fall	-10.8 ± 0.1	3.4 ± 0.4	14.2 ± 0.4	5
Syringodium				
Summer	-7.6 ± 0.4	2.8 ± 0.6	14.3 ± 0.0	2
Fall	-7.6	3.8	18.6	1
Thalassia				
Spring	-9.0 ± 0.4	4.8 ± 0.4	15.7 ± 0.6	5
Summer	-10.1 ± 0.2	5.0 ± 0.4	11.6 ± 0.5	4
Fall	-10.7 ± 0.4	5.0 ± 0.2	12.0 ± 0.4	4
		Epiphytes		
on *Halodule*				
Spring	-13.2 ± 0.7	3.3 ± 0.4	10.0 ± 0.3	5
Summer	-12.3 ± 1.5	5.5 ± 1.1	8.5 ± 0.9	4
Fall	-10.4 ± 1.1	4.7 ± 0.4	15.3 ± 1.3	5
on *Syringodium*				
Summer	-12.0 ± 0.8	5.7 ± 0.7	8.9 ± 0.7	2
on *Thalassia*				
Spring	-12.8 ± 1.5	4.0 ± 0.8	9.1 ± 1.4	4
Summer	-10.1 ± 0.8	4.7 ± 0.4	9.3 ± 0.4	4
Fall	-7.8 ± 0.3	3.9 ± 0.4	14.8 ± 1.0	5
		Macroalgae		
Spring	-15.5 ± 0.9	4.5 ± 0.6	24.7 ± 5.4	5
Summer	-16.4 ± 0.9	6.3 ± 0.2	15.9 ± 0.9	10
Fall	-16.8 ± 1.4	5.9 ± 0.3	19.9 ± 2.1	3

Table 27. Average isotopic composition for seagrasses, epiphytes and macroalgae in Port Bay and East Flats, May – Nov 2011. Mean ± SE.

	$\delta^{13}C$	$\delta^{15}N$	C:N	N
		Port Bay		
		Seagrass		
Halodule	-16.6 ± 0.3	4.0 ± 0.3	13.5 ± 0.5	(25)
Ruppia	-17.3 ± 0.2	5.1 ± 0.6	11.7 ± 0.1	(2)
		Epiphytes		
on Halodule	-16.7 ± 0.9	5.3 ± 0.8	7.1 ± 0.2	(9)
		Macroalgae		
Gracilaria	-22.1 ± 0.8	7.4 ± 0.3	14.7 ± 1.1	(11)
		East Flats		
		Seagrass		
Halodule	-11.1 ± 0.2	3.8 ± 0.3	17.1 ± 0.7	(17)
Thalassia	-9.9 ± 0.3	4.9 ± 0.2	13.3 ± 0.6	(13)
Syringodium	-7.6 ± 0.2	3.1 ± 0.5	15.7 ± 1.5	(3)
		Epiphytes		
on Halodule	-12.0 ± 0.7	4.4 ± 0.4	11.5 ± 1.0	(14)
on Thalassia	-10.0 ± 0.8	4.2 ± 0.3	11.4 ± 1.0	(13)
on Syringodium	-12.0 ± 0.8	5.7 ± 0.7	8.9 ± 0.7	(2)
		Macroalgae		
Various	-16.2 ± 0.6	5.7 ± 0.3	19.0 ± 1.8	(18)

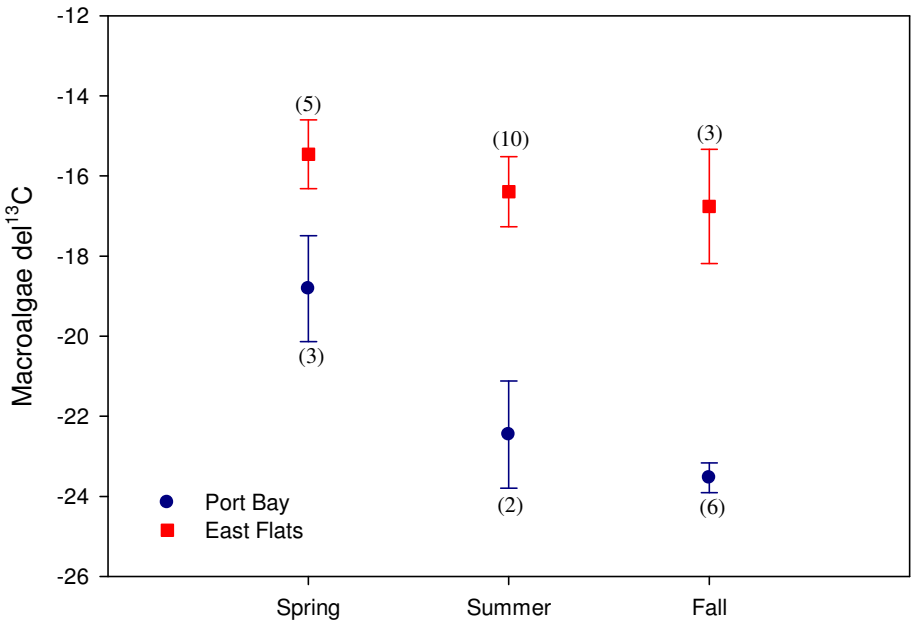


Figure 47. Stable isotope ratio, $\delta^{13}C$, for macroalgae from Port Bay and East Flats, May – Nov 2010. Mean ± SE (N).

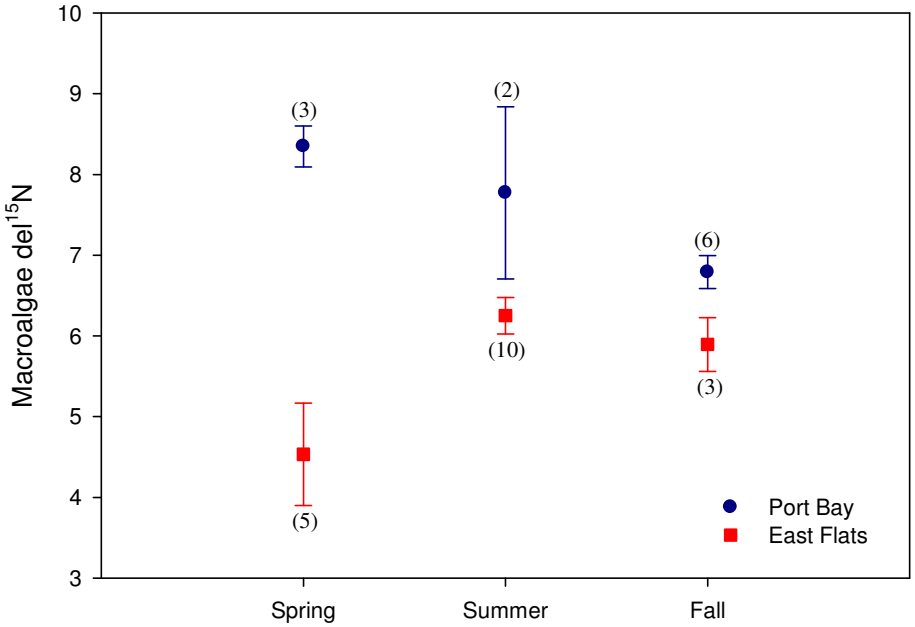


Figure 48. Stable isotope ratio, $\delta^{15}N$, for macroalgae from Port Bay and East Flats, May – Nov 2010. Mean ± SE (N).

Videography

Each transect at Port Bay and East Flats was videotaped using an underwater video camera, with the exception of the summer trip at Port Bay and the fall trip at East Flats, when the camera or recorder malfunctioned. Obtaining clear underwater images was a challenge due to water clarity (Figure 49). At Port Bay the water was often stained as well as turbid. The instrument is an automatic focus camera, which requires a steady hand to avoid jerkiness in the video image. The best images were obtained when wave action was minimal, water clarity was good, and the camera was moved very slowly along the transect line. We experimented with wading with the camera rather than shooting from the boat to avoid jerkiness due to wave motion. Video files were archived and can be compared with future videotapes of the transects. With better-quality video, features that could potentially be analyzed include seagrass species, seagrass coverage and presence and coverage of macroalgae.

Figure 49. Videocamera still image from Port Bay T3, Oct 2010.

Discussion

TPWD planned and executed a seagrass monitoring program with two goals – evaluate impacts of a new wastewater discharge on an existing seagrass bed, and evaluate usefulness of proposed seagrass monitoring methods for a statewide monitoring program. The study design was based on the Dunton *et al.* 2007 proposal for seagrass monitoring. Their proposal encompasses a three-tier sampling regime. Tier 1 consists of acquiring aerial photography of seagrass beds at a large scale (1:24,000). Tier 2 is a rapid assessment approach designed to measure a variety of environmental and seagrass parameters at a large number of points on an annual basis (at the time of peak biomass in the fall). Tier 3 is the most intensive sampling regime, intended to not only characterize environmental and seagrass conditions in an area of interest, but to determine causation if degradation of the seagrass bed is detected. Tier 3 calls for the same parameters as Tier 2 sampling, but with more replication (N=3 to N=30), and is based on a framework of three 50 m transects roughly perpendicular to shore that follow the bottom slope and encompass the deep edge of the seagrass bed. Since one of the project goals was to determine impacts of a new wastewater discharge, the Tier 3 study design was selected for testing. This choice allowed testing of protocols for a statewide monitoring program, since the Tier 3 study design employed all the parameters and protocols for Tier 2, but with more replication. This makes it possible to infer the level of effort that would be needed to implement a Tier 2 program up and down the coast, and have some expectations about the kind of information that could be derived from collecting these parameters, and the spatial and temporal variability of that information.

Wastewater Impact

Because the wastewater plant was not built on Port Bay during the study, it is not possible to evaluate the impacts of wastewater on the seagrass community there. One positive outcome to the delay in construction of the wastewater plant is that three seasons of data were collected that may be considered baseline data (prior to commencement of the discharge). Having results from May through October enables some understanding of seasonal variability in the parameters collected. In addition, most of the environmental and biological parameters collected through this study have a fairly high degree of variability, and a larger data set provides more robustness to the estimates derived from this effort. Texas bays experience major changes in freshwater inflow from year to year. Port Bay, being a secondary bay, is highly subject to terrestrial influences and large swings in salinity based on inflow. TPWD hopes to re-sample Port Bay within the next few years, even if the wastewater plant is not yet constructed. This will double the amount of data available for the area of interest in Port Bay, and enhance understanding of interannual variation.

Completing the sample collection and analysis at Port Bay allowed TPWD to characterize the seagrass community in the vicinity of the proposed wastewater treatment plant discharge. In turn, this led to evaluation of the sample design as a tool in detecting any wastewater impacts in a future data collection effort. Collecting the same data in the same way at the same locations after the wastewater plant is online will obviously provide the most relevant information. Thus TPWD recommends that any follow-up efforts to this study sample the same transects using the same methodology. This study involved a seasonal component, so repeated sampling should take into account the time of year when baseline samples were collected. Many of the parameters collected in this study are known to exhibit seasonal variation, especially seagrass condition indicators. Whether seasonality was observed or not in our results, this must be a consideration in timing future sampling events.

One aspect of the study design that may deserve reconsideration is the use of reference sites. Originally East Flats was proposed as a reference site for Port Bay. The two sites are 22 km apart, and subject to different salinity regimes and other environmental variables. Also, the seagrass community at Port Bay is dominated by *H. wrightii* with small amounts of *R. maritima*, but that at East Flats is a multi-species assemblage of *T. testudinum*, *H. wrightii*, *S. filiforme*, and *R. maritima*. Given the differences between the two bays, in future work it seems more appropriate to sample an area within Port Bay that would be minimally affected by the wastewater discharge, and use data from that area as a reference.

Although Tier 3 sampling design considers all three transects as replicates, at Port Bay T3 was selected as an internal reference in case the wastewater discharge commenced before or during the study sampling period. T3 was chosen because it was relatively far from the permitted wastewater outfall, while T1 and T2 bracket the outfall. The thought was that T3 would not be affected by wastewater. However, results from the study show that T3 might not be an ideal reference transect for the area of interest in Port Bay. T3 differs from T1 and T2 in several ways. T3 had almost 100% seagrass coverage during the study, while T1 and T2 had more bare area. Shoot density and biomass appeared higher at T3 than at T1 and T2 during the study. T3 is on a shallow shelf that extends far from shore almost to the middle of the bay, while T1 and T2 are closer to shore.

When Port Bay is resampled, these differences must be kept in mind. One option would be to go ahead and resample the same three transects, gaining the advantage of sampling the same sites and benefitting from the statistical power of repeated sampling design. Another option would be to move the third transect so that it samples an area that more closely resembles the area close to the discharge, perhaps farther south of the permitted wastewater outfall. Since the predominant wind direction in Port Bay is from the southeast, south of the outfall could be considered the best location for a reference site that would be little affected by wastewater impacts. Another option would be to add another transect that may be a better reference site and to continue to resample the existing three transects to take advantage of the effort that has already been expended at those sites.

Some parameters collected under Tier 3 may be more useful than others in identifying wastewater impacts. Salinity or specific conductance would be important because domestic wastewater represents a freshwater addition to the bay. How widespread or dramatic the impact of salinity changes would be depends on the discharge rate, current salinity in the bay, and hydrologic conditions such as the prevailing winds/currents. Dissolved nutrients (nitrogen and phosphorus) are present in wastewater and have known impacts on seagrass growth. Total suspended solids and chlorophyll-*a* are important parameters with respect to seagrass growth because they reflect the amount of light available to the seagrass bed. Total suspended solids are already regulated in domestic wastewater permits. Chlorophyll-*a* can reflect algal blooms, which can have a deleterious effect on seagrass beds. While instantaneous measurements represent only a snapshot of the environmental conditions under which seagrass is growing, a wastewater discharge will have a fairly constant concentration and input of nutrients into the area, which will need to be characterized. In addition, it will be important to monitor sediment porewater ammonia, which represents both an important nutrient source to seagrass and a potential stressor, and may change with the discharge of wastewater effluent in the area. Sediment total organic carbon is important in characterizing sediment quality and may change with nutrient loading. Grain size is important in characterizing sediment quality with respect to seagrass growth.

Within the seagrass bed itself, important parameters to measure include coverage and condition indicators (biomass, shoot density, leaf morphometrics), which represent basic information about what seagrasses are in place and their health. Stable isotope ratios and C:N ratio are tools that are increasingly being used to track flow of nutrients into seagrasses, especially using the characteristic higher $\delta^{15}N$ of wastewater as a tracer. Macroalgae biomass and epiphyte biomass may increase in areas receiving nutrient loading. Furthermore, excessive macroalgae and epiphytes may have direct negative impacts on seagrass health. Aerial imagery will also be useful in looking at wastewater impacts because of its ability to detect landscape-level changes in the seagrass bed over time. Aerial imagery may also be used to obtain information about macroalgae accumulations that is not available from analysis of quadrats.

Considering that every measurement collected in the field has some type of cost attached to it, some of the parameters collected in this study may be less useful in looking at wastewater impacts to seagrass beds. Many of the water quality parameters collected in this study are important to seagrass condition, as mentioned above. However, collecting this information as a grab sample in conjunction with collection of biological samples may not be the best way to

understand water quality impacts. Seagrass growth integrates environmental conditions over preceding months and even years, so long-term measurements of salinity, light availability, and dissolved nutrients would better characterize the environmental conditions of a seagrass bed and help explain changes. Videography may eventually prove to be a useful tool in evaluating seagrass health, but at present there is no methodology for analyzing video files on seagrass transects for useful indications of seagrass condition.

Statewide Seagrass Monitoring

Developing a statewide seagrass monitoring program is the keystone of seagrass management in Texas. Resource managers must have accurate information of the status and trends of seagrass beds along the Texas coast. Regulatory decisions must be science-based. An ideal monitoring program would focus the state's limited resources on collecting the data that best describes seagrass condition and gives sufficient information to understand the environmental stressors affecting seagrasses. Some components of a statewide monitoring program that must be considered include: best time of year to conduct monitoring (that is, to define an "index period"), essential parameters to sample, level of effort, cost, laboratory capability and applicability of the program to all parts of the coast where seagrasses grow.

Confining biological sampling events to an index period is integral to regulatory assessment in Texas (TCEQ 2005). One benefit of confining sampling to an index period is to reduce seasonal variation in the data. This study found wide temporal variability in some of the biological parameters measured. Peak seagrass biomass in fall has been proposed as the optimal time to monitor seagrasses (Dunton *et al.* 2007). Another reason for establishing an index period for monitoring is to collect data during a time when anthropogenic and climatic stresses should be most severe (Neckles, ed., 1994). Based on this study, TPWD would support establishing an index period for annual seagrass monitoring. In timing seagrass sampling, it is important to note that leaves begin to slough off as winter approaches. For a coastwide monitoring program, peak biomass may occur at different times based on latitude or local environmental factors. An initial estimate of an index period for statewide monitoring might be September 1 through November 30.

Monitoring parameters should be related to seagrass condition in a way that can be quantified and interpreted (Neckles, ed., 1994). Parameters measured in this study were quantifiable, although some parameters were easier to interpret than others. Instantaneous physicochemical measurements and water and sediment chemistry are straightforward, but interpretation of seagrass condition indicators at this time is more difficult. It is not hard to accept the concept that seagrass indicators like shoot density, canopy height and biomass will increase under favorable environmental conditions and decline under unfavorable ones. However, determining whether those conditions arise directly from human activities, like excessive nutrient loading, or natural causes, such as low temperatures due to early cold fronts, is more problematic. This confounds the interpretation of these seagrass indicators. While the use of stable isotopes and C:N ratios as environmental tracers is developing, it is similarly difficult to interpret results. Overall, it is likely that sampling on a much wider geographic basis, including a variety of impacted and least-impacted systems, will be needed to develop robust protocols for interpreting seagrass condition data.

Stressor measurements are similarly challenging to interpret. For example, excessive growth of macroalgae can be caused by anthropogenic nutrient loading. However, macroalgae can also move around the bays with wind and current. It can get caught on top of the seagrass canopy, and can accumulate in bay bottom depressions and prop scars. Thus a number of factors control the distribution and density of macroalgae in a seagrass area. As noted above, aerial imagery may be the best, albeit more expensive tool, for understanding macroalgae abundance and dynamics in bays.

Likewise, the concept that epiphyte load may increase in response to nutrient enrichment is not hard to understand, but other environmental and biological factors can confound the interpretation. For example, seagrasses in Port Bay consistently had lower normalized epiphyte loads than those in East Flats. It is difficult to understand this in terms of nutrient concentrations, since Port Bay had higher instantaneous ortho-phosphate and nitrate+nitrite concentrations than East Flats. However, Port Bay also had higher chlorophyll-*a* concentrations, demonstrating the need to consider all available nutrient uptake pathways in evaluating biological indicators. Another complicating factor related to higher chlorophyll-*a* concentrations is that increased phytoplankton density may reduce light availability to seagrass beds. In addition, the lower salinities and limited water clarity at Port Bay could have inhibited epiphyte growth. Further, this study did not measure biological interactions that might counteract epiphyte biomass accumulation, such as grazing activity.

Conducting this study gave TPWD project participants the opportunity to develop a feel for the level of difficulty, amount of time, and approximate cost of collecting and analyzing a variety of environmental and seagrass parameters. TPWD project participants had little to no previous experience in collecting seagrass parameters. In order to adhere to the chosen study design and methodology, TPWD staff were trained by seagrass experts affiliated with the UTMSI who have studied seagrasses for two decades along the Texas coast. TPWD staff collected samples in the field and also processed samples in the laboratory, with the exception of water and sediment chemistry, stable isotope and C:N ratio samples. This suggests that it will be possible to conduct statewide seagrass sampling using aquatic biologists that have been given appropriate training.

In fact, this effort has already borne fruit in the form of a pilot statewide seagrass monitoring project initiated by TCEQ. Once again with the help of experts from UTMSI, TPWD shared expertise gained in conducting this study with staff at TCEQ. In November 2009, the two state agencies jointly began a two-year pilot project, sampling 11 sites along the coast, from the Galveston Bay system to the Corpus Christi Bay system.

When evaluating the implications of this study for development of a statewide monitoring program, it is important to note that the level of effort reported here reflected the more-intense Tier 3 of the statewide monitoring program proposed by Dunton *et al.* 2007. If statewide seagrass monitoring takes a form more like that proposed by those authors as Tier 2, it would involve many fewer replicates than Tier 3, but essentially the same list of parameters. Based on experience in this study and recent collaboration with TCEQ noted above, TPWD estimates that it would take approximately 30-45 minutes for a seasoned crew of three to sample a station with these parameters and frequencies: one instantaneous physicochemical measurement (datasonde),

water and sediment chemistry (one sample each type), seagrass coverage (four quadrats observed), seagrass core collection for biomass, shoot density, and leaf morphometrics (2 cores), seagrass shoot collection for epiphyte biomass, stable isotope and C:N ratio (two samples), and macroalgae biomass (one sample). With travel time between stations factored in, about 6 to 8 stations within a bay can be sampled per day. Samples from 6 to 8 stations would take an experienced team of three 2 to 3 days in the laboratory to process (seagrass cores, shoots for epiphyte biomass, and macroalgae biomass).

Because of the level of effort involved in collecting so many parameters for this study, expanding to a statewide monitoring program is likely to require a reduction in the number of parameters. For instance, light availability is an all-important component of seagrass growth, but currently it is expensive to buy and maintain equipment for long-term measurements, such as those conducted in East Flats. However, instantaneous measurements can be part of a statewide program and both TPWD and TCEQ have light meters available for coastal studies. Likewise instantaneous physicochemical measurements are easily obtained with existing equipment. Grab samples for water chemistry analysis are easily obtained, but expensive to analyze. TCEQ already maintains a network of routine water sampling stations under its SWQM program. A state seagrass monitoring program could rely on this existing data collection effort, perhaps with supplementation by TCEQ or local partners in areas where stations are too far apart to adequately characterize an area. This would allow a statewide seagrass monitoring program to focus on biological parameters associated with seagrass beds.

With respect to biological sampling, a relatively easy and useful measure is the visual examination of quadrats for seagrass coverage. It is quick and inexpensive. Multiple replicates can be made at a site to increase confidence in the result. Seagrass cores are quick and easy to collect, but take hours to process in the laboratory. Cleaning cores, counting shoots and separating above-ground from below-ground material takes time. With seagrass species such as *Halodule* that have numerous long fragile leaves, scraping and cleaning the leaves is the most time-consuming task involved in processing the cores. This is one of the reasons for exploring fluorescence techniques for estimating epiphyte load. For Port Bay epiphyte load samples, TPWD measured the leaf area scraped to estimate epiphyte load by surface area scraped, and also weighed the leaf area scraped to get a potentially more accurate measure for those species that do not have perfectly flat leaves. As it turned out there was good agreement ($R^2 = 0.85$) between the two measures, so either one may provide a reasonable estimate of epiphyte load, without a need to measure it both ways. Macroalgae biomass is quick and inexpensive to obtain.

Videography was difficult and did not yield much useful information for this study. Given the cost of camera equipment, this component does not seem feasible to include in a statewide monitoring program.

Laboratory capability needs to be explored in developing statewide monitoring. TCEQ may only use data from NELAC-certified labs when making regulatory decisions, for parameters where NELAC certification is available. (NELAC certification is available for most water and sediment chemistry parameters, but not for biological measurements such as biomass.) Decisions about laboratories and methods must take this into account. Another challenge is developing laboratory capability in the area of marine methods. One advantage to the use of

marine methods is that samples do not have to be diluted because of saltwater interferences that are found in some tests. When samples must be diluted the detection limit is increased by the factor of dilution, *i.e*., it becomes harder to detect nutrients at low concentration in a sample. When very small concentrations of nutrients are being measured this can be a problem. While NELAC certification is not available for stable isotope and C:N ratio methods, it may be possible to develop quality assurance protocols that meet state requirements. For sediment porewater ammonia, the LCRA laboratory was able to run analyses using a NELAC-certified method for ammonia, but had to develop a protocol for centrifuging sediment cores to extract the porewater.

It is important to note that seagrass beds have been studied intensively for many years in only two areas of the coast – the central coast, especially East Flats and Redfish Bay, and the upper Laguna Madre. While a large database exists to document typical conditions in those areas, including estimates of seasonal and interannual variation, similar data does not exist for seagrass areas in other parts of the coast. A statewide monitoring program must sample seagrasses along the entire coast. It must be demonstrated that the parameters proposed in Dunton *et al.* 2007 are feasible to sample in other areas of the coast and will yield meaningful information about seagrass and environmental conditions.

Aerial Imagery

Method

Several advantages and disadvantages of the semi-automated method of image classification became apparent during this study. One benefit was that the method was relatively quick when compared to manually digitizing habitat throughout the entire study area. Also, as the classification in Port Bay exemplifies, using the intensity and saturation bands can be useful for editing out the effects of plumes or turbidity. Additionally, the results cover the entire study area, so the researcher can see landscape trends if comparing multiple years of classified imagery. Classifications of East Flats imagery from 2005 and 2009 showed that comparisons were informative and convenient with this technique (results from that study are beyond the scope of this work and are not reported here) (Pulich and Summers 2010).

In contrast, the method presented several challenges throughout the project. For example, the entire classification process is iterative and must be executed multiple times to find the optimal solution. Pixel threshold, AOIs, and interim products are evaluated solely by visual inspection and are subjectively accepted or rejected. Analysts not only need to be experienced with the technique but also with the study area to best interpret the results.

This study revealed that ease-of-use of the classification method differs by site location. Classification of East Flats was straight-forward and required minimal analysis time, largely based on prior experience with this site. Port Bay, however, as a new site required more complicated AOIs and several more iterations to produce an acceptable product. Additionally, this method was not helpful in distinguishing algae deposits from seagrass in either study area.

Additional studies would be needed to determine the suitability of this method at other sites. Future work could also focus on interpretation of the landscape metrics to draw clearer parallels between landscape graphics, virtual transect indices, and Patch Analyst indices and to establish if

significant correlations exist between landscape and field methods, such as determining seagrass coverage by inspection of quadrats.

Comparison of 2009 and 2010 Imagery Analysis

Since not all analyses were performed for both 2009 and 2010 imagery (Table 28), only the virtual transects and visual interpretations can be compared between the two time periods for both sites. In addition, results of image classification analysis can be compared for Port Bay 2009 and 2010 images.

Table 28. 2009 and 2010 imagery analysis.

	East Flats 2009	East Flats 2010	Port Bay 2009	Port Bay 2010
Classification	√	-	√	√
Patch analysis	√	-	√	-
Transect analysis	√	√	√	√

The Port Bay study area and the northern part of the East Flats study area both appeared to have increased vegetation in 2010 when compared to the 2009 imagery. Both of these are primarily *Halodule* seagrass flats.

Comparison of vegetated and bare areas obtained from image classification for Port Bay in 2009 and 2010 indicate a 24% increase in vegetation and a 36% decrease in bare area between the two years of imagery. The most obvious explanation would be the one-month difference in acquisition time for the photography. By late December 2009, there would have been significant die-off of seagrass due to weather conditions, while in mid-November of 2010, there would still have been substantial seagrass present. Moreover, groundtruthing data suggests a possible difference in species composition between the two years. *Ruppia* was reported in November 2010, but was not was reported in December 2009. *Ruppia* is an annual that sometimes reaches high biomass in late fall, but is not present all years. When present in the fall, it then dies off quickly under cold temperatures, and is also readily consumed by waterfowl. Since groundtruthing in 2009 did not report *Ruppia* (and *Halodule* was reported in March 2010), 2010 may have been an unusually productive year for *Ruppia*. Note that comparison of the 2009 and 2010 analyses shows a difference in the total area (or total number of pixels). This small difference of about one percent may result from the analysis being conducted by two analysts using different software packages, one summing results from six areas of interest and one using a single region of interest.

Table 29. Comparison of Port Bay vegetated and bare areas from 2009 and 2010 images.

	2009 acreage	2010 acreage	Percent change
Vegetation	128	158	+ 24 %
Bare	92	59	- 36 %
Total	220	217	

East Flats virtual transects from 2009 were placed only in the southwestern portion of the study area primarily consisting of *Thalassia* beds. There were, on average, eleven transitions in 2009

and eight transitions in 2010 per 100 m transect. In Port Bay in 2009, the average number of transitions per 100 m transect was ten, whereas the average in 2010 was five. The number of transitions in 2009 for both study sites, however, are not robust enough for statistical comparison.

The depth range at deep edge for both study sites remained similar between years. Care should be taken in the interpretation of results, however, due to the difference in sampling periods. Data was collected during April 2010 for the first year and during November 2010 for the second year of analysis.

Conclusions

Baseline data has been collected prior to initiation of wastewater discharge in Port Bay. TPWD intends to resample Port Bay following completion of the wastewater discharge plant. An internal reference site, along with comparison to baseline data, will probably give the most accurate information about wastewater impacts.

This work demonstrated that state staff can accurately and efficiently conduct monitoring and analyze seagrass samples. Seagrass protection in the state will benefit from continued development of this expertise and expansion of sampling efforts coastwide. Seagrass sampling techniques will need to be refined to address limited state resources, while at the same time minimizing redundancy among methods and optimizing the ability to detect real change in seagrass communities. This will most likely be possible after a variety of minimally-impacted and impacted sites have been sampled. In particular, aerial imagery analysis techniques need refinement, due to their high cost not only in capital, but also in GIS analyst time. To maximize use of data, it will be important that all seagrass sampling be conducted according to state-accepted protocols, including having appropriate quality assurance documentation (QAPPs), adhering to SWQM guidance and using NELAC-certified laboratories. State staff will benefit from continuing dialogue with academic seagrass experts to develop breadth and depth of knowledge in interpreting data.

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Appendix A. UTMSI Protocols

Percent Surface Irradiance and Light Attenuation

Ken Dunton and Kim Jackson
Revised December 2009

Field Measurements

Measurements of percent surface irradiance (% SI) and the diffuse light attenuation coefficient (*k*) are made from simultaneous measurements of surface (ambient) and underwater irradiance. Measurements of photosynthetically active radiation (PAR = ca. 400 to 700 nm wavelength) are collected on the surface using an LI-190SA quantum-sensor that provides input to a Li-Cor datalogger (LI-COR Inc., Lincoln, Nebraska, USA). Underwater measurements are made using a LI-192SA or LI-193SA sensor. Measurements of % SI and *k* are based on three or more replicate determinations of instantaneous PAR collected by surface and underwater sensors and recorded by the datalogger. Care is taken to reduce extraneous sources of reflected light (from boats or clothing).

Light attenuation will be calculated using the transformed Beer Lambert equation:

$$K_d = -[\ln(I_z/I_0)]/z$$

where *k* is the attenuation coefficient (m^{-1}) and I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively. Percent surface irradiance available at the seagrass canopy will be calculated as follows:

$$\% \text{ SI} = (I_z/I_0) \times 100$$

where I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively.

Nutrient Methods: Nitrate+Nitrite, Phosphate, Silicate, Ammonia

UTMSI SOP 0201

1. Introduction

1.1 Modern colorimetric assays are based on a limited number of modifications of the same basic chemistry. In the following text, the Lachat protocol will be described with figures included to show detection limits and precision.

1.2 Nutrients will be analyzed using a Lachat Quikchem 8000. The system is fully automated and generates data reports that contain standard curve, sampling statistics and sample concentrations. The latter are reported as both volts and concentration units. The software is capable of ignoring refractive index peaks by gating the integration window to periods where only the sample is in the optical cell. The electronics of this system are so stable that the system can utilize a 1 cm cell and achieve the same detection levels as older 5 cm cells on segmented flow analysis systems.

1.3 The Lachat protocol differs from the methodology described in current EPA methods require the use of segmented flow analysis. The nutrient methods described below use flow injection technology proprietary to Zellweger Analytics. The sensitivity and precision of flow injection is the same as that of segmented flow, however, the data processing and actual plumbing of the sampling manifold is proprietary to the manufacturer (Zellweger Analytics).

2. Field Procedures

2.1 Sample Collection:

2.1.1 At each station, water samples will be collected at the surface.

2.1.2 Two 10 ml sub-samples will be collected and filtered on site using a hand syringe and ~0.7 µm glass fiber filter.

2.2 Sample Preservation:

2.2.1 Samples will be stored in 15 ml capped tubes on dry ice while in the field and frozen on return to the lab.

2.2.2 Samples will be kept frozen until analyzed, no more than 30 days. Analysis is typically within 10 days of sample collection.

3. Sample Handling

3.1 Samples will be thawed prior to analysis. Typically, the sample tube is placed directly into the Lachat manifold. This minimizes sample handling and the introduction of potential error.

4. Analytical Methods

4.1 Standards and Blanks -- The calibration curve for each nutrient species analyzed is checked beyond the linearity criteria using verifiable and traceable second source standards where available. Recoveries of all nutrient analytes from the seawater matrix are tested and documented.

4.1.1 Each sample run consists of 5 standards

4.1.2 Two samples of deionized water (to establish a baseline)

4.1.3 Two samples of Gulf of Mexico water (lowest nutrient seawater available used to check software integration)

4.1.4 Two samples of Gulf of Mexico seawater amended with nitrate to equal the midrange standard (matrix spike to check recovery of nitrate from seawater)

4.1.5 A nitrite sample equal to the midrange standard (to be compared with the nitrate sample in order to verify Cd column efficiency)

4.1.6 The standard addition sample (matrix spike) and the Gulf of Mexico blank are run every 20 sample to verify instrument performance. The matrix spike will include nitrate, nitrite, phosphate, silicate, and/or ammonia as appropriate to the analysis being conducted.

4.2 Nitrate and/or Nitrite in Brackish Waters or Seawater (Lachat Quikchem method 31-107-04-1-A)

4.2.1 Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrate) is then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion is coupled with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting pink dye absorbs at 520 nm. This is the same chemistry used in EPA method 353.4.

4.2.2 Though this method is written for seawater and brackish water, it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of

varying salinity (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 540 nm. The salt effect is less than 2%. The applicable range is 0.03 to 5.0 µM. The method detection limit is 0.03 µM N. The method throughput is 48 injections per hour.

4.2.3 Standard curves are linear (Fig. 1) and will be accepted only when the r^2 ≥0.995.

4.2.4 Precision exceeds 1% at the 1.25 µM level in analysis of 10 samples in an optimal laboratory system. Field handling reduces precision to about 5% (Fig. 2). Carryover is negligible.

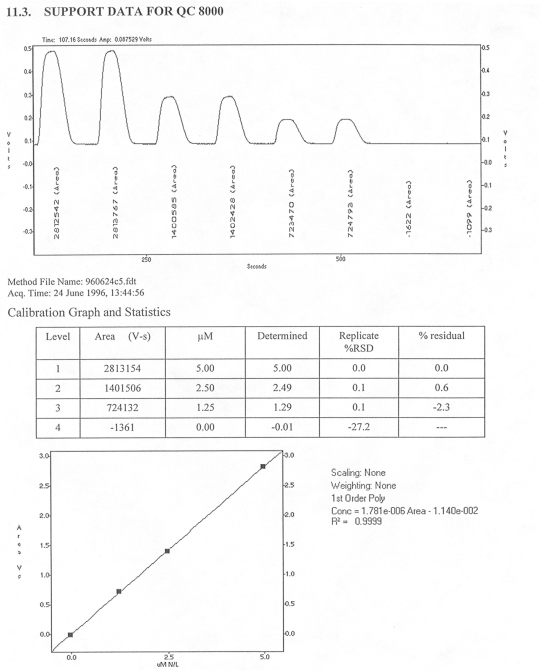


Fig. 1. Standard curve data for nitrate+nitrite analysis using Quikchem method 31-107-04-1-A.

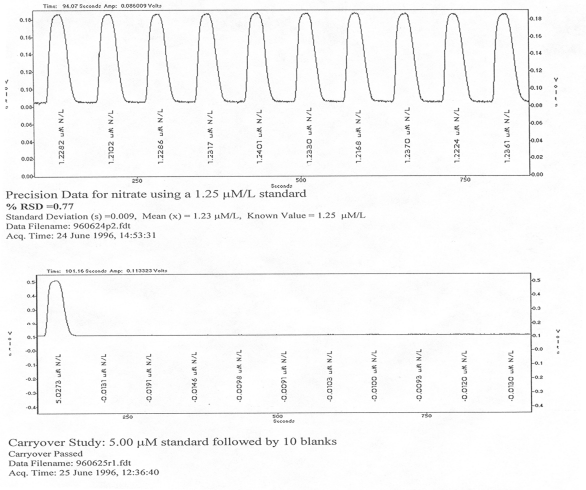


Fig. 2. Precision and carryover of nitrate+nitrite analysis using Quikchem method 31-107-04-1-A.

4.3 Phosphate in Brackish Water or Seawater(Lachat Quikchem method 31-115-01-3-A)

4.3.1 Orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a yellow complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The ascorbic acid and molybdate reagents are merged on the chemistry manifold and the reagent stream is then merged with the carrier stream. The sample zone appears at the detector less than 10 seconds after injection. The absorbance is

proportional to the concentration of orthophosphate in the sample. This method is written for seawater and brackish water but is also applicable to non-saline sample matrixes. The applicable range is 0.03 to 2.00 µM. The method detection limit is 0.03 µM. The method throughput is 48 injections per hour.

4.3.2 The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinity (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 880 nm. The salt effect was less than 2% as measured in Sargasso seawater.

4.3.3 Standard curves are linear (Fig. 1) and will be accepted only when the r^2 $\geq$0.995.

4.3.4 Precision exceeds 1% at the 0.5 µM level in analysis of 10 samples in an optimal laboratory system. Field handling will reduce precision to roughly 5% (Fig. 2). Carryover is negligible.

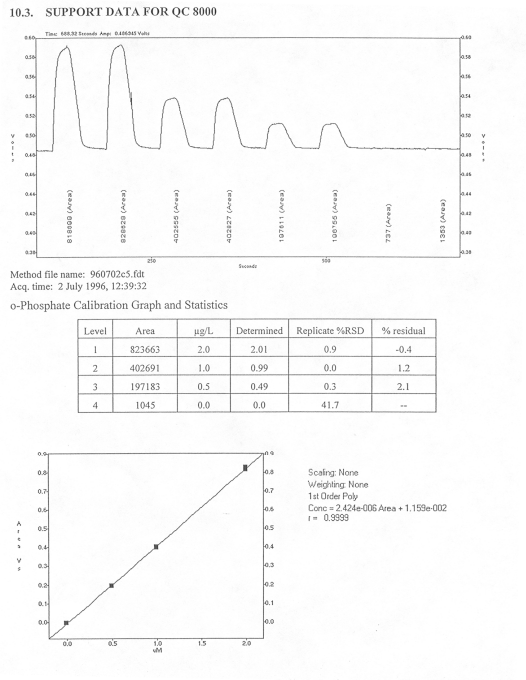


Fig. 1. Standard curve data for orthophosphate analysis using Quikchem method 31-115-01-3-A.

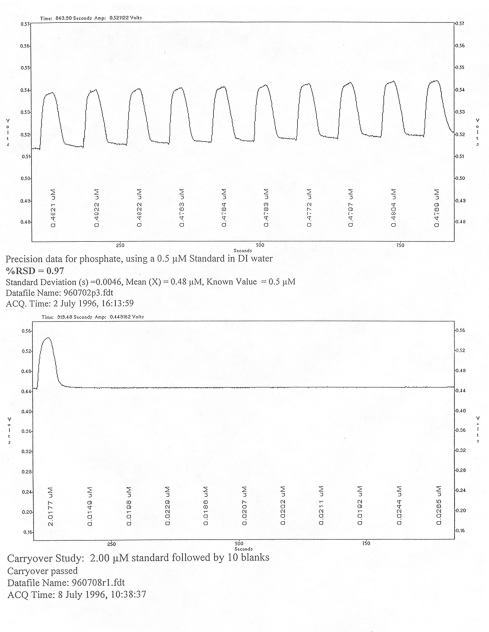


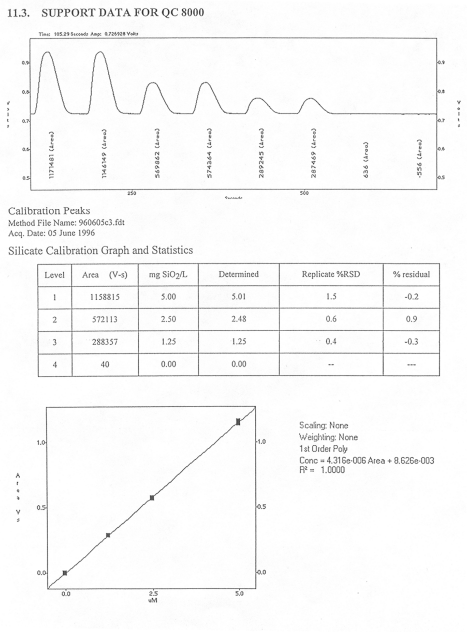
Fig. 2. Precision and carryover of orthophosphate analysis using Quikchem method 31-115-01-3-A.

4.4 Silicate in Brackish or Seawater (Lachat Quikchem method 31-114-27-1-B)

4.4.1 Soluble silica species (silicic acid) react with molybdate at 37* C and pH of 1.2 to form a yellow silicamolybdate complex. This complex is subsequently reduced with stannous chloride to form a heteropoly blue complex which has an absorbance maximum at 820 nm. The intensity of the color is proportional to the concentration of molybdate reactive silica. Though the method is written for Brackish and Seawater, it is also applicable to non-saline sample matrixes.

4.4.2 The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 820 nm. The applicable range is 0.03 to 5.00 µM SiO_2 L^{-1}. The method detection limit is 0.03 µM SiO_2 L^{-1}. The method throughput is 48 injections per hour.

4.4.3 Precision exceeds 1% at the 1.25 µM level in analysis of 10 samples in an optimal laboratory system (Fig. 2). Field handling will reduce to roughly 5%. Carryover is negligible.



Level	Area (V-s)	mg SiO_2/L	Determined	Replicate %RSD	% residual
1	1158815	5.00	5.01	1.5	-0.2
2	572113	2.50	2.48	0.6	0.9
3	288357	1.25	1.25	0.4	-0.3
4	40	0.00	0.00	--	---

Fig. 1. Standard curve data for silicic acid (soluble silica) analysis using Quikchem method 31-114-27-1-B.

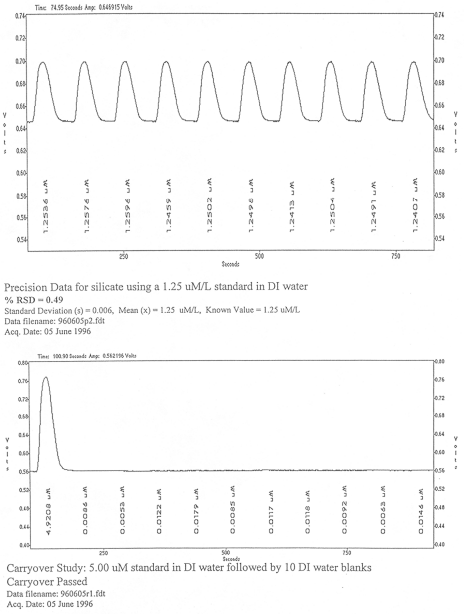


Fig. 2. Precision and carryover of silicic acid (soluble silica) analysis using Quikchem method 31-114-27-1-B.

4.5 Ammonia in Brackish Water or Seawater (Lachat Quikchem method 31-107-06-1-A)

4.5.1 This method is based on the Berthelot reaction. Ammonia reacts in alkaline solution with hypochlorite to form monochloramine which, in the presence of phenol, catalytic amounts of nitroprasside (nitroferricyanide) and excess hypochlorite, gives indophenol blue. The formation of monochloramine requires a pH between 8 and 11.5. At higher pH, ammonia may begin to oxidize to nitrate. At pH greater than 9.6, some precipitation of calcium and magnesium as hydroxides and carbonates occurs in seawater, but these ions may be held in solution by complexing them with EDTA. The indophenol blue measured at 630 nm is proportional to the original ammonia concentration. This is the same chemistry as in EPA method 349.0 modified for flow injection analysis. Though the method is written for Seawater and Brackish water, it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 630 nm. The salt effect was less than 2% as measured in tropical Pacific surface seawater.

4.5.2 At seawater pH, ammonia (NH_3) exists as the monovalent cation ammonium (NH_4^+). The analytical method measures both as ammonium.

4.5.3 Standard curves are linear (Fig. 1) and will be accepted only when the $r^2 \geq 0.995$.

4.5.4 Precision exceeds 1% at the 0.5 µM level in analysis of 10 samples in an optimal laboratory system. Handling in the field will reduce this to a nominal value of 5% (Fig. 2). Carryover is negligible.

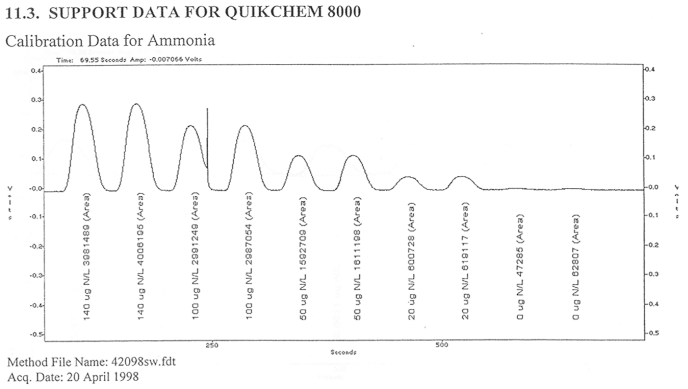
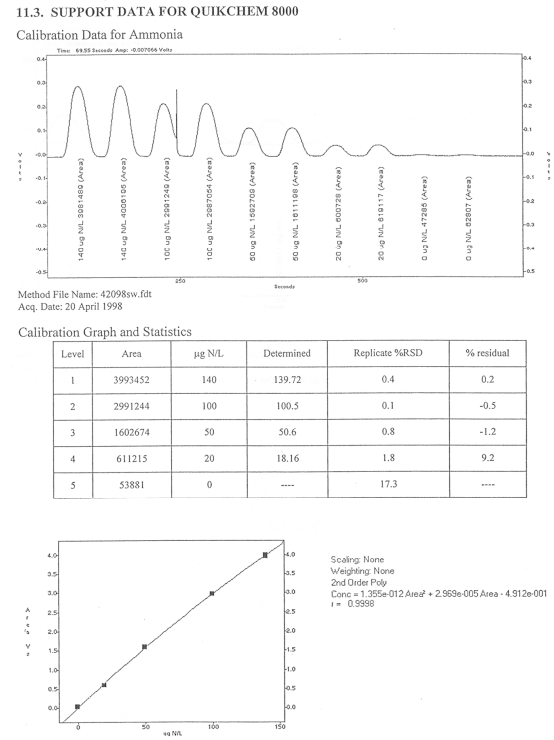


Fig. 1. Standard curve data for ammonium analysis using QuikChem Method 31-107-06-1-A.



Level	Area	µg N/L	Determined	Replicate %RSD	% residual
1	3993452	140	139.72	0.4	0.2
2	2991244	100	100.5	0.1	-0.5
3	1602674	50	50.6	0.8	-1.2
4	611215	20	18.16	1.8	9.2
5	53881	0	----	17.3	----

Fig. 2. Precision and carryover of ammonium analysis using QuikChem Method 31-107-06-1-A

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Water Column Chlorophyll a Extraction

Updated November 2009
Ken Dunton/Kim Jackson

Adapted from: ESS Method 150.1: Chlorophyll – Spectrophotometric, Environmental Sciences Section, Inorganic Chemistry Unit, Wisconsin State Lab of Hygiene, 465 Henry Mall, Madison, WI 53706. Equation for chlorophyll *a* from Jeffrey and Humphrey (1975).

1.0 Introduction

Chlorophyll *a*, a characteristic algal pigment, constitutes approximately 1% to 2% (dry weight) of planktonic algal biomass. This feature makes chlorophyll *a* a convenient indicator of algal biomass. This method is applicable to most surface waters.

2.0 Summary of Method

Algal cells are concentrated by filtering a known volume of water through a membrane filter (25 mm, 0.45 µm pore size nitrocellulose filter). The pigments are extracted from the concentrated algal sample in an aqueous solution of 90% acetone. The chlorophyll *a* concentration is determined spectrophotometrically by measuring the absorbance or optical density (OD) of the extract at various wavelengths. The resulting absorbance measurements are then applied to a standard equation.

3.0 Sample Preservation and Preparation

1) Chlorophyll *a* samples are placed in a dark cooler and packed on ice at the time of collection.
2) All chlorophyll work is carried out in low light conditions (all overhead lights must be off) since light degrades chlorophyll pigments. Arrange the filtering manifold, seawater trap, and vacuum pump (or aspirator) on the lab bench.
3) Using forceps, place a 0.45 µm pore size nitrocellulose filter on each filtering funnel, and filter a known volume (measure with a graduated cylinder) of sample (in the dark), applying vacuum until the sample is dry. The amount of sample required depends on the phytoplankton density in the water sample. For coastal waters, filter in 50 ml increments. When water flow begins to slow, continue to filter small amounts of water until flow almost ceases.
4) Record the volume filtered for each sample.
5) If samples are spectrophotometrically at a later date, fold the filter in half and wrap in pre-labeled aluminum foil or opaque tubes (or wrap test tube wrap with black plastic bag) and freeze. If samples are run immediately, proceed to step 4.0.

4.0 Procedure

1) Place the filter containing the concentrated algal sample in a pre-labeled test tube.
2) Add 5 mL of 90% acetone solution (i.e., 900 ml of acetone mixed with 100 ml of double distilled or ultrapure water).
3) Cap tightly, vortex or shake until filter dissolves.
4) Repeat until the all samples are processed.
5) Create two blanks using 5 ml acetone solution and new unused filter.

6) Wrap test tube rack in a black plastic and place samples in a freezer. Allow extraction to occur overnight (up to 24 hr).
7) Remove samples from freezer. Keep samples covered in low light conditions at all times.
8) Clarify extract by centrifuging samples for 15 minutes at approximately 5000 g. Remember to balance the centrifuge (i.e., put equal number of samples on each side).
9) Turn on spectrophotometer and allow to stabilize while samples are centrifuging.
10) Remove samples from centrifuge. DO NOT SHAKE! Rewrap test tube wrap in black plastic and take samples to spectrophotometer.
11) Carefully transfer the two blanks to the two 1.0 cm cuvettes. Pour using a continuous motion.
12) Set up the spec to measure absorbances at: 750, 664, 647, 630, and 600 nm.
13) Auto zero the spec with the blanks (make sure clear sides of cuvettes are facing away from you when you place in spec).
14) Remove closest cuvette. Empty contents into waste container. Pour first sample into cuvette. Do not shake and only pour once into cuvette after centrifuging.
15) Place cuvette into the slot vacated by the blank. Push Read Sample.
16) Repeat for remaining samples.
17) When finished save output file on computer hard drive and on a floppy disk.

5.0 Calculation

Subtract the absorbance at 750 nm from the 630, 647, and 664 nm values for the turbidity correction, and then use the corrected values in this equation:

$$\mu g \text{ chl } a \text{ } L^{-1} = \frac{S\,[11.85\,(Abs664) - 1.54\,(Abs647) - 0.08\,(Abs630)]}{V}$$

Where S = volume of acetone used for the extraction (mL)
V = volume of water filtered (L)
L = cell path length (cm; this is normally 1 cm for the cuvettes we use)

Total Suspended Solids

Updated November 2009
Ken Dunton/Kim Jackson

Adapted from: EPA METHOD #: 160.2

1.0 Scope and Application
This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of the determination is 4 mg/L to 20,000 mg/L.

2.0 Summary of Method
A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C. The filtrate from this method may be used for Residue, Filterable. Residue, and Non-Filterable. These are defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103-105°C.

3.0 Sample Handling and Preservation
Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result. Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

4.0 Interferences
Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results. Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.

5.0 Procedure
1) Place the glass fiber filter (i.e., Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent Our lab uses 47 mm GF/F 0.7 micron retention on the membrane filter apparatus. NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", collection efficiencies and effective retention are used to define this property in glass fiber filters.

2) Dry new filters at 60C in oven prior to use.

3) Weigh filter immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.

4) For a 47 mm diameter filter, filter 100 mL of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 mL/cm of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above. Note: If filtering clear pristine water, start with 1L. If filtering turbid water start with 100 m.

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five mL increments for timing are suggested. Continue to record the time and volume increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

5) Assemble the filtering apparatus and begin suction.
6) Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected to the filter using a graduated cylinder. Pour into funnel.
7) Remove all traces of water by continuing to apply vacuum after sample has passed through.
8) With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of distilled rinse water used should equal no less than 50mls following complete filtration of sample volume.

9) Carefully remove the filter from the filter support.
10) Dry at least one hour at 103-105°C. Overnight insures accurate filter weight.
11) Cool in a desiccator and weigh.
12) Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

6.0 Calculations

Calculate non-filterable residue as follows, where:

A = weight of filter (or filter and crucible) + residue in mg
B = weight of filter (or filter and crucible) in mg
C = mL of sample filtered

$$1000*(A-B)*1000/C=TSS\ mg/L$$

Assay for Sediment and Water Column Ammonium

Ken Dunton and Kim Jackson
Revised December 2009

Adapted from: Parsons, T.R., Y. Maita and C.M. Lilli. A Manual of Chemical and Biological Methods for Seawater Analysis. Determination of Ammonia (The Alternative Method). Pergammon Press, New York, 1st edition, 173 pp.

Field Procedures

Collection and Storage: Water
Collection bottles are acid washed before use. At the sampling station, bottles and their lids with sample water are rinsed several times prior to filling with actual sample. Leave approximately 1 cm of air space at the top of bottle to prevent water expansion during freezing from cracking the bottle. Immediately place samples on ice in field, and freeze as soon as possible upon return to the lab. DO NOT STORE SAMPLES IN REFRIGERATOR. Allow samples to thaw completely before taking a sub-sample. Bottles can be thawed overnight in a refrigerator. Standard curve and reagents are calculated for 2.5 ml samples. Larger or smaller volumes may be used, but reagent volumes must be multiplied or divided accordingly.

Collection and storage: Sediment
A 2.5 cm core is taken from the top 10 cm of sediment. Extrude the sample into a 50 ml polycarbonate bottle. If using Whirl Pacs, remove the air from the bag, and seal the bag tightly. Immediately place samples on ice in field, and freeze as soon as possible upon return to the lab. DO NOT STORE SAMPLES IN REFRIGERATOR. Allow samples to thaw completely. Samples can be thawed overnight in a refrigerator.

Laboratory Procedures

Once thawed, homogenize samples by stirring with glass rod or squeezing the bag with your hands several times. Fill an individually pre-labeled plastic centrifuge tube ~ ¾ full of sample. Weigh each sample + centrifuge tube to the nearest 0.1 g and match with another sample weighing the same. This assures that the centrifuge is balanced prior to use. Cap each centrifuge tube immediately after weighing to prevent evaporation from the sediment sample. Place the two samples weighing the same opposite each other in the centrifuge. Continue until all eight places are full. Hand tighten the centrifuge lid, shut the door, and set the centrifuge to run for 10-20 min (depending on soil moisture) at 10,000 rpm.

Reagents

1) Ultra pure water: Double deionized reverse osmosis water found in Tracy Villareal's lab.
2) Phenol alcohol: Dissolve 5 g reagent-grade phenol in 50 ml 95% ethanol. Store in dark and in refrigerator.

3) Sodium nitroprusside solution: Dissolve 0.5 g sodium nitroprusside in 100 ml ultra pure water. Store in dark and in refrigerator. Solution is stable for at least one month (a color change to brown indicates that the solution should be re-made).
4) Alkaline solution: Dissolve 80 g sodium citrate and 4.0 g NaOH in 400 ml ultra pure water. Solution is stable indefinitely.
5) Sodium hypochlorite: Use commercially available hypochlorite (e.g. Ultra Clorox-do not use cheap brands) which should be about 1.5 N. The solution decomposes slowly and should be checked periodically (see note 4). A new bottle of bleach should be purchased approximately every month, or before running a new standard curve.
6) Oxidizing solution: Mix 5 ml alkaline solution with 1.25 ml of sodium hypochlorite. This makes enough for ~25 samples. Adjust volumes for larger sample sizes. Keep covered when not in use and prepare fresh each day samples are run (i.e. do not use from previous days).

Standard Curve

Add 133.7 mg NH_4Cl (FW = 53.49 g $mole^{-1}$) to a volumetric flask and bring to 500 ml with ultra pure water (= 5 mM). Add 1 ml of this solution to a volumetric flask and bring to 100 ml with ultra pure water (= 50 µM). Standard curve and reagents are calculated for 2.5 ml samples. Larger or smaller volumes may be used, but reagent volumes must be multiplied or divided accordingly. Dilute stock solution as follows:

Stock solution	Blue Water	µg NH_4^+/2.5 ml	µM NH_4^+
0.0 ml	2.5 ml	0.00	0
0.5 ml	2.0 ml	0.45	10
1.0 ml	1.5 ml	0.90	20
1.5 ml	1.0 ml	1.35	30
2.0 ml	0.5 ml	1.80	40
2.5 ml	0.0 ml	2.26	50

Run at least three replicates (n = 3) for each concentration.

µM concentrations can be calculated from µg NH_4^+/2.5 ml by multiplying by 22.2.

For example, (1 µg NH_4^+/2.5 ml) x (1000 ml/L) x (1 µmole NH_4^+/18 µg NH_4^+) = 22.2 µmole/L

Samples

1) Rinse a test tube rack (use rack with small holes)
2) Set up test tubes in rack (label with site, replicate # if necessary)
3) Add 2.5 mL of water sample or standard (use 5000 µL pipette set to 250; use new pipette tip for each water sample) to corresponding test tube. Dilute as necessary with low-ammonia seawater (i.e. "blue water"). For example, for sediments, dilute 0.5 ml sample with 2.0 ml "blue water". Make sure to run two blue water blanks with your samples and two standards.
4) FROM THIS POINT ON, SAMPLES SHOULD BE PROCESSED IN THE HOOD.
5) Add 0.1 ml phenol alcohol. (Use digital pipette. Hit F, then 2, then make sure it says 1000 µL, hit enter, enter 100 µL, hit enter, enter 10, hit enter, place pipette into phenol alcohol, hold down button on side until it beeps, put pipette in first sample, push down button

until it beeps, then move to next sample and do same, after dispensing in all ten samples, put pipette over phenol and hit the C button to clear any left in pipette, repeat for next set of samples); vortex and wait one minute. (Note: rinse the pipette with the phenol alcohol first to prevent dripping; i.e. withdraw some up into pipette and empty back into phenol container; use the digital pipette.)

6) 0.1 ml sodium nitroprusside solution (use digital pipette); vortex and wait one minute.
7) 0.25 ml oxidizing solution (use digital pipette set to 4 dispenses of 250 µL); vortex and wait one minute.
8) Mix samples thoroughly. Cap or cover with parafilm and allow to develop for 1 hr in the dark and at room temperature.
9) Record absorbance at 640 nm on spectrophotometer. Make sure to auto zero spectrophotometer to blue water blanks that have had chemicals added to them as well. Read standards first and again every 10 samples to ensure spec is running properly.
10) Regress samples absorbance to standard curve (take into account the dilution factor).

Notes

1) Use glassware which has been cleaned with 10% HCl and rinsed thoroughly with DIN free RO water.
2) Use 10 ml disposable test tubes for ease with vortexing and cleaning.
3) Freezing sediment cores may alter the distribution of NH_4^+ because ice crystals may change pore size and as the core freezes, salinity increases in unfrozen porewater and NH_4^+ may be pulled off the sediment and detrital particles.
4) See Parsons *et al*., 1984. Use only Clorox bleach, no "off" brands. When having difficulties with low readings, check age of bleach.
5) Great care is necessary to reduce contamination from external sources. Latex gloves should be worn at all times and regularly changed during the analyses. No use of ammonia for other purposes should be allowed in the same lab. Do not move back and forth between ammonium and nitrate+nitrite sample processing stations, particularly when using stock solution of ammonium. This can contaminate work areas. Do not transfer scissors, sharpies, etc. between workstations.
6) Sample color is stable for ~ 24 hr after the reaction period, if kept out of direct light.
7) Do not dilute samples with deionized distilled water (use "blue water"); this could upset the ion balance and affect NH_4^+ concentration.
8) If the colorimetric reaction seems somehow not right, check the pH and make sure that it does not exceed 9.8. Also, more than 0.25 ml of oxidizing solution may have to be added.
9) Range: 0.1 to 50 µM. It's important to remember this range when diluting samples, especially sediment porewater.

Sediment Grain Size

Updated December 2009
Ken Dunton/Kim Jackson
(adapted from Rick Kalke, Paul Carangelo and Dr. E.W. Behrens)

Field

Three replicate sediment samples are collected at each station using a plastic syringe (2.5 cm diameter, 10 cm length) driven up to 10 cm into the seafloor (depth is determined by sediment compactness). These samples are placed in pre-labeled Whirl Pak bags and immediately placed on ice for transport to the lab.

Lab

Samples are either immediately frozen or processed upon return to the lab. If samples are frozen, the samples will be thawed overnight in a refrigerator prior to processing.

To determine sediment grain size, sand/silt/clay ratios are determined following the methods of Folk (1964). Percent contribution by weight is measured for four components: rubble, sand, silt, and clay. A 20-ml sediment sample is mixed with 100 ml of 3% hydrogen peroxide and 75 ml of de-ionized water to digest organic material in the sample. The sample is then wet sieved through a 62µm mesh stainless steel screen using a vacuum pump and a Millipore Hydrosol SST filter holder to separate rubble and sand from silt and clay. After drying, the rubble and sand will be separated on a 250µm screen. The silt and clay fractions will be measured using pipette analysis. Briefly, the settling velocity will be used to classify the particles and to determine the percent composition of each fraction, based on weight.

Step by Step Procedure

1. Wash all glassware with detergent (Alconox) and rinse with distilled or deionized water.
2. Extract 20 cc of homogenized sample using a wide-mouth syringe, or fill syringe using a spatula.
3. Place sample in a labeled beaker and add 100 ml 3% hydrogen peroxide. Mix with a rubber policeman and let sit until liquid is clear (several days). This step is to digest the organics in the sample.
4. Weigh labeled aluminum weighing pans and 62µm stainless steel filters; one each for each sediment sample.
5. Decant excess hydrogen peroxide (as much as possible without stirring up the sample). Add approximately 100 ml deionized water to the sample; stir and filter using a vacuum pump and a Millipore Hydrosol SST Filter Holder with 62um screen. Repeat two more times or until water is more or less clear. Dump the remaining sand and rubble onto the filter; rinse beaker and rubber policeman. (Don't use more than 900 ml water in this step).
6. Place sand and screen in weighing pan and dry at 100-130 degrees C for at least 24 hours.
7. Pour the filtrate in a 1-liter graduated cylinder. Add 10 ml 10% Calgon dispersant and dilute to 1 liter. After all samples have sat long enough to be close to room temperature, take water

temperature and refer to temperature/fall distance table to determine the length of time between the first and second withdrawals.

8. Weigh and label two beakers (A and B) for each sample.
9. Stir the samples uniformly, working with one sample at a time. Twenty seconds after stirring, insert a pipette to a depth of 20 cm and withdraw 20 ml of the suspension. Put first withdrawal in the pre-weighed A beaker. Rinse pipette with deionized water into A beaker. Proceed with other samples.
10. Second 20 ml withdrawal is taken from a depth of 10 cm at the time indicated on the temperature/fall distance table and placed in the pre-weighed B beaker. Rinse pipette with deionized water.
11. Place A and B beakers into the drying oven and dry at 100-130 degrees C for at least 24 hours.
12. After pans of sand and beakers have dried completely, remove from oven and allow them to cool to room temperature and equilibrate with the humidity in the atmosphere (one to two hours).
13. Weigh each beaker to the nearest 0.001 g.
14. Weigh one aluminum pan (no screen) to the nearest 0.001 g. This pan will be used to weigh the sand fraction of each sample and can be used repeatedly without re-weighing each time. (Just be sure you get all of the sand out).
15. Weigh each pan + screen + sand + rubble to the nearest 0.001 g for each sample. Dump pan contents into a 250µm geological sieve, making sure to get all the sand out of the pan and off the screen. Sieve the sample. Pour the sand into the pre-weighed aluminum pan from step 14; weigh to the nearest 0.001 g.

Notes

1. (Weight of silt/clay in the A beaker) - 0.02 (weight of dispersant/20 ml) x 50 = total weight of silt/clay (call this F) in the total sample. Percent silt equals 100 x (F- weight of clay)/(S + F).

2. (Weight of clay in the B beaker) – 0.02 (weight of dispersant) x 50 = total clay in sample. Percent clay = 100 x weight of clay/(S +F).

3. The percent of sand in the sample is 100S/(S + F), where S is the sand fraction.

Reference

Folk, R.L. 1961. Petrology of Sedimentary Rocks. Hemphills Press. Austin, Texas. 154 p.

Sediment Organic Carbon

Updated December 2009
Ken Dunton and Kim Jackson

Field Procedures

Three replicate sediment samples are acquired at each station using a plastic 60cc syringe (2.5 cm diameter, 10 cm length with end removed) for sampling. Push the syringe into the sediment 10 cm deep. The samples are placed in pre-labeled Whirl Pak bags and immediately placed on ice for transport to the lab.

Lab Procedures

Samples are either immediately frozen or processed upon return to the lab. If samples are frozen, the samples are thawed overnight (in the refrigerator) prior to processing. Samples are homogenized, placed in aluminum weighing tins and dried in a 105 ºC oven (to remove water) for 12-24 hr. Samples are then removed from the oven and placed in a dessicator (to prevent moisture from the air changing the sample weight) to cool to room temperature. Once cooled, samples are weighed to the nearest 0.1 g, and placed in a muffle furnace to combust organic material, at 550 ºC for 4 hr. After cooling samples to room temperature in a dessicator, the samples are reweighed and Loss on Ignition (LOI) calculated using the following formula, where DW is sample dry weight (in grams):

LOI_{550} (as a percentage) = $((DW_{105} - DW_{550}) / DW_{105}) \times 100$

The weight loss is proportional to the amount of organic carbon contained in the sample.

Quantitative Measurements of Seagrass and their Algal Epiphytes

Ken Dunton, Kim Jackson, and Chris Wilson
Revised December 2009

Field Procedures

Transect Lines and Calculation of Seagrass Cover

We can employ three different techniques to assess vegetative percent cover. The first is the Braun-Blanquet method, which can be applied to both seagrasses and benthic macroalgae. At each site a 50-m transect is established by extending a meter tape along the bottom (preferably in an up-current direction in the absence of a depth gradient). Ten quadrats (0.25 m^2) are placed along each transect at pre-determined random distances from the marker rods at the "0 meter" mark. A new set of random sampling positions are chosen before each visit to a site. Each quadrat is examined underwater by a diver. All seagrass species occurring in the quadrat are listed, and a score based on the cover of the species in that quadrat is assigned (Table 1). Cover is defined as the fraction of the total quadrat area that is obscured by a particular species when viewed from directly above.

A second more quantitative measure employs 0.25 m^2 quadrats subdivided into 100 5 x 5 cm cells to estimate percent cover of each seagrass species and bare area along the 50 m transect. The transect line will extend (1) perpendicular to an existing depth gradient from shallower to deeper water, *or in the absence of a discernable depth gradient*, (2) perpendicular from the shoreline outwards into the bay. The "0 meter" mark will be oriented at the shallow edge or at the shoreline and the "50 meter" mark will be positioned toward deeper water or towards the bay.

Leaf Area Index (LAI) is a third measure to assess vegetation cover. It is calculated as a product of blade width measurements, shoot length, and shoot density. These data are retrieved from the analysis of seagrasses in cores collected for biomass (see below) at three or more random locations within 5 m of the transect line.

Table 1. Braun-Blanquet abundance scores (S). Each seagrass species will be scored in each quadrat according to this scale.

S	Interpretation
0	Species absent from quadrat
0.1	Species represented by a solitary short shoot, < 5 % cover
0.5	Species represented by a few (< 5%) short shoots, < 5% cover
1	Species represented by a many (> 5%) short shoots, < 5% cover
2	Species represented by many (> 5%) short shoots 5%-25% cover
3	Species represented by many (> 5) short shoots, 25%-50% cover
4	Species represented by many (> 5) short shoots, 50%-75% cover
5	Species represented by many (> 5) short shoots, 75%-100% cover

Seagrass Biomass
Three replicate cores are used for estimates of above- and below-ground biomass. A 15 cm (ID) diameter corer is used to sample *Thalassia,* and a 9 cm ID diameter core is used to sample *Halodule, Syringodium, Ruppia,* and *Halophila*. Samples are taken of each species present within 5 m of the transect line at each site. Species present (i.e. seagrass species composition) will be determined by visual *in situ* analysis of plants observed within a 25 m radius of each site. Samples are placed in pre-labeled Ziploc bags and immediately placed on ice. A PVC or polycarbonate core is used for the collection of belowground and above ground material. Care is taken to keep only the shoots that actually belong in the core.

Following placement of the large 15-cm core on the seabed, the rubber stopper is removed from the top of the core. For both 9-cm and 15-cm cores, before pressing the corer into the sediment, the diver runs their fingers carefully around the bottom of the core. If grass has been pulled under the core, it is removed. The diver then presses and twists the core down into the sediment 10-15 cm. The stopper is re-installed in the 15-cm core, and the core rocked back and forth. The diver then works their hand under the core and removes it from the grass bed, making sure to keep their hand under the bottom of the core (to prevent loss of sample).

After emptying the core into the sieve, broken shoots are removed since these are likely exterior shoots that were cut by the core tube. Samples are placed in pre-labeled Ziploc bags and immediately placed on ice.

Drift Macroalgal Biomass
Drift macroalgal biomass is determined from the collection of all algal material within ten 0.0625 m^2 quadrats. Material from each replicate is placed in sealed plastic bags and then transported to the laboratory in cooled containers. If algal samples will not be sorted to genus (or species), an additional sample will be obtained for elemental composition (C:N) and stable isotope ratio (^{13}C and ^{15}N) analysis.

Laboratory Procedures

Seagrass and Drift Algal Biomass
Aboveground tissue will include leaves (including sheath material) and floral parts, while below-ground tissues will include root and rhizome material. This process begins with the separation of shoots from non-photosynthetic tissues (where the blade turns white). Leaves are carefully cleaned of all attached biota by scraping with a wet cloth or razor blade prior to analysis. Shoots are carefully counted to obtain accurate estimates of density (number of shoots per square meter). The roots and rhizomes are kept separate from the above-ground tissue and placed in separate aluminum envelopes for drying. Sample labels include information on site, species, date collected, shoots or R/R, and number of shoots. Dead plant material will be discarded. The live tissue (shoots, roots, and rhizomes) is dried to a constant weight (60 °C) and weighed to the nearest milligram. The drying process takes 3-5 days. The biomass values for above- and below-ground biomass are used to calculate a root:shoot ratio.

For benthic macroalgae, samples from each quadrat will be cleaned of debris and non-algal material and may be sorted and identified to genus (and species when possible). Samples (sorted

or unsorted) will be dried to a constant weight, weighed, and archived. If sorted, blade tissues from individual species will be dried, weighed, and archived separately. If not sorted, then cleaned samples will be dried and weighed to obtain a total weight.

C:N:P Ratios

Algal blade tissues and seagrass leaf tissue samples obtained directly adjacent to biomass cores is used for C:N:P determination. Tissue samples for C:N:P ratios must be processed within three days of collection or dried at 60 °C for long-term storage. For seagrasses, newly formed leaves (the youngest leaf in a shoot bundle) are gently scraped and rinsed in deionized water to remove algal and faunal epiphytes. Algal tissues must appear healthy and free of epiphytes and debris. These rinsed samples will be dried to a constant weight at 60 °C and homogenized by grounding to a fine powder using a mortar and pestle. Total carbon and nitrogen contents in blade or leaf tissues will be determined from two replicates of each sample by oxidation in a Carlo Erba model EA 1109 CHN elemental analyzer. Phosphorus content will be measured with a modification of the method of Solorzano and Sharp (1980) as described by Fourqurean *et al*. (1992). Molar C:P, C:N, and N:P ratios are then calculated for evaluation of temporal and spatial trends.

Epiphyte Quantification

Estimates of algal epiphytic biomass will be made from separate leaf samples of entire shoots taken directly adjacent to the biomass cores. Leaf samples for epiphytic biomass must be processed within three days of collection. In the laboratory epiphytes are separated from the leaf surface by scraping with a scalpel. Scraped material is then collected and retained on pre-weighed glass fiber filters. The collected epiphytic biomass and scraped seagrass leaves are then dried to a constant weight at 60 °C for determination of dry weight biomass. Algal epiphytic dry weight biomass will be expressed as a percent of total dry weight biomass of seagrass tissue scraped. Estimates of epiphyte biomass made on an areal basis are only possible with *Thalassia* and require accurate measurements of the length and width of the area scraped. This is not a valid procedure for leaves of *Halodule* or *Syringodium*. This is based on the fact that *Halodule* and *Syringodium* leaves are both essentially terete (Fig. 1) and require knowledge of the radius (or diameter) of the leaf for an accurate determination of the surface area, which in turn requires high resolution three-dimensional (e.g. CT) imagery or microscopy.

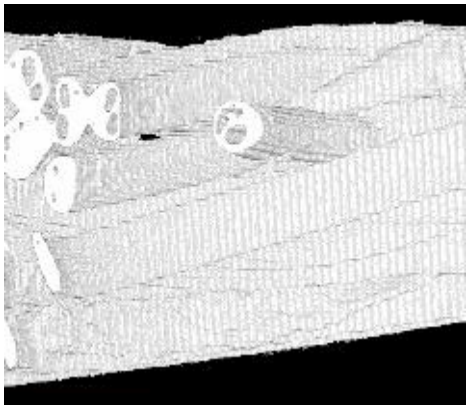

Figure 1. CT imagery of *Halodule* leaves illustrating their tube-like morphology (from Chris Wilson).

Stable Isotope Analysis: Protocol and Procedures

Ken Dunton, Kim Jackson and Patty Garlough
Updated November 2009

General instructions for stable isotope analysis

We analyze excised portions of tissue (for plants) or excised muscle tissue (for animals). When organisms are too small to excise tissues, the entire sample is prepared and combusted for ^{15}N or ^{13}C determination. Gloves are worn at all times for this analysis and organisms must be identified to species or appropriate taxonomic level prior to analysis. Small sample sizes increase the risk of contamination; therefore this process dictates extreme cleanliness. Work areas are kept organized, uncluttered and entirely spotless. Scattered pieces of sample must be removed following each preparation. All utensils are wiped with ethyl alcohol. Field bags, vials, and labels are detailed with organism identity, date, and site. Voucher specimens are often collected to confirm taxonomic identity.

Seagrasses

Samples are collected in well labeled (site, date, rep#, type of sample, species) Whirlpac bags, placed on ice and normally processed within two days upon returning from the field. Blades are scraped and cleaned of epiphytic material using gloved fingers or a paper towel. Scalpels are used to remove encrusting algae or heavily covered epiphytes. Tissue samples are normally taken from base of the shoot, usually the area above white non-photosynthetic section of the blade sheath. Dead or senescent portions of blades or blades or areas with heavy epiphyte coverage are avoided. Tissue samples are rinsed with milli-Q water to remove any loose materials. Each sample, which includes five clean replicate blades from different plants, is placed in a 10 ml labeled vial and dried. After drying for 48 hrs, blades are ground using a Wiggle Bug. All used parts of the Wiggle Bug are cleaned with ethyl alcohol before and after each sample preparation. Ground samples are returned to the vial and placed in a Ziploc bag to maintain dryness.

Algae

Algal samples are collected, cleaned, and catalogued as described for seagrass leaves. Sample sizes are approximately the size of a dime. Place the clean sample in a 10 ml labeled vial. For calcareous algae, half of the sample is acidified since these algae contain calcium carbonate that must be removed prior to isotopic analysis to obtain an accurate C^{13} measurement (two vials, one containing acidified tissue and one not containing acidified tissue, are needed for each sample replicate). The non-acidified sample vials are placed in a Petri dish and dried in oven. Samples are acidified in a dish containing 3% HCL /90% milli-Q water with just enough 3% acid to cover the tissue. Samples are soaked until bubble formation ceases, then decanted of acid and soaked with milli-Q water for 5 min. After excess water is removed, tissues are dried in an oven for minimum of 24 hours at 60C. A mortar and pestle is used to grind samples with all instruments (i.e. mortar, pestle, and spatula) cleaned with ethyl alcohol using Kimwipes. Ground samples are return to vials and placed in a Ziploc bag to maintain dryness.

All samples are run on a Finnigan MATT Delta Plus isotope ratio mass spectrometer (IRMS) interfaced to a Carlo Erba 1500 elemental analyzer.

References

Dunton, K.H. and D.M. Schell. 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: ^{13}C evidence. *Marine Biology* 93:615-625

Dunton, K.H. 2001. $\delta^{15}N$ and $\delta^{13}C$ measurements of Antarctic peninsular fauna: trophic relationships and assimilation of benthic seaweeds. *American Zoologist* 41(1):99-112.

Appendix B. TAMU-CC Standard Operating Procedures

Fluorescence Assessment of Seagrass Epiphyte Accumulation

Introduction

This section describes the procedures used to measure abundance and accumulation profiles of epiphytes on seagrasses. The abundance of epiphytes is often considered to be an integrated measure of nutrient conditions in a seagrass bed, but is the result of complex interactions between a variety of factors (discussed in Borum 1985; Lin *et al.* 1986; Frankovich and Fourqurean 1997; Moore and Wetzel 2000; Hays 2005; Heck and Valentine 2007; Peterson *et al.* 2007; Burkholder *et al.* 2007). Eutrophication affects growth of epiphytes and seagrass leaves directly via nutrition and indirectly by stimulation of phytoplankton and changes in top-down control by grazers and predators. The method described here measures fluorescence of photosynthetic accessory pigments as a proxy for epiphyte abundance. This measure achieves significantly greater spatiotemporal resolution compared to traditional measures of epiphyte biomass. The accumulation profile, a plot of incremental epiphyte abundance along the age gradient of the seagrass leaf, will provide an historical record of epiphyte recruitment and growth *relative* to the growth of the seagrass leaf. This relationship is expected to change with increased eutrophication. The relative leaf areas of seagrass samples can also be estimated with the described method. Fluorescence images of epiphytes can be archived for subsequent development of additional analytical tools such as comparisons of the predominant morphotypes of fluorescent epiphytes.

This novel fluorescence method digitally images and analyzes epiphytic organisms which contain photosynthetic accessory pigments absorbing light in the green range of the visible spectrum (532 nm) and emitting fluorescence at wavelengths between 550 nm and 610 nm (Cammarata 2008). These organisms include cyanobacteria, red algae, diatoms, cryptomonads, brown algae and dinoflagellates (Raven *et al.* 2005; Frouin 2006; Robertson 2009). The pigments primarily responsible for this absorption are phycobilins, fucoxanthin and peridinin (French and Young 1952; Dawson *et al.* 1986). The method is based on the preferential excitation and fluorescence emission signatures of the accessory pigments in epiphytes relative to those of the underlying seagrass leaf which contains only chlorophylls and lutein-based carotenoids.

Scanning seagrass leaves by this method does not quantify green algal components of seagrass epiphytes specifically, because the red excitation light needed to excite the chlorophylls of the green algae also excites the leaf pigments. However, if the epiphytes are removed from the seagrass blade by scraping, then removed epiphytes can be fluoresced and quantified using *both* red and green excitation wavelengths. This will provide a measure that includes all of the different types of epiphytic algae, including green (Cammarata *et al.,* 2009). Perhaps more importantly, any changes in the relative contributions of green and red algae to total epiphyte abundance can be captured by comparing the ratio of red-excited fluorescence to green-excited fluorescence. Previous work has documented changes in primary producer composition by nutrient addition (see for example Armitage *et al.* 2005), so it will be useful to monitor for such changes.

The procedures outlined in this section are designed to produce data of consistent quality meeting an objective of not more than 15% total error in fluorescence quantification of a given sample. In addition, long term comparisons of fluorescence measurements will be facilitated by normalization to measurements of fluorescence standards.

Sample Harvesting

There will be three transects at each of the Port Bay and East Flats study sites. Samples for epiphyte fluorescence measurements will be collected near quadrats representing the shallow end, middle and deep end of each transect. Three replicate samples will be obtained for each significant seagrass species at each quadrat (nine samples per seagrass species for each transect). Seagrass samples will be obtained from locations displaced "up-current" from the transects in order to avoid excessive disturbance caused by other sampling activities. Sampling and fluorescence epiphyte measurements will be performed separately for each seagrass species whose abundance is estimated to exceed 20% of the total seagrass coverage at a quadrat site. Thus, if two species are present at >20% of the total seagrass coverage, separate samples will be obtained for each seagrass species. Single-species seagrass shoot samples (up to 50) will be obtained by gently pinching or cutting off shoots near their base, handling only at the base to avoid disturbing attached epiphytes and transferring to widemouth sample bottles.

Unique identifier numbers/letters will be placed on sample containers and recorded on field sheets. This information will be transferred to a sample log that will accompany samples.

At an appropriate time, samples will be transferred from the field team to a laboratory courier to effect prompt delivery of samples, under the appropriate storage conditions, to the laboratory.

Sample Handling and Storage

Sample processing starts with reception of samples in the laboratory. Upon receipt, each sample identification number will be checked against a copy of the sample log sample identification record. Any discrepancy between sample identification numbers and the sample log, or any missing or damaged samples will be reported to the Project Manager within 24 hours, verbally or in writing.

Within the laboratory, all samples will be carefully tracked by sample number using a laboratory log. It will be the responsibility of the laboratory to keep accurate and timely records of the status of all samples in their custody.

Samples will be stored between 0˚C and 40˚C to avoid freezing and retard evaporative drying. Samples will be stored away from direct sunlight. Samples exposed to environmental extremes or potentially subject to drying due to loosely-fitting or damaged containers will be flagged and described in the sample log. Stored samples must be easily retrieved and protected from environmental extremes.

In the laboratory, samples will be stored at < 20˚C in either an ice chest or a refrigerator. The maximum holding time for sample storage prior to fluorescence scanning will be 72 hours from the time of harvest.

Sample Preparation for Fluorescence Measurements

Seagrass leaves, harvested and stored in sample bottles as described above, will be gently removed from sample bottles and transferred into a shallow tray of distilled water to briefly (< 1 minute) and gently wash off sediments or other unattached debris. Seagrass leaves are to be handled gently and at the base only, to avoid disturbing attached epiphytes. Individual blades will be severed from the shoot at the ligule and transferred to the platen of the scanning fluorescence imager. The number of whole shoots to be scanned will depend on the seagrass species and blade length, but will be at least ten (10) *Halodule* or *Syringodium* shoots, or five (5) *Thalassia* shoots, and not more than 20 of any one species. Only living (green) blades will be scanned. Blades of more than one seagrass species from a single quadrat site may be scanned collectively, but in such case they will be sorted according to species to facilitate separate post-scan analyses. Blades will be positioned lying flat and in a parallel, non-overlapping orientation. Sample washing, transfer to the scanning platen and initiation of scanning will occur within ten minutes to prevent excessive drying. Any blades exhibiting curling will be re-wetted with a few drops of distilled water. The scanning compartment lid will be closed immediately upon loading. The scanned seagrass blades will be scraped to remove epiphytes as described below. The epiphyte-free blades will be dried at 60^{o}C to constant weight for biomass determination.

For measurements capturing the green algal seagrass epiphyte components, and to detect potential shifts in algal family composition, seagrass epiphyte samples removed from the seagrass blades will be transferred into optical 96-well microplates and measured for epiphyte fluorescence. Three (3) to ten (10) whole shoots will be gently washed as described above, transferred to a clean tray containing deionized water (10-50 mL), and gently scraped with the edge of a glass microscope slide to remove the epiphytes from both sides of each blade. Removed epiphytes will be quantitatively transferred to a capped tube and the final volume will be adjusted to a standard volume (typically 14 mL) and stored at 0 – 5 ºC. Fluorescence assays will be made after vigorously vortexing the samples and transferring 50 µL aliquots into the wells of 96-well optical microplates.

Fluorescence Scanning and Quantification

Instrument Settings and Adjustments

For each sample, two types of scans will be obtained. "Green Scans" will utilize green 532 nm laser excitation to excite fluorescence from accessory photosynthetic pigments and "Red Scans" will utilize green 633 nm laser excitation to excite fluorescence from chlorophyll photosynthetic pigments. Standard default instrument settings will be as follows, with any deviation so noted in the laboratory log.

Green Scans

The X:Y coordinates of the platen area to be scanned will be selected.
Acquisition Mode: Fluorescence
Setup Parameters:

- Laser: 532 nm (green) excitation
- Emission Filter: 580 bp 30nm emission filter
- PMT: 360 V

Sensitivity: Normal
Orientation: "R"
Pixel Size: 200 µm for typical quantification/ 10 µm for high resolution archival images
Press: Yes
Focal Plane: Platen (use +3 mm for samples in 96-well plates)

Red Scans
The X:Y coordinates of the platen area to be scanned will be selected.
Acquisition Mode: Fluorescence
Setup Parameters:
Laser: 633 nm (red) excitation
Emission Filter: 670 bp 30nm emission filter
PMT: 360 V
Sensitivity: Normal
Orientation: "R"
Pixel Size: 200 µm for typical quantification/ 10 µm for high resolution archival images
Press: Yes
Focal Plane: Platen (use +3 mm for samples in 96-well plates)

Measurement Calibration with Reference Fluorophores
To facilitate long term data comparisons, it will be important to understand the sensitivity of the fluorescence detection. Excitation laser power and photomultiplier tube (PMT) sensitivity may change over time, so periodic characterization will enable normalization of quantitative fluorescence data. This characterization will be obtained by recording scans of reference fluorophores. Selected reference fluorophores are B-phycoerythrin or eosin Y. Solutions of these will be prepared in 50 mM Tris-Cl buffer, pH 7.5 and quantified by absorbance measurements ($E_M^{545\ nm} = 2.41 \times 10^6 M^{-1}cm^{-1}$; $E_M^{517\ nm} = 78{,}200\ M^{-1}cm^{-1}$; for phycoerythrin and eosin-Y, respectively).

Initial solution concentrations of the reference fluorophores will be adjusted to final concentrations of 0.1 M (phycoerythrin), 0.1 mM (eosin Y) with buffer, and then used to prepare five, 5-fold serial dilutions to span the *relative concentration* range of 1 X to 1/3,125 X. Standard solutions will be aliquotted and stored appropriately (4 ˚C or -20 ˚C, respectively, dark).

For fluorescence measurement characterization, 50 µL of each reference dilution will be pipetted into the 96-well optical plates. Aliquots of buffer will be included as a control. Both red and green scan images will be obtained using the normal sample scanning parameters outlined above. Total fluorescence signal for each standard concentration will be obtained as described below, and plotted to determine linearity and sensitivity. This data will be entered into the QC Summary Sheet for seasonal sampling.

Sample Measurements
For each sample, two types of scans will be obtained. "Green Scans" will utilize green 532 nm laser excitation to excite fluorescence from accessory photosynthetic pigments, and "Red Scans" will utilize red 633 nm laser excitation to excite fluorescence from chlorophyll photosynthetic

pigments. For general quantitative purposes, scans will be obtained using a 200 µm pixel size. In addition, at least one representative high resolution archival image will be obtained for each major seagrass species from a sample obtained near the middle of each transect. The archival images may be useful to identify epiphyte species morphologies. A 10-25 µm pixel size will be used to obtain high resolution green-excited scans.

Upon sample scan completion, the platen will be rigorously cleaned as follows: All samples are removed and debris is gently blotted away with lint-free laboratory wipers. The platen is washed and wiped clean using, in all cases, non-scratching optical wipers with a defined sequence of solutions: distilled water, 70% ethanol, 10% H_2O_2 and distilled water. (Appropriate safety equipment for use of 10% H_2O_2 includes gloves, eye protection and lab coat).

File Naming, Data Storage and Data Backup

Epiphyte image data obtained at 200 µm resolution will be labeled by the unique sample identification number with "red" or "green" appended as a suffix. Data labels for scans performed at 10 µm resolution will additionally be appended with "10u". Raw image data is obtained in a ".gel" file format. Scan data will be saved in this format, and additionally in ".tif" file format. All scan data will be backed up onto a portable hard drive device following scanning of all samples for an individual sampling trip. Sample data will additionally be backed up onto DVD media as well.

Data Processing

Epiphyte image data obtained at 200 µm resolution will initially be quantified from a ".gel" file format using "ImageQuant" software. The image area(s) to be analyzed is (are) delineated with an object box. Then, for each seagrass species on a scan, the following parameters will be determined:

- Total signal above background from green-excited fluorescence
- Total signal above background from red-excited fluorescence
- Total number of pixels above background from red-excited fluorescence

Numerical values will be recorded into a spreadsheet for calculations and analysis.

Epiphyte image data obtained at 10 µm resolution will be archived in both ".gel" and ".tif" file formats for possible future analyses, if warranted, of epiphyte morphologies.

Accumulation profiles indicative of epiphyte recruitment and growth rates, relative to seagrass leaf growth rate, will be quantified as follows: First, the leaf length of representative full-length undamaged blades will be determined from the red scan image by drawing a line from leaf base to leaf tip and multiplying the number of pixels by 200 µm. This length will be divided to obtain the number of standard sized (1-10 mm) optical slices into which the image will be divided. A single-column grid with this number of rows will then be positioned on the leaf image. This grid will then be copied and pasted onto the corresponding green image (representing epiphytes). A volume report will quantify the total green epiphyte signal in each segment of the image ("total signal above background"). The profile of epiphyte accumulation can be obtained by plotting the fluorescence signal versus the position from the leaf base. Plots of accumulation profiles may, if necessary, be smoothed by the "sliding windows" technique that averages quantitative data for groups of windows.

Data Analysis
Data for the following parameters will be compiled into a spreadsheet which will serve as the data quantification log and compared for each site, sampling event, and seagrass species.

- Total red-excited fluorescence signal above background
- Total number pixels red-excited fluorescence above background (an estimate of scanned leaf area for normalizing epiphyte signal measurements; also a proxy for leaf biomass)
- Total green-excited fluorescence signal above background (a measure of total epiphyte load in shoot samples)
- Total green-excited fluorescence signal above background divided by total number pixels red-excited fluorescence above background (a *normalized* measure of epiphyte accumulation based on scanned leaf area)
- The profile of epiphyte accumulation from the leaf base (a measure of epiphyte recruitment and growth relative to the growth of the seagrass leaf)
- The ratio of the total red-excited fluorescence signal above background divided by the total green-excited fluorescence signal above background (only for microplate assays of removed epiphytes)

For example, data on the analyzed parameters will be plotted by sampling date, by site and by seagrass species to observe apparent trends or differences. Statistical analyses will be applied to determine significance.

Processed data will be transmitted to the Project Manager in electronic format, within 30 days of scanning, for collective analysis of total project data as described elsewhere. All processed data will additionally be compiled in the Final Report.

Quality Assurance and Quality Control
Various quality control (QC) procedures will be implemented to ensure consistent production of high quality data. A minimum of 5% of all samples will be re-scanned to produce a parallel set of data for analysis and comparison. The objective is not more than 15% total error in fluorescence quantification of a given sample. For samples scanned directly on the platen, following the first set of green and red scans, the instrument settings will be returned to those of the first scan (*i.e.* green) and then the whole scanning process will be repeated. Duplicate analyses will be documented and noted in the laboratory log.

The results of sample re-scans may require that certain actions be taken. If results from duplicate scans differ by more than 15%, all of the samples from that particular sampling site or transect (to which that duplicate scan applies) will be re-scanned and a random sample selected again for a duplicate scan. If QC criteria are met, sample residues may be discarded. Re-scan results will be summarized on a QC Summary Sheet.

We anticipate continuation of this project, necessitating long term comparisons of fluorescence measurements. These comparisons will be accompanied by comparison of and normalization to measurements of fluorescence reference samples. We cannot predict if, or how much, instrument sensitivity may change over time. But the reference fluorophore measurements

described above will provide a valuable measure of any such changes and permit data normalization for long term comparisons.

Data Management, Reporting and Deliverables

All sample information and numerical data generated in the laboratory are recorded directly onto standardized data forms. Image scans will be archived as described above for future re-analysis. Data forms and sample/file labeling contain all necessary information, so that data is recorded clearly and unambiguously. Completed data forms are kept in notebooks arranged by form type. All sample logs will be digitally recorded by document scanning and saved along with other data. Archived image scans will be stored on an external hard drive and CD/DVD media upon completion of a seasonal sampling event.

The Project Manager will receive electronic copies of this data within 30 days of scanning. Numerical data and forms will be in spreadsheet format while archival pictures will be in ".tif" or ".gel" file formats. A Final Report will be prepared and delivered in electronic format to the Project Manager no later than January 31, 2011.

Data Forms

This section lists example data forms that are used for sample tracking and data recording. Example forms are appended to this document. Examples of data forms are presented in the following order:

Sample Log
Laboratory Log
Quantification Log
QC Summary Sheet
Data Log

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Appendix: Data Forms

Sample Log
Sample ID
Date
Time
Location
Collector
Description
    Site conditions
    Seagrass Species Present
    Collection Container Type
    Observations
Notes
    Damage ?

Laboratory Log
Sample ID
    Checked With Sample Log ?
    Exposure To Environmental Extremes ?
Location, sample type and sample description
Date & Time Received
Receiver
Scan Date & Time
    Scan conditions: Default or Different ?
    Collection Container Type
    Associated Filenames : Default or Different ?
Duplicate Analysis Samples
Notes

Data Quantification Log
Sample ID
    Scan Date & Time
    Associated Filenames
        Total # Pixels Red
        Total Green Fluorescence Above Background
        Total Red Fluorescence Above Background
        Total Green Fluorescence Above Background/ Total # Pixels Red
        Epiphyte Accumulation Profile
        Ratio Total Red Fluorescence Above Background divided by Total Green
    Fluorescence Above Background for microplate assays of removed epiphytes

QC Summary Sheet
Results of Duplicate Scans
    Sample ID & Filenames
        Total # Pixels Red
        Total Green Fluorescence Above Background
        Total Red Fluorescence Above Background
        Total Green Fluorescence Above Background/ Total # Pixels Red

Appendix C. Aerial Imagery Analysis

Methods for Analysis of Aerial Imagery

Ashley C. Summers, TPWD Information Technology Division
January 13, 2011

Analysis of aerial imagery in this study included image classification, generation of landscape metrics and depth limit analysis.

Classification

Image classification refers to creating a thematic map of discrete habitats from continuous photography. In this study, the 2009 images were split into vegetated and bare categories using a semi-automated method developed by Fletcher *et al*. (2009). This method uses saturation and intensity information from the imagery to classify pixels into different habitats.

To prepare the data, imagery tiles for each study site were mosaicked (if necessary) and clipped to a rectangular area of interest (AOI) to reduce file size. The original images, in RGB format, were then transformed to IHS and the bands were separated into individual files. Both the saturation and intensity bands were rescaled to an 8-bit pixel depth to produce pixel values from 0 to 255.

Using ESRI's ArcMap, the bare and vegetated areas were sampled and pixel values from the intensity and saturation bands were recorded. The "pixel threshold" represents the value (between 0 and 255) at which habitat changes between bare (coded as 1) and vegetation (coded as 2). In both Port Bay and East Flats, the threshold between habitats was different depending on location in the image. To compensate, multiple AOIs were created to enable processing several sections of the image with the most appropriate threshold for that area (Table 1 and Table 2). Thresholds, the bands associated with them, and the AOI boundaries were chosen by the analyst after an iterative trial-and-error process and visual interpretation of interim results.

Appendix C. Table 1. East Flats pixel thresholds.

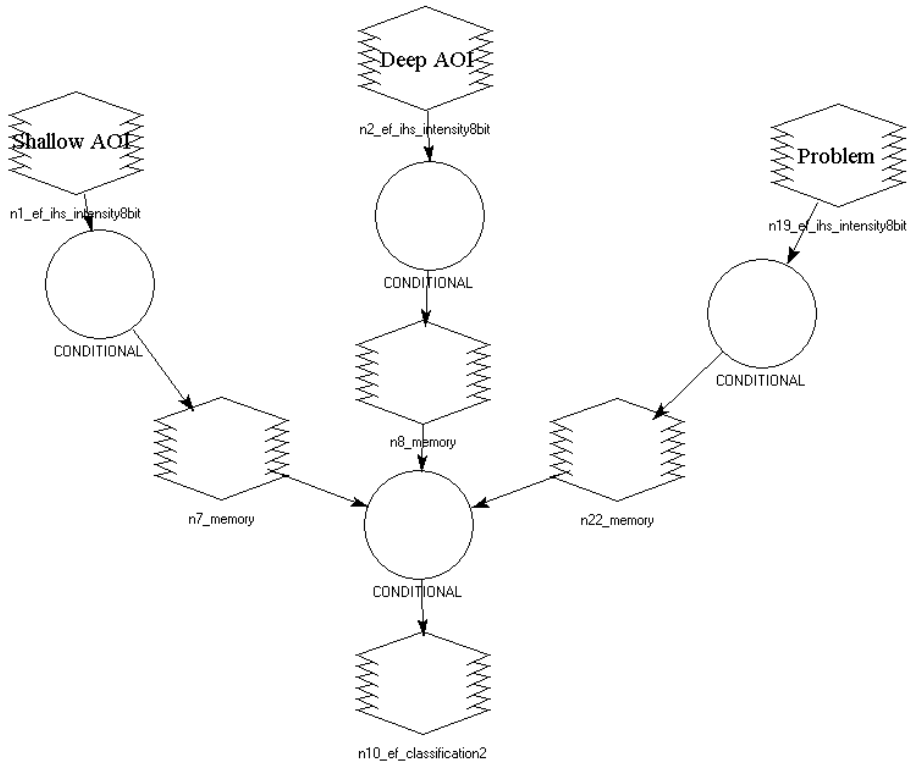
AOI	Pixel threshold	Formula
Shallow	140	CONDITIONAL {($n1_ef_ihs_intensity8bit >= 140) 1, ($n1_ef_ihs_intensity8bit < 140 and $n1_ef_ihs_intensity8bit > 0) 2 }
Deep	135	CONDITIONAL {($n2_ef_ihs_intensity8bit >= 135) 1, ($n2_ef_ihs_intensity8bit < 135 and $n2_ef_ihs_intensity8bit > 0) 2 }
Problem	116	CONDITIONAL {($n19_ef_ihs_intensity8bit >= 116) 1, ($n19_ef_ihs_intensity8bit < 116 and $n19_ef_ihs_intensity8bit > 0) 2 }

Appendix C. Table 2. Port Bay pixel thresholds.

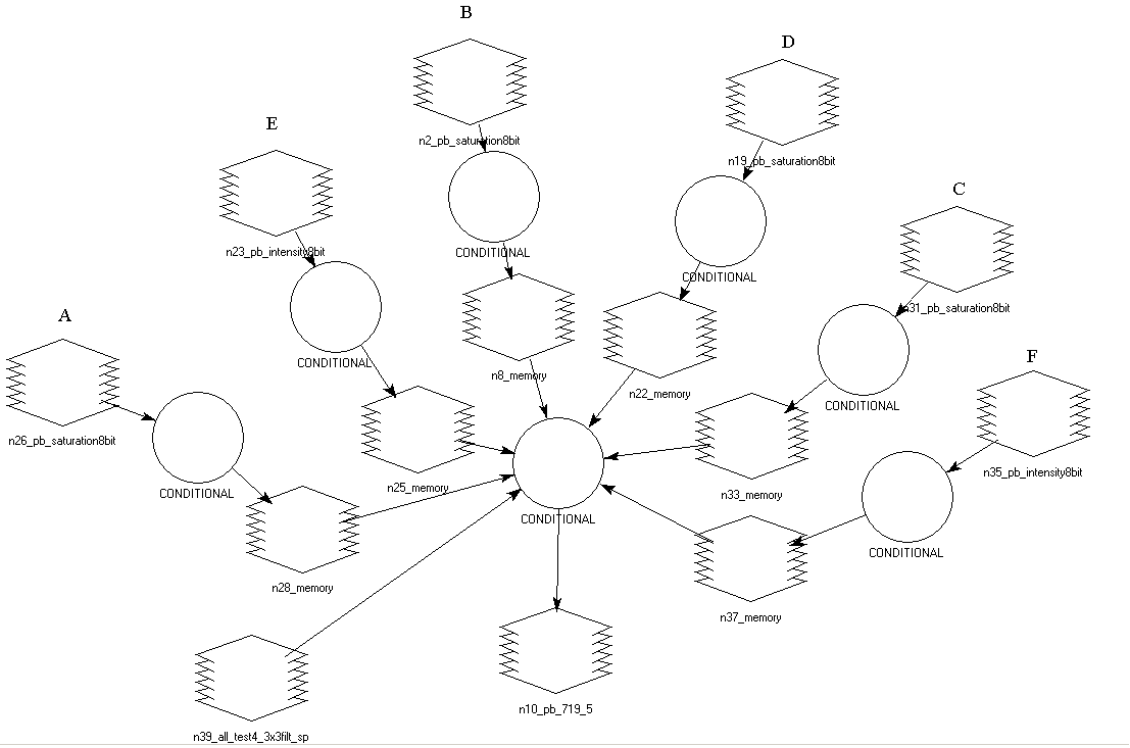
AOI	Pixel threshold	Formula
A	61	CONDITIONAL {($n26_pb_saturation8bit >= 61) 1, ($n26_pb_saturation8bit < 61 and $n26_pb_saturation8bit > 0) 2}
B	63	CONDITIONAL {($n2_pb_saturation8bit >= 63) 1, ($n2_pb_saturation8bit < 63 and $n2_pb_saturation8bit > 0) 2}
C	77	CONDITIONAL {($n31_pb_saturation8bit >= 77) 1, ($n31_pb_saturation8bit < 77 and $n31_pb_saturation8bit > 0) 2}

AOI	Pixel threshold	Formula
D	37	CONDITIONAL {($n19_pb_saturation8bit >= 37) 1, ($n19_pb_saturation8bit < 37 and $n19_pb_saturation8bit >0) 2}
E	77	CONDITIONAL {($n23_pb_intensity8bit >= 77) 1, ($n23_pb_intensity8bit < 77 and $n23_pb_intensity8bit > 0) 2}
F	77	CONDITIONAL {($n35_pb_intensity8bit >= 77) 1, ($n35_pb_intensity8bit < 77 and $n35_pb_intensity8bit > 0) 2}

An Erdas Spatial Model (Figure 1 and Figure 2) was used to process the image in each AOI and then join the AOIs together into a single binary output. In each study area, the final output was filtered with a 5x5 neighborhood majority filter to reduce noise in the data. In Port Bay, the interior AOI (A) was filtered an additional two times prior to being added to the final product. The final thematic image was then evaluated against the original imagery and the field collection points to determine accuracy.



Appendix C. Figure 1. Erdas spatial model for East Flats.



Appendix C. Figure 2. Erdas spatial model for Port Bay.

Vegetated and bare habitat area calculations were generated by counting pixels on the final classified image. Algae coverage could not be reliably identified using the above the method. To compensate, areas of excessive algae deposits (>85%) were manually delineated at a 1:600 scale and validated by visual interpretation.

Field Collection and Accuracy Assessment

The accuracy assessment incorporated points collected in the field and points generated by visual inspection of the imagery. Ground-truthing efforts should typically take place as close in time to the imagery acquisition date as possible. However, poor weather conditions around the acquisition date in 2009 delayed the possibility of field work until the habitats of interest were in their dormant season. For this reason, many field points were collected when the growing season resumed the following April. Field points used to validate the 2010 imagery were collected in the same month as the imagery acquisition (November 2010).

Field points in 2009 were chosen prior to acquiring imagery by generating a stratified random sample (one point randomly placed inside a 100mx100m grid overlay of the study area). A few of the points were removed or moved to more suitable locations based on prior field knowledge. Points were collected with a Trimble GeoXT 2005 Series Pocket PC with a custom data dictionary. Data recorded at the point included habitat type (bare, seagrass, algae), species of vegetation, if present, and water depth. All points were collected with a maximum Position Dilution of Precision (PDOP) of 5 and were differentially corrected for sub-meter accuracy in Pathfinder Office software. The 2010 field sampling effort attempted to revisit the 2009 sites but

was limited in time due to weather. Fewer points were collected in 2010 and the focus was more on deep edge points than point collection in the interior of the study area.

In areas where field points were not collected in 2009 and the habitat was obvious from the imagery, the analyst added more points to aid in accuracy assessment. Accuracy assessments for the 2009 classifications were done by viewing the points on the classification and determining if the habitats matched. Only bare and vegetated habitats were evaluated. Algae and seagrass categories recorded in the field were combined into the vegetation category and a one-meter buffer was used around the points to compensate for GPS error. Accuracies were recorded in an error matrix that reports user's, producer's and overall accuracy (Congalton 1991). The minimum overall accuracy for each study area was 80%. There were no classifications produced in 2010 and thus the 2010 field points were used to train the analyst for virtual transect analysis.

Landscape Metrics

Landscape metrics in this study refer to vegetated patch characteristics. To achieve discrete patches, the classified image showing bare and vegetated area was converted from a raster image to vector polygons using ESRI's Spatial Analyst. In this tool, adjacent pixels of the same value are joined together into a discrete polygon, or patch. To identify patchy and continuous areas spatially, a threshold based on patch area was chosen to differentiate the habitats. Patches less than 2 m^2 were excluded from the landscape metrics analysis. The threshold was determined by photo-interpretation and ecological knowledge of the study areas. In most areas along the Texas coast, anything larger than 10-25 m^2 can be considered continuous from an ecological point-of-view (Pulich 2010).

After choosing the threshold, a graphic of the spatial distribution of patchy and continuous habitat was created. Each habitat type was further analyzed with Patch Analyst 4 software to generate average shape index, average perimeter-to-area ratio and average size. Shape index and perimeter-to-area ratio analysis both measure shape complexity. Perimeter-to-area ratio is calculated by dividing the sum of each patch's perimeter by its area, while shape index is calculated by dividing each patch's perimeter by the square root of the patch area and then adjusted for a circular standard (when all polygons are perfect circles, the mean shape index is 1). Increased complexity may be related to increased patchiness over time. Number of patches and the total area were also recorded for the patchy, continuous and bare habitat.

Virtual transects were used as an additional way to attain landscape metrics. Transect locations were randomly chosen by creating a grid roughly perpendicular to the shoreline, in which each 100 m-wide gridlet began at the shore and extended to the opposite edge of the study area. In 2009, after randomly choosing three gridlets, transects were drawn within them. This method resulted in three transects in Port Bay and four transects in East Flats in 2009 (two transects were constructed in one of the gridlets to capture seagrass beds on either side of a deepwater area). In East Flats, a stratified random sample was required based on seagrass type (transects should run through one species only). Only *Thalassia testudinum* beds were sampled with the virtual transect technique in 2009. In 2010, fifteen transects for East Flats and eleven transects for Port Bay were sampled.

Each transect crossed the entire vegetated area, from shallow to deep edge. Transitions between vegetation and bare area were identified along each transect by interpreting the original photography. The number of transitions, the transect length and the overall length of bare patches were recorded. For the 2009 imagery, three transects were chosen to test the labor requirements and utility of the method. The number of transects analyzed was expanded in 2010. Approximately 11-12 transects have been used in other studies for statistically significant results (Gaeckle *et al*. 2008).

Accuracy of the landscape metrics were inferred from visual inspection and, for 2009, from the accuracy of the overall classification. Field validation was not practical due to the large number of points required and the inability for field crews to define patchy areas at the same scale as photo-interpretation.

Depth Limit Analysis

Determining the deepest water at which the seagrass grows is the goal of the depth limit analysis. Through photo-interpretation, the estimated edge between vegetation and open water was delineated and points were generated along this line for field validation. Field crews aimed to collect eleven points at each site, but were only able to collect eight in East Flats and nine in Port Bay due to field conditions in 2009. Five points were collected at each study site in 2010. In the field, researchers adjusted their location to reflect the actual deepest edge and collected a depth measurement. Depths were adjusted for tide using "Meters above Mean Sea Level" data from the Texas Coastal Ocean Observation Network (TCOON) for the gauge closest to each study area ("Ingleside 006" for East Flats; "Copano Bay 036" for Port Bay). This analysis assumes that tide at the gauges is the same as tide at the study areas.

Results were displayed as a graphic showing the locations of the adjusted depths, as well as with descriptive statistics (mean, standard deviation, and range). For best comparison, subsequent years should attempt to collect points at or near those collected in the first year.

References

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Appendix D. Data Tables

Appendix D. Table 1. Photosynthetically-active radiation (PAR) measurements for Port Bay, May - Oct 2010. Data collected using Li-Cor LI-193 spherical sensor.

Date	Transect	Depth at seagrass (m)	PAR surface (μmol m^{-2} s^{-1})	PAR seagrass canopy (μmol m^{-2} s^{-1})	Weather	Days since last rain
18 May 2010	T1	1.1	1290	132.4	Light breeze, partly cloudy	-
			1111	150.2		
			1112	158.5		
			1112	142.0		
	T2	1.1	1054	175.3	Light breeze, partly cloudy	-
			1044	186.8		
			1058	195.1		
			1023	194.5		
	T3	0.6	86.8	37.4	Overcast	-
			87.5	40.2		
			92.6	40.5		
			88.6	33.4		
15 Jul 2010	T1	0.75	699	66.4	Light wind, partly cloudy, warm	6
			788.3	67.3		
			693.3	69.93		
			859.2	145.2		
	T2	0.8	985.7	220.5	Partly cloudy, light breeze	6
			956	221.0		
			971.7	217.8		
			948.2	219.5		
	T3	0.8	1454	505.9	Warm, partly cloudy, light breeze	6
			1492	484.5		
			1412	496.5		
			1340	500.1		
06 Oct 2010	T1	1	1473	197.0	No clouds	>7
			1599	290.8		
			1601	290.4		
			1498	212.3		
	T2	1	1424	207.9	No clouds	>7
			1482	212.2		
			1398	213.2		
			1391	207.6		
	T3	1.1	1342	205.2	Clear, 70's, breezy, ~ 8 mph north wind	>7
			1132	212.5		
			1470	207.5		
			1225	214.7		

Appendix D. Table 2. Photosynthetically-active radiation (PAR) measurements for East Flats, Jun - Nov 2010.

Date	Transect	Depth at seagrass (m)	PAR surface ($\mu mol\ s^{-1}\ m^{-2}$)	PAR seagrass canopy ($\mu mol\ s^{-1}\ m^{-2}$)	Weather	Days since last rain
01 Jun 2010	T1	0.32	2498	2076	Hazy, some fluffy clouds	-
			2499	2019		
			2565	2118		
			2492	2225		
	T2	1	2263	1554	-	-
			2285	1375		
			2049	1434		
			1890	1490		
	T3	1.3	1586	427.8	-	-
			1571	438.6		
			1592	399.9		
			1521	417.8		
02 Aug 2010	T1	0.4	2284	2281	Few clouds, low wind	7
			2320	2204		
			2264	2283		
			2350	2342		
	T2	1.2	2194	1169	Hot, low wind, few clouds	7
			2149	1069		
			2182	1005		
			2176	1017		
	T3	1.1	1541	803.0	Hot, little wind, few clouds	7
			1406	716.0		
			1447	781.0		
			1548	792.0		
09 Nov 2010	T1	0.3	1562	1275	Slightly windy	>7
			1864	1460		
			1460	1333		
			1537	1248		
	T2	0.75	1868	1462	Slightly windy	>7
			1840	1501		
			1959	1458		
			1890	1475		
	T3	1.1	2033	1093	Windy	>7
			2018	1040		
			2229	1060		
			2011	1107		

Appendix D. Table 3. Water chemistry values for Port Bay, May - Oct 2010.
"J" indicates reported value greater than the method detection limit (MDL) and less than or equal to the reporting level (RL) or practical quantitation level (PQL). "ND" indicates non-detect value.

Date	Analyte	Units	Method	Rep	T1		T2		T3	
18 May 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	0.10	J	1.72		0.07	J
	Ammonia	µmol L^{-1}	UTMSI SOP	2	0.10	J	0.19		0.06	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	1.4		24.1		1.0	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	1.3		2.7		0.8	J
	Chlorophyll-*a*	µg L^{-1}	E445.0	1	5.00		3.60		2.90	
	Chlorophyll-*a*	µg L^{-1}	E445.0	2	3.70		4.40		3.10	
	Chlorophyll-*a*	µg L^{-1}	E445.0	3	--		4.50		--	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	0.42		0.50		0.43	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	0.38		0.41		0.41	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	5.9		7.0		6.0	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	5.4		5.7		5.8	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	0.119		0.126		0.156	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	0.143		0.147		0.119	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	3.7		3.9		4.8	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	4.4		4.6		3.7	
	Pheophytin-*a*	µg L^{-1}	E445.0	1	1.7		1.1		0.7	
	Pheophytin-*a*	µg L^{-1}	E445.0	2	2.2		1.1		0.9	
	Pheophytin-*a*	µg L^{-1}	E445.0	3	--		1.5		--	
	Silicate	µmol L^{-1}	UTMSI SOP	1	210.9		220.1		248.4	
	Silicate	µmol L^{-1}	UTMSI SOP	2	223.7		219.5		220.6	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	5.91		6.16		6.96	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	6.26		6.15		6.18	
	Total suspended solids	mg L^{-1}	SM2540D	1	31.6		30.0		21.7	
	Total suspended solids	mg L^{-1}	SM2540D	2	39.6		30.4		24.6	
15 Jul 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	< 0.10	ND	< 0.10	ND	< 0.10	ND
	Ammonia	µmol L^{-1}	UTMSI SOP	2	< 0.10	ND	< 0.10	ND	< 0.10	ND
	Ammonia	µmol L^{-1}	UTMSI SOP	3	< 0.10	ND	--		--	
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	< 1.4	ND	< 1.4	ND	< 1.4	ND
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	< 1.4	ND	< 1.4	ND	< 1.4	ND
	Ammonia-N	µg L^{-1}	UTMSI SOP	3	< 1.4	ND	--		--	
	Chlorophyll-*a*	µg L^{-1}	E445.0	1	8.20		6.90		6.40	
	Chlorophyll-*a*	µg L^{-1}	E445.0	2	9.00		9.10		6.00	
	Chlorophyll-*a*	µg L^{-1}	E445.0	3	9.40		--		--	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	1.29		0.22		< 0.03	ND
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	1.27		0.32		< 0.03	ND
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	3	1.24		--		--	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	18.1		3.1		< 0.4	ND
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	17.7		4.4		< 0.4	ND
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	3	17.4		--		--	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	1.249		0.766		0.328	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	1.266		0.733		0.313	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	3	1.208		--		--	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	38.7		23.7		10.2	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	39.2		22.7		9.7	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	3	37.4		--		--	
	Pheophytin-*a*	µg L^{-1}	E445.0	1	1.9		1.5		1.1	

Date	Analyte	Units	Method	Rep	T1		T2		T3	
	Pheophytin-*a*	µg L^{-1}	E445.0	2	1.6		1.9		0.9	
	Pheophytin-*a*	µg L^{-1}	E445.0	3	1.9		--		--	
	Silicate	µmol L^{-1}	UTMSI SOP	1	90.9		86.1		129.6	
	Silicate	µmol L^{-1}	UTMSI SOP	2	88.2		94.0		144.0	
	Silicate	µmol L^{-1}	UTMSI SOP	3	85.5		--		--	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	2.54		2.41		3.63	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	2.47		2.63		4.03	
	Silicate-Si	mg L^{-1}	UTMSI SOP	3	2.39		--		--	
	Total suspended solids	mg L^{-1}	SM2540D	1	16.4		14.8		13.4	
	Total suspended solids	mg L^{-1}	SM2540D	2	15.2		15.8		12.8	
	Total suspended solids	mg L^{-1}	SM2540D	3	--		15.1		--	
06 Oct 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	0.23	J	0.40	J	0.36	J
	Ammonia	µmol L^{-1}	UTMSI SOP	2	0.23	J	0.25	J	0.27	J
	Ammonia	µmol L^{-1}	UTMSI SOP	1	0.35	J	--		--	
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	3.3	J	5.7	J	5.0	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	3.2	J	3.4	J	3.8	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	4.9	J	--		--	
	Chlorophyll-*a*	µg L^{-1}	E445.0	1	9.40		15.30		7.20	
	Chlorophyll-*a*	µg L^{-1}	E445.0	2	11.00		14.10		6.80	
	Chlorophyll-*a*	µg L^{-1}	E445.0	3	13.00		--		--	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	0.75		0.06		0.63	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	0.73		< 0.03	ND	0.70	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	3	0.77		--		--	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	10.5		0.8		8.9	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	10.2		< 0.4	ND	9.9	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	3	10.8		--		--	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	1.606		1.41		1.622	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	1.648		1.386		1.637	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	3	1.599		--		--	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	49.8		43.7		50.3	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	51.1		43.0		50.7	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	3	49.6		--		--	
	Pheophytin-*a*	µg L^{-1}	E445.0	1	3.8		4.6		3.8	
	Pheophytin-*a*	µg L^{-1}	E445.0	2	5.1		4.2		3.7	
	Pheophytin-*a*	µg L^{-1}	E445.0	3	5.6		--		--	
	Silicate	µmol L^{-1}	UTMSI SOP	1	200.1		206.0		219.1	
	Silicate	µmol L^{-1}	UTMSI SOP	2	238.7		191.3		308.2	
	Silicate	µmol L^{-1}	UTMSI SOP	3	197.7		--		--	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	5.60		5.77		6.13	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	6.68		5.36		8.63	
	Silicate-Si	mg L^{-1}	UTMSI SOP	3	5.54		--		--	
	Total suspended solids	mg L^{-1}	SM2540D	1	19.8		14.0		16.8	
	Total suspended solids	mg L^{-1}	SM2540D	2	17.6		13.2		15.9	
	Total suspended solids	mg L^{-1}	SM2540D	3	--		14.6		--	
	Volatile suspended solids	mg L^{-1}	E160.4	1	1.8		3.2		3.6	
	Volatile suspended solids	mg L^{-1}	E160.4	2	1.8		2.4		3.3	
	Volatile suspended solids	mg L^{-1}	E160.4	3	--		4.2		--	

Appendix D. Table 4. Water chemistry values for East Flats, Jun - Nov 2010.
"J" indicates reported value greater than the method detection limit (MDL) and less than or equal to the reporting level (RL) or practical quantitation level (PQL). "ND" indicates non-detect value.

Date	Analyte	Units	Method	Rep	T1		T2		T3	
01 Jun 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	0.19		0.15		0.02	J
	Ammonia	µmol L^{-1}	UTMSI SOP	2	0.09	J	0.05	J	0.02	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	2.6		2.1		0.3	J
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	1.3		0.7	J	0.3	J
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	1	2.96		2.11		1.98	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	2	2.11		2.97		2.97	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	0.33		0.44		0.37	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	0.32		0.38		0.22	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	4.7		6.2		5.2	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	4.4		5.3		3.1	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	< 0.030	ND	0.093		< 0.030	ND
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	< 0.030	ND	< 0.030	ND	< 0.030	ND
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	< 0.9	ND	2.9		< 0.9	ND
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	< 0.9	ND	< 0.9	ND	< 0.9	ND
	Silicate	µmol L^{-1}	UTMSI SOP	1	57.7		44.1		53.9	
	Silicate	µmol L^{-1}	UTMSI SOP	2	56.1		43.5		53.2	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	1.62		1.24		1.51	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	1.57		1.22		1.49	
	Total suspended solids	mg L^{-1}	UTMSI SOP	1	8.4		18.9		19.1	
	Total suspended solids	mg L^{-1}	UTMSI SOP	2	10.6		15.3		16.9	
02 Aug 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	< 0.10	ND	< 0.10	ND	7.18	
	Ammonia	µmol L^{-1}	UTMSI SOP	2	< 0.10	ND	2.04		5.19	
	Ammonia	µmol L^{-1}	UTMSI SOP	3	< 0.10	ND	--		--	
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	< 1.4	ND	< 1.4	ND	100.5	
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	< 1.4	ND	28.6		72.7	
	Ammonia-N	µg L^{-1}	UTMSI SOP	3	< 1.4	ND	--		--	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	1	4.58		2.38		3.56	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	2	4.74		3.56		3.56	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	3	4.74		--		--	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	< 0.03	ND	< 0.03	ND	< 0.03	ND
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	< 0.03	ND	< 0.03	ND	< 0.03	ND
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	3	0.05		--		--	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	< 0.4	ND	< 0.4	ND	< 0.4	ND
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	< 0.4	ND	< 0.4	ND	< 0.4	ND
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	3	0.6		--		--	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	< 0.030	ND	< 0.030	ND	< 0.030	ND
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	< 0.030	ND	< 0.030	ND	< 0.030	ND
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	3	< 0.030	ND	--		--	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	< 0.9	ND	< 0.9	ND	< 0.9	ND
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	< 0.9	ND	< 0.9	ND	< 0.9	ND
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	3	< 0.9	ND	--		-- --	
	Silicate	µmol L^{-1}	UTMSI SOP	1	57.6		65.9		60.1	
	Silicate	µmol L^{-1}	UTMSI SOP	2	54.5		67.3		60.4	

Date	Analyte	Units	Method	Rep	T1		T2		T3	
	Silicate	µmol L^{-1}	UTMSI SOP	3	54.8		--		--	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	1.61		1.84		1.68	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	1.53		1.89		1.69	
	Silicate-Si	mg L^{-1}	UTMSI SOP	3	1.54		--		--	
	Total suspended solids	mg L^{-1}	UTMSI SOP	1	20.3		18.4		18.4	
	Total suspended solids	mg L^{-1}	UTMSI SOP	2	22.2		18.8		21.8	
	Total suspended solids	mg L^{-1}	UTMSI SOP	3	25.0		--		--	
09 Nov 2010	Ammonia	µmol L^{-1}	UTMSI SOP	1	0.45	J	0.54		0.61	
	Ammonia	µmol L^{-1}	UTMSI SOP	2	0.84		1.04		--	
	Ammonia-N	µg L^{-1}	UTMSI SOP	1	6.3	J	7.6		8.5	
	Ammonia-N	µg L^{-1}	UTMSI SOP	2	11.7		14.6		--	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	1	4.19		2.66		1.13	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	2	3.45		2.26		1.58	
	Chlorophyll-*a*	µg L^{-1}	UTMSI SOP	3	2.71		--		--	
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	1	< 0.05	ND	< 0.05	ND	0.08	J
	Nitrate + nitrite	µmol L^{-1}	UTMSI SOP	2	0.08	J	< 0.05	ND	--	
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	1	< 0.7	ND	< 0.7	ND	1.1	J
	Nitrate-N + nitrite-N	µg L^{-1}	UTMSI SOP	2	1.1	J	< 0.7	ND	--	
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	1	< 0.050	ND	0.094	J	0.120	J
	Ortho-phosphate	µmol L^{-1}	UTMSI SOP	2	0.059	J	< 0.050	ND	--	
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	1	< 1.6	ND	2.9	J	3.7	J
	Ortho-phosphate-P	µg L^{-1}	UTMSI SOP	2	1.8	J	< 1.6	ND	--	
	Silicate	µmol L^{-1}	UTMSI SOP	1	25.9		41.2		35.3	
	Silicate	µmol L^{-1}	UTMSI SOP	2	24.1		43.6		--	
	Silicate-Si	mg L^{-1}	UTMSI SOP	1	0.72		1.15		0.99	
	Silicate-Si	mg L^{-1}	UTMSI SOP	2	0.67		1.22		--	
	Total suspended solids	mg L^{-1}	UTMSI SOP	1	19.6		11.0		7.6	
	Total suspended solids	mg L^{-1}	UTMSI SOP	2	20.1		13.2		6.6	
	Total suspended solids	mg L^{-1}	UTMSI SOP	3	16.7		--		--	

Appendix D. Table 5. Sediment characterization for Port Bay and East Flats, May - Nov 2010.
"J" indicates reported value greater than the method detection limit (MDL) and less than or equal to the reporting level (RL) or practical quantitation level (PQL). "ND" indicates non-detect value.

Date	Analyte	Units	Method	T1		T2		T3	
			Port Bay						
15 Jul 2010	Clay (<0.002mm)	%	E600/2-78-54	4.8		10.8		2.9	
	Silt (0.002-0.05mm)	%	E600/2-78-54	6.0		4.0		2.0	
	Sand (0.05-2.0mm)	%	E600/2-78-54	89.1		85.1		95.0	
	Gravel (>0.0787")	%	E600/2-78-54	0.2		0.1		0.1	
	Total organic carbon	mg kg-1	SW9060	1230	J	1660		1270	J
			East Flats						
09 Nov 2010	Clay (<0.002mm)	%	E600/2-78-54	1.6		1.6		0.0	ND
	Silt (0.002-0.05mm)	%	E600/2-78-54	6.0		0.0	ND	2.0	
	Sand (0.05-2.0mm)	%	E600/2-78-54	92.2		98.2		97.9	
	Gravel (>0.0787")	%	E600/2-78-54	0.2		0.2		0.1	
	Clay	%	UTMSI SOP	4.8		1.5		2.0	
	Silt	%	UTMSI SOP	1.2		3.2		2.3	
	Sand	%	UTMSI SOP	92.8		94.0		94.5	
	Rubble	%	UTMSI SOP	1.2		1.4		1.1	
	Total organic carbon	mg kg-1	SW9060	3080		1380	J	< 500	ND
	Total organic carbon	mg kg-1	UTMSI SOP	12302		8291		6238	
	Total organic carbon	mg kg-1	UTMSI SOP	13837		8342		5878	
	Total organic carbon	mg kg-1	UTMSI SOP	13341		9048		5779	

Appendix D. Table 6. Sediment porewater ammonia values for Port Bay, May - Oct 2010. Distance was measured from deep end of transect.

		T1		T2		T3	
Date	Distance (m)	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}
17 May 2010	1	1.41	100.93	2.18	155.64	1.72	122.82
	4	1.82	130.21	1.49	106.25	1.30	92.95
	5	1.49	106.55	1.36	96.79	2.13	152.39
	7	0.75	53.32	1.58	112.76	1.85	131.98
	9	0.99	71.06	1.18	84.08	0.71	50.36
	12	1.21	86.74	2.17	155.34	1.34	95.61
	14	1.10	78.75	1.57	112.17	1.46	104.18
	34	1.46	104.48	1.35	96.50	1.19	84.67
	41	0.95	67.81	1.29	92.06	1.32	94.13
	42	1.12	80.23	1.45	103.89	1.20	85.55
15 Jul 2010	1	1.17	83.45	1.07	76.33	1.80	128.82
	2	0.98	70.10	0.89	63.28	1.87	133.27
	3	1.27	90.56	0.77	55.27	1.19	85.23
	5	1.21	86.71	0.81	57.65	0.96	68.32
	24	1.04	74.25	0.69	49.34	1.82	130.30
	25	0.84	60.02	0.86	61.21	1.50	107.17
	31	0.95	68.03	0.48	34.22	1.17	83.74
	35	0.80	57.35	0.65	46.08	1.53	109.25
	48	0.70	49.94	0.58	41.63	-	-
	49	0.61	43.41	0.55	39.26	3.34	238.24
06 Oct 2010	6	0.73	52.42	2.82	201.11	0.72	51.47
	7	0.71	50.52	1.71	122.02	0.93	66.34
	9	1.15	82.16	1.27	90.38	0.40	28.69
	12	1.64	116.96	1.19	84.69	1.33	95.13
	13	1.77	126.45	1.69	120.76	0.60	42.93
	16	1.31	93.55	2.26	161.25	0.91	65.07
	27	2.48	177.39	1.93	137.84	0.99	70.77
	28	2.59	184.66	2.13	152.08	0.66	47.04
	38	1.40	100.19	1.90	135.62	0.54	38.50
	41	3.17	226.42	1.42	101.14	1.09	77.73

Appendix D. Table 7. Sediment porewater ammonia values for East Flats, Jun - Nov 2010. Distance was measured from shallow end of transect.

		T1		T2		T3	
Date	Distance (m)	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}
01 Jun 2010	6	2.25	160.80	1.24	88.71	1.44	103.20
	8	1.38	98.82	2.45	174.95	1.62	116.00
	9	2.00	142.95	1.60	113.98	1.24	88.71
	15	1.46	104.21	1.99	141.94	1.62	116.00
	20	1.93	137.89	1.41	100.84	1.45	103.53
	24	1.16	82.99	1.12	80.29	0.95	67.83
	25	1.72	123.07	1.29	92.08	1.40	100.17
	26	2.02	144.63	1.92	136.88	1.25	89.39
	37	1.56	111.62	1.69	121.05	1.24	88.38
	41	1.72	122.74	1.42	101.18	1.33	95.11
02 Aug 2010	0	1.65	117.63	2.18	155.66	2.03	145.01
	4	3.49	249.05	3.47	248.14	1.49	106.68
	8	1.93	137.71	2.72	194.60	2.95	210.72
	10	1.60	114.59	1.63	116.72	3.73	266.08
	15	1.99	142.27	1.36	96.95	2.85	203.72
	19	1.66	118.24	0.78	55.88	2.08	148.66
	29	1.58	112.76	2.23	159.61	-	-
	30	2.16	154.44	3.05	217.72	-	-
	38	0.85	61.05	3.18	227.15	1.99	142.27
	47	1.84	131.32	2.78	198.85	2.40	171.17
09 Nov 2010	2	0.55	39.26	0.58	41.52	0.60	43.21
	6	0.72	51.39	0.51	36.72	2.20	157.48
	16	0.61	43.49	1.31	93.43	2.61	186.26
	23	0.64	46.03	0.78	55.63	1.70	121.65
	31	0.24	17.26	1.34	95.41	1.70	121.08
	36	0.75	53.65	1.38	98.23	0.95	68.04
	41	0.35	25.15	1.08	77.35	-	-
	45	0.41	29.39	0.65	46.32	1.13	81.02
	46	0.56	40.11	1.28	91.74	1.22	87.23
	48	0.28	19.79	0.56	39.83	-	-

Appendix D. Table 8. Seagrass percent coverage and Braun-Blanquet scores by species for Port Bay, May - Oct 2010. Distance was measured from deep end of transect.

			Percent coverage						Braun-Blanquet score				
Date	Transect	Distance (m)	*Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
17 May 2010	T1	1	85	0	0	0	15	85	5	0	0	0	5
		4	1	0	0	0	99	1	1	0	0	0	1
		5	1	0	0	0	99	1	1	0	0	0	1
		7	0	0	0	0	100	0	0	0	0	0	0
		9	1	0	0	0	99	1	1	0	0	0	1
		12	30	0	0	0	70	30	3	0	0	0	3
		14	25	0	0	0	75	25	3	0	0	0	3
		34	20	0	0	0	80	20	2	0	0	0	2
		41	40	0	0	0	60	40	3	0	0	0	3
		42	75	0	0	0	25	75	5	0	0	0	5
	T2	1	1	0	0	0	99	1	1	0	0	0	1
		4	12	0	0	0	88	12	2	0	0	0	2
		5	45	0	0	0	55	45	3	0	0	0	3
		7	65	0	0	0	35	65	4	0	0	0	4
		9	65	0	0	0	35	65	4	0	0	0	4
		12	85	0	0	0	15	85	5	0	0	0	5
		14	45	0	0	0	55	45	3	0	0	0	3
		34	15	0	0	0	85	15	2	0	0	0	2
		41	90	0	0	0	10	90	5	0	0	0	5
		42	95	0	0	0	5	95	5	0	0	0	5
	T3	1	85	0	0	0	15	85	5	0	0	0	5
		4	95	0	0	0	5	95	5	0	0	0	5
		5	95	0	0	0	5	95	5	0	0	0	5
		7	90	0	0	0	10	90	5	0	0	0	5
		9	98	0	0	0	2	98	5	0	0	0	5
		12	95	0	0	0	5	95	5	0	0	0	5
		14	90	0	0	0	10	90	5	0	0	0	5
		34	100	0	0	0	0	100	5	0	0	0	5
		41	100	0	0	0	0	100	5	0	0	0	5
		42	90	0	0	0	10	90	5	0	0	0	5
15 Jul 2010	T1	1	85	0	0	0	15	85	5	0	0	0	5
		2	40	0	0	0	60	40	3	0	0	0	3

Date	Transect	Distance (m)	Percent coverage						Braun-Blanquet score				
			Halodule	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
		3	40	0	0	0	60	40	3	0	0	0	3
		5	0	0	0	1	99	1	0	0	0	1	1
		24	1	0	0	0	99	1	1	0	0	0	1
		25	0	0	0	1	99	1	0	0	0	1	1
		31	98	0	0	2	0	100	5	0	0	1	5
		35	0	0	0	15	85	15	0	0	0	2	2
		48	100	0	0	0	0	100	5	0	0	0	5
		49	100	0	0	0	0	100	5	0	0	0	5
	T2	1	0	0	0	20	80	20	0	0	0	2	2
		2	10	0	0	45	45	55	2	0	0	3	4
		3	0	0	0	40	60	40	0	0	0	3	3
		5	20	0	0	0	80	20	2	0	0	0	2
		24	60	0	0	0	40	60	4	0	0	0	4
		25	0	0	0	30	70	30	0	0	0	3	3
		31	100	0	0	0	0	100	5	0	0	0	5
		35	50	0	0	50	0	100	4	0	0	4	5
		48	60	0	0	40	0	100	4	0	0	3	5
		49	60	0	0	40	0	100	4	0	0	3	5
	T3	1	100	0	0	0	0	100	5	0	0	0	5
		2	100	0	0	0	0	100	5	0	0	0	5
		3	100	0	0	0	0	100	5	0	0	0	5
		5	100	0	0	0	0	100	5	0	0	0	5
		24	100	0	0	0	0	100	5	0	0	0	5
		25	100	0	0	0	0	100	5	0	0	0	5
		31	100	0	0	0	0	100	5	0	0	0	5
		35	100	0	0	0	0	100	5	0	0	0	5
		48	100	0	0	0	0	100	5	0	0	0	5
		49	100	0	0	0	0	100	5	0	0	0	5
06 Oct 2010	T1	6	1	0	0	0	99	1	1	0	0	0	1
		7	0	0	0	0	100	0	0	0	0	0	0
		9	1	0	0	9	90	10	1	0	0	2	2
		12	15	0	0	60	25	75	2	0	0	4	5
		13	0	0	0	0	100	0	0	0	0	0	0

Date	Transect	Distance (m)	Percent coverage						Braun-Blanquet score				
			Halodule	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
		16	1	0	0	5	94	6	1	0	0	2	2
		27	0	0	0	0	100	0	0	0	0	0	0
		28	85	0	0	10	5	95	5	0	0	2	5
		38	90	0	0	0	10	90	5	0	0	0	5
		41	1	0	0	0	99	1	1	0	0	0	1
	T2	6	40	0	0	45	15	85	3	0	0	3	5
		7	45	0	0	0	55	45	3	0	0	0	3
		9	80	0	0	0	20	80	5	0	0	0	5
		12	40	0	0	0	60	40	3	0	0	0	3
		13	60	0	0	0	40	60	4	0	0	0	4
		16	20	0	0	0	80	20	2	0	0	0	2
		27	0	0	0	60	40	60	0	0	0	4	4
		28	10	0	0	40	50	50	2	0	0	3	4
		38	60	0	0	0	40	60	4	0	0	0	4
		41	70	0	0	0	30	70	4	0	0	0	4
	T3	6	85	0	0	0	15	85	5	0	0	0	5
		7	98	0	0	0	2	98	5	0	0	0	5
		9	100	0	0	0	0	100	5	0	0	0	5
		12	100	0	0	0	0	100	5	0	0	0	5
		13	100	0	0	0	0	100	5	0	0	0	5
		16	100	0	0	0	0	100	5	0	0	0	5
		27	100	0	0	0	0	100	5	0	0	0	5
		28	98	0	0	0	2	98	5	0	0	0	5
		38	75	0	0	0	25	75	5	0	0	0	5
		41	100	0	0	0	0	100	5	0	0	0	5

Appendix D. Table 9. Seagrass percent coverage and Braun-Blanquet scores by species for East Flats, Jun - Nov 2010. Distance was measured from shallow end of transect.

			Percent coverage						Braun-Blanquet score				
Date	Transect	Distance (m)	*Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*BB Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
01 Jun 2010	T1	6	0	100	0	0	0	100	0	5	0	0	5
		8	5	89	0	0	6	94	2	5	0	0	5
		9	25	75	0	0	0	100	3	5	0	0	5
		15	100	0	0	0	0	100	5	0	0	0	5
		20	95	0	0	0	5	95	5	0	0	0	5
		24	100	0	0	0	0	100	5	0	0	0	5
		25	100	0	0	0	0	100	5	0	0	0	5
		26	100	0	0	0	0	100	5	0	0	0	5
		37	0	100	0	0	0	100	0	5	0	0	5
		41	100	0	0	0	0	100	5	0	0	0	5
	T2	6	15	85	0	0	0	100	2	5	0	0	5
		8	0	100	0	0	0	100	0	5	0	0	5
		9	0	100	0	0	0	100	0	5	0	0	5
		15	0	100	0	0	0	100	0	5	0	0	5
		20	0	100	0	0	0	100	0	5	0	0	5
		24	0	100	0	0	0	100	0	5	0	0	5
		25	0	100	0	0	0	100	0	5	0	0	5
		26	0	100	0	0	0	100	0	5	0	0	5
		37	80	0	20	0	0	100	5	0	2	0	5
		41	0	0	100	0	0	100	0	0	5	0	5
	T3	6	100	0	0	0	0	100	5	0	0	0	5
		8	100	0	0	0	0	100	5	0	0	0	5
		9	100	0	0	0	0	100	5	0	0	0	5
		15	96	0	4	0	0	100	5	0	1	0	5
		20	0	0	100	0	0	100	0	0	5	0	5
		24	0	25	65	0	10	90	0	3	4	0	5
		25	0	5	95	0	0	100	0	2	5	0	5
		26	0	0	100	0	0	100	0	0	5	0	5
		37	70	0	0	0	30	70	4	0	0	0	4
		41	100	0	0	0	0	100	5	0	0	0	5
02 Aug 2010	T1	0	0	100	0	0	0	100	0	5	0	0	5
		4	100	0	0	0	0	100	5	0	0	0	5

Date	Transect	Distance (m)	Percent coverage						Braun-Blanquet score				
			Halodule	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*BB Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
		8	100	0	0	0	0	100	5	0	0	0	5
		10	100	0	0	0	0	100	5	0	0	0	5
		15	100	0	0	0	0	100	5	0	0	0	5
		19	100	0	0	0	0	100	5	0	0	0	5
		29	50	50	0	0	0	100	4	4	0	0	5
		30	25	75	0	0	0	100	3	5	0	0	5
		38	0	95	0	0	5	95	0	5	0	0	5
		47	0	100	0	0	0	100	0	5	0	0	5
	T2	0	100	0	0	0	0	100	5	0	0	0	5
		4	100	0	0	0	0	100	5	0	0	0	5
		8	0	0	100	0	0	100	0	0	5	0	5
		10	0	0	96	0	4	96	0	0	5	0	5
		15	0	0	100	0	0	100	0	0	5	0	5
		19	0	100	0	0	0	100	0	5	0	0	5
		29	0	100	0	0	0	100	0	5	0	0	5
		30	0	100	0	0	0	100	0	5	0	0	5
		38	0	100	0	0	0	100	0	5	0	0	5
		47	5	95	0	0	0	100	2	5	0	0	5
	T3	0	100	0	0	0	0	100	5	0	0	0	5
		4	100	0	0	0	0	100	5	0	0	0	5
		8	100	0	0	0	0	100	5	0	0	0	5
		10	100	0	0	0	0	100	5	0	0	0	5
		15	50	0	50	0	0	100	4	0	4	0	5
		19	0	0	75	0	25	75	0	0	5	0	5
		29	0	0	70	0	30	70	0	0	4	0	4
		30	0	0	45	0	55	45	0	0	3	0	3
		38	100	0	0	0	0	100	5	0	0	0	5
		47	2	0	0	0	98	2	1	0	0	0	1
09 Nov 2010	T1	2	25	75	0	0	0	100	3	5	0	0	5
		6	100	0	0	0	0	100	5	0	0	0	5
		16	99	1	0	0	0	100	5	1	0	0	5
		23	100	0	0	0	0	100	5	0	0	0	5
		31	95	5	0	0	0	100	5	2	0	0	5

Date	Transect	Distance (m)	Percent coverage						Braun-Blanquet score				
			Halodule	*Thalassia*	*Syringodium*	*Ruppia*	Bare substrate	Total seagrass	*BB Halodule*	*Thalassia*	*Syringodium*	*Ruppia*	Total seagrass
		36	95	5	0	0	0	100	5	2	0	0	5
		41	0	100	0	0	0	100	0	5	0	0	5
		45	0	100	0	0	0	100	0	5	0	0	5
		46	0	100	0	0	0	100	0	5	0	0	5
		48	0	96	0	0	4	96	0	5	0	0	5
	T2	2	100	0	0	0	0	100	5	0	0	0	5
		6	50	0	50	0	0	100	4	0	4	0	5
		16	0	0	95	0	5	95	0	0	5	0	5
		23	0	100	0	0	0	100	0	5	0	0	5
		31	0	90	0	0	10	90	0	5	0	0	5
		36	0	70	0	0	30	70	0	4	0	0	4
		41	0	90	0	0	10	90	0	5	0	0	5
		45	0	88	0	0	12	88	0	5	0	0	5
		46	4	86	0	0	10	90	1	5	0	0	5
		48	8	77	0	0	15	85	2	5	0	0	5
	T3	2	100	0	0	0	0	100	5	0	0	0	5
		6	87	0	0	0	13	87	5	0	0	0	5
		16	2	0	0	0	98	2	1	0	0	0	1
		23	1	0	99	0	0	100	1	0	5	0	5
		31	100	0	0	0	0	100	5	0	0	0	5
		36	100	0	0	0	0	100	5	0	0	0	5
		41	65	0	0	0	35	65	4	0	0	0	4
		45	0	0	0	0	100	0	0	0	0	0	0
		46	50	0	0	0	50	50	4	0	0	0	4
		48	0	0	0	0	100	0	0	0	0	0	0

Appendix D. Table 10. Macroalgae biomass for Port Bay, May - Oct 2010.
Distance was measured from deep end of transect.

		T1		T2		T3	
Date	Distance (m)	Biomass ($g\ m^{-2}$)	Notes	Biomass ($g\ m^{-2}$)	Notes	Biomass ($g\ m^{-2}$)	Notes
17 May 2010	1	84.9		2.8		5.3	
	4	68.7		0	no algae present	0	no algae present
	5	0	no algae present	29.4		0	no algae present
	7	0	no algae present	12.9		0	no algae present
	9	0	no algae present	51.9		0	no algae present
	12	24.3		0	no algae present	0	no algae present
	14	10.0		0	no algae present	0	no algae present
	34	0	no algae present	38.6		0	no algae present
	41	66.3		0	no algae present	0	no algae present
	42	33.5		0	no algae present	21.1	
15 Jul 2010	1	0	no algae present	137.7		0	no algae present
	2	0	no algae present	0.4		0	no algae present
	3	3.6		0	no algae present	0	no algae present
	5	51.0		81.7		0	no algae present
	24	16.4		0	no algae present	0	no algae present
	25	54.9		121.6		0	no algae present
	31	10.5		0	no algae present	0	no algae present
	35	118.9		0	no algae present	0	no algae present
	48	0	no algae present	0	no algae present	0	no algae present
	49	0	no algae present	0	no algae present	0	no algae present
06 Oct 2010	6	147.7		0	no algae present	0	no algae present
	7	120.8		10.5		0	no algae present
	9	7.9		35.3		0	no algae present
	12	128.1		0	no algae present	0	no algae present
	13	187.3		0	no algae present	0	no algae present
	16	92.1		57.7		3.2	
	27	18.4		6.1		3.1	
	28	1.7		1.1		0	no algae present
	38	1.1		15.6		0	no algae present
	41	188.0		0	no algae present	0	no algae present

Appendix D. Table 11. Macroalgae biomass for East Flats, Jun - Nov. 2010.
Distance was measured from shallow end of transect.

		T1		T2		T3	
Date	Distance (m)	Biomass ($g\ m^{-2}$)	Notes	Biomass ($g\ m^{-2}$)	Notes	Biomass ($g\ m^{-2}$)	Notes
01 Jun 2010	6	13.7		0	no algae present	35.8	
	8	0	no algae present	68.8		2.2	
	9	0	no algae present	0	no algae present	0	no algae present
	15	4.6		0	no algae present	6.3	
	20	0	no algae present	0	no algae present	4.1	
	24	15.5		1.3		20.7	
	25	0	no algae present	0	no algae present	14.5	
	26	1.8		0	no algae present	7.2	
	37	8.0		10.0		0	no algae present
	41	13.5		21.4		2.5	
02 Aug 2010	0	56.8		22.4		32.8	
	4	51.1		16.8		21.9	
	8	2.8		105.8		1.2	
	10	2.4		118.9		1.3	
	15	0		210.8		2.8	
	19	1.7		0	no algae present	46.0	
	29	5.8		0	no algae present	15.5	
	30	9.9		0	no algae present	0	no algae present
	38	102.1		0	no algae present	0	no algae present
	47	0	no algae present	4.8		0	no algae present
09 Nov 2010	2	102.6		0	no algae present	4.6	
	6	94.9		0	no algae present	0	no algae present
	16	18.9		7.5		0	no algae present
	23	0	no algae present	2.5		4.2	
	31	14.9		0	no algae present	6.4	
	36	3.7		0	no algae present	0	no algae present
	41	0	no algae present	0	no algae present	23.8	
	45	2.0		0	no algae present	0	no algae present
	46	0	no algae present	0	no algae present	0	no algae present
	48	6.9		0	no algae present	0	no algae present

Appendix D. Table 12. Seagrass condition indicators for Port Bay, May - Oct 2010.

Date	Transect	Species	Core diameter (cm)	Seagrass core number	Shoot density (number m^{-2})	Above-ground biomass (g m^{-2})	Below-ground biomass (g m^{-2})	Total biomass (g m^{-2})	Root:shoot
17 May 2010	T1	*Halodule*	9	1	4401	21.3	23.4	44.7	1.10
		Halodule	9	2	4873	27.1	33.7	60.8	1.24
		Halodule	9	3	8488	66.2	40.9	107.1	0.62
	T2	*Halodule*	9	1	2043	9.8	17.3	27.1	1.77
		Halodule	9	2	6602	22.9	48.2	71.1	2.11
		Halodule	9	3	6602	44.3	59.0	103.3	1.33
	T3	*Halodule*	9	1	16662	101.9	189.0	290.9	1.85
		Halodule	9	2	16191	143.8	135.9	279.7	0.94
		Halodule	9	3	13518	83.1	149.9	233.0	1.80
15 Jul 2010	T1	*Halodule*	9	1	1258	16.3	11.5	27.8	0.71
		Ruppia	9	1	1729	4.5	2.3	6.8	0.52
		Halodule	9	2	9589	153.6	89.5	243.1	0.58
		Halodule	9	3	2201	10.2	17.4	27.6	1.70
		Ruppia	9	3	629	3.0	4.0	7.1	1.33
	T2	*Halodule*	9	1	2829	26.3	17.2	43.5	0.66
		Halodule	9	2	3930	56.1	29.0	85.0	0.52
		Ruppia	9	2	472	8.4	2.0	10.4	0.24
		Halodule	9	3	10375	74.9	86.1	160.9	1.15
	T3	*Halodule*	9	1	6130	53.9	74.5	128.5	1.38
		Halodule	9	2	10689	46.3	154.5	200.9	3.34
		Halodule	9	3	10532	64.4	155.7	220.2	2.42
06 Oct 2010	T1	*Halodule*	9	1	10375	24.4	82.0	106.4	3.36
		Halodule	9	2	2358	37.7	20.0	57.7	0.53
		Halodule	9	3	1100	8.0	16.5	24.5	2.06
	T2	*Halodule*	9	1	6130	34.8	43.5	78.2	1.25
		Ruppia	9	1	472	25.4	18.6	44.0	0.73
		Halodule	9	2	3458	38.6	91.0	129.7	2.36
		Halodule	9	3	3930	53.4	62.2	115.6	1.16
	T3	*Halodule*	9	1	2515	40.6	74.3	114.9	1.83
		Halodule	9	2	7545	92.4	119.8	212.2	1.30
		Ruppia	9	2	472	3.5	1.1	4.6	0.33
		Halodule	9	3	8803	109.1	172.2	281.3	1.58

Appendix D. Table 13. Seagrass condition indicators for East Flats, Jun - Nov 2010.

Date	Transect	Species	Core diameter (cm)	Seagrass core number	Shoot density (number m^{-2})	Above-ground biomass (g m^{-2})	Below-ground biomass (g m^{-2})	Total biomass (g m^{-2})	Root:shoot
01 Jun 2010	T1	*Thalassia*	15	1	566	168.8	388.4	557.2	2.30
		Halodule	15	2	7639	156.7	276.2	432.9	1.76
		Ruppia	15	2	57	7.1	1.2	8.4	0.17
		Halodule	15	3	2716	30.4	111.0	141.5	3.65
		Thalassia	15	3	226	22.1	49.5	71.6	2.24
	T2	*Thalassia*	15	1	1528	227.4	403.8	631.2	1.78
		Thalassia	15	2	2207	148.1	287.7	435.8	1.94
		Syringodium	9	3	5659	376.9	247.4	624.4	0.66
	T3	*Halodule*	9	1	4087	169.8	90.9	260.6	0.54
		Syringodium	9	1	3458	133.8	121.8	255.6	0.91
		Syringodium	9	2	1415	80.0	65.5	145.6	0.82
		Halodule	9	3	1729	134.9	158.3	293.2	1.17
02 Aug 2010	T1	*Thalassia*	15	1	962	174.2	752.0	926.2	4.32
		Thalassia	15	2	1471	140.8	234.4	375.2	1.66
		Halodule	15	3	283	4.1	30.0	34.1	7.38
		Thalassia	15	3	792	249.7	407.7	657.4	1.63
	T2	*Syringodium*	9	1	3144	101.9	258.9	360.8	2.54
		Thalassia	15	2	1019	276.4	849.1	1125.5	3.07
		Thalassia	15	3	1188	150.5	746.5	897.0	4.96
	T3	*Halodule*	9	1	7545	188.5	155.8	344.2	0.83
		Halodule	9	2	3458	96.0	231.5	327.6	2.41
		Halodule	9	3	4087	38.0	99.8	137.9	2.62
09 Nov 2010	T1	*Halodule*	15	1	736	6.8	9.6	16.4	1.40
		Thalassia	15	1	1075	241.6	411.3	653.0	1.70
		Halodule	15	2	453	3.9	12.6	16.5	3.23
		Thalassia	15	2	1019	138.2	498.1	636.3	3.60
		Halodule	15	3	57	0.0	0.3	0.3	undefined
		Thalassia	15	3	1471	200.8	597.3	798.1	2.98
	T2	*Halodule*	9	1	4401	49.7	146.8	196.5	2.96
		Thalassia	15	2	566	64.5	419.6	484.1	6.50
		Halodule	15	3	57	0.5	0.5	1.0	0.89
		Thalassia	15	3	1302	88.3	511.4	599.7	5.79
	T3	*Halodule*	9	1	3773	51.2	157.5	208.7	3.07
		Halodule	9	2	157	0.0	1.4	1.4	undefined
		Syringodium	9	2	2043	151.4	167.1	318.5	1.10
		Halodule	9	3	4873	92.9	160.0	252.9	1.72

Appendix D. Table 14. Leaf measurements for Port Bay, May - Oct 2010.
"Seagrass core number" refers to Appendix D. Table 12.

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
17 May 2010	T1	*Halodule*	1	1	4	11.4	1
		Halodule	1	2	3	11.9	1
		Halodule	1	3	3	7.9	1
		Halodule	1	4	4	14.2	1
		Halodule	1	5	2	14.8	1
		Halodule	2	1	3	14	1
		Halodule	2	2	3	16.2	1
		Halodule	2	3	2	13.7	1
		Halodule	2	4	2	13.5	1
		Halodule	2	5	3	12.3	1
		Halodule	3	1	2	11.3	1
		Halodule	3	2	3	13.9	1
		Halodule	3	3	3	17.5	1
		Halodule	3	4	2	17.2	1
		Halodule	3	5	2	22.1	1
	T2	*Halodule*	1	1	3	10.4	1
		Halodule	1	2	3	16	1
		Halodule	1	3	3	7.6	1
		Halodule	1	4	3	11.4	1
		Halodule	1	5	2	10.5	1
		Halodule	2	1	2	11	1
		Halodule	2	2	3	9.7	1
		Halodule	2	3	4	14.2	1
		Halodule	2	4	2	12.1	1
		Halodule	2	5	2	7.9	1
		Halodule	3	1	2	7.6	1
		Halodule	3	2	3	10.5	1
		Halodule	3	3	2	12.6	1
		Halodule	3	4	3	14.2	1
		Halodule	3	5	2	13.7	1
	T3	*Halodule*	1	1	3	8.9	1
		Halodule	1	2	3	21.3	1
		Halodule	1	3	4	12.5	1
		Halodule	1	4	2	13.4	1
		Halodule	1	5	3	15.7	1
		Halodule	2	1	2	13.5	1
		Halodule	2	2	3	6.4	1
		Halodule	2	3	2	11.2	1
		Halodule	2	4	2	13.6	1
		Halodule	2	5	2	12.8	1
		Halodule	3	1	3	12.8	1
		Halodule	3	2	3	9.8	1
		Halodule	3	3	3	15.9	1
		Halodule	3	4	2	12.9	1
		Halodule	3	5	2	17.3	1
14 Jul 2010	T1	*Halodule*	1	1	2	20	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Halodule	1	2	2	12	1
		Halodule	1	3	2	14	1
		Halodule	1	4	1	16	1
		Halodule	1	5	2	23	1
		Ruppia	1	1	4	11	1
		Ruppia	1	2	4	5	1
		Ruppia	1	3	4	8	1
		Ruppia	1	4	6	8	1
		Ruppia	1	5	6	8.5	1
		Halodule	2	1	2	23.6	1
		Halodule	2	2	3	22.2	1
		Halodule	2	3	3	25.1	1
		Halodule	2	4	3	22.4	1
		Halodule	2	5	3	24.3	1
		Halodule	3	1	3	9	1
		Halodule	3	2	2	15	1
		Halodule	3	3	1	16.5	1
		Halodule	3	4	2	9.9	1
		Halodule	3	5	1	20	1
		Ruppia	3	1	9	9	1
		Ruppia	3	2	4	4	1
		Ruppia	3	3	6	9	1
		Ruppia	3	4	4	8	1
	T2	*Halodule*	1	1	3	10	1
		Halodule	1	2	2	18.5	1
		Halodule	1	3	3	20.4	1
		Halodule	1	4	3	15.8	1
		Halodule	1	5	2	13.9	1
		Halodule	2	1	2	25.1	1
		Halodule	2	2	2	24.9	1
		Halodule	2	3	2	25.4	1
		Halodule	2	4	2	10.3	1
		Halodule	2	5	1	13	1
		Ruppia	2	1	6	10.5	1
		Ruppia	2	2	2	12.5	1
		Ruppia	2	3	3	11.7	1
		Ruppia	2	4	3	7.1	1
		Ruppia	2	5	4	7	1
		Halodule	3	1	3	26	1
		Halodule	3	2	2	21.5	1
		Halodule	3	3	2	16.8	1
		Halodule	3	4	2	24.6	1
		Halodule	3	5	3	16.5	1
	T3	*Halodule*	1	1	2	19.6	1
		Halodule	1	2	1	19.7	1
		Halodule	1	3	2	23.8	1
		Halodule	1	4	4	36.1	1
		Halodule	1	5	4	26.1	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Halodule	2	1	3	22.9	1
		Halodule	2	2	4	24.5	1
		Halodule	2	3	3	9.7	1
		Halodule	2	4	2	9.6	1
		Halodule	2	5	3	9.2	1
		Halodule	3	1	3	21.4	1
		Halodule	3	2	2	23.5	1
		Halodule	3	3	3	25.3	1
		Halodule	3	4	3	23	1
		Halodule	3	5	2	28.4	1
06 Oct 2010	T1	*Halodule*	1	1	2	20.1	1
		Halodule	1	2	2	18	1
		Halodule	1	3	3	13.5	1
		Halodule	1	4	3	26.1	1
		Halodule	1	5	2	22.5	1
		Halodule	2	1	2	15.1	1
		Halodule	2	2	2	6	1
		Halodule	2	3	2	6.8	1
		Halodule	2	4	2	17.3	1
		Halodule	2	5	3	20.5	1
		Halodule	3	1	2	18	1
		Halodule	3	2	3	25.4	1
		Halodule	3	3	2	22.6	1
		Halodule	3	4	2	14.4	1
		Halodule	3	5	2	16.7	1
	T2	*Halodule*	1	1	2	29.1	1
		Halodule	1	2	2	21	1
		Halodule	1	3	2	15.3	1
		Halodule	1	4	2	16	1
		Halodule	1	5	3	9.5	1
		Ruppia	1	1	15	9	1
		Ruppia	1	2	6	10.3	1
		Ruppia	1	3	27	12.1	1
		Halodule	2	1	2	29.9	1
		Halodule	2	2	2	22.3	1
		Halodule	2	3	2	27.9	1
		Halodule	2	4	2	21.7	1
		Halodule	2	5	2	24.2	1
		Halodule	3	1	2	17.5	1
		Halodule	3	2	2	7.3	1
		Halodule	3	3	2	8.1	1
		Halodule	3	4	2	5.5	1
		Halodule	3	5	2	5.8	1
	T3	*Halodule*	1	1	3	13.5	1
		Halodule	1	2	2	22.1	1
		Halodule	1	3	2	14	1
		Halodule	1	4	2	17.5	1
		Halodule	1	5	2	17.4	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Halodule	2	1	3	20.2	1
		Halodule	2	2	2	25.2	1
		Halodule	2	3	3	19.1	1
		Halodule	2	4	3	24.2	1
		Halodule	2	5	3	27.1	1
		Ruppia	2	1	6	7.1	1
		Ruppia	2	2	15	8	1
		Ruppia	2	3	3	5.9	1
		Halodule	3	1	2	31.8	1
		Halodule	3	2	3	22.2	1
		Halodule	3	3	3	26.3	1
		Halodule	3	4	2	25.8	1
		Halodule	3	5	2	22.8	1

Appendix D. Table 15. Leaf measurements for East Flats, Jun - Nov 2010.
"Seagrass core number" refers to Appendix D. Table 13.

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
01 Jun 2010	T1	*Thalassia*	1	1	4	28	9
		Thalassia	1	2	3	22.5	7
		Thalassia	1	3	3	23.1	5
		Thalassia	1	4	4	24.6	9
		Thalassia	1	5	3	19.6	5
		Halodule	2	1	3	28.9	1
		Halodule	2	2	2	27	1
		Halodule	2	3	3	25.2	1
		Halodule	2	4	2	25.4	1
		Halodule	2	5	2	23.2	1
		Ruppia	2	1	1	25	1
		Thalassia	3	1	2	11.9	4
		Thalassia	3	2	2	15	8
		Thalassia	3	3	4	14	4
		Thalassia	3	4	3	8	4
		Halodule	3	1	2	15.1	1
		Halodule	3	2	2	9	1
		Halodule	3	3	3	27	1
		Halodule	3	4	2	15	1
		Halodule	3	5	3	22	1
	T2	*Thalassia*	1	1	3	24.5	4.5
		Thalassia	1	2	3	15.9	4
		Thalassia	1	3	3	34	4.5
		Thalassia	1	4	3	18.1	5
		Thalassia	1	5	3	23.1	4.5
		Thalassia	2	1	4	17.1	7
		Thalassia	2	2	3	17.4	6.5
		Thalassia	2	3	3	12	6
		Thalassia	2	4	4	7.1	7
		Thalassia	2	5	4	14.7	5.5
		Syringodium	3	1	1	18	1
		Syringodium	3	2	1	23	1.5
		Syringodium	3	3	1	54	1
		Syringodium	3	4	1	50	1
		Syringodium	3	5	1	35	1
	T3	*Halodule*	1	1	2	23.9	1
		Halodule	1	2	2	21.5	1
		Halodule	1	3	3	29.8	1
		Halodule	1	4	3	24.4	1
		Halodule	1	5	3	26.6	1
		Syringodium	1	1	1	25	1
		Syringodium	1	2	1	18.2	1
		Syringodium	1	3	1	26	1
		Syringodium	1	4	1	18.4	1
		Syringodium	1	5	2	19.5	1
		Syringodium	2	1	2	29	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Syringodium	2	2	1	25	1
		Syringodium	2	3	2	29	1
		Syringodium	2	4	1	31	1
		Syringodium	2	5	1	23.5	1
		Halodule	3	1	2	25.5	1
		Halodule	3	2	2	38.5	1
		Halodule	3	3	2	38	1
		Halodule	3	4	3	29	1
		Halodule	3	5	3	39	1
02 Aug 2010	T1	*Halodule*	-	1	3	27.8	1
		Halodule	-	2	2	37	1
		Halodule	-	3	3	35.8	1
		Halodule	-	4	2	33	1
		Halodule	-	5	2	26.1	0.9
		Thalassia	-	1	3	35	8
		Thalassia	-	2	4	19.5	8
		Thalassia	-	3	4	38.5	6
		Thalassia	-	4	3	37.1	10
		Halodule	-	1	3	28.4	1
		Halodule	-	2	3	29.2	1
		Halodule	-	3	3	33	1
		Halodule	-	4	2	32	1
		Halodule	-	5	3	29.1	1
		Thalassia	-	1	3	42.2	6
		Thalassia	-	2	3	32	7
		Thalassia	-	3	3	64	7
		Thalassia	-	4	3	52	7
		Thalassia	-	5	4	50	8
	T2	*Halodule*	-	1	3	19	1.5
		Halodule	-	2	2	27.4	2
		Halodule	-	3	3	27.2	1
		Halodule	-	4	3	25.3	1.5
		Halodule	-	5	4	22.1	2
		Thalassia	-	1	2	77	5.5
		Thalassia	-	2	3	71.6	7
		Thalassia	-	3	3	46	6
		Thalassia	-	4	2	30	3
		Thalassia	-	5	3	28.3	6
		Thalassia	-	1	2	49.1	4
		Thalassia	-	2	3	53	5
		Thalassia	-	3	3	77.5	6
		Thalassia	-	4	2	78	5
		Thalassia	-	5	3	62	6
	T3	*Syringodium*	-	1	1	53	1
		Syringodium	-	2	1	40	1
		Syringodium	-	3	2	32.4	1
		Syringodium	-	4	1	32	1
		Halodule	-	1	3	33.3	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Halodule	-	2	2	24	1
		Halodule	-	3	3	30	1
		Halodule	-	4	4	33.8	1
		Halodule	-	5	4	28.4	1
		Syringodium	-	1	1	25.2	2
		Syringodium	-	2	1	23	1.5
		Syringodium	-	3	1	29	1
		Syringodium	-	4	2	19	2
		Syringodium	-	5	1	24.5	1
		Halodule	-	1	3	28.2	1
		Halodule	-	2	2	24	1
		Halodule	-	3	1	16	1
		Halodule	-	4	3	19.1	1
		Halodule	-	5	3	25.3	1
09 Nov 2010	T1	*Thalassia*	-	1	2	25.5	7.5
		Thalassia	-	2	2	43	7
		Thalassia	-	3	2	50	7
		Thalassia	-	4	2	22	4
		Thalassia	-	5	2	18	6
		Halodule	-	1	2	18	1
		Halodule	-	2	3	19.5	1
		Halodule	-	3	3	18.5	1
		Halodule	-	4	2	10	1
		Halodule	-	5	2	16.7	1
		Thalassia	-	1	4	37	8.8
		Thalassia	-	2	3	37.5	7.9
		Thalassia	-	3	3	31.8	6.8
		Thalassia	-	4	2	29.3	6
		Thalassia	-	5	2	21.5	6
	T2	*Halodule*	-	1	4	22.1	1.3
		Halodule	-	2	2	18.2	1.1
		Halodule	-	3	3	12.61	1
		Halodule	-	4	2	21.71	1.1
		Halodule	-	5	3	22.2	1.1
		Thalassia	-	1	3	29.4	6.5
		Thalassia	-	2	3	31.3	6.5
		Thalassia	-	3	2	30.2	6.4
		Thalassia	-	4	2	35.5	7
		Thalassia	-	5	3	30	6.3
		Thalassia	-	1	3	16.4	6.9
		Thalassia	-	2	3	27.7	6.2
		Thalassia	-	3	3	32	6.2
		Thalassia	-	4	3	45.8	6.9
		Thalassia	-	5	2	23.35	6.1
		Halodule	-	1	2	10	1
		Halodule	-	2	3	6.45	1
		Halodule	-	3	3	15.12	1
		Halodule	-	4	3	10.5	1

Date	Transect	Seagrass species	Seagrass core number	Shoot number	Number of blades (shoot^{-1})	Leaf length (cm)	Leaf width (mm)
		Halodule	-	5	2	6.9	1
	T3	*Halodule*	-	1	4	18.1	1.1
		Halodule	-	2	3	15.4	1.1
		Halodule	-	3	3	13.7	1
		Halodule	-	4	4	20.2	1.2
		Halodule	-	5	3	17.5	1.1
		Thalassia	-	1	3	26.6	5.8
		Thalassia	-	2	3	23.64	6.7
		Thalassia	-	3	2	16.85	6.1
		Halodule	-	1	2	12.7	1
		Halodule	-	2	3	16.4	1.1
		Halodule	-	3	3	11.1	1
		Halodule	-	4	2	10.7	1
		Halodule	-	5	3	11.8	1

Appendix D. Table 16. Epiphyte load determined by scraping (TPWD lab) for Port Bay, May - Oct 2010.

Date	Transect	Depth	Seagrass species	Rep	Epiphyte load (mg cm^{-2})	Epiphyte load (mg g^{-1} dry seagrass)
17 May 2010	T1	Various	*Halodule*	1	0.32	280
	T2	Various	*Halodule*	1	0.31	221
	T3	Various	*Halodule*	1	0.21	205
14 Jul 2010	T1	Shallow	*Halodule*	1	0.04	52
		Middle	*Halodule*	2	0.08	117
		Deep	*Halodule*	3	0.13	244
	T2	Shallow	*Halodule*	1	0.18	174
		Middle	*Halodule*	2	0.16	165
		Deep	*Halodule*	3	0.12	142
	T3	Shallow	*Halodule*	1	0.11	109
		Middle	*Halodule*	2	0.02	17
		Deep	*Halodule*	3	0.00	-4
05 Oct 2010	T1	Shallow	*Halodule*	1	0.04	41
		Middle	*Halodule*	2	0.02	15
		Deep	*Halodule*	3	0.02	44
	T2	Shallow	*Halodule*	1	0.04	37
		Middle	*Halodule*	2	0.09	82
		Deep	*Halodule*	3	0.06	55
	T3	Shallow	*Halodule*	1	0.05	48
		Middle	*Halodule*	2	0.07	84
		Deep	*Halodule*	3	0.06	63

Appendix D. Table 17. Normalized epiphyte load determined by scraping (UTMSI lab) for East Flats, Jun - Nov 2010.

Date	Transect	Depth	Seagrass species	Rep	Epiphyte load (mg cm^{-2})	Epiphyte load (mg g^{-1} dry seagrass)
01 Jun 2010	T1	Shallow	*Thalassia*	1	1.46	-
		Shallow	*Thalassia*	1	0.70	-
		Shallow	*Thalassia*	1	1.40	-
		Shallow	*Thalassia*	1	1.28	-
		Shallow	*Thalassia*	1	0.93	-
		Shallow	*Halodule*	2	0.32	-
		Shallow	*Halodule*	2	0.88	-
		Shallow	*Halodule*	2	2.17	-
		Shallow	*Halodule*	2	0.26	-
		Shallow	*Halodule*	2	0.22	-
		Middle	*Thalassia*	1	0.14	-
		Middle	*Thalassia*	1	0.18	-
		Middle	*Thalassia*	1	0.49	-
		Middle	*Thalassia*	1	0.95	-
		Middle	*Thalassia*	1	2.09	-
		Middle	*Halodule*	2	0.45	-
		Middle	*Halodule*	2	0.15	-
		Middle	*Halodule*	2	0.57	-
		Middle	*Halodule*	2	0.15	-
		Middle	*Halodule*	2	0.90	-
		Deep	*Thalassia*	1	2.17	-
		Deep	*Thalassia*	1	0.31	-
		Deep	*Thalassia*	1	0.56	-
		Deep	*Thalassia*	1	0.41	-
		Deep	*Thalassia*	1	0.54	-
	T2	Shallow	*Halodule*	1	1.03	-
		Shallow	*Halodule*	1	0.51	-
		Shallow	*Halodule*	1	0.35	-
		Shallow	*Halodule*	1	0.82	-
		Shallow	*Halodule*	1	0.98	-
		Middle	*Thalassia*	2	0.16	-
		Middle	*Thalassia*	2	0.14	-
		Middle	*Thalassia*	2	0.67	-
		Middle	*Thalassia*	2	0.73	-
		Middle	*Thalassia*	2	0.22	-
		Deep	*Halodule*	3	0.29	-
		Deep	*Halodule*	3	0.78	-
		Deep	*Halodule*	3	0.85	-
		Deep	*Halodule*	3	0.53	-
		Deep	*Halodule*	3	0.63	-
	T3	Shallow	*Thalassia*	1	0.70	-
		Shallow	*Thalassia*	1	0.40	-
		Shallow	*Thalassia*	1	0.09	-
		Shallow	*Thalassia*	1	0.47	-
		Shallow	*Thalassia*	1	0.93	-

Date	Transect	Depth	Seagrass species	Rep	Epiphyte load (mg cm^{-2})	Epiphyte load (mg g^{-1} dry seagrass)
		Shallow	*Halodule*	2	0.14	-
		Shallow	*Halodule*	2	0.11	-
		Shallow	*Halodule*	2	0.17	-
		Shallow	*Halodule*	2	0.23	-
		Shallow	*Halodule*	2	0.13	-
		Middle	*Halodule*	3	0.17	-
		Middle	*Halodule*	3	0.44	-
		Middle	*Halodule*	3	0.18	-
		Middle	*Halodule*	3	0.17	-
		Middle	*Halodule*	3	0.19	-
		Deep	*Halodule*	4	0.23	-
		Deep	*Halodule*	4	0.53	-
		Deep	*Halodule*	4	0.63	-
		Deep	*Halodule*	4	0.50	-
		Deep	*Halodule*	4	0.33	-
02 Aug 2010	T1	Shallow	*Halodule*	1	0.35	-
		Shallow	*Halodule*	1	1.13	-
		Shallow	*Halodule*	1	0.18	-
		Shallow	*Halodule*	1	1.78	-
		Shallow	*Halodule*	1	0.68	-
		Shallow	*Thalassia*	1	1.21	-
		Shallow	*Thalassia*	1	1.11	-
		Middle	*Thalassia*	2	1.15	-
		Middle	*Thalassia*	2	0.83	-
		Middle	*Thalassia*	2	0.53	-
		Middle	*Thalassia*	2	0.70	-
		Middle	*Thalassia*	2	1.60	-
		Middle	*Halodule*	2	0.90	-
		Middle	*Halodule*	2	0.32	-
		Middle	*Halodule*	2	0.55	-
		Middle	*Halodule*	2	0.70	-
		Middle	*Halodule*	2	0.50	-
		Deep	*Thalassia*	3	0.83	-
		Deep	*Thalassia*	3	1.02	-
		Deep	*Thalassia*	3	0.81	-
		Deep	*Thalassia*	3	0.72	-
		Deep	*Thalassia*	3	0.63	-
	T2	Shallow	*Syringodium*	1	0.48	-
		Shallow	*Syringodium*	1	0.19	-
		Shallow	*Syringodium*	1	0.29	-
		Shallow	*Syringodium*	1	2.74	-
		Shallow	*Syringodium*	1	1.56	-
		Middle	*Thalassia*	2	1.00	-
		Middle	*Thalassia*	2	0.52	-
		Middle	*Thalassia*	2	0.19	-
		Middle	*Thalassia*	2	0.50	-
		Middle	*Thalassia*	2	0.59	-
		Deep	*Thalassia*	3	0.49	-

Date	Transect	Depth	Seagrass species	Rep	Epiphyte load (mg cm^{-2})	Epiphyte load (mg g^{-1} dry seagrass)
		Deep	*Thalassia*	3	0.65	-
		Deep	*Thalassia*	3	0.85	-
		Deep	*Thalassia*	3	0.12	-
		Deep	*Thalassia*	3	1.15	-
	T3	Shallow	*Halodule*	1	0.48	-
		Shallow	*Halodule*	1	0.65	-
		Shallow	*Halodule*	1	0.38	-
		Shallow	*Halodule*	1	0.60	-
		Shallow	*Halodule*	1	0.50	-
		Middle	*Syringodium*	2	0.25	-
		Middle	*Syringodium*	2	0.45	-
		Middle	*Syringodium*	2	2.20	-
		Middle	*Syringodium*	2	0.73	-
		Middle	*Syringodium*	2	0.16	-
		Deep	*Halodule*	3	0.18	-
		Deep	*Halodule*	3	1.63	-
		Deep	*Halodule*	3	0.43	-
		Deep	*Halodule*	3	0.98	-
		Deep	*Halodule*	3	0.48	-
09 Nov 2010	T1	Shallow	*Thalassia*	1	2.47	-
		Middle	*Halodule*	2	2.65	-
		Deep	*Thalassia*	3		-
	T2	Shallow	*Halodule*	1	3.25	-
		Middle	*Thalassia*	2	6.06	-
		Deep	*Halodule*	3	2.03	-
		Deep	*Thalassia*	3	4.60	-
	T3	Shallow	*Halodule*	1	2.92	-
		Middle	*Thalassia*	2	2.79	-
		Deep	*Halodule*	3	2.40	-

Appendix D. Table 18. Epiphyte fluorescence data and epiphyte load determined by scraping (TAMU-CC lab) for Port Bay, May - Oct 2010.

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
17 May 2010	T1	shallow	*Halodule*	9.19E+05	0.053	4.29E+06	17.54	4.7	8.10E+07	331	5.16E+02	4.77E+03	9.3
		shallow	*Halodule*	1.32E+06	0.083	5.54E+06	8.26	4.2	6.68E+07	100	5.45E+02	6.54E+03	12.0
		shallow	*Halodule*	1.34E+06	0.077	7.63E+06	13.87	5.7	9.91E+07	180	7.84E+02	8.47E+03	10.8
		middle	*Halodule*	8.56E+05	0.050	3.12E+06	12.14	3.6	6.25E+07	243	3.39E+02	5.50E+03	16.2
		middle	*Halodule*	8.97E+05	0.060	3.87E+06	16.12	4.3	6.45E+07	269	4.69E+02	9.21E+03	19.6
		middle	*Halodule*	8.84E+05	0.098	3.08E+06	9.18	3.5	3.14E+07	94	4.31E+02	9.37E+03	21.8
		deep	*Halodule*	1.21E+06	-	3.75E+06	24.90	3.1	-	-	2.12E+02	5.87E+03	27.7
		deep	*Halodule*	1.08E+06	0.070	4.02E+06	14.90	3.7	5.74E+07	356	2.63E+02	8.40E+03	32.0
		deep	*Halodule*	7.47E+05	0.033	1.83E+06	5.92	2.5	5.55E+07	179	1.51E+02	1.90E+03	12.6
	T2	shallow	*Halodule*	6.22E+05	0.036	4.71E+06	8.16	7.6	1.31E+08	227	5.05E+02	1.03E+04	20.5
		shallow	*Halodule*	6.29E+05	0.047	4.56E+06	4.39	7.2	9.70E+07	93	6.50E+02	9.92E+03	15.3
		shallow	*Halodule*	5.96E+05	0.039	4.87E+06	5.71	8.2	1.25E+08	146	6.90E+02	1.09E+04	15.8
		middle	*Halodule*	9.20E+05	0.059	5.10E+06	12.14	5.5	8.64E+07	206	5.45E+02	1.32E+04	24.2
		middle	*Halodule*	6.08E+05	0.040	2.50E+06	6.02	4.1	6.25E+07	150	3.74E+02	1.18E+04	31.7
		middle	*Halodule*	1.28E+06	0.096	7.17E+06	33.56	5.6	7.47E+07	350	8.33E+02	1.99E+04	23.9
		deep	*Halodule*	1.07E+06	0.050	3.37E+06	17.75	3.2	6.74E+07	355	3.89E+02	1.20E+04	30.8
		deep	*Halodule*	4.50E+05	0.033	2.21E+06	17.44	4.9	6.70E+07	529	3.85E+02	1.25E+04	32.4
		deep	*Halodule*	7.84E+05	0.051	2.75E+06	15.91	3.5	5.40E+07	312	5.90E+02	1.63E+04	27.6
	T3	shallow	*Halodule*	1.07E+06	0.081	1.61E+07	5.51	15.1	1.99E+08	68	3.09E+03	1.35E+04	4.4
		shallow	*Halodule*	5.97E+05	0.046	8.38E+06	4.69	14.0	1.82E+08	102	2.67E+03	1.20E+04	4.5
		shallow	*Halodule*	9.28E+05	0.063	1.32E+07	8.98	14.2	2.09E+08	142	3.99E+03	1.81E+04	4.5
		middle	*Halodule*	1.24E+06	0.097	1.64E+07	6.73	13.2	1.69E+08	69	3.51E+03	1.66E+04	4.7
		middle	*Halodule*	7.95E+05	0.064	1.18E+07	7.14	14.9	1.85E+08	112	2.20E+03	1.04E+04	4.7
		middle	*Halodule*	9.15E+05	0.077	1.48E+07	4.28	16.2	1.93E+08	56	3.15E+03	1.32E+04	4.2
		deep	*Halodule*	9.33E+05	0.063	1.01E+07	7.75	10.8	1.60E+08	123	2.34E+03	1.50E+04	6.4
		deep	*Halodule*	1.10E+06	0.086	1.36E+07	14.59	12.4	1.59E+08	170	2.42E+03	2.14E+04	8.8
		deep	*Halodule*	1.21E+06	0.050	9.67E+06	6.12	8.0	1.93E+08	122	1.65E+03	1.19E+04	7.2
14 Jul 2010	T1	shallow	*Halodule*	1.50E+06	0.075	3.45E+06	10.71	2.3	4.62E+07	143	2.22E+02	5.84E+03	26.3
		shallow	*Halodule*	1.41E+06	0.120	3.10E+06	12.55	2.2	2.59E+07	105	2.96E+02	1.12E+04	38.1
		shallow	*Halodule*	1.42E+06	0.057	2.74E+06	11.53	1.9	4.84E+07	204	2.17E+02	7.94E+03	36.5
		middle	*Halodule*	1.23E+06	0.087	3.18E+06	19.18	2.6	3.67E+07	221	3.26E+02	1.36E+04	41.8

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
		middle	*Halodule*	1.90E+06	0.083	4.18E+06	15.10	2.2	5.04E+07	182	3.22E+02	1.34E+04	41.7
		middle	*Halodule*	1.41E+06	0.157	3.29E+06	16.01	2.3	2.10E+07	102	3.81E+02	1.36E+04	35.7
		deep	*Halodule*	7.16E+05	0.039	1.01E+06	2.45	1.4	2.57E+07	62	1.82E+02	2.02E+03	11.1
		deep	*Halodule*	1.61E+06	0.072	3.68E+06	23.36	2.3	5.11E+07	324	3.48E+02	1.34E+04	38.6
		deep	*Halodule*	1.26E+06	0.044	2.40E+06	10.81	1.9	5.48E+07	247	1.69E+02	3.35E+03	19.9
	T2	shallow	*Halodule*	1.24E+06	0.067	2.42E+06	12.14	2.0	3.63E+07	182	2.65E+02	1.82E+04	68.6
		shallow	*Halodule*	1.54E+06	0.078	2.61E+06	15.40	1.7	3.34E+07	197	3.58E+02	3.48E+04	97.3
		shallow	*Halodule*	5.92E+05	0.091	3.04E+06	14.69	5.1	3.35E+07	162	4.26E+02	2.20E+04	51.7
		middle	*Halodule*	1.23E+06	0.074	1.91E+06	10.61	1.6	2.57E+07	143	1.77E+02	2.23E+04	125.9
		middle	*Halodule*	1.28E+06	0.057	2.19E+06	10.10	1.7	3.83E+07	177	1.97E+02	1.58E+04	80.3
		middle	*Halodule*	6.67E+05	0.076	2.18E+06	16.32	3.3	2.86E+07	214	2.63E+02	2.38E+04	90.5
		deep	*Halodule*	6.98E+05	0.075	3.11E+06	9.18	4.5	4.16E+07	123	2.17E+02	1.47E+04	67.8
		deep	*Halodule*	5.91E+05	0.049	2.40E+06	11.02	4.1	4.88E+07	224	2.17E+02	1.44E+04	66.4
		deep	*Halodule*	1.11E+06	0.058	2.71E+06	6.02	2.4	4.65E+07	103	2.11E+02	9.38E+03	44.5
	T3	shallow	*Halodule*	1.54E+06	0.080	7.80E+06	9.69	5.1	9.82E+07	122	1.48E+03	6.04E+04	40.7
		shallow	*Halodule*	1.62E+06	0.087	1.02E+07	12.75	6.3	1.17E+08	147	1.55E+03	6.34E+04	40.9
		shallow	*Halodule*	1.44E+06	0.083	6.67E+06	13.67	4.6	8.02E+07	164	1.82E+03	7.31E+04	40.1
		middle	*Halodule*	8.58E+05	0.064	5.12E+06	7.75	6.0	7.98E+07	121	8.19E+02	3.70E+04	45.2
		middle	*Halodule*	1.27E+06	0.067	6.34E+06	7.55	5.0	9.51E+07	113	8.96E+02	3.85E+04	43.0
		middle	*Halodule*	1.06E+06	0.082	7.87E+06	8.98	7.4	9.65E+07	110	1.13E+03	5.13E+04	45.6
		deep	*Halodule*	6.10E+05	0.084	6.41E+06	16.01	10.5	7.65E+07	191	2.02E+03	8.50E+04	42.0
		deep	*Halodule*	6.98E+05	0.084	7.04E+06	10.71	10.1	8.42E+07	128	1.80E+03	7.21E+04	40.0
		deep	*Halodule*	9.29E+05	0.069	5.29E+06	6.32	5.7	7.65E+07	91	1.06E+03	4.24E+04	39.9
05 Oct 2010	T1	deep	*Halodule*	2.17E+06	0.063	3.27E+06	6.22	1.5	5.17E+07	98	2.11E+02	2.15E+03	10.2
		deep	*Halodule*	1.66E+06	0.043	1.69E+06	5.30	1.0	3.98E+07	125	1.79E+02	2.96E+03	16.5
		middle	*Halodule*	2.20E+06	0.039	2.81E+06	5.81	1.3	7.18E+07	149	1.49E+02	2.18E+03	14.6
		shallow	*Halodule*	9.76E+05	0.040	2.04E+06	7.85	2.1	5.09E+07	196	2.00E+02	1.22E+03	6.1
		shallow	*Halodule*	1.04E+06	0.064	2.13E+06	6.73	2.1	3.32E+07	105	2.22E+02	2.39E+03	10.8
		middle	*Halodule*	1.48E+06	0.069	2.26E+06	7.85	1.5	3.29E+07	114	1.82E+02	2.09E+03	11.5
		middle	*Halodule*	2.15E+06	0.050	3.36E+06	3.57	1.6	6.74E+07	72	1.56E+02	2.78E+03	17.8
		deep	*Halodule*	2.96E+06	0.078	7.00E+06	6.22	2.4	9.00E+07	80	1.57E+02	3.96E+03	25.3
		shallow	*Halodule*	7.92E+05	0.058	1.05E+06	7.04	1.3	1.83E+07	122	1.91E+02	1.25E+03	6.5
	T2	middle	*Halodule*	2.64E+06	0.083	4.90E+06	6.22	1.9	5.90E+07	75	1.87E+02	4.68E+03	25.0

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
		deep	*Halodule*	2.37E+06	0.060	4.23E+06	12.24	1.8	7.01E+07	203	2.41E+02	1.20E+04	49.5
		deep	*Halodule*	2.09E+06	0.060	2.60E+06	6.22	1.2	4.31E+07	103	2.11E+02	6.05E+03	28.7
		middle	*Halodule*	1.98E+06	0.057	3.15E+06	12.04	1.6	5.57E+07	213	1.59E+02	3.74E+03	23.5
		middle	*Halodule*	2.09E+06	0.088	3.72E+06	20.60	1.8	4.24E+07	234	2.04E+02	1.16E+04	56.6
		shallow	*Halodule*	2.14E+06	0.077	3.29E+06	21.01	1.5	4.26E+07	273	2.27E+02	1.47E+04	65.1
		shallow	*Halodule*	2.12E+06	0.064	2.45E+06	12.95	1.2	3.84E+07	203	2.43E+02	1.27E+04	52.3
		shallow	*Halodule*	2.29E+06	0.093	3.95E+06	9.49	1.7	4.25E+07	102	2.60E+02	1.20E+04	46.1
		deep	*Halodule*	2.07E+06	0.076	3.46E+06	8.26	1.7	4.56E+07	109	2.62E+02	5.89E+03	22.5
	T3	middle	*Halodule*	2.08E+06	0.058	2.98E+06	5.10	1.4	5.16E+07	88	3.23E+02	1.68E+04	51.9
		shallow	*Halodule*	2.37E+06	0.069	4.36E+06	9.18	1.8	6.29E+07	132	6.88E+02	4.71E+04	68.4
		shallow	*Halodule*	2.54E+06	0.077	5.59E+06	17.95	2.2	7.26E+07	233	6.89E+02	6.73E+04	97.7
		shallow	*Halodule*	2.68E+06	0.080	6.17E+06	16.42	2.3	7.72E+07	206	5.18E+02	3.32E+04	64.1
		middle	*Halodule*	1.79E+06	0.060	2.92E+06	11.42	1.6	4.85E+07	190	3.89E+02	2.06E+04	53.0
		deep	*Halodule*	2.26E+06	0.069	3.74E+06	12.04	1.7	5.44E+07	175	3.36E+02	1.58E+04	47.2
		middle	*Halodule*	2.44E+06	0.078	4.40E+06	8.06	1.8	5.65E+07	103	1.13E+03	6.88E+04	60.8
		deep	*Halodule*	2.32E+06	0.062	3.70E+06	4.39	1.6	6.02E+07	71	3.65E+02	1.03E+04	28.1
		deep	*Halodule*	2.07E+06	0.074	2.76E+06	9.28	1.3	3.72E+07	125	3.77E+02	1.01E+04	26.8

Appendix D. Table 19. Epiphyte fluorescence data and epiphyte load determined by scraping (TAMU-CC lab) for East Flats, Jun - Nov 2010.

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
01 Jun 2010	T1	shallow	*Halodule*	1.13E+06	0.107	1.91E+08	40.60	168.9	1.78E+09	379	5.55E+04	5.14E+04	0.9
		shallow	*Halodule*	1.23E+06	0.105	2.35E+08	53.86	191.0	2.24E+09	513	3.85E+04	5.72E+04	1.5
		shallow	*Halodule*	1.58E+06	-	3.55E+08	60.49	224.8	-	-	1.13E+05	1.00E+05	0.9
		shallow	*Thalassia*	1.62E+06	0.912	3.91E+08	191.45	242.2	4.29E+08	210	8.17E+04	1.45E+05	1.8
		shallow	*Thalassia*	1.99E+06	0.433	1.81E+08	361.39	91.1	4.60E+06	835	5.86E+04	1.48E+05	2.5
		shallow	*Thalassia*	1.55E+06	0.513	1.27E+08	236.74	81.6	2.47E+08	461	1.02E+05	1.36E+05	1.3
		middle	*Halodule*	8.05E+05	0.086	8.37E+07	16.12	104.0	9.73E+08	187	1.93E+04	2.81E+04	1.5
		middle	*Halodule*	8.31E+05	0.072	1.12E+08	62.93	135.0	1.56E+09	874	3.35E+04	2.53E+04	0.8
		middle	*Halodule*	5.90E+05	0.042	3.56E+07	6.32	60.4	8.48E+08	151	2.78E+03	4.74E+03	1.7
		middle	*Thalassia*	1.30E+06	0.785	5.65E+08	362.60	435.2	7.20E+08	462	-	-	
		middle	*Thalassia*	1.14E+06	0.692	3.80E+08	194.41	333.7	5.49E+08	281	8.94E+04	1.49E+05	1.7
		middle	*Thalassia*	1.27E+06	-	4.50E+08	221.95	353.4	-	-	7.45E+04	1.38E+05	1.9
		deep	*Thalassia*	1.94E+06	1.183	5.72E+08	449.20	295.2	4.84E+08	380	1.63E+05	1.42E+05	0.9
		deep	*Thalassia*	9.47E+05	0.408	2.16E+08	127.50	227.9	5.29E+08	313	1.19E+05	9.51E+04	0.8
		deep	*Thalassia*	9.43E+05	0.488	3.25E+08	204.53	344.9	6.66E+08	419	4.29E+04	5.94E+04	1.4
	T2	shallow	*Halodule*	6.40E+05	0.069	1.63E+08	14.18	254.7	2.36E+09	205	9.98E+03	1.56E+04	1.6
		shallow	*Halodule*	8.08E+05	0.156	2.84E+08	43.25	351.4	1.82E+09	277	8.25E+04	8.08E+04	1.0
		middle	*Thalassia*	4.70E+05	0.158	4.33E+08	91.29	921.8	2.74E+09	578	2.82E+05	7.10E+04	0.3
		middle	*Thalassia*	2.73E+06	1.251	2.96E+09	373.12	1085.2	2.36E+09	298	8.66E+05	4.17E+05	0.5
		middle	*Thalassia*	3.15E+06	1.453	2.84E+09	487.46	900.9	1.95E+09	335	1.04E+06	5.38E+05	0.5
		deep	*Halodule*	1.01E+06	0.076	1.46E+08	9.28	143.8	1.92E+09	122	9.53E+03	1.00E+04	1.1
		deep	*Halodule*	1.44E+06	0.249	1.11E+09	66.81	771.7	4.46E+09	268	6.23E+04	4.82E+04	0.8
		deep	*Thalassia*	1.54E+06	0.589	8.40E+08	150.76	544.7	1.43E+09	256	2.31E+05	9.38E+04	0.4
		deep	*Thalassia*	2.85E+05	-	2.41E+07	4.18	84.5	-	-	1.79E+03	1.77E+03	1.0
		deep	*Thalassia*	1.28E+06	0.638	1.15E+09	186.97	897.8	1.80E+09	293	3.41E+05	1.35E+05	0.4
	T3	shallow	*Halodule*	1.13E+06	0.159	4.14E+08	43.96	365.6	2.60E+09	276	3.99E+04	3.98E+04	1.0
		shallow	*Halodule*	1.13E+06	0.137	3.25E+08	25.70	287.2	2.37E+09	188	3.03E+04	2.84E+04	0.9
		shallow	*Halodule*	6.26E+05	0.152	2.50E+08	35.50	399.5	1.64E+09	234	3.57E+04	4.24E+04	1.2
		middle	*Halodule*	6.03E+05	0.083	1.55E+08	14.59	257.3	1.87E+09	176	1.32E+04	1.03E+04	0.8
		middle	*Halodule*	1.34E+06	0.247	6.28E+08	50.08	468.9	2.54E+09	203	5.30E+04	4.98E+04	0.9
		middle	*Halodule*	1.15E+06	0.152	3.04E+08	52.53	265.6	2.00E+09	346	6.90E+04	4.06E+04	0.6

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
		deep	*Halodule*	1.12E+06	0.147	3.99E+08	26.11	355.1	2.72E+09	178	3.31E+04	3.31E+04	1.0
		deep	*Halodule*	1.32E+06	0.191	4.70E+08	39.98	355.7	2.46E+09	209	3.94E+04	4.24E+04	1.1
		deep	*Halodule*	1.15E+06	0.179	4.67E+08	68.14	405.3	2.61E+09	381	1.06E+05	8.38E+04	0.8
02 Aug 2010	T1	shallow	*Halodule*	6.43E+05	0.122	2.31E+08	54.06	358.4	1.89E+09	442	5.31E+04	7.28E+04	1.4
		shallow	*Halodule*	2.05E+06	0.244	2.91E+08	136.27	141.9	1.19E+09	559	6.21E+04	1.29E+05	2.1
		shallow	*Thalassia*	2.78E+06	1.050	5.09E+08	532.44	183.2	4.85E+08	507	5.47E+05	3.67E+05	0.7
		shallow	*Thalassia*	1.83E+06	1.283	9.83E+08	600.47	538.1	7.67E+08	468	3.24E+05	4.02E+05	1.2
		middle	*Halodule*	1.70E+06	0.109	2.41E+08	55.28	142.0	2.22E+09	509	5.19E+04	6.50E+04	1.3
		middle	*Halodule*	1.00E+06	0.095	1.36E+08	43.04	135.6	1.42E+09	452	1.33E+04	2.73E+04	2.0
		middle	*Thalassia*	2.75E+06	1.035	9.85E+08	358.22	357.9	9.52E+08	346	3.25E+05	3.24E+05	1.0
		deep	*Thalassia*	2.42E+06	0.945	1.53E+09	483.89	633.4	1.62E+09	512	7.57E+05	4.58E+05	0.6
		deep	*Thalassia*	2.08E+06	0.476	1.29E+09	318.75	621.6	2.72E+09	670	7.81E+05	3.94E+05	0.5
		deep	*Thalassia*	2.01E+06	0.592	1.13E+09	310.79	563.2	1.91E+09	525	5.91E+05	2.67E+05	0.5
	T2	shallow	*Halodule*	1.94E+06	0.176	4.06E+08	109.55	209.6	2.30E+09	621	9.85E+04	8.24E+04	0.8
		shallow	*Syringodium*	1.97E+06	0.247	6.21E+08	105.16	315.7	2.51E+09	426	4.04E+05	1.59E+05	0.4
		shallow	*Syringodium*	9.93E+05	0.755	5.68E+08	130.05	572.3	7.53E+08	172	1.26E+05	1.03E+05	0.8
		middle	*Thalassia*	1.23E+06	0.731	1.64E+09	794.27	1329.5	2.25E+09	1087	2.61E+06	5.41E+05	0.2
		middle	*Thalassia*	1.75E+06	0.879	3.47E+09	682.28	1981.5	3.95E+09	776	2.16E+06	5.24E+05	0.2
		middle	*Thalassia*	1.80E+06	0.694	1.59E+09	1201.87	883.8	2.29E+09	1731	3.74E+06	6.27E+05	0.2
		deep	*Halodule*	1.25E+06	0.074	3.21E+08	35.39	257.5	4.31E+09	476	6.39E+04	2.31E+04	0.4
		deep	*Thalassia*	-	0.818	1.94E+09	470.73	-	2.37E+09	575	1.88E+06	4.42E+05	0.2
		deep	*Thalassia*	2.81E+06	0.717	4.04E+09	401.27	1435.0	5.63E+09	560	1.91E+06	3.61E+05	0.2
	T3	shallow	*Halodule*	2.36E+06	0.137	4.34E+08	64.57	183.5	3.16E+09	471	7.00E+04	4.17E+04	0.6
		shallow	*Halodule*	2.16E+06	0.144	4.32E+08	70.89	200.3	2.99E+09	491	9.82E+04	4.94E+04	0.5
		shallow	*Halodule*	1.84E+06	1.183	4.65E+08	70.07	252.7	3.93E+08	59	1.32E+05	6.13E+04	0.5
		middle	*Syringodium*	2.74E+06	0.252	1.37E+08	39.27	49.9	5.43E+08	156	2.58E+04	3.71E+04	1.4
		middle	*Syringodium*	2.85E+06	0.274	8.52E+07	52.02	29.9	3.11E+08	190	4.71E+04	4.41E+04	0.9
		middle	*Syringodium*	1.91E+06	0.231	1.39E+08	36.41	72.6	6.00E+08	158	3.08E+04	3.41E+04	1.1
		deep	*Halodule*	1.85E+06	0.058	3.33E+08	61.81	179.9	5.78E+09	1073	9.97E+04	4.39E+04	0.4
		deep	*Halodule*	1.55E+06	0.095	1.60E+08	19.48	103.2	1.68E+09	205	5.28E+04	2.57E+04	0.5
		deep	*Halodule*	1.27E+06	0.069	2.09E+08	26.62	164.9	3.04E+09	388	7.15E+04	3.24E+04	0.5
09 Nov 2010	T1	shallow	*Halodule*	1.79E+06	0.092	2.02E+08	91.49	112.8	2.20E+09	998	2.71E+05	1.22E+05	0.5
		shallow	*Halodule*	1.50E+06	0.093	2.18E+08	80.17	145.5	2.35E+09	865	2.54E+05	1.09E+05	0.4

Date	Transect	Depth	Seagrass species	Scanned leaf area (number of pixels of red-excited fluorescence)	Leaf dry weight (g)	Green-excited fluorescence (fluorescence units)	Scraped epiphyte dry weight (mg)	Normalized epiphyte load (fluorescence units pixel^{-1})	Normalized epiphyte load (fluorescence units g^{-1})	Normalized epiphyte load (scraped epiphyte dry weight per leaf dry weight (mg g^{-1}))	Green-excited fluorescence of scraped epiphytes (fluorescence units)	Red-excited fluorescence of scraped epiphytes (fluorescence units)	Ratio of red-excited fluorescence to green-excited fluorescence of scraped epiphytes
		shallow	*Thalassia*	3.01E+06	1.287	1.39E+09	1440.65	460.0	1.08E+09	1119	1.25E+06	9.16E+05	0.7
		middle	*Halodule*	1.43E+06	0.037	1.76E+08	46.00	123.6	4.84E+09	1260	4.42E+04	4.63E+04	1.0
		middle	*Halodule*	7.64E+05	0.066	2.32E+08	28.15	303.2	3.49E+09	425	5.59E+04	3.79E+04	0.7
		middle	*Halodule*	1.10E+06	0.071	1.81E+08	63.04	164.5	2.56E+09	893	5.36E+04	7.12E+04	1.3
		deep	*Thalassia*	1.90E+06	0.428	7.10E+08	575.99	374.5	1.66E+09	1345	9.84E+05	5.43E+05	0.6
		deep	*Thalassia*	2.13E+06	0.516	7.16E+08	742.15	335.9	1.39E+09	1439	7.97E+05	5.80E+05	0.7
		deep	*Thalassia*	2.30E+06	0.585	8.18E+08	791.93	355.1	1.40E+09	1354	1.12E+06	8.05E+05	0.7
	T2	shallow	*Halodule*	1.73E+06	0.107	2.85E+08	179.01	165.3	2.66E+09	1668	3.27E+05	2.21E+05	0.7
		shallow	*Halodule*	2.07E+06	0.085	2.32E+08	142.60	112.1	2.72E+09	1672	4.72E+05	2.26E+05	0.5
		shallow	*Halodule*	1.50E+06	0.078	1.84E+08	101.29	122.5	2.37E+09	1307	2.25E+05	1.41E+05	0.6
		middle	*Thalassia*	2.99E+06	1.018	2.69E+09	2284.19	899.2	2.64E+09	2244	3.71E+06	9.96E+05	0.3
		middle	*Thalassia*	3.00E+06	0.886	1.83E+09	1339.06	610.8	2.06E+09	1511	3.94E+06	9.05E+05	0.2
		middle	*Thalassia*	2.67E+06	0.692	1.49E+09	1337.73	556.9	2.15E+09	1932	3.37E+06	8.96E+05	0.3
		deep	*Halodule*	1.88E+06	0.156	3.13E+08	101.90	166.4	2.01E+09	654	1.15E+06	2.68E+05	0.2
		deep	*Thalassia*	2.18E+06	0.709	8.43E+08	814.67	387.2	1.19E+09	1149	2.51E+06	6.18E+05	0.2
		deep	*Thalassia*	2.52E+06	0.509	9.64E+08	468.49	382.9	1.89E+09	921	5.09E+06	8.97E+05	0.2
	T3	shallow	*Halodule*	2.02E+06	0.147	3.48E+08	84.97	172.2	2.38E+09	580	1.59E+05	7.44E+04	0.5
		shallow	*Halodule*	2.01E+06	0.107	2.65E+08	77.42	131.9	2.47E+09	724	1.04E+05	5.24E+04	0.5
		shallow	*Halodule*	2.31E+06	0.165	4.40E+08	85.27	190.5	2.67E+09	518	1.81E+05	7.56E+04	0.4
		middle	*Thalassia*	2.88E+06	0.630	5.66E+08	325.69	196.6	8.99E+08	517	2.98E+05	1.24E+05	0.4
		middle	*Thalassia*	2.08E+06	0.539	3.50E+08	320.48	168.3	6.49E+08	595	3.09E+05	1.27E+05	0.4
		middle	*Syringodium*	2.92E+06	0.200	1.80E+08	78.74	61.5	8.99E+08	394	1.45E+05	7.36E+04	0.5
		deep	*Halodule*	2.01E+06	0.149	4.37E+08	50.90	217.1	2.93E+09	341	9.38E+04	3.80E+04	0.4
		deep	*Halodule*	1.51E+06	0.130	3.35E+08	71.20	221.6	2.58E+09	549	1.65E+05	6.62E+04	0.4
		deep	*Halodule*	1.47E+06	0.078	1.75E+08	37.94	119.1	2.23E+09	484	7.24E+04	3.35E+04	0.5

Appendix D. Table 20. Isotopic composition of seagrasses for Port Bay, May - Oct 2010.

Date	Transect	Depth	Seagrass species	del13C	del15N	C:N
17 May 2010	T1	various	*Halodule*	-15.65	5.58	14.64
		various	*Halodule*	-16.16	5.64	13.46
		various	*Halodule*	-15.64	4.27	13.32
	T2	various	*Halodule*	-16.41	1.54	12.28
		various	*Halodule*	-15.68	4.12	13.62
		various	*Halodule*	-15.62	4.71	13.91
	T3	various	*Halodule*	-14.23	6.32	17.02
		various	*Halodule*	-14.31	6.28	16.18
		various	*Halodule*	-14.56	6.71	16.53
15 Jul 2010	T1	shallow	*Halodule*	-16.66	3.03	16.31
		middle	*Ruppia*	-17.14	5.68	11.57
		deep	*Ruppia*	-17.49	4.48	11.82
	T2	shallow	*Halodule*	-16.02	5.08	15.01
		middle	*Halodule*	-16.31	4.33	16.31
		deep	*Halodule*	-16.43	4.22	13.35
	T3	shallow	*Halodule*	-16.07	5.34	16.24
		middle	*Halodule*	-16.33	5.37	16.95
		deep	*Halodule*	-17.14	5.54	13.89
06 Oct 2010	T1	shallow	*Halodule*	-16.56	3.53	10.26
		middle	*Halodule*	-17.31	3.80	12.09
		deep	*Halodule*	-18.40	0.17	9.63
	T2	shallow	*Halodule*	-18.56	3.73	12.40
		middle	*Halodule*	-17.35	2.71	11.14
		deep	*Halodule*	-17.84	3.15	10.04
	T3	shallow	*Halodule*	-18.04	3.05	10.61
		middle	*Halodule*	-18.99	1.37	11.04
		deep	*Halodule*	-18.01	1.63	10.51

Appendix D. Table 21. Isotopic composition of seagrasses for East Flats, Jun - Nov 2010.

Date	Transect	Depth	Seagrass species	del13C	del15N	C:N
01 Jun 2010	T1	shallow	*Thalassia*	-7.95	5.04	16.17
		shallow	*Halodule*	-9.70	3.33	21.14
		middle	*Thalassia*	-8.14	3.87	17.22
		middle	*Halodule*	-10.12	1.67	19.08
		deep	*Thalassia*	-9.04	3.93	14.89
	T2	shallow	*Halodule*	-11.17	5.85	19.61
		middle	*Thalassia*	-10.06	5.71	13.98
		deep	*Halodule*	-10.26	4.12	19.18
	T3	shallow	*Thalassia*	-10.00	5.27	16.24
		shallow	*Halodule*	-11.16	5.18	20.69
		middle	*Halodule*	-11.29	4.71	20.09
		deep	*Halodule*	-11.05	3.75	21.92
02 Aug 2010	T1	shallow	*Halodule*	-12.01	2.53	15.16
		middle	*Halodule*	-12.31	3.23	15.92
		middle	*Thalassia*	-10.44	4.33	12.18
		deep	*Thalassia*	-10.42	4.47	12.22
	T2	shallow	*Halodule*	-11.38	4.53	15.40
		middle	*Thalassia*	-9.80	5.25	10.11
		deep	*Thalassia*	-9.83	5.84	11.72
	T3	shallow	*Syringodium*	-7.98	3.37	14.26
		shallow	*Halodule*	-12.93	4.13	15.47
		middle	*Syringodium*	-7.13	2.22	14.26
		deep	*Halodule*	-11.57	3.76	15.07
09 Nov 2010	T1	shallow	*Thalassia*	-9.82	4.48	12.30
		middle	*Halodule*	-11.00	2.64	14.81
		deep	*Thalassia*	-10.83	5.34	12.50
	T2	shallow	*Halodule*	-10.47	2.33	12.68
		middle	*Thalassia*	-10.36	5.15	10.76
		deep	*Halodule*	-10.99	4.71	14.96
	T3	shallow	*Halodule*	-10.77	3.93	14.37
		middle	*Thalassia*	-11.65	5.18	12.58
		middle	*Syringodium*	-7.61	3.79	18.64
		deep	*Halodule*	-10.80	3.52	14.29

Appendix D. Table 22. Isotopic composition of epiphytes for Port Bay and East Flats, May - Nov 2010.

Date	Transect	Depth	Seagrass species	del13C	del15N	C:N
			Port Bay			
17 May 2010	T1	shallow	*Halodule*	-16.18	7.52	6.33
		middle	*Halodule*	-16.38	7.78	6.65
	T2	shallow	*Halodule*	-16.01	7.36	6.42
		middle	*Halodule*	-15.34	6.78	7.24
	T3	shallow	*Halodule*	-13.87	4.94	7.14
		middle	*Halodule*	-13.38	5.84	6.84
06 Oct 2010	T1	shallow	*Halodule*	-20.66	2.29	7.18
	T2	shallow	*Halodule*	-20.40	2.21	7.87
	T3	shallow	*Halodule*	-18.32	2.86	8.63
			East Flats			
01 Jun 2010	T1	shallow	*Halodule*	-15.64	3.83	9.21
		shallow	*Thalassia*	-14.15	3.51	8.93
		middle	*Thalassia*	-11.94	2.58	8.75
	T2	shallow	*Halodule*	-13.34	3.51	9.90
		middle	*Thalassia*	-16.21	6.43	6.07
		deep	*Halodule*	-12.97	2.21	9.71
	T3	shallow	*Thalassia*	-8.99	3.28	12.85
		shallow	*Halodule*	-11.95	2.61	9.86
		middle	*Halodule*	-12.15	4.49	11.30
02 Aug 2010	T1	shallow	*Thalassia*	-9.52	4.17	8.96
		shallow	*Halodule*	-10.53	4.11	8.91
		middle	*Thalassia*	-8.70	4.14	9.64
		middle	*Halodule*	-12.28	3.29	8.78
	T2	shallow	*Syringodium*	-12.80	6.45	8.24
		middle	*Thalassia*	-12.32	4.93	8.27
		deep	*Thalassia*	-9.71	5.73	10.31
	T3	shallow	*Halodule*	-9.99	6.56	10.37
		middle	*Syringodium*	-11.18	4.96	9.64
		deep	*Halodule*	-16.46	7.87	5.92
09 Nov 2010	T1	shallow	*Thalassia*	-8.03	4.12	10.93
		middle	*Halodule*	-13.73	3.30	10.76
		deep	*Thalassia*	-7.31	2.31	14.89
	T2	shallow	*Halodule*	-9.38	4.48	16.18
		middle	*Thalassia*	-8.61	4.28	15.87
		deep	*Halodule*	-12.46	4.88	14.59
		deep	*Thalassia*	-8.17	3.87	15.62
	T3	shallow	*Halodule*	-8.38	5.80	17.08
		middle	*Thalassia*	-6.72	4.95	16.84
		deep	*Halodule*	-8.08	4.92	18.03

Appendix D. Table 23. Isotopic composition of macroalgae for Port Bay and East Flats, May - Nov 2010.

Date	Transect	Macroalgae species	del13C	del15N	C:N
		Port Bay			
17 May 2010	T1	*Gracilaria graclus*	-21.34	7.88	13.17
	T2	*Gracilaria graclus*	-18.23	8.41	15.37
	T3	*Gracilaria graclus*	-16.88	8.75	17.36
15 Jul 2010	T1	*G. tikvahiae*	-23.80	8.84	15.64
	T2	*G. tikvahiae*	-21.12	6.70	24.11
06 Oct 2010	T1	*G. tikvahiae*	-24.33	7.08	11.21
		G. tikvahiae	-22.21	6.98	11.41
	T2	*G. tikvahiae*	-23.34	6.27	15.41
		G. tikvahiae	-22.88	6.09	15.15
	T3	*G. tikvahiae*	-23.90	6.96	11.35
		G. tikvahiae	-24.57	7.37	11.62
		East Flats			
01 Jun 2010	T1	*Chondria*	-16.10	5.50	26.90
		Gracilaria	-16.90	3.91	43.97
		Hypnea	-13.50	2.35	11.72
	T2	*macroalgae*	-17.47	5.82	19.95
	T3	*macroalgae*	-13.35	5.08	21.19
02 Aug 2010	T1	*D. simplex*	-16.69	4.89	10.18
		G. deblis	-17.80	6.93	17.67
		G. tikvahiae	-18.81	6.09	16.07
	T2	*C. poiteaui*	-13.93	6.33	12.16
		G. tikvahiae	-19.59	7.30	17.24
		J. capillacia	-12.46	5.72	17.40
	T3	*A. suloulata*	-16.26	6.75	15.83
		G. patens	-17.41	6.03	15.65
		G. tikvahiae	-19.13	6.79	15.80
		J. capillacea	-11.86	5.69	20.52
09 Nov 2010	T1	*G. deblis,G. tikvahiae*	-16.80	5.45	20.75
	T2	*H. cornuta*	-14.28	5.69	15.83
	T3	*G. tikvahiae*	-19.21	6.55	23.07

Appendix D. Table 24. Comparison of water and sediment chemistry results from field splits, laboratory splits, and replicates. Relative percent difference (RPD) is defined as (x-y)/((x+y)/2). "J" indicates reported value greater than the method detection limit (MDL) and less than or equal to the reporting level (RL) or practical quantitation level (PQL). "ND" indicates non-detect value.

Parameter	Units	Matrix	Type	Sample Location	Sampling Event	Sample tag	Lab		Sample results		Split tag	Lab		Split results		RPD
Ammonia	mg L^{-1}	water	Field split	EF1	summer	1085	UTMSI	<	0.0014	ND	1086	UTMSI	<	0.0014	ND	0.0%
Ammonia	mg L^{-1}	water	Field split	PB1	summer	1078	UTMSI	<	0.0014	ND	1079	UTMSI	<	0.0014	ND	0.0%
Chlorophyll-*a*	µg L^{-1}	water	Field split	PB2	summer	1055	LCRA		9.00		1056	LCRA		9.40		-4.3%
Chlorophyll-*a*	µg L^{-1}	water	Field split	EF1	summer	1065	UTMSI		4.74		1070	UTMSI		4.74		0.0%
Nitrate+nitrite	mg L^{-1}	water	Field split	EF1	summer	1085	UTMSI	<	0.0004	ND	1086	UTMSI		0.0006		-40.4%
Nitrate+nitrite	mg L^{-1}	water	Field split	PB1	summer	1078	UTMSI		0.0177		1079	UTMSI		0.0174		1.9%
Ortho-phosphate	mg L^{-1}	water	Field split	EF1	summer	1085	UTMSI	<	0.0009	ND	1086	UTMSI	<	0.0009	ND	0.0%
Ortho-phosphate	mg L^{-1}	water	Field split	PB1	summer	1078	UTMSI		0.0392		1079	UTMSI		0.0374		4.7%
TSS	mg L^{-1}	water	Field split	PB1	summer	1040	LCRA		15.8		1041	LCRA		15.1		4.5%
TSS	mg L^{-1}	water	Field split	EF1	summer	1047	UTMSI		20.3		1053	UTMSI		25.0		-20.8%
Ammonia	mg L^{-1}	water	Lab split	PB1	summer	1074	LCRA		0.0050	J	1077	UTMSI	<	0.0014	ND	112.5%
Ammonia	mg L^{-1}	water	Lab split	PB2	summer	1075	LCRA		0.0230		1080	UTMSI	<	0.0014	ND	177.0%
Ammonia	mg L^{-1}	water	Lab split	PB3	summer	1076	LCRA	<	0.0050	ND	1082	UTMSI	<	0.0014	ND	112.5%
Chlorophyll-*a*	µg L^{-1}	water	Lab split	EF1	summer	1061	LCRA		4.80		1064	UTMSI		4.58		4.7%
Chlorophyll-*a*	µg L^{-1}	water	Lab split	EF2	summer	1062	LCRA		5.90		1066	UTMSI		2.38		85.1%
Chlorophyll-*a*	µg L^{-1}	water	Lab split	EF3	summer	1063	LCRA		5.30		1068	UTMSI		3.56		39.2%
Nitrate+nitrite	mg L^{-1}	water	Lab split	PB1	summer	1071	LCRA		0.0560	ND[a]	1077	UTMSI		0.0181		102.3%
Nitrate+nitrite	mg L^{-1}	water	Lab split	PB2	summer	1072	LCRA		0.0600	ND[b]	1080	UTMSI		0.0031		180.5%
Nitrate+nitrite	mg L^{-1}	water	Lab split	PB3	summer	1073	LCRA		0.0360	ND[c]	1082	UTMSI	<	0.0004	ND	195.4%
Ortho-phosphate	mg L^{-1}	water	Lab split	PB1	summer	1071	LCRA		0.0280	J	1077	UTMSI		0.0387		-32.1%
Ortho-phosphate	mg L^{-1}	water	Lab split	PB2	summer	1072	LCRA	<	0.0100	ND	1080	UTMSI		0.0237		-81.5%
Ortho-phosphate	mg L^{-1}	water	Lab split	PB3	summer	1073	LCRA	<	0.0100	ND	1082	UTMSI		0.0102		-1.6%
TSS	mg L^{-1}	water	Lab split	EF1	summer	1044	LCRA		37.0		1047	UTMSI		20.3		58.3%
TSS	mg L^{-1}	water	Lab split	EF2	summer	1045	LCRA		23.2		1049	UTMSI		18.4		23.1%
TSS	mg L^{-1}	water	Lab split	EF3	summer	1046	LCRA		45.8		1051	UTMSI		18.4		85.4%
Clay (<0.002mm)	%	sediment	Lab split	EF1	fall	2009	LCRA		1.6		2012	UTMSI		4.8		-102.1%
Clay (<0.002mm)	%	sediment	Lab split	EF2	fall	2010	LCRA		1.6		2013	UTMSI		1.5		6.0%
Clay (<0.002mm)	%	sediment	Lab split	EF3	fall	2011	LCRA	<	0	ND	2014	UTMSI		2.0		-200.0%
Silt (0.002-0.05mm)	%	sediment	Lab split	EF1	fall	2009	LCRA		6.0		2012	UTMSI		1.2		133.8%
Silt (0.002-0.05mm)	%	sediment	Lab split	EF2	fall	2010	LCRA	<	0	ND	2013	UTMSI		3.2		-200.0%

Parameter	Units	Matrix	Type	Sample Location	Sampling Event	Sample tag	Lab		Sample results		Split tag	Lab	Split results	RPD
Silt (0.002-0.05mm)	%	sediment	Lab split	EF3	fall	2011	LCRA		2.0		2014	UTMSI	2.3	-15.4%
Sand (0.05-2.0mm)	%	sediment	Lab split	EF1	fall	2009	LCRA		92.2		2012	UTMSI	92.8	-0.6%
Sand (0.05-2.0mm)	%	sediment	Lab split	EF2	fall	2010	LCRA		98.2		2013	UTMSI	94.0	4.4%
Sand (0.05-2.0mm)	%	sediment	Lab split	EF3	fall	2011	LCRA		97.9		2014	UTMSI	94.5	3.5%
Gravel (>0.0787")	%	sediment	Lab split	EF1	fall	2009	LCRA		0.2		2012	UTMSI	1.2	-145.1%
Gravel (>0.0787")	%	sediment	Lab split	EF2	fall	2010	LCRA		0.2		2013	UTMSI	1.4	-151.1%
Gravel (>0.0787")	%	sediment	Lab split	EF3	fall	2011	LCRA		0.1		2014	UTMSI	1.1	-179.9%
Porewater ammonia	mg L^{-1}	sediment	Lab split	PB1	summer	5060	LCRA		1.38		5068	UTMSI	0.84	48.6%
Porewater ammonia	mg L^{-1}	sediment	Lab split	PB2	summer	5061	LCRA		1.63		5078	UTMSI	0.86	62.2%
Porewater ammonia	mg L^{-1}	sediment	Lab split	PB3	summer	5062	LCRA		1.98		5088	UTMSI	1.50	27.6%
Total organic carbon	mg kg-1	sediment	Lab split	EF1	fall	2015	LCRA		3080		2018	UTMSI	12302	-119.9%
Total organic carbon	mg kg-1	sediment	Lab split	EF2	fall	2016	LCRA		1380	J	2019	UTMSI	8291	-142.9%
Total organic carbon	mg kg-1	sediment	Lab split	EF3	fall	2017	LCRA	<	500	ND	2020	UTMSI	6238	-170.3%
Total organic carbon	mg kg-1	sediment	Lab split	EF1	fall				3080		2018	UTMSI	13837	-127.2%
Total organic carbon	mg kg-1	sediment	Lab split	EF1	fall				3080		2018	UTMSI	13341	-125.0%
Total organic carbon	mg kg-1	sediment	Lab split	EF2	fall				1380		2019	UTMSI	8342	-143.2%
Total organic carbon	mg kg-1	sediment	Lab split	EF2	fall				1380		2019	UTMSI	9048	-147.1%
Total organic carbon	mg kg-1	sediment	Lab split	EF3	fall				500		2020	UTMSI	5878	-168.6%
Total organic carbon	mg kg-1	sediment	Lab split	EF3	fall				500		2020	UTMSI	5779	-168.2%
Porewater ammonia	mg L-1	sediment	Replicate	PB	fall	3373	LCRA		1.34		5183	UTMSI	0.75	NA
Porewater ammonia	mg L-1	sediment	Replicate	PB	fall	3374	LCRA		1.24		5184	UTMSI	1.00	NA
Porewater ammonia	mg L-1	sediment	Replicate	PB	fall	3375	LCRA		1.30		5185	UTMSI	1.38	NA
Porewater ammonia	mg L-1	sediment	Replicate	PB	fall	mean	LCRA		1.29		mean	UTMSI	1.04	21.3%

a - LCRA value is sum of separate determinations of NO_3 and NO_2, NO_3 was 0.044 mg L^{-1}. NO_2 was <0.012 mg L^{-1} and was added as 0.012 mg L^{-1}.

b - LCRA value is sum of separate determinations of NO_3 and NO_2, NO_3 was 0.048 mg L^{-1}. NO_2 was <0.012 mg L^{-1} and was added as 0.012 mg L^{-1}.

c - LCRA value is sum of separate determinations of NO_3 and NO_2, NO_3 was 0.024 mg L^{-1}. NO_2 was <0.012 mg L^{-1} and was added as 0.012 mg L^{-1}.

Appendix E. UTMSI Report

Baseline Studies of Seagrass Condition and Water Quality in East Flats, Corpus Christi Bay: Spring, Summer and Fall 2010

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Executive Summary

The purpose of this monitoring study was two-fold: first, to compare baseline seagrass condition data from a reference site (East Flats) to a site in Port Bay where residential development and a wastewater plant are planned, and second, to test elements of a seagrass monitoring program recently proposed by Dunton *et al*. (2007) and outlined in the Quality Assurance Project Plan (QAPP) for Seagrass Response to Wastewater Inputs (Radloff 2010). We monitored East Flats, an area within Corpus Christi Bay, Texas, from June–November, 2010 using the methods described by Dunton *et al*. (2007) and Radloff (2010), including discrete and continuous monitoring of water, sediment and seagrass condition indicators along three replicate 50-m transects set roughly perpendicular to shore and extending to the deep edge of the seagrass bed.

A series of water and sediment quality indicators proposed by Dunton *et al*. (2007) to correlate with seagrass condition were measured throughout the study according to the QAPP (Radloff 2010). Discrete and continuous light measurements were gathered to determine the light environment at the seagrass canopy. Water and sediment quality indicators that directly affect seagrass condition were measured over the course of three sampling events (spring, summer and fall). Measurements included nutrients, total suspended solids, chlorophyll *a* and porewater ammonium. During the fall sampling period, sediment grain size and total organic carbon were also measured. Water and sediment quality measurements suggest that the areas monitored in East Flats are relatively unimpacted and provide suitable conditions for seagrass growth. The average chlorophyll *a* concentration was 3.0 ± 0.2 µg L^{-1} (range: 1.1 µg L^{-1} – 4.7 µg L^{-1}) and concentrations of total suspended solids were consistently low among sampling dates (usually <20 mg L^{-1}). Nitrate + nitrite, ortho-phosphate, and ammonium were often near or below the lower limits of detection.

We also monitored the condition of the two dominant seagrass species in East Flats, *Halodule wrightii* and *Thalassia testudinum.* Throughout the study, we monitored seagrass percent cover, above- and below-ground biomass, root:shoot ratios (RSR) of biomass, leaf area index, leaf nutrients and isotopic composition, and epiphyte and macroalgal biomass and composition. Our results indicate that *Halodule* and *Thalassia* meadows at East Flats are healthy with biomass changes that match the natural seasonal patterns along the Texas coast. These patterns are characterized by peak biomass in summer (*Halodule*: 211.1 ± 75.3 g m^{-2}, *Thalassia*: 793.6 ± 129.8 g m^{-2}) and a gradual decline in areal cover and an increase in epiphyte cover in the fall season. Mean percent seagrass cover was nearly 100% during spring and summer sampling, but dropped to 83.6% during fall. During the fall sampling, average epiphyte cover on *Thalassia was* 39.8 ± 7.5 g m^{-2} and on *Halodule* was 26.5 ± 2.1 g m^{-2}.

Water and sediment quality and seagrass condition results from this study indicate that East Flats is a relatively unimpacted area characterized by seagrass meadows that extend to depths of 1.3 m. We were able to effectively implement a basic Tier 3 *in situ* transect sampling effort. Of the parameters measured, turbidity measurements were of least value since units are relative and cannot be correlated with *in situ* PAR or effective water transparency.

Introduction

The condition of seagrasses often reflects inputs to the system. Inputs can range from runoff to directed discharges and can be constant or intermittent additions to the system. A wastewater treatment plant is planned in conjunction with a residential development on the shores of Port Bay. Since Port Bay has extensive seagrass meadows dominated by *Halodule wrightii* and *Ruppia maritima,* it is important to document the condition of the seagrasses in relation to present water quality prior to development. The purpose of this study was thus two-fold: first, to obtain baseline seagrass condition data from a reference site (East Flats) that is unimpacted by a wastewater outfall, and second, to test the applicability of a seagrass monitoring program for the Texas coast proposed by Dunton *et al*. (2007) and outlined in the QAPP (Radloff 2010).

Sampling for this study was conducted from June–November 2010 in the East Flats area of Corpus Christi Bay. Discrete seasonal sampling was based on recommendations from Dunton *et al*. (2007) and included measurements of water quality (nutrients, total suspended solids, chlorophyll *a*) and sediment quality (porewater ammonium, grain size, total organic carbon) indicators that directly affect seagrass condition. Seagrass condition indicators were also measured as percent cover, above- and below-ground biomass, the ratio of root:shoot biomass, leaf area index, leaf nutrients, and epiphyte and macroalgal biomass. We also monitored seagrass, epiphyte and macroalgae stable isotopes to identify carbon and nitrogen sources to the primary producers.

Each of these indicators and seagrass condition indicators were measured along three replicate 50-m transects in East Flats. Transects were roughly perpendicular to shore and extended to the deep edge of the seagrass bed. We selected transect locations after reviewing an aerial photograph of East Flats. Transects were located such that each transect spanned a depth gradient. A ground-truthing visit to the site verified these depth gradients. Each transect included the deep edge of the seagrass bed. A light meter was placed at the deep edge of a seagrass bed to continuously measure photosynthetically active radiation (PAR). We also measured salinity, temperature, depth and turbidity continuously throughout the entire study period with a YSI sonde.

Study Location

East Flats is a shallow embayment located in the Nueces estuary within Corpus Christi Bay (Appendix E. Figure 1). It is near the southeast side of a small inlet and is bordered by Mustang Island. East Flats has minimal human influence and is remotely located from any wastewater outfalls (Appendix E. Figure 1). Although East Flats contains all five seagrass species found along the Texas coast, *Thalassia testudinum* and *Halodule wrightii* are the most abundant. Consequently, this study focused on those two species. Although *Thalassia* and *Halodule* can form monospecific stands, they are generally found in mixed beds (Dunton 1996).

Appendix E. Figure 1. Location of East Flats (yellow outline), permitted wastewater outfalls and monitoring stations.

Inset is the location of East Flats in relation to Port Bay. From Whisenant *et al*. (2010).

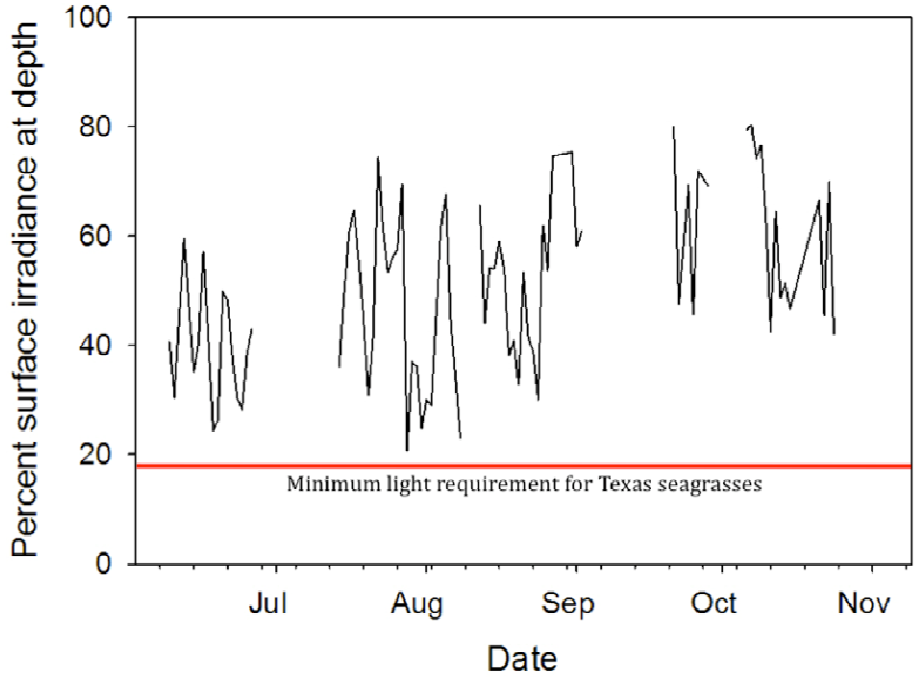
Measurements of the Seagrass Light Environment

Seagrasses have unique adaptations that require high light levels to ensure survival in a marine environment. Light limitation is common in seagrasses and is often the result of high water levels or decreased water clarity due to algal blooms or turbid conditions. To assess the light environment of seagrass meadows within East Flats, discrete and continuous light measurements were gathered throughout the study. Discrete light measurements were made (n = 4) at each of the seasonal sampling periods (spring, summer, fall) and a LI-COR continuously-recording light meter (LI-COR, Nebraska, USA) was placed at the deep edge of a seagrass bed. Average water depth at the sensor location was 110 cm (range: 100–130 cm) and represented an intermediate deep-edge depth among the three replicate transects.

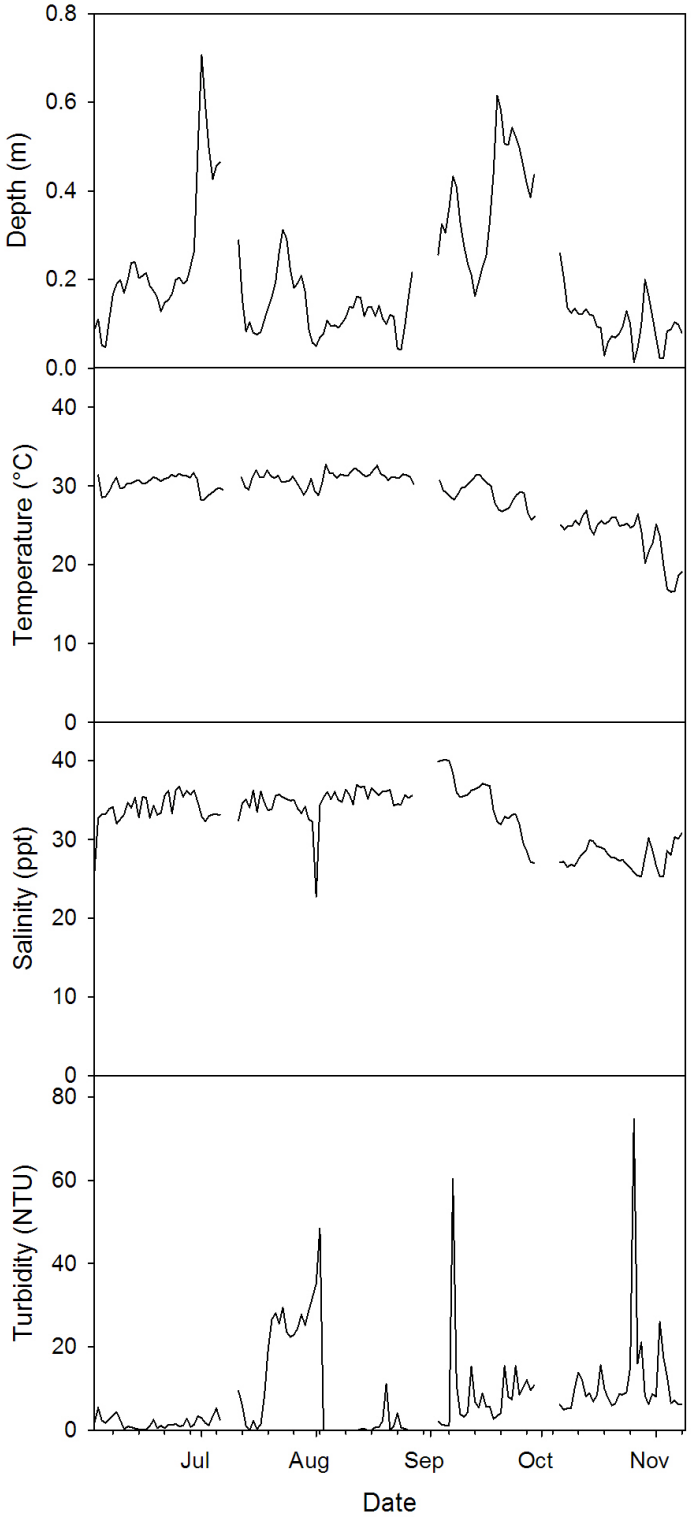
Discrete light measurements were gathered during the three seasonal sampling trips. At all times measured, the amount of light penetrating through the water column was sufficient for seagrass growth. In June, the percent surface irradiance at depth averaged 60.1 ± 7.4% (range: 25–89%); in August, 66.6 ± 6.9% (range: 46–99%); and in November, 71.1 ± 4.2% (range: 47–91%). There was no difference in percent surface irradiance at depth over time. Secchi depth often represented the depth of the seagrass canopy.

Continuous light measurements further supported the observation that light availability was consistently above the minimum light requirement needed for seagrasses (Czerny and Dunton 1995) (Appendix E. Figure 2). The average daily maximum percent surface irradiance at depth was 50.6 ± 1.7% (range: 20.7–80.2 %). The average light attenuation coefficient (K_d) during peak daylight hours (1000 – 1400) was 1.4 ± 0.58 and was within the range previously reported for the area (Kopecky and Dunton 2006).

Turbidity was also monitored continuously throughout the study with a YSI sonde and, despite a few peaks following storm events, was low (Appendix E. Figure 3). Average daily turbidity was 8.2 ± 0.9 NTU (range: 0–48.2). Turbidity measurements were of limited value in this study since units are relative and cannot be correlated with *in situ* PAR or effective water transparency. Current turbidimeters are not designed to measure any fundamental scattering properties of water. In addition, NTUs are essentially arbitrary units based off a comparison to an artificial standard, which makes turbidity difficult to correlate with other measures of water clarity (Kirk 1983). Consequently, turbidity indices are most valuable when simultaneously collected with measures of the inherent optical properties of water (Aumack *et al*. 2007).



Appendix E. Figure 2. Daily maximum percent surface irradiance at East Flats at sensor depth (average 0.8 m) throughout the study.



Appendix E. Figure 3. Daily averages of water depth, temperature, salinity and turbidity, measured continuously with a YSI sonde.

Although hourly measurements were recorded, we chose to plot daily averages because it allows trends to be seen more easily.

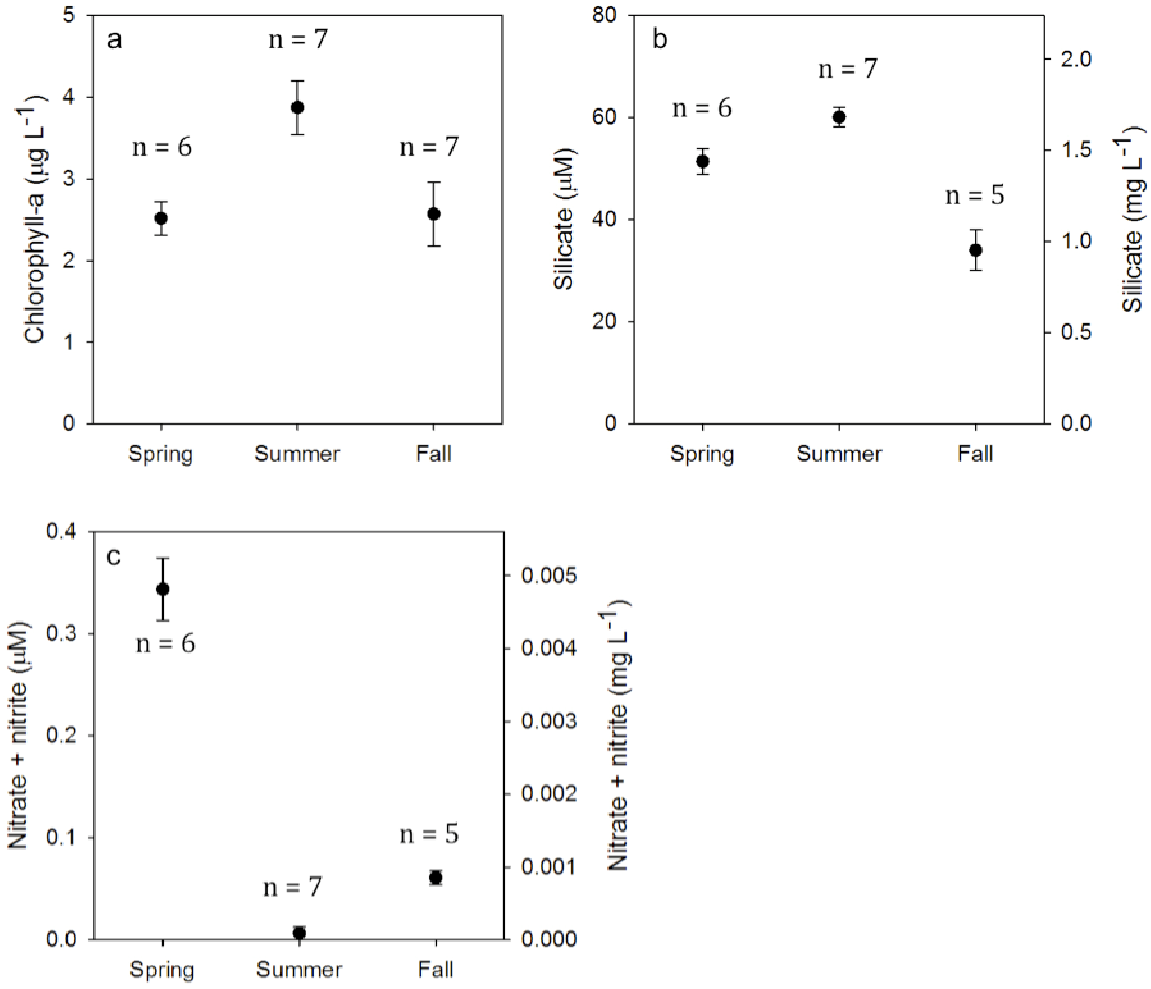
Measurements of Water and Sediment Quality

Water Quality

Scattering and absorption of light in the water column are generally attributed to high concentrations of chlorophyll *a* and total suspended solids. Both of these parameters were measured during the three seasonal sampling periods near the deep edge of seagrass beds.

Concentrations of chlorophyll *a* were low compared to the range encountered in coastal bays and estuaries (Onuf 1996). The average chlorophyll *a* concentration was 3.0 ± 0.2 μg L^{-1} (range: 1.1 μg L^{-1} – 4.7 μg L^{-1}) and during all sampling events, concentrations were not sufficient to effectively reduce light reaching the seagrass (Larkum *et al*. 2006 and chapters within) (Appendix: Table 1). Chlorophyll *a* varied over time, and was highest during the summer sampling date (3.9 ± 0.3 μg L^{-1}) (Appendix E. Figure 4a). Concentrations of total suspended solids were consistently low among sampling dates and were usually less than 20 mg L^{-1}. The measured concentrations of total suspended solids were not sufficient to block light from reaching the seagrasses.

Water column nutrient concentrations were measured during the three seasonal sampling periods near the deep edge of the seagrass beds and were also low throughout the study period (Appendix: Table 1). Similar to chlorophyll *a* concentrations, silicate, which is present in siliceous diatoms, varied with time and peaked during our summer sampling (60.1 ± 1.9 μM). (Appendix E. Figure 4b). Nitrate + nitrite also varied with time, but peaked during our spring sampling (0.3 ± 0.03 μM) (Appendix E. Figure 4c). Despite obvious peaks, silicate and nitrate + nitrite were still considerably low. Nitrate + nitrite, ortho-phosphate, and ammonium were often near or below the lower limits of detection. This, combined with low levels of chlorophyll *a* and low total suspended solids indicates that East Flats did not receive substantial nutrient runoff during the time frame of our study. Water column data for all nutrients are within ranges normally observed in East Flats (Mutchler and Dunton 2007).

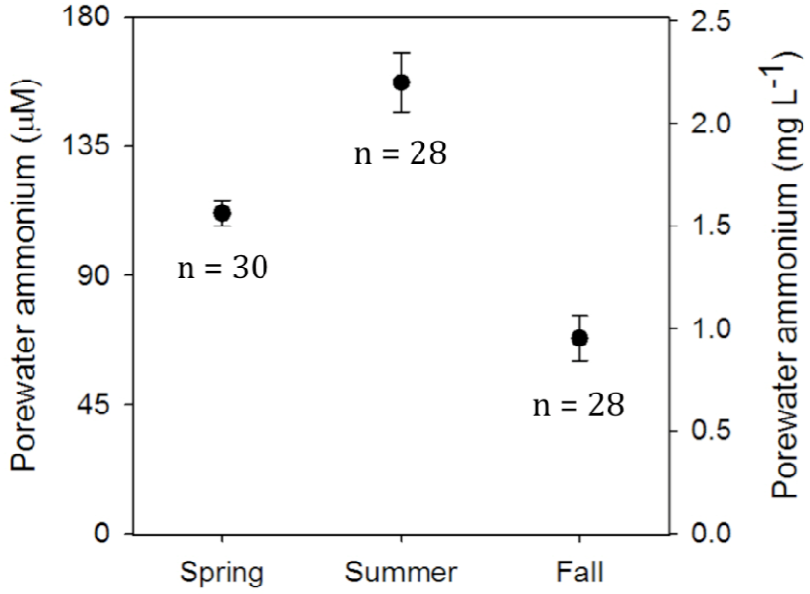


Appendix E. Figure 4. Chlorophyll *a* (*a*) , silicate (*b*), and nitrate + nitrite (*c*) concentrations during the study period. Values represent mean ± SE.

Sediment Quality

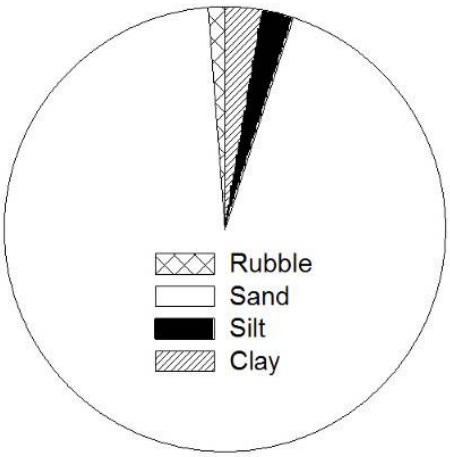
Seagrasses utilize sediments for nutrient uptake and stabilization. Sediment quality was characterized during this study by measuring porewater ammonium, grain size and total organic carbon.

Porewater ammonium was measured at 10 locations along the 3 transects during each seasonal sampling event and varied among sampling times (Appendix E. Figure 5). The highest values were observed in summer (157.3 ± 10.4 µM) and the lowest in fall (68.1 ± 7.8 µM) (Appendix E. Figure 5, Appendix E. Table 1). Data are within the range of reported values for East Flats (Czerny and Dunton 1995) and other seagrass beds (Larkum *et al*. 2006 and chapters within). There was no clear trend with depth.



Appendix E. Figure 5. Porewater ammonium concentrations during the study period. Values represent mean ± SE.

Grain size was measured during the fall sampling event. Sand composed an average of 92% of the samples. The remaining 8% was a mix of rubble, clay and silt (Appendix E. Figure 6). Although Mutchler and Dunton (2007) reported that *T. testudinum* shoot density is negatively correlated with percent sand in sediment, the composition measured in this study is representative of sediments from healthy *T. testudinum* and *H. wrightii* beds in East Flats (Mutchler and Dunton 2007) and other Texas estuaries (Dunton 1990). Total organic carbon (TOC) represents the amount of organic material in the sediments and was also measured during the fall sampling event. Average TOC was 0.92 ± 0.11% (range: 0.57 – 1.38%) (Appendix E. Table 1). The TOC measured in this study is higher than has been reported for East Flats (Mutchler and Dunton 2007).



Appendix E. Figure 6. Grain size composition of sediment samples during the fall sampling event.

Measurements of Seagrass Condition Indicators

Seagrass condition indicators were measured during all three sampling events. Average total biomass (above-ground and below-ground) was higher for *Thalassia* (631.2 ± 67 g m^{-2}) than *Halodule* (158.3 ± 36.3 g m^{-2}) and was similar to values previously reported for the area (Kopecky and Dunton 2006). Total biomass for both *Thalassia* and *Halodule* peaked during the summer sampling (Appendix E. Table 2).

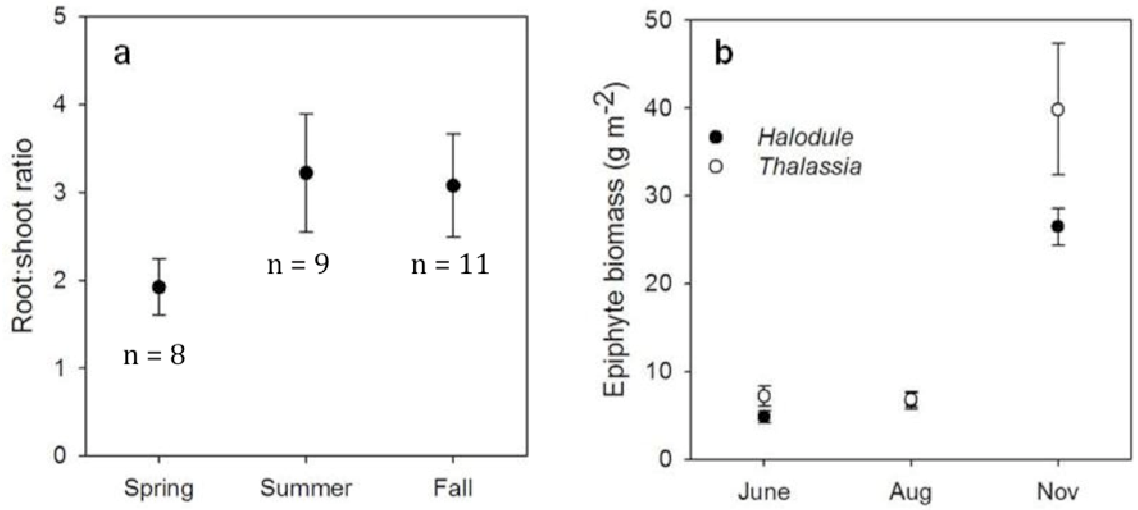
We found similar root:shoot ratios between *Halodule* and *Thalassia*. For both species, root:shoot ratios were highest in summer and fall (Appendix E. Figure 7a). During summer and into the beginning of fall, seagrass biomass and nutrient storage peaks in Texas. This is represented by high root:shoot ratios. The lowest root:shoot ratio value was observed for *Halodule* in spring (0.5) and the highest value observed was for *Thalassia* in fall (5.8) (Appendix E. Table 2). The majority of root:shoot ratios were greater than 1, indicating that seagrasses were healthy with a large amount of belowground structure. Root:shoot ratios did not vary by water depth.

Seagrass percent cover varied among sites and was dominated by *Halodule* and *Thalassia* (Appendix E. Table 3). Mean percent seagrass cover was nearly 100% during spring and summer sampling, but dropped to 83.6% during fall. This represents the fall die-back of seagrasses in Texas due to decreasing water temperatures and daylight.

Epiphyte biomass varied among sampling dates for both *Halodule* and *Thalassia.* The highest epiphyte biomass was observed in fall (Appendix E. Figure 7b). This is commonly seen along the Texas coast, and is the result of leaf age and declining seagrass growth in the fall allowing heavier epiphyte accumulation. During the fall sampling, *Thalassia* had greater epiphyte loading (39.8 ± 7.5 g m^{-2}) than *Halodule* (26.5 ± 2.1 g m^{-2}) (Appendix E. Table 2). Epiphyte loading was within the range previously reported for this area (Kopecky and Dunton 2006). Epiphyte biomass did not vary with water depth.

Leaf Area Index (LAI) is an additional measure to assess vegetation cover, and is calculated as the product of leaf length, leaf width and shoot density. The LAI was highest for both *Halodule* and *Thalassia* during summer sampling (Appendix E. Table 2). *Thalassia* had a higher overall LAI (2.20 ± 0.18) than *Halodule* (0.64 ± 0.09) when averaged over all sampling events.

Macroalgal biomass varied with depth, although no consistent trends were evident. During spring and summer sampling, macroalgae were present along the entirety of the transects, whereas only 17 of the 30 transects (56%) during fall contained macroalgae. Macroalgal biomass peaked during summer sampling (Appendix E. Table 2). Ten genera of macroalgae were represented: *Agardhiella, Canistrocarpus, Ceramium, Chondria, Digenia, Gracilaria, Hypnea, Jania, Polysiphonia*, and *Spyridia*. Of these, *Gracilaria, Hypnea* and *Jania* were the most abundant and were present in 42%, 23%, and 21% of the quadrats, respectively (Appendix E. Table 4).



Appendix E. Figure 7. Measurements of seagrass condition over the study period: (*a*) root:shoot ratios for *Halodule* and *Thalassia* combined, (*b*) epiphyte cover on *Halodule* and *Thalassia*. Values represent mean ± SE.

Number of epiphyte biomass replicates (b) for *Halodule* are: June – 35, Aug – 20, Nov – 5; For *Thalassia* are: June – 25, Aug – 22, Nov – 8.

Stable Isotope Measurements

Stable isotopic analysis of plant tissue can provide useful data for evaluating plant health and human impacts on a particular system (Peterson and Fry 1987). Stable isotopic data is presented in delta (δ) notation, which represents the ratio of heavy to light isotope in a sample compared to the ratio of heavy to light isotope in a standard, multiplied by 1000. In this study, we measured both carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes of seagrass, epiphytes, and macroalgae in order to trace the ultimate source of carbon and nitrogen used by these primary producers. Variation in $\delta^{13}C$ or $\delta^{15}N$ values can indicate different sources of inorganic dissolved carbon (DIC) or nitrogen (DIN), or differences in plant physiology, such as photosynthetic pathways (Fry 2006). We can also use the data gathered in stable isotope analysis to construct tissue C:N ratios, which gives us information about nutrient availability or limitation in these primary producers (Duarte 1990).

Stable isotope composition and C:N ratios were determined in seagrasses, epiphytes and macroalgae collected from East Flats and Port Bay. Samples were collected during all seasonal sampling events. Seagrass and epiphyte samples were collected along a depth gradient at each transect and a composite macroalgal sample was collected from each transect that included all depths.

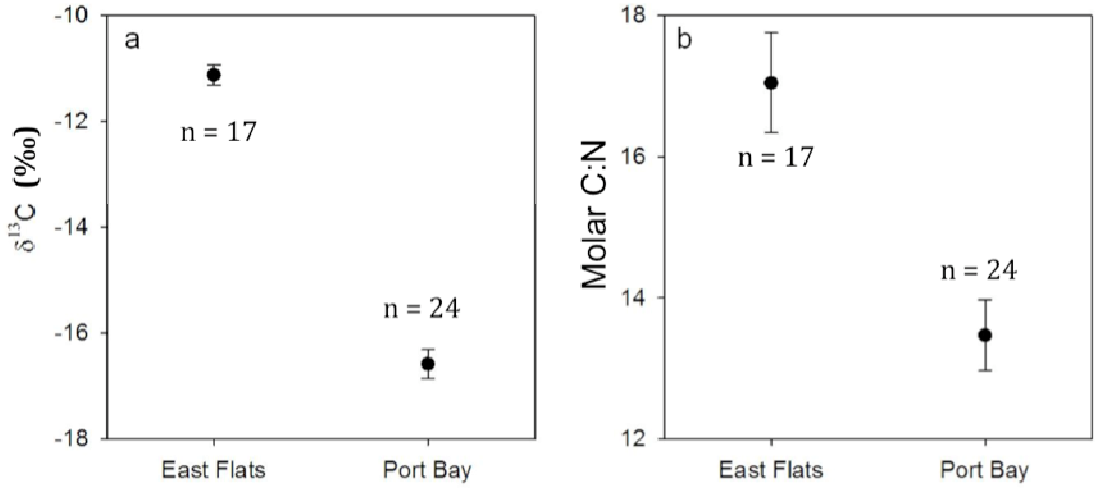
Both *Halodule* and *Thalassia* were present in East Flats. We found no difference in the $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios between seagrass species or by season (Appendix E. Table 5). Similarly, epiphyte isotopic composition and C:N ratios were similar among seagrass species and season (Appendix E. Table 5). Macroalgal isotopic composition and C:N ratios did not vary by season (Appendix E. Table 5).

Only *Halodule* was present in Port Bay. The seagrass and epiphyte isotopic compositions and C:N ratios did not vary by depth or season (Appendix E. Table 5). Likewise, macroalgal isotopic composition and C:N ratio remained constant throughout the study (Appendix E. Table 5). These data indicate no significant shifts in ultimate carbon source or nutrient inputs between seasons in East Flats and Port Bay.

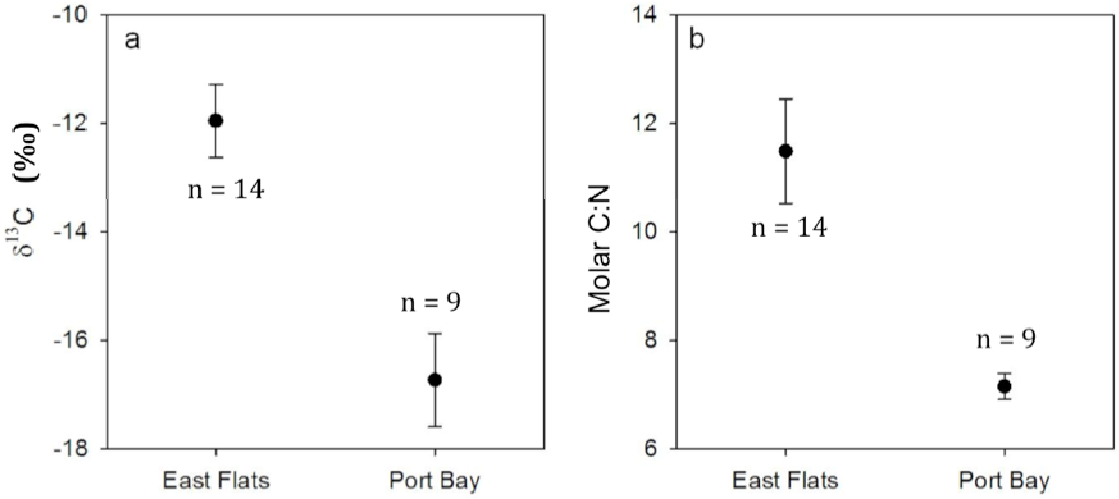
Seagrass $\delta^{13}C$ isotopic composition varied by location. The average $\delta^{13}C$ value for *Halodule* East Flats was -11.1 ± 0.2 ‰ and in Port Bay was -16.6 ± 0.3 ‰ (Appendix E. Figure 8a). The C:N ratios between locations were also different. The average C:N ratio in East Flats was 17.0 ± 0.7 and the average C:N in Port Bay was 13.5 ± 0.5 (Appendix E. Figure 8b). Similarly, epiphyte isotopic $\delta^{13}C$ composition and C:N ratio for *Halodule* were different between sites. The average $\delta^{13}C$ and C:N ratio values for East Flats were -12.0 ± 0.7 ‰ and 11.5 ± 1.0, respectively, and for Port Bay, were -16.7 ± 0.9 ‰ and 7.2 ± 0.2, respectively (Appendix E. Figure 9 a,b). These isotopic variations are likely related to differences in the dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN) pools at both sites, as well as a higher availability of inorganic-N for the plants at Port Bay compared to East Flats.

Macroalgal $\delta^{13}C$ and $\delta^{15}N$ differed by location, but the C:N ratio did not (Appendix E. Table 5). In East Flats, the $\delta^{13}C$ and $\delta^{15}N$ values were -16.2 ± 0.6 ‰ and 5.7 ± 0.3 ‰, respectively, and in Port Bay were -22.0 ± 0.8 ‰ and 7.4 ± 0.3 ‰, respectively (Appendix E. Figure 10a,b). Again, these differences are associated with the isotopic signatures of the DIC and DIN pools at each site, which are a product of any number of interrelating factors related to water column and sediment chemistry. $\delta^{15}N$ values of macroalgae collected in both locations fall within the normal range of marine macrophytes (Fry 2006). While $\delta^{15}N$ values are slightly elevated at Port Bay, these measurements do not reflect a terrestrial or anthropogenic signal.

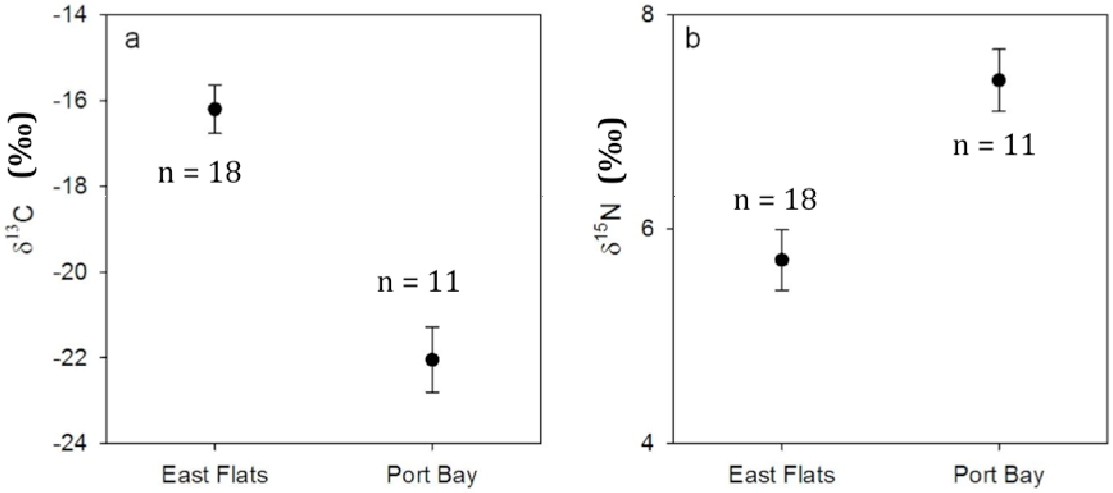
The primary producers in East Flats and Port Bay exhibit a moderate amount of natural variability. Differences in $\delta^{13}C$ values between classes of primary producers (seagrasses vs. macroalgae) highlight the different physiological processes of carbon uptake possessed by these organisms. Differences in primary producer $\delta^{13}C$ and $\delta^{15}N$ values between Port Bay and East Flats can be attributed to the two sites having distinct DIC and DIN pools. The $\delta^{15}N$ values of primary producers in both sample locations vary slightly but remain within the expected range for marine macrophytes



Appendix E. Figure 8. The $\delta^{13}C$ (a) and C:N ratio (b) values for *Halodule* from East Flats and Port Bay. Values represent mean ± SE.



Appendix E. Figure 9. The $\delta^{13}C$ (a) and C:N ratio (b) values for epiphytes on *Halodule* from East Flats and Port Bay. Values represent mean ± SE.



Appendix E. Figure 10. The $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values for macroalgae collected from East Flats and Port Bay. Values represent mean ± SE.

Conclusion

The purpose of this monitoring study was two-fold: first, to compare baseline seagrass condition data from a reference site (East Flats) to a site in Port Bay where residential development and a wastewater plant are planned, and second, to test elements of a seagrass monitoring program recently proposed by Dunton *et al*. (2007) and outlined in the Quality Assurance Project Plan (QAPP) for Seagrass Response to Wastewater Inputs (Radloff 2010).

Water and sediment quality results from this study indicate that East Flats is a relatively unimpacted area characterized by healthy seagrass meadows. During our monitoring study, East Flats had low water column nutrients and total suspended solids. There was adequate percent surface irradiance at sensor depth to sustain maximum diel seagrass production. More eutrophic conditions are denoted by considerably higher water column nutrients, chlorophyll *a*, total suspended solids and lower water transparency than observed in this study at East Flats. Such conditions usually result in higher epiphyte cover and lower percent surface irradiance at depth, which produces decreased seagrass shoot density, root:shoot ratio and leaf percent cover. We were able to effectively implement a basic Tier 3 *in situ* transect sampling effort. The data collected in this study provide a valuable baseline to assess change in a relatively pristine Corpus Christi Bay seagrass meadow.

Appendix

Appendix E. Table 1. Water and sediment quality indicators.
Values represent mean ± SE (range). Measurements that were non-detectable were considered zero. Detection limits are: Nitrate + Nitrite: 0.03 µM; Ammonium: 0.1 µM; Silicate: 0.03 µM; Orthophosphate: 0.03 µM.

Parameter	Spring	Summer	Fall
Water Quality			
Chlorophyll *a* ($\mu g\ L^{-1}$)	2.52 ± 0.2 (2.98 - 2.97)	3.87 ±0.3 (2.39 - 4.74)	2.57 ± 0.4 (1.13 - 4.19)
Total Suspended Solids ($mg\ L^{-1}$)	14.86 ± 1.8 (8.4 - 19.1)	20.74 ± 0.9 (18.4 - 25)	13.54 ± 2.1 (7.57 - 20.14)
Nitrate + Nitrite (µM)	0.34 ± 0.03 (0.22 - 0.44)	0.006 ± 0.006 (0 - 0.0452)	0.06 ± 0.007 (0 - 0.08)
Nitrate + Nitrite ($mg\ L^{-1}$)	0.005 ± 0.0004 (0.003 - 0.006)	0.00009 ± 0.000009 (0 - 0.0006)	0.0009 ± 9.5e^-5 (0 - 0.001)
Ammonium (µM)	0.087 ± 0.03 (0.018 - 0.18)	2.06 ± 1.12 (0 – 7.18)	0.7 ± 0.11 (0.007 - 0.014)
Ammonium ($mg\ L^{-1}$)	0.001 ± 0.0004 (0.0002 – 0.0026)	0.03 ± 0.021 (0 - 0.1)	0.01 ± 0.003 (0.006 – 0.015)
Ortho-phosphate (µM)	0.015 ± 0.01 (0 - 0.09)	Below detection limits	0.05 ± 0.02 (0 - 0.12)
Ortho-phosphate ($mg\ L^{-1}$)	0.0006 ± 0.0005 (0 - 0.003)	Below detection limits	0.002 ± 0.0007 (0 - 0.0037)
Silicate (µM)	51.42 ± 2.50 (43.47 - 57.69)	60.10 ± 1.90 (54.54 - 67.34)	34.0 ± 3.95 (24.1 - 43.6)
Silicate ($mg\ L^{-1}$)	1.44 ± 0.07 (1.22 - 1.61)	1.68 ± 0.05 (1.61 - 1.89)	0.95 ± 0.11 (0.67 - 1.22)
Sediment Quality			
Porewater Ammonium (µM)	111.66 ± 4.58 (67.83 – 174.95)	157.31 ± 10.38 (55.88 – 266.08)	68.13 ± 7.83 (17.26 – 186.26)
Porewater Ammonium ($mg\ L^{-1}$)	1.56 ± 0.1 (0.95 - 2.45)	2.2 ± 0.1 (0.78 - 3.49)	0.95 ± 0.1 (0.28 - 2.61)
Total Organic Carbon (% dry weight)	-	-	0.92 ± 0.11%

Appendix E. Table 2. Seagrass condition indicators.
Values represent mean ± SE (range).

Parameter	Spring	Summer	Fall
Halodule wrightii			
Total Dry Weight ($g\ m^{-2}$)	282.18 ± 59.96 (141.54 – 433.12)	211.06 ± 75.31 (34.14 - 344.42)	86.76 ± 39.33 (0.28 – 253.05)
Aboveground Biomass ($g\ m^{-2}$)	123.02 ± 31.68 (30.46 – 169.85)	81.70 ± 40.37 (4.08 – 188.57)	21.55 ± 11.80 (0 – 92.95)
Belowground Biomass ($g\ m^{-2}$)	159.16 ± 41.52 (90.90 – 276.29)	129.36 ± 42.72 (30.06 – 231.66)	48.51 ± 23.85 (0.28 – 160.10)
Root:Shoot	1.78 ± 0.67 (0.53 - 3.65)	3.31 ± 1.41 (0.83 - 7.34)	2.21 ± 0.41 (0.89 - 3.23)
Epiphyte Biomass ($g\ m^{-2}$)	4.84 ± 0.70 (1.08 - 21.7)	6.68 ± 0.96 (1.75 - 17.75)	26.49 ± 2.10 (20.25 - 32.5)
Leaf Area Index	0.761 ± 0.165 (0.159 – 2.209)	0.838 ± 0.207 (0.067 – 2.552)	0.386 ± 0.078 (0.004 – 1.265)
Thalassia testudinum			
Total Dry Weight ($g\ m^{-2}$)	424.16 ± 124.23 (71.62 - 557.45)	793.60 ± 129.76 (375.43 - 1126.06)	634.57 ± 50.50 (484.36 - 798.53)
Aboveground Biomass ($g\ m^{-2}$)	141.70 ± 43.25 (22.14 – 227.54)	195.37 ± 26.02 (140.92 – 276.57)	146.76 ± 33.32 (64.54 – 241.76)
Belowground Biomass ($g\ m^{-2}$)	282.46 ± 81.82 (49.48 – 403.96)	598.23 ± 117.79 (234.51 – 849.48)	487.80 ± 34.05 (411.55 – 597.65)
Root:Shoot	2.06 ± 0.12 (1.94 - 2.24)	3.15 ± 0.61 (1.66 - 4.96)	4.12 ± 0.89 (1.70 - 5.79)
Epiphyte Biomass ($g\ m^{-2}$)	7.24 ± 7.48 (0.88 - 21.7)	6.80 ± 0.85 (0 - 15.96)	39.79 ± 7.48 (24.6 - 60.59)
Leaf Area Index	1.2074 ± 0.18 (0.725 – 2.64)	3.08 ± 0.32 (0.32 – 5.53)	2.3 ± 0.27 (1.07 – 4.79)
Macroalgae			
Biomass (g dry weight m^{-2})	8.39 ± 2.61 (0 – 68.78)	27.79 ± 8.82 (0 - 210.82)	9.76 ± 4.55 (0 – 102.61)

Appendix E. Table 3. Percent seagrass cover in 0.25 m2 quadrats.
The shallow end of the transect is represented by a distance of 0 m.

Transect 1

Date	Distance (m)	*Halodule*	*Syringodium*	*Thalassia*	Total % Seagrass Cover
6/1/10	6	0	0	100	100
6/1/10	8	5	0	89	94
6/1/10	9	25	0	75	100
6/1/10	15	100	0	0	100
6/1/10	20	95	0	0	95
6/1/10	24	100	0	0	100
6/1/10	25	100	0	0	100
6/1/10	26	100	0	0	100
6/1/10	37	0	0	100	100
6/1/10	41	100	0	0	100
8/2/10	0	0	0	100	100
8/2/10	4	100	0	0	100
8/2/10	8	100	0	0	100
8/2/10	10	100	0	0	100
8/2/10	15	100	0	0	100
8/2/10	19	100	0	0	100
8/2/10	29	50	0	50	100
8/2/10	30	25	0	75	100
8/2/10	38	0	0	95	95
8/2/10	47	0	0	100	100
11/9/10	2	25	0	75	100
11/9/10	6	100	0	0	100
11/9/10	16	99	0	1	100
11/9/10	23	100	0	0	100
11/9/10	31	95	0	5	100
11/9/10	36	95	0	5	100
11/9/10	41	0	0	100	100
11/9/10	45	0	0	100	100
11/9/10	46	0	0	100	100
11/9/10	48	0	0	96	96

Table 3, Continued

Transect 2

Date	Distance (m)	*Halodule*	*Syringodium*	*Thalassia*	Total % Seagrass Cover
6/1/10	6	15	0	85	100
6/1/10	8	0	0	100	100
6/1/10	9	0	0	100	100
6/1/10	15	0	0	100	100
6/1/10	20	0	0	100	100
6/1/10	24	0	0	100	100
6/1/10	25	0	0	100	100
6/1/10	26	0	0	100	100
6/1/10	37	80	20	0	100
6/1/10	41	0	100	0	100
8/2/10	0	100	0	0	100
8/2/10	4	100	0	0	100
8/2/10	8	0	100	0	100
8/2/10	10	0	96	0	96
8/2/10	15	0	100	0	100
8/2/10	19	0	0	100	100
8/2/10	29	0	0	100	100
8/2/10	30	0	0	100	100
8/2/10	38	0	0	100	100
8/2/10	47	5	0	95	100
11/9/10	2	100	0	0	100
11/9/10	6	50	50	0	100
11/9/10	16	0	95	0	95
11/9/10	23	0	0	100	100
11/9/10	31	0	0	90	90
11/9/10	36	0	0	70	70
11/9/10	41	0	0	90	90
11/9/10	45	0	0	88	88
11/9/10	46	4	0	86	90
11/9/10	48	8	0	77	85

Table 3, Continued

Transect 3

Date	Distance (m)	*Halodule*	*Syringodium*	*Thalassia*	Total % Seagrass Cover
6/1/10	6	100	0	0	100
6/1/10	8	100	0	0	100
6/1/10	9	100	0	0	100
6/1/10	15	96	4	0	100
6/1/10	20	0	100	0	100
6/1/10	24	0	65	25	90
6/1/10	25	0	95	5	100
6/1/10	26	0	100	0	100
6/1/10	37	70	0	0	70
6/1/10	41	100	0	0	100
8/2/10	0	100	0	0	100
8/2/10	4	100	0	0	100
8/2/10	8	100	0	0	100
8/2/10	10	100	0	0	100
8/2/10	15	50	50	0	100
8/2/10	19	0	75	0	75
8/2/10	29	0	70	0	70
8/2/10	30	0	45	0	45
8/2/10	38	100	0	0	100
8/2/10	47	2	0	0	2
11/9/10	2	100	0	0	100
11/9/10	6	87	0	0	87
11/9/10	16	2	0	0	2
11/9/10	23	1	99	0	100
11/9/10	31	100	0	0	100
11/9/10	36	100	0	0	100
11/9/10	41	65	0	0	65
11/9/10	45	0	0	0	0
11/9/10	46	50	0	0	50
11/9/10	48	0	0	0	0

Appendix E. Table 4. Macroalgal composition by genus in 0.0625 m^2 quadrats.

Date	Transect	Distance (m)	*Agardhiella*	*Canistrocarpus*	*Ceramium*	*Chondria*	*Digenia*	*Gracilaria*	*Hypnea*	*Jania*	*Polysiphonia*	*Spyridia*
6/1/10	1	6						X				
6/1/10	1	15						X				
6/1/10	1	24						X				
6/1/10	1	26								X		
6/1/10	1	37				X		X	X		X	
6/1/10	1	41									X	
6/1/10	2	8								X		X
6/1/10	2	24							X			
6/1/10	2	37							X	X		
6/1/10	2	41						X	X	X		
6/1/10	3	6						X	X	X		
6/1/10	3	8						X	X	X		
6/1/10	3	15						X	X			
6/1/10	3	20							X	X		
6/1/10	3	24						X	X	X		
6/1/10	3	25							X	X		
6/1/10	3	26							X	X		
6/1/10	3	41							X	X		
8/1/10	1	0						X				
8/1/10	1	4						X				
8/1/10	1	8						X				
8/1/10	1	10						X				
8/1/10	1	19						X				
8/1/10	1	29					X					
8/1/10	1	30					X	X				
8/1/10	1	38		X			X	X				
8/1/10	2	0	X	X				X		X		
8/1/10	2	4		X				X	X			
8/1/10	2	8	X	X				X				
8/1/10	2	10						X	X	X		
8/1/10	2	15					X	X		X		

Table 4, Continued

Date	Transect	Distance (m)	*Agardhiella*	*Canistrocarpus*	*Ceramium*	*Chondria*	*Digenia*	*Gracilaria*	*Hypnea*	*Jania*	*Polysiphonia*	*Spyridia*
8/1/10	2	47						X				
8/1/10	3	0						X		X		
8/1/10	3	4		X				X		X		
8/1/10	3	8		X				X	X			
8/1/10	3	10						X				
8/1/10	3	15		X					X	X		
8/1/10	3	19	X	X				X	X	X		
8/1/10	3	29		X				X		X		
11/9/10	1	2					X	X				
11/9/10	1	6	X					X				
11/9/10	1	16						X				
11/9/10	1	31						X				
11/9/10	1	36						X				
11/9/10	1	45						X				
11/9/10	1	48						X				
11/9/10	2	16							X			
11/9/10	2	23							X			
11/9/10	3	2						X				
11/9/10	3	23			X				X			
11/9/10	3	31		X				X				
11/9/10	3	41					X		X			

Appendix E. Table 5. Isotopic composition of seagrasses, epiphytes and macroalgae.

	East Flats				Port Bay			
	del13C (‰)	**del15N (‰)**	**C:N**	**n**	**del13C (‰)**	**del15N (‰)**	**C:N**	**n**
Seagrasses								
Halodule								
Spring	-10.68 ± 0.24	4.087 ± 0.52	20.24 ± 0.40	7	-15.36 ± 0.27	5.02 ± 0.53	14.55 ± 0.55	9
Summer	-12.04 ± 0.28	3.64 ± 0.35	15.41 ± 0.15	5	-16.42 ± 0.17	4.78 ± 0.39	15.78 ± 0.46	6
Fall	-10.81 ± 0.10	3.43 ± 0.43	14.22 ± 0.41	5	-17.90 ± 0.25	2.57 ± 0.42	10.86 ± 0.31	9
Thalassia								
Spring	-9.039 ± 0.45	4.76 ± 0.37	15.7 ± 0.57	5	-	-	-	-
Summer	-10.12 ± 0.18	4.97 ± 0.35	11.55 ± 0.50	4	-	-	-	-
Fall	-10.66 ± 0.39	5.04 ± 2.52	12.03 ± 0.43	4	-	-	-	-
Epiphytes								
Halodule								
Spring	-13.21 ± 0.66	3.33 ± 0.41	9.99 ± 0.35	5	-15.19 ± 0.52	6.70 ± 0.45	6.77 ± 0.15	6
Summer	-12.31 ± 1.46	5.46 ± 1.06	8.49 ± 0.93	4	-	-	-	-
Fall	-10.40 ± 1.14	4.68 ± 0.40	15.33 ± 1.27	5	-19.8 ± 0.74	2.46 ± 0.20	7.89 ± 0.42	3
Thalassia								
Spring	-12.82 ± 1.55	3.95 ± 0.85	9.15 ± 1.40	4	-	-	-	-
Summer	-10.06 ± 0.78	4.74 ± 0.38	9.29 ± 0.44	4	-	-	-	-
Fall	-7.77 ± 0.34	3.90 ± 0.44	14.83 ± 1.02	5	-	-	-	-
Macroalgae								
Spring	-15.46 ± 0.86	4.53 ± 0.63	24.7 ± 5.38	5	-18.82 ± 1.32	8.35 ± 0.25	15.30 ± 1.21	3
Summer	-16.39 ± 0.87	6.25 ± 0.23	15.85 ± 0.92	10	-22.46 ± 1.34	7.77 ± 1.07	19.88 ± 4.24	2
Fall	-16.76 ± 1.42	5.89 ± 0.33	19.88 ± 2.13	3	-23.54 ± 0.37	6.79 ± 0.20	12.69 ± 0.82	6

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Appendix F. TAMU-CC Report

Seagrass Response to Wastewater Inputs: Implementation of a Seagrass Monitoring Program in Two Texas Estuaries

Seagrass Epiphyte Fluorescence Measurement Final Report

February 25, 2011

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GLO contract number: 10-049-000-3745
TPWD contract number: 211946

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Introduction

Project Objectives

The study protocol followed a recent proposal by Dunton and Pulich to the Seagrass Monitoring Work Group (Landscape Monitoring and Biological Indicators for Seagrass Conservation in Texas Coastal Waters, draft, Dunton *et al.* 2007) and included three components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) seagrass epiphyte fluorescence analysis. Findings from the third objective are presented here.

Background

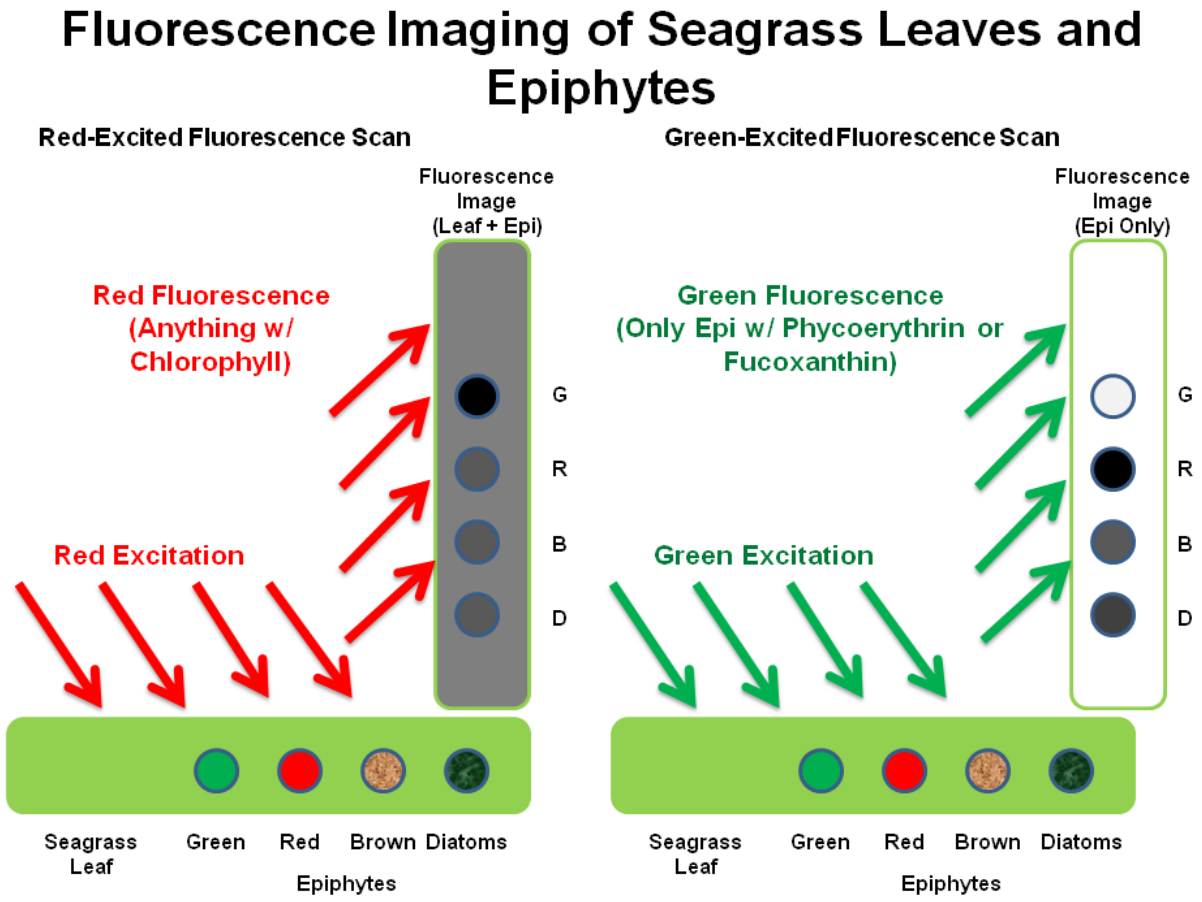
In nutrient-enriched waters, epiphytic algae growth may increase; at some point interfering with photosynthesis or having other effects, and potentially causing seagrass loss. The relationship between epiphyte accumulation and seagrass impairment is not fully understood (Burkholder *et al.* 2007), but eutrophication can be associated with increased epiphyte loading (Worm and Sommer 2000; Mutchler and Dunton 2007; Burkholder *et al.* 2007; Peterson *et al.* 2007). As nutrient levels increase through eutrophication, grazing by crustaceans, gastropods and other organisms may counter-balance the increased growth of epiphytes up to some threshold level when epiphyte growth overwhelms the grazing rate (Hays 2005; Burkholder *et al.* 2007; Heck and Valentine 2007). The abundance of epiphytes is believed to be an integrated measure of nutrient conditions in a seagrass bed. In addition, both algal epiphyte species composition and morphology can change with nutrient availability (Armitage *et al.* 2005; Frankovich *et al.* 2009).

Measurements of epiphytic algal density, expressed on a normalized basis relative to seagrass leaf area or biomass, are commonly used to quantify epiphyte loading as a sensitive way to detect impacts of increased nutrient loadings. While traditional biomass measures are simple and inexpensive to perform, they are somewhat tedious and fail to account for species composition or morphology changes and thus provide an incomplete picture of epiphyte dynamics in response to nutrient loading. Changes in seagrass growth rate and turnover time may additionally need to be considered.

This study compared traditional measurements of epiphytic algal biomass obtained from leaf scrapings to novel epiphyte fluorescence measurements made in Cammarata's lab (Cammarata *et al.* 2009). The method measures fluorescence of photosynthetic accessory pigments as a proxy for epiphyte abundance and provides greater spatiotemporal resolution of epiphyte accumulation than the traditional leaf-scraping method. Plotting incremental epiphyte abundance along the age gradient of the seagrass leaf reveals an historical record of epiphyte recruitment and growth relative to the growth of the seagrass leaf. This relationship is expected to change with increased eutrophication. Morphometrics, including number of blades per whole shoot, leaf length and width, and a proxy for leaf area for a seagrass sample can also be estimated. Fluorescence images of epiphytes can be archived for subsequent development of new analytical tools.

The fluorescence method, shown conceptually in Appendix F. Figure 1, digitally images epiphytes which absorb light in the green range of the visible spectrum (532 nm) and emit fluorescence at wavelengths between 550 nm and 610 nm (French and Young 1952). These

organisms include cyanobacteria, red algae, diatoms, cryptomonads, brown algae and dinoflagellates (Appendix F. Table 1; Raven *et al*. 2005; Frouin 2006; Robertson 2009). The method is based on the preferential excitation and fluorescence emission of epiphytes relative to the underlying seagrass leaf. Image analysis quantifies fluorescence intensity and areal coverage (number of image pixels of known size). Two fluorescence images are obtained for each sample: a green-excited fluorescence (Green F) image that preferentially images epiphytes, and a red-excited fluorescence (Red F) image used to visualize the seagrass leaf *plus epiphytes* (Appendix F. Figure 1). Red wavelengths of light fluoresce all chlorophyll-containing organisms to some degree (Appendix F. Table 1).



Appendix F. Figure 1. Conceptual illustration of red- vs. green-excited fluorescence from seagrass leaves and different types of epiphytes.

Chlorophyll pigments in seagrass leaves or epiphytes will fluoresce when excited with red wavelengths of light (Red F). Photosynthetic accessory pigments unique to certain classes of epiphytes (see Appendix F. Table 1) fluoresce when excited with green wavelengths of light (Green F). Red F images show seagrass blades plus epiphytes, whereas Green F images show only epiphytes.

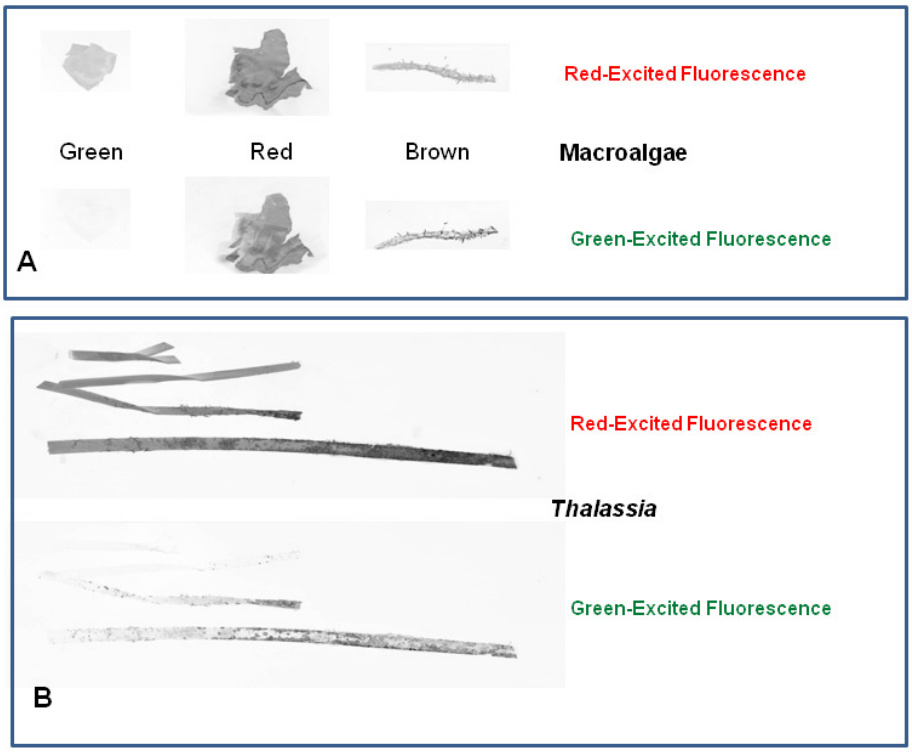
Appendix F. Table 1. Algal epiphyte pigments and fluorescence properties.

Class of Algal Epiphytes	Photosynthetic Pigments Contributing to Fluorescence	Green-Excited Fluorescence*	Red-Excited Fluorescence*
Green Algae	Chlorophylls *a, b*	Very Weak	**Very Strong**
Red Algae	Chlorophylls *a, c*; Phycobilins**	**Very Strong**	Weak
Diatoms	Chlorophylls *a, c*; Fucoxanthin	**Weak**	Weak
Brown Algae	Chlorophylls *a, c*; Fucoxanthin	**Weak**	Weak
Dinoflagellates	Chlorophylls *a, c*; Peridinin	**Weak**	Weak
Cyanobacteria	Chlorophyll *a*; Phycobilins**	**Weak**	Weak

* Variable

** Phycoerythrin specifically contributes to green-excited fluorescence

An illustrative example of the selective imaging properties of Green F compared to Red F is shown in Appendix F. Figure 2. Images of green, red and brown macroalgae are easily observed with Red F, but the Green F preferentially visualizes only the red and brown macroalgae by virtue of their accessory pigments. Likewise, all blades of the *Thalassia* whole shoot are visualized by Red F, but only the epiphytes are seen in the Green F image. Very young blades and the youngest parts of older blades do not fluoresce with green excitation because only chlorophyll is present.



Appendix F. Figure 2. Red- and green-excited fluorescence images of example macroalgae and a seagrass whole shoot.

All images darkened by 7% to enhance reproduction. (A) Macroalgae scanned for red- and green-excited fluorescence using standard scanning conditions used in this study (200 µm pixels, 360 V). Note that all algal types visible by Red F, but green algal image barely visible by Green F. (B) Three blades and base of a whole shoot of *Thalassia testudinum* scanned as in (A). Seagrass blades and their epiphytes are clearly visible by Red F, but only epiphytes visualized by Green F. Note near absence of epiphytes on youngest blade and at bottom of older blades.

Scanning seagrass leaves by this method does not quantify green algal components of seagrass epiphytes, because the red light needed to excite the chlorophylls of the green algae also excites the seagrass leaf pigments (Appendix F. Figure 1; Appendix F. Table 1). However, if epiphytes are first removed from the seagrass blade by scraping, then removed epiphytes can be fluoresced and quantified using *both* red and green excitation wavelengths. This provides a measure that includes all of the different types of epiphytic algae, including green. Changes in the relative contributions of green and red algae to total epiphyte abundance can be captured by comparing the ratio of red-excited fluorescence to green-excited fluorescence.

Methods

The Quality Assurance Project Plan (QAPP) (Radloff 2010) describes procedures that were followed in data acquisition and analysis for all components of this work.

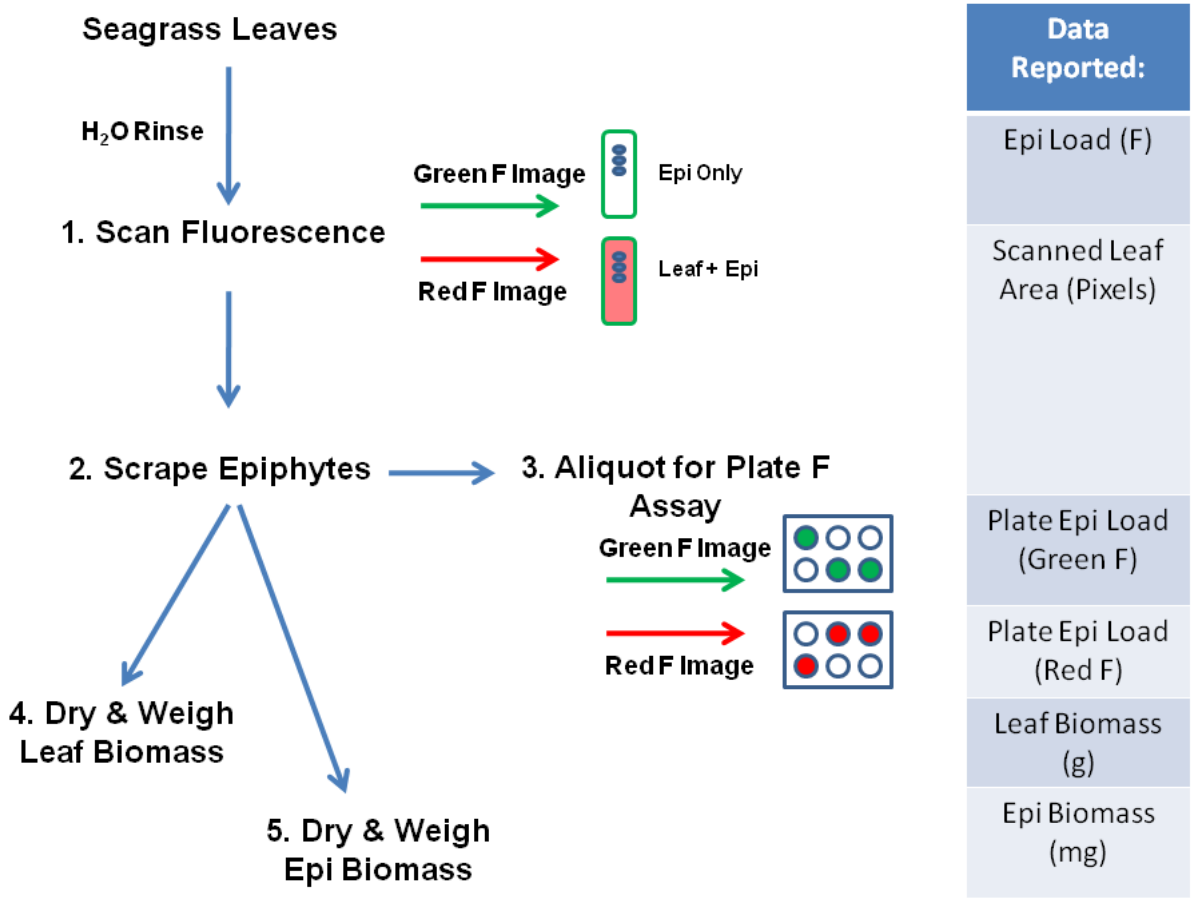
Samples for epiphyte fluorescence measurements were collected (by UTMSI and TPWD staff) near quadrats representing the shallow end, middle and deep end of each transect. Three replicate samples were obtained for each significant seagrass species at each depth (generally nine samples for *Halodule* and fewer for *Thalassia* and *Syringodium* for each transect). Sampling and fluorescence epiphyte measurements were typically performed only for the predominant seagrass species collected at each depth. Samples comprised whole-shoots (up to 50) obtained by gently pinching or cutting off shoots near their base, handling only at the base and transferring to widemouth sample bottles without water to avoid disturbing attached epiphytes.

A conceptual summary of the analytical workflow for this portion of the project is shown in Appendix F. Figure 3. Five to fifteen seagrass whole shoots were rinsed in water, separated into individual blades and scanned for fluorescence using standard procedures described in the Cammarata protocol/QAPP. Two scans (Green F and Red F) were obtained for each sample. Epiphytes were then removed from the scanned seagrass blades by scraping into a small volume of water. An aliquot of the removed epiphytes was scanned for fluorescence in the Plate Assay (both Green F and Red F). The remaining epiphytes and the epiphyte-free blades were then dried to constant weight for biomass measurements. In addition, for one blade from each sample collection site, a "high resolution" (10 µm pixel size) scan of epiphyte Green F was recorded to document the morphology and colonization patterns of the epiphytes.

A summary of the types of data reported, including performance specifications, is provided in Appendix F. Table 2.

Evaluation of the fluorescence-based methodology for assessment of epiphyte accumulation entails three considerations:

1. How do fluorescence measurements correlate with traditional seagrass condition measurements, particularly biomass?
2. Are apparent seasonal, depth or location (transect) trends consistent for both fluorescence and biomass measurements?
3. Does the fluorescence-based methodology provide new insights not previously possible?



Appendix F. Figure 3. Analytical workflow for seagrass epiphyte fluorescence and associated measurements.

Seagrass leaves were rinsed in water and scanned for fluorescence using standard procedures described in the Cammarata protocol/QAPP. Epiphytes were then removed from the scanned seagrass blades by scraping into a small volume of water. An aliquot of the removed epiphytes was scanned for fluorescence in the Plate Assay. The remaining epiphytes and the epiphyte-free blades were dried to constant weight for biomass measurements.

Appendix F. Table 2. Seagrass epiphyte fluorescence measurement performance specifications.

Analysis	Matrix	Units	STORET Code	Analytical Method	Sensitivity	Precision	Expected Range
Red-excited fluorescence	plant	Arbitrary Fluorescence Units (F.U.)	NA	Red-excited fluorescence signal Cammarata protocol/QAPP	5 F.U.	±15%	NA
Scanned Leaf Area	plant	Number of pixels @ 200 µm	NA	Number of pixels of red-excited fluorescence Cammarata protocol/QAPP	NA	±15%	NA
Epiphyte Load	plant	F. U.	NA	Green-excited fluorescence Cammarata protocol/QAPP	5 F.U.	±15%	NA
Normalized Epiphyte Load	plant	F.U./pixel and F.U./g dry biomass	NA	Calculated Cammarata protocol/QAPP	NA	±15%	1-300
Epiphyte recruitment and growth	plant	F.U. per unit leaf length	NA	Profile of epiphyte accumulation from leaf base Cammarata protocol/QAPP	NA	±15%	NA
Relative contribution green vs. red algal Epiphyte Load	plant	NA	NA	Calculated ratio of red-excited fluorescence signal to green-excited signal in removed epiphytes Cammarata protocol/QAPP	NA	NA	1-100

Results

Examples of Fluorescence Imaging

Representative examples of fluorescence images, organized by sampling site, season and seagrass species, are presented in Appendix F. Figure 4 to Appendix F. Figure 17. Note that the images are not all on the same scale in these figures in order to accommodate the complete images. Pairs of images from red-excited and green-excited fluorescence reveal, respectively, the seagrass blades scanned and the epiphyte images obtained. Each image in this report was artificially darkened by 7% to enhance visual observation of green excited epiphyte images in samples with relatively low epiphyte loads. Note that the 2-4 blades typically observed for each whole shoot are grouped together to allow future analyses based on individual whole shoots. For the blades comprising a whole shoot, it is generally very easy to distinguish the older blades with heavier epiphyte accumulations from the younger blades with less accumulation and a steep accumulation gradient of epiphytes, representing initial colonization of the leaf blade. It is also visually obvious that *Halodule* from East Flats have greater accumulations in general, and especially for filamentous epiphytes, compared to Port Bay.

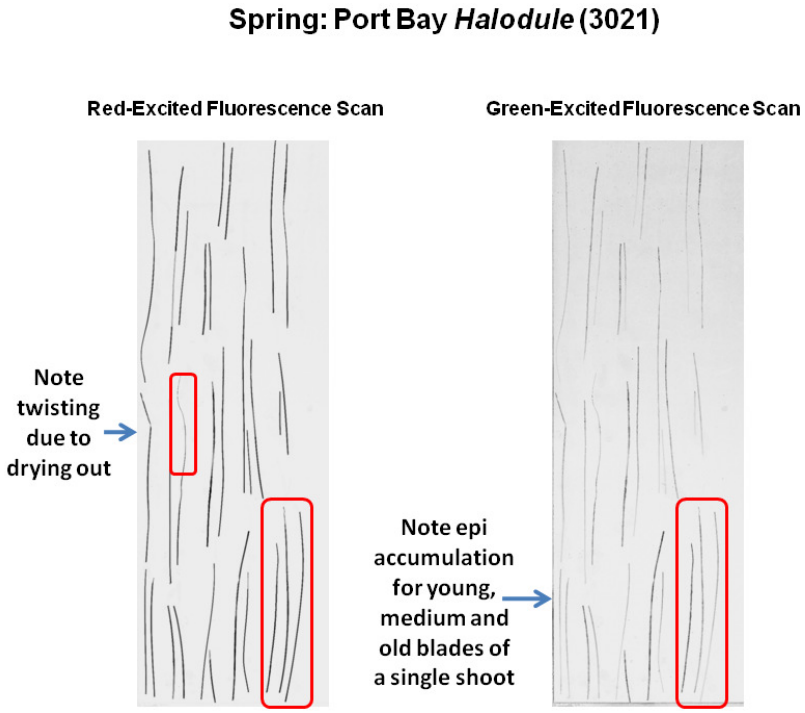
Seagrass leaf and epiphyte images also show two problems encountered in the scanning methodology: 1) *Thalassia,* particularly long old blades, often have a tendency to twist; and 2)

Halodule, particularly young thin blades, have a tendency to curl because of drying induced by the heat of the laser excitation. Despite the fact that the lid on the scanner platen can be set to "press" the samples, both issues still present difficulties for scanning and quantification. We have devised a strategy which greatly minimizes the problem, though does not eliminate it. If seagrass blades are soaked in a solution of 5-20% glycerol (5 min prior to scanning), the drying is prevented and it is easier to get *Thalassia* to lie flat. Glass microscope slides can also be used to help weigh down the blades. Unfortunately, this strategy could not be employed in this study because the experimental design called for weighing the leaves and the epiphytes scraped from the leaves after scanning. The presence of glycerol would have interfered with this process. But for future work where weighing the epiphytes may not be necessary, these procedural modifications are recommended.

Appendix F. Figure 4 to Appendix F. Figure 17 are examples of the image data archived as TIFF files. All images can be accessed for future data mining by image processing. In support of this concept, high resolution Green F epiphyte images were additionally obtained for one representative blade at the medium depth of each transect for each sampling event. These high resolution images were obtained using a 10 µm pixel size (as opposed to the standard 200 µm pixel size used for all other quantitative work), and examples from the fall season are seen in Appendix F. Figure 13 to Appendix F. Figure 15. The images in this report were artificially darkened by 12 % for visual observation because the small pixel size (100 μm^2 vs. 40,000 μm^2) results in low fluorescence intensities. No quantitative metrics were determined for these high resolution images, but archiving will facilitate any future efforts.

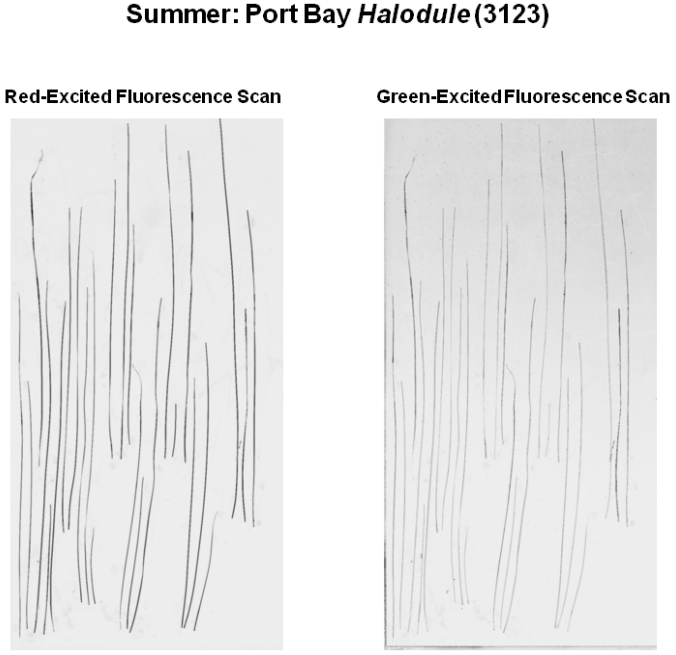
Qualitatively, there are large differences in accumulation levels and primary morphology of the imaged epiphytic algae when comparing Port Bay *Halodule* to East Flats *Halodule* to East Flats *Thalassia*. Consistent with all other findings, East Flats epiphyte accumulations are much greater than Port Bay accumulations. Port Bay *Halodule* blades have little filamentous or distinct colonial forms, but rather a general background accumulation of what are probably very small and/or weakly fluorescing epiphytes, perhaps diatoms. In contrast, East Flats *Halodule* has distinct colonial or crustose forms on young blades, and massive amounts of filamentous epiphytes on the older blades, illustrating the succession of epiphytes. East Flats *Thalassia* epiphytes were predominantly crustose forms of varying fluorescence intensities. A general gradient of accumulation is observed from the youngest base of the blade to the oldest tip of the blade, a property that is exploited for analysis below.

Examples of the Plate Assay of *removed* epiphytes show typical results for samples and reference sample dilution series analyzed by red- and green-excited fluorescence (Appendix F. Figure 16 and Appendix F. Figure 17). The pure reference standards are not fluorescent with red-excitation. Most notable is the contrast between Port Bay and East Flats samples in a comparison of red- and green-excited fluorescence. Port Bay samples from the fall season show little green-excited fluorescence, but significant red-excited fluorescence, resulting in a high ratio of Red F to Green F (R/G). In contrast, East Flats samples show higher and relatively similar levels of fluorescence from red- vs. green- excitation, resulting in low R/G ratios. This is an important metric unique to the fluorescence-based method. It is proposed that this metric provides insight into the relative classes of epiphytic algae which are predominant (*e.g.* red vs. green algae).



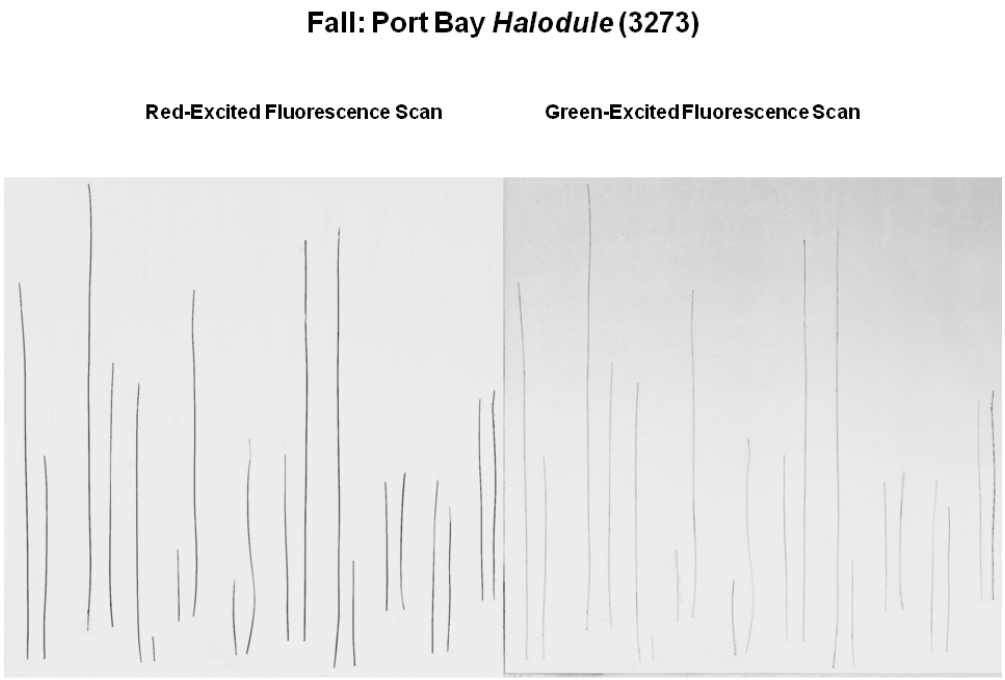
Appendix F. Figure 4. Representative red- and green-excited fluorescence scans.

Whole shoots (15) of PB *Halodule* from spring sampling (sample 3021) were scanned under standard conditions. Note arrangement of blades from each whole shoot, and different epiphyte accumulation on each. In some cases, the oldest blades are shorter than younger blades and very fine blades may dry out and curl (see text).



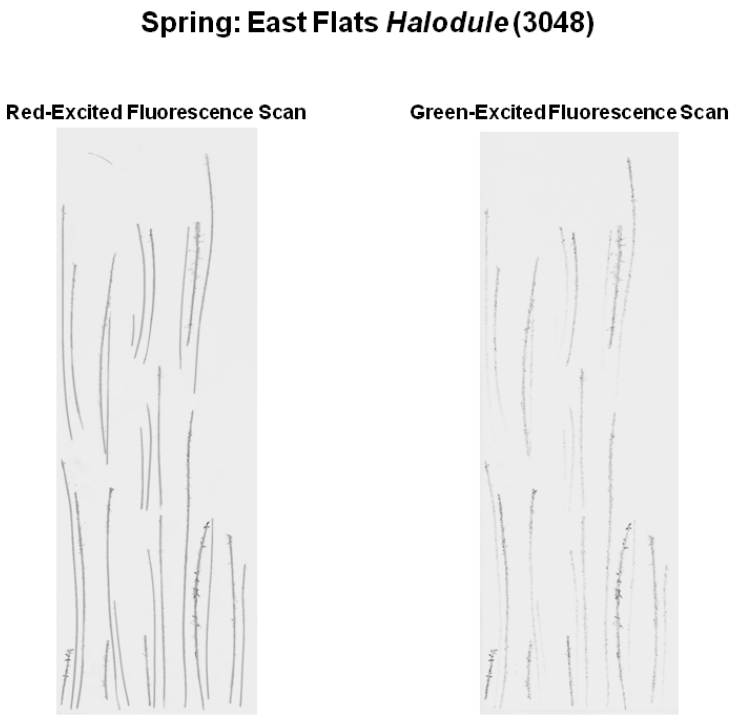
Appendix F. Figure 5. Representative red- and green-excited fluorescence scans.

Whole shoots (10) of PB *Halodule* from summer sampling (sample 3123) were scanned under standard conditions.



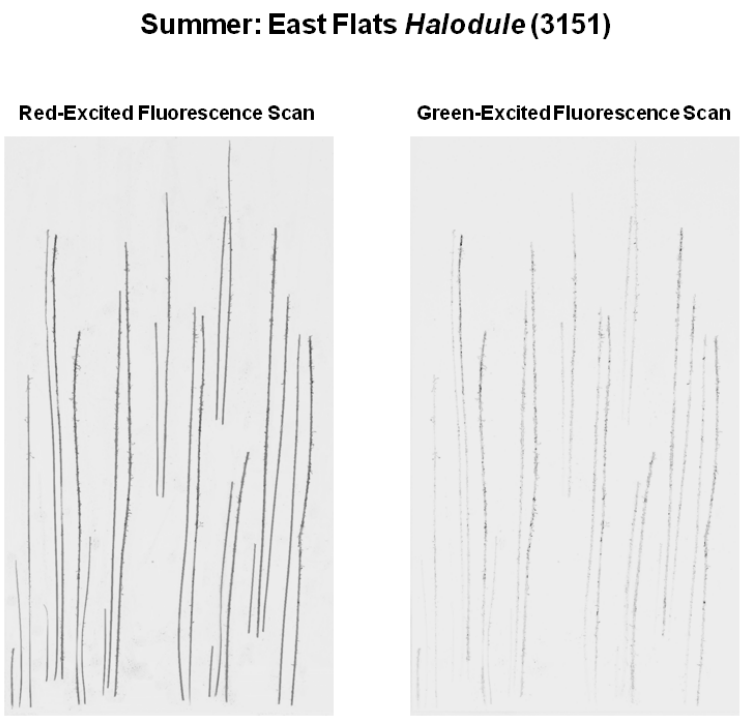
Appendix F. Figure 6. Representative red- and green-excited fluorescence scans.

Whole shoots (10) of PB *Halodule* from fall sampling (sample 3273) were scanned under standard conditions.



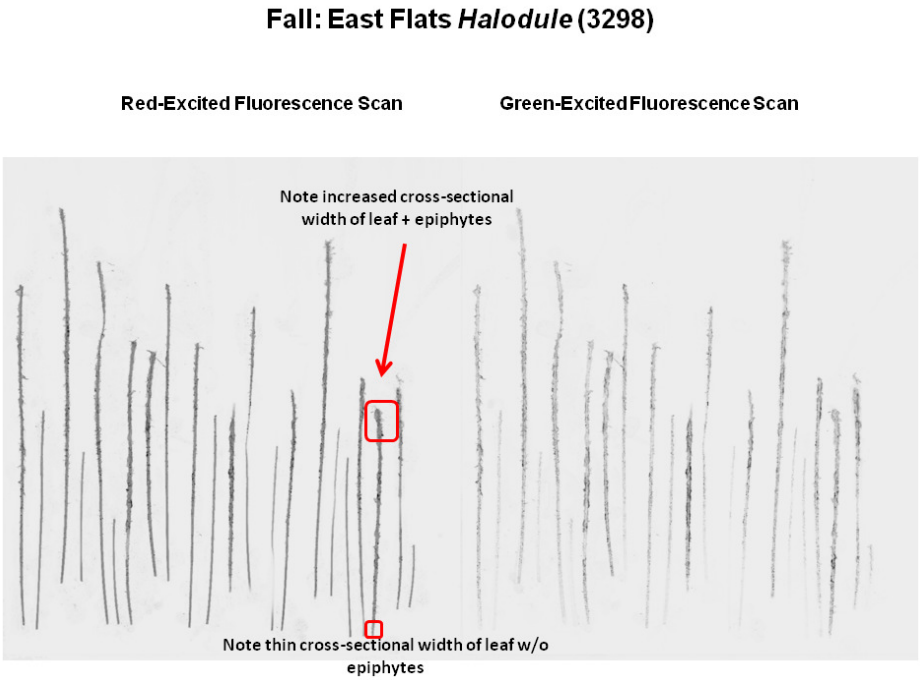
Appendix F. Figure 7. Representative red- and green-excited fluorescence scans.

Whole shoots (10) of EF *Halodule* from spring sampling (sample 3048) were scanned under standard conditions. Note heavy accumulation of filamentous epiphytes.



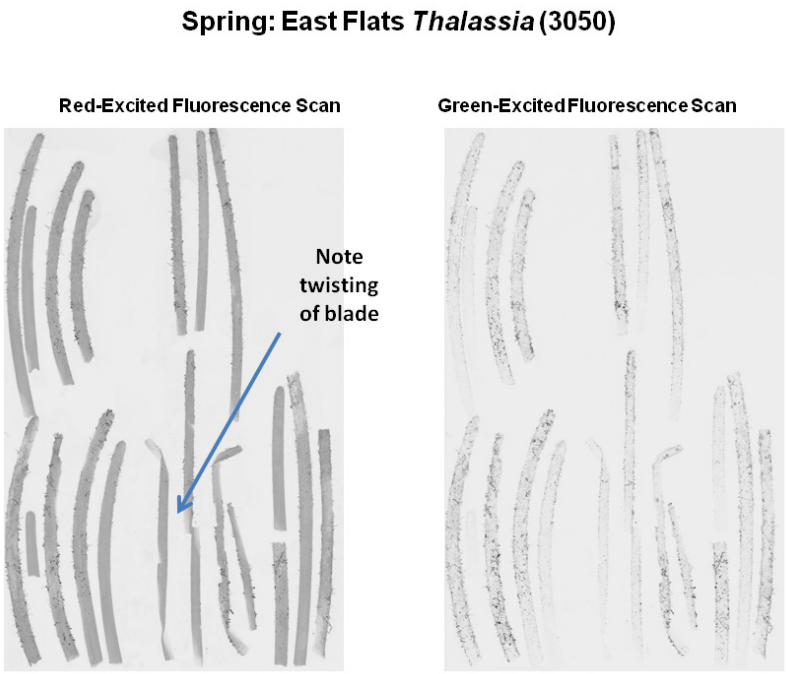
Appendix F. Figure 8. Representative red- and green-excited fluorescence scans.

Whole shoots (10) of EF *Halodule* from summer sampling (sample 3151) were scanned under standard conditions.



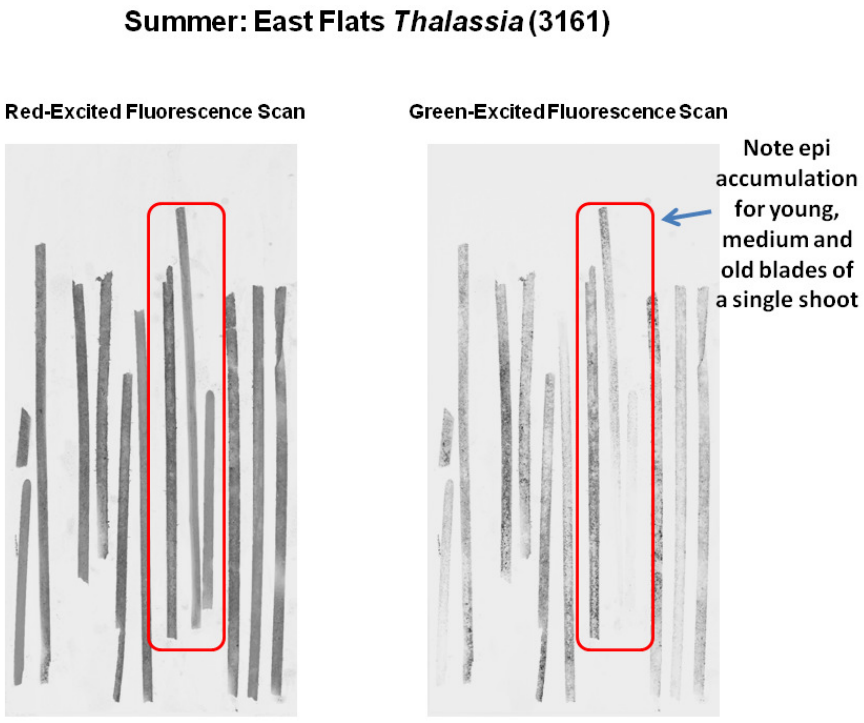
Appendix F. Figure 9. Representative red- and green-excited fluorescence scans.

Whole shoots (10) of EF *Halodule* from fall sampling (sample 3298) were scanned under standard conditions. Note that heavy epiphyte accumulations increase cross-sectional width of leaf as estimated from the Red F scan.



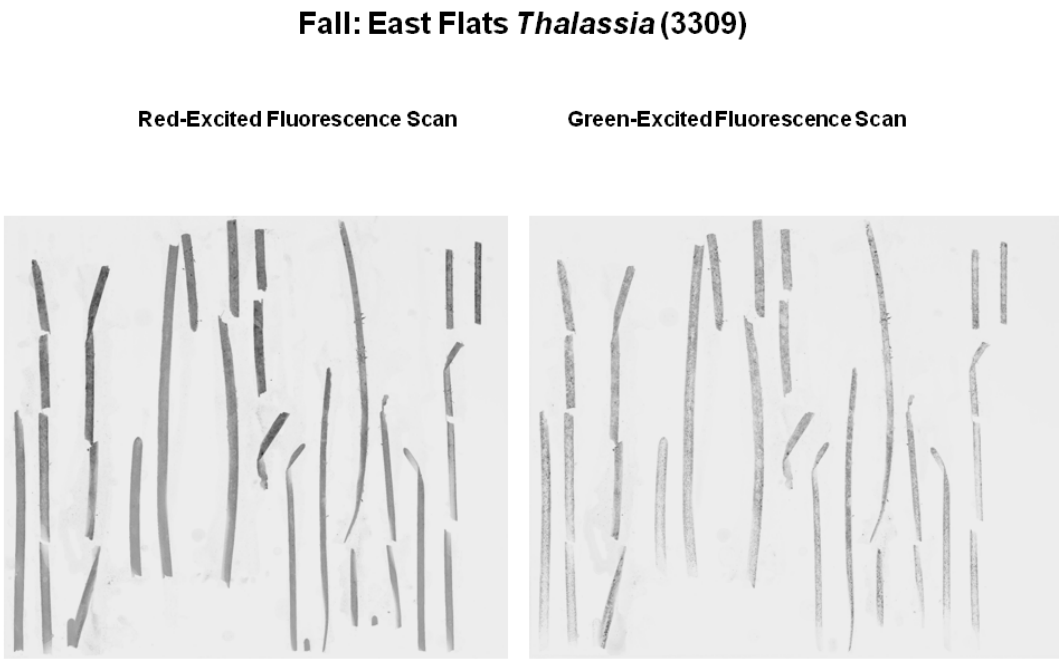
Appendix F. Figure 10. Representative red- and green-excited fluorescence scans.

Whole shoots (5) of EF *Thalassia* from spring sampling (sample 3050) were scanned under standard conditions. Note severe twisting of *Thalassia* blades in some cases.



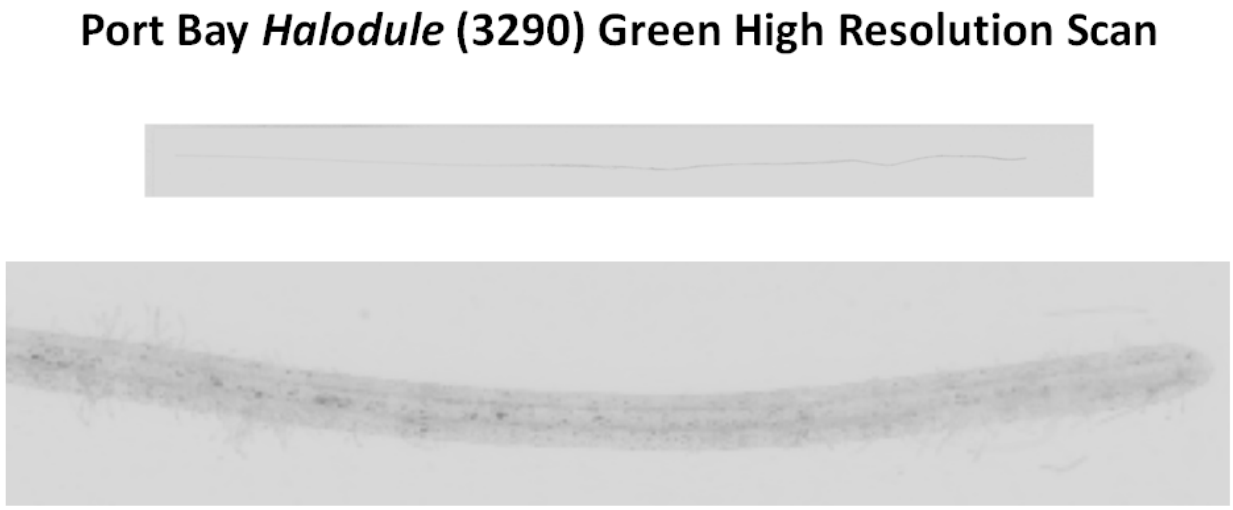
Appendix F. Figure 11. Representative red- and green-excited fluorescence scans.

Whole shoots (5) of EF *Thalassia* from summer sampling (sample 3161) were scanned under standard conditions. Note arrangement of blades from each whole shoot, and different epiphyte accumulation on each. In some cases, the oldest blades are shorter than younger blades.



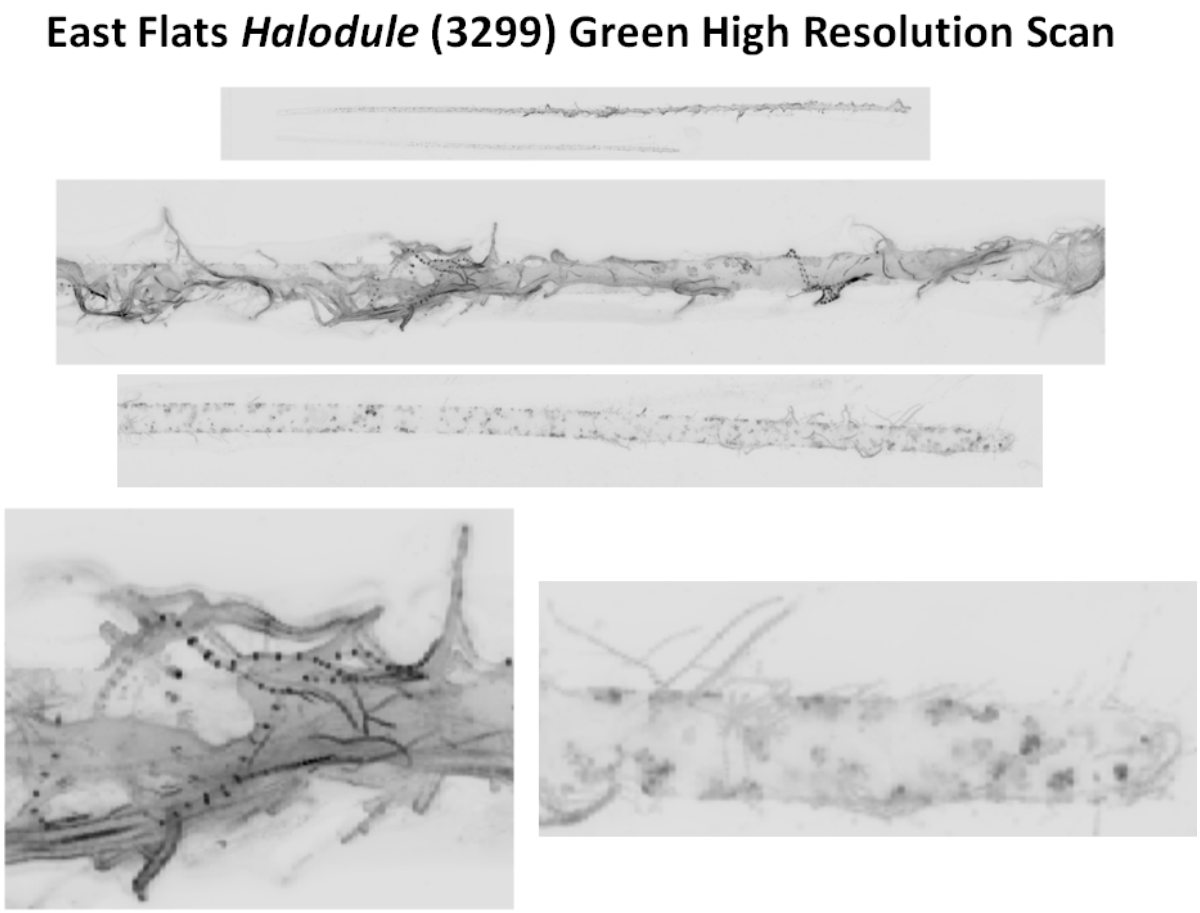
Appendix F. Figure 12. Representative red- and green-excited fluorescence scans.

Whole shoots (5) of EF *Thalassia* from fall sampling (sample 3309) were scanned under standard conditions.



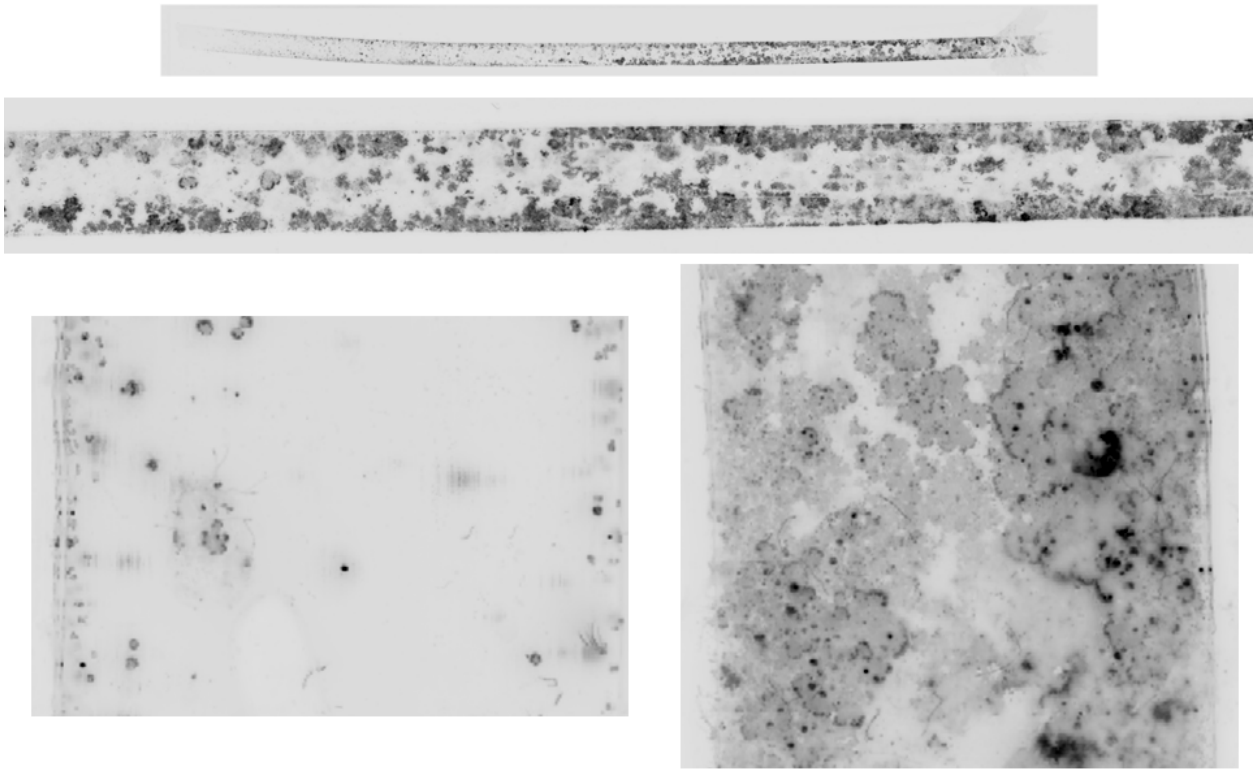
Appendix F. Figure 13. Representative green-excited fluorescence scan at high resolution.

A representative blade from PB *Halodule* from a middle depth of the fall sampling (sample 3290) was scanned at 10 µm pixel size. Top: Whole blade. Bottom: Tip section of the same whole blade. Note the mostly homogeneous coverage of non-distinct green epiphyte coverage with occasional colonial or filamentous epiphytes visible.



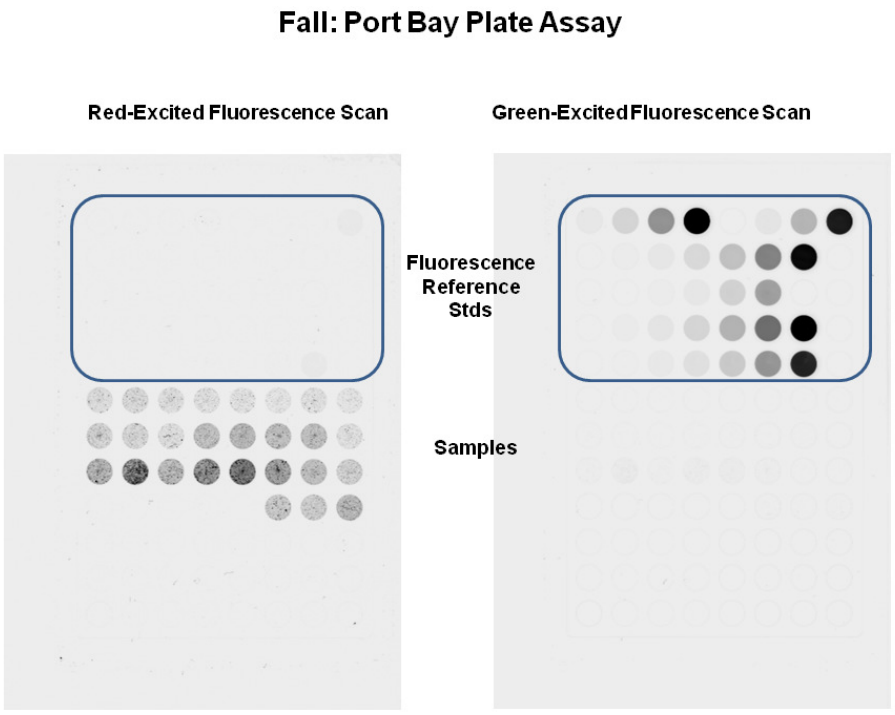
Appendix F. Figure 14. Representative green-excited fluorescence scan at high resolution.

A representative shoot from EF *Halodule* from a middle depth of the fall sampling (sample 3299) was scanned at 10 µm pixel size. Top: Older and younger blade from a whole shoot. Middle: Closer view of sections of same old and young blades. Lower: Closest view of sections of same old (left) and young (right) blades. Note differences between old and young blades suggestive of successional patterns of epiphyte accumulation. The older blade is completely covered with filamentous epiphytes and the younger is covered by an abundance of colonial/crustose and some filamentous epiphytes. Despite heavy accumulation on the older blade, a gradient of abundance is apparent from the leaf base to the tip.



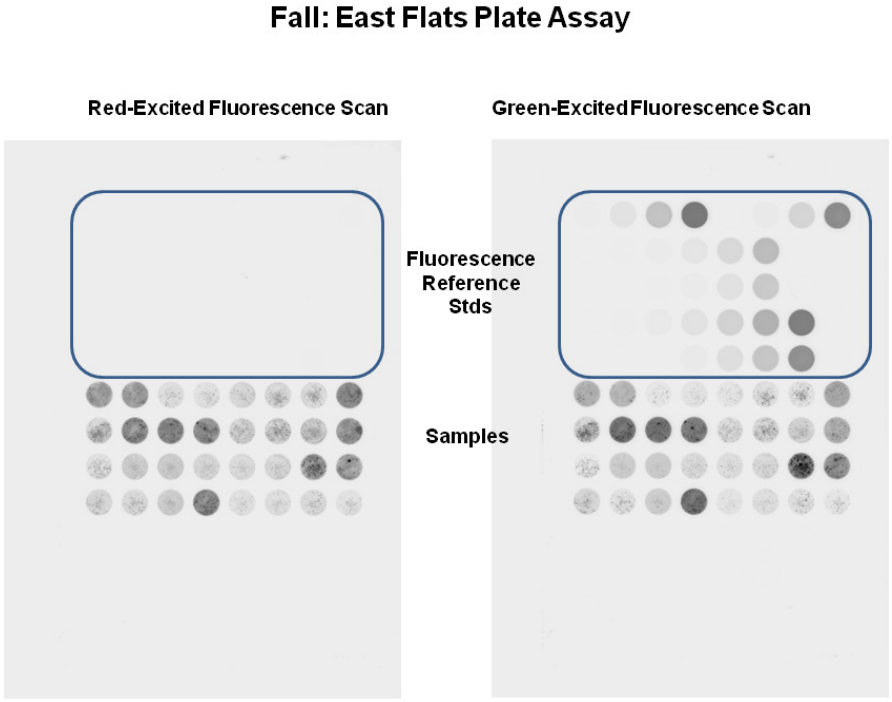
Appendix F. Figure 15. Representative green-excited fluorescence scan at high resolution.

A representative blade from EF *Thalassia* from a middle depth of the fall sampling (sample 3317) was scanned at 10 µm pixel size. Top: Whole blade. Middle: Closer view of a section of the same whole blade. Lower: Closest view of lower (left) and upper (right) sections of the same whole blade. Note the predominance of colonial/crustose epiphytes with very different fluorescence intensities. A gradient of coverage along the blade ranges from little to nearly complete.



Appendix F. Figure 16. Representative red- and green-excited fluorescence scans of Plate Assay.

PB *Halodule* samples from the fall sampling were scanned in a 96-well optical plate under standard conditions (scanner focused at +3 mm). Note that the Eosin Y and B-type phycoerythrin standards are not observed on the Red F scan. PB samples typically had high Red F and relatively low Green F.



Appendix F. Figure 17. Representative red- and green-excited fluorescence scans of Plate Assay.

EF *Halodule* and *Thalassia* samples from the fall sampling were scanned in a 96-well optical plate under standard conditions (scanner focused at +3 mm). EF samples typically had high Green F and lower Red F.

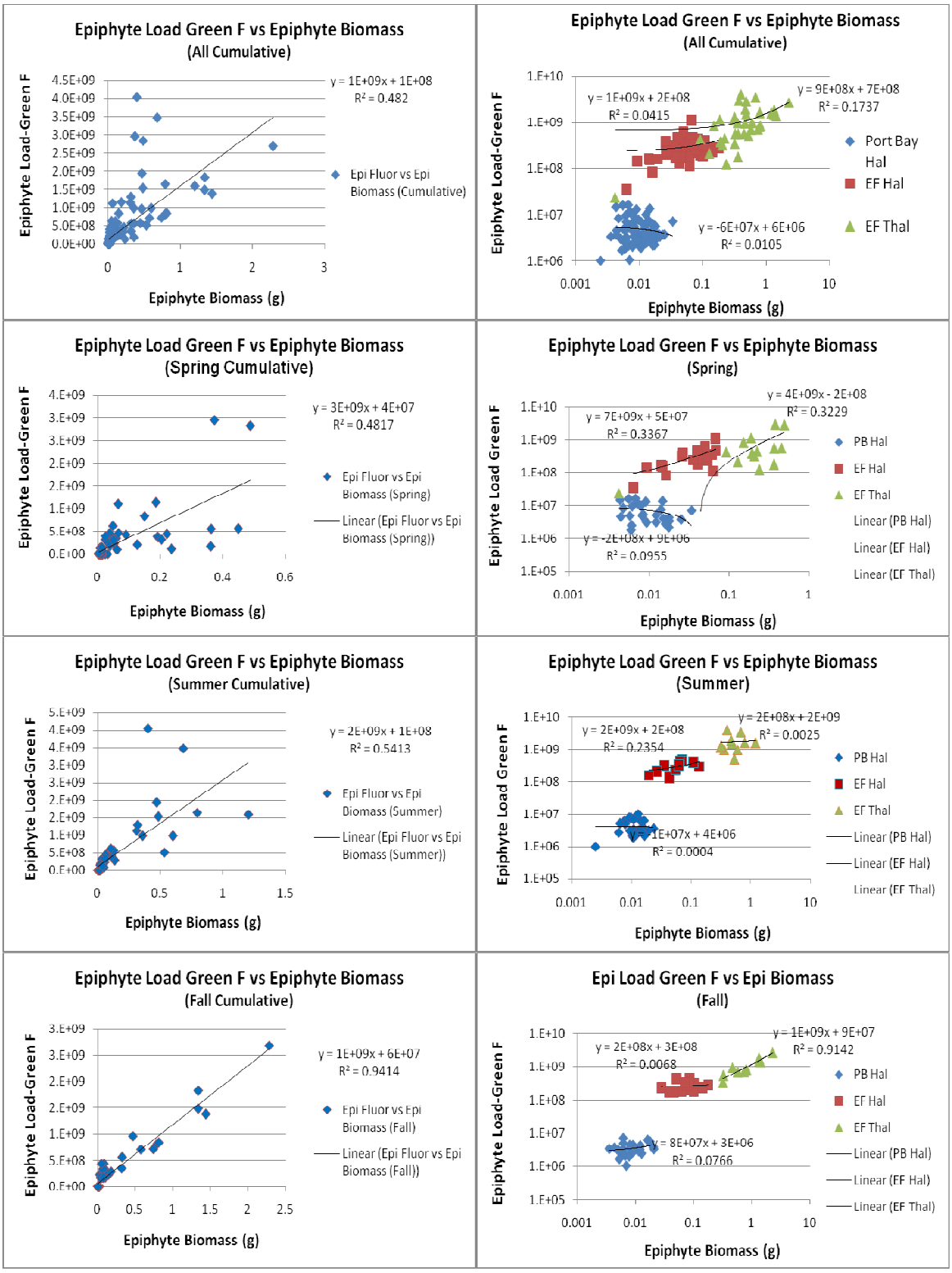
Correlations of Fluorescence Measures

Appendix F. Figure 18 to Appendix F. Figure 24 explore correlation of the fluorescence measures of epiphyte load ("Epiphyte Load") and leaf area ("Scanned Leaf Area") with the biomass-based measures "Epiphyte Biomass" and "Leaf Biomass." Correlations are reported as determination coefficients (r^2) for linear regressions, and were generally explored for total cumulative data (all seasons, all sites, all species) and then broken down by species, seasons, and sites. Structure in the widely ranging data is observed in greater detail using logarithmic scales for both axes. The determination coefficients for these comparisons are summarized in Appendix F. Table 3.

Epiphyte Load Estimated by Green-Excited Fluorescence

Comparison of the direct measures of epiphyte abundance (Appendix F. Figure 18: Epiphyte Load vs. Epiphyte Biomass) reveals correlations varying from 0.48 to 0.94 for data from all sites and species over all or specific seasons. Log-Log plots uncover structure in the data, consisting of a clustering by site and species, regardless of season. Clustering is observed for data from both measures, and is most pronounced for both measures between the Port Bay and East Flats sites, and least pronounced for *Halodule* vs. *Thalassia* fluorescence at East Flats. Plots for individual species at individual sites for each season have correlations ranging from -0.96 to 0.91. There was never a correlation for epiphyte fluorescence and biomass at the Port Bay site, for any or all seasons. But the PB data do contribute to the cumulative correlations, as particularly illustrated by the summer data showing an overall correlation of 0.54, whereas the EF *Thalassia*, EF *Halodule*, and PB *Halodule* individual correlations are, respectively, 0, 0.24, and 0.

A frequently recurring observation through all of the data is an inconsistency in correlations at different scales. In the above example, a complete lack of correlation for each individual site/species/season still produces a reasonable correlation for the overall cumulative data. In other cases, there may be a lack of correlation at the cumulative level, but good correlation within an individual site/species/season. Similar observations were made for purely biomass data (Appendix F. Figure 20) and for purely fluorescence-based measures (Appendix F. Figure 21), suggesting that the clustering phenomenon was not an artifact of a particular measurement. Correlations were generally poorest in the summer season as well.



Appendix F. Figure 18. Epiphyte Load (Green F) vs. Epiphyte Biomass.

Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species.

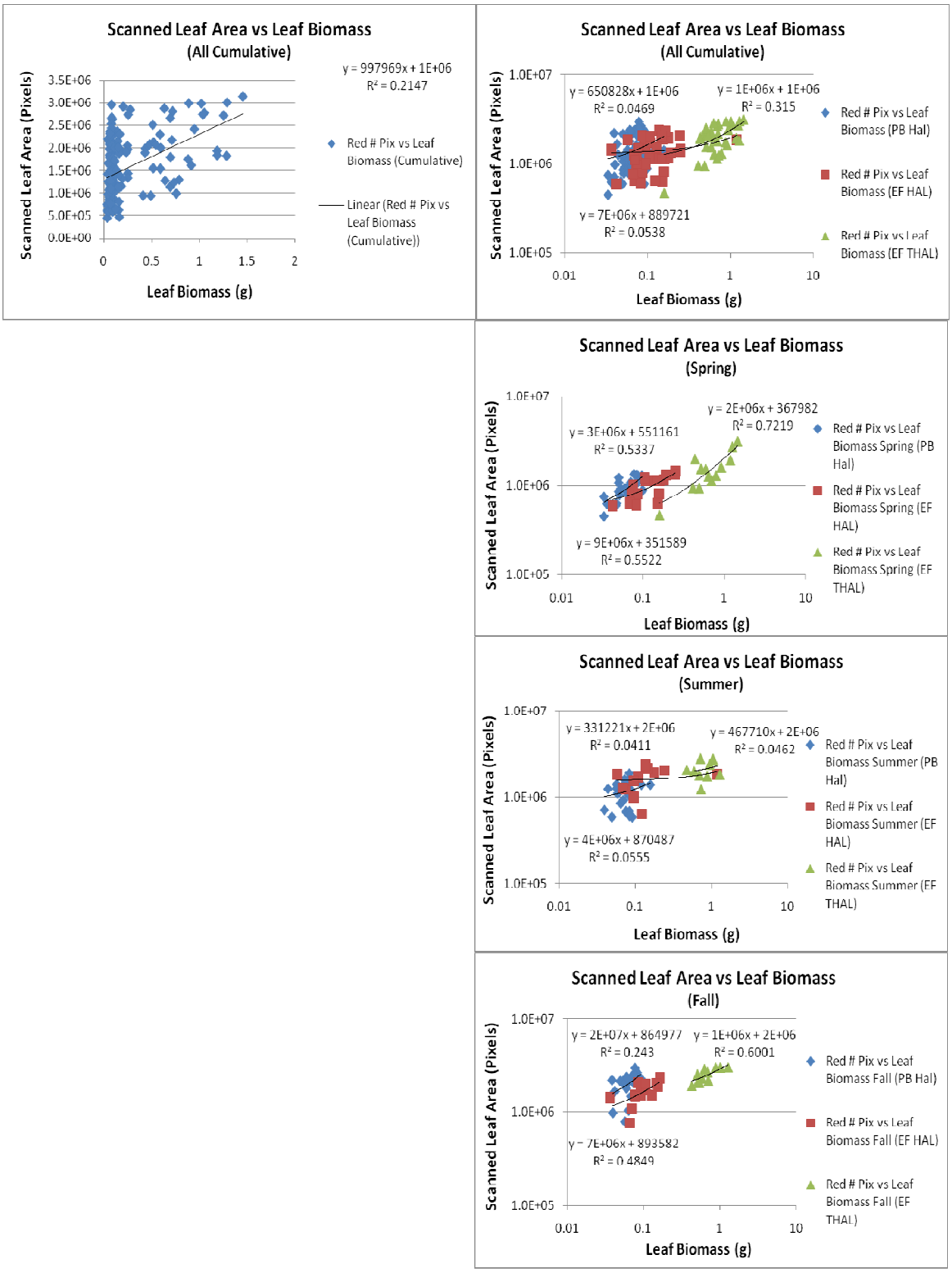
Scanned Leaf Area Estimated from Red Number of Pixels

The second critical measure proposed to be obtained from the fluorescence measurements is Scanned Leaf Area, determined by the number of image pixels of known size (200 µm x 200 µm) where fluorescence signal is above background. Red-excited fluorescence images are used to calculate this because bare, un-epiphytized seagrass leaves exhibit red-excited fluorescence by virtue of their chlorophyll. All epiphytes have chlorophyll and are imaged as well, so this number represents a 2-D cross-sectional area of the seagrass blades plus their epiphytes. The 2-D cross-sectional area of a leaf should be related to the surface area of a leaf, as well as the overall leaf biomass. The 2-D cross-sectional area cannot be said to truly measure leaf surface area or biomass due to biological variation from the terete nature of some seagrasses or to differences in the organic carbon content of seagrass cells, respectively. However, there should be a generally good correlation between leaf area and biomass (Dunton *et al*. 2007).

Correlation between Scanned Leaf Area and Leaf Biomass was poor (0.21) for total cumulative data from all species/all sites/all seasons, and varied from 0.04 to 0.72 when broken out into seasons, sites or species. This provides an example where the overall large-scale correlation was poor, but individual correlations were sometimes quite good. Again, distinct clusters were observed and correlations were poorest in the summer season.

One observation which helps to account for the poor overall cumulative correlation is the greatly increased epiphyte accumulation, especially of filamentous-type epiphytes, at East Flats vs. Port Bay (See Appendix F. Figure 9 and Appendix F. Figure 4). The base of the blades in the red-excited fluorescence images reveals the seagrass blade width, which is approximately constant (there are some minor variations) along the length of the blade. However, *Halodule* blade images from East Flats in the fall season (Appendix F. Figure 14) increase in width approximately two-fold from the base to the leaf tip, due to very heavy accumulations of epiphytes, which show up in the Scanned Leaf Area metric. Moreover, in some cases, the epiphyte biomass actually exceeded the leaf biomass.

An alternative metric in future work should be calculation of leaf area from the blade length and base width measurements, which are readily obtained from the images. We have observed (Cammarata *et al.* unpublished) that there is good correlation between scanned leaf area and biomass for a given seagrass sample that lacks heavy accumulations of epiphytes.



Appendix F. Figure 19. Scanned Leaf Area vs. Leaf Biomass.

Scanned leaf cross-sectional area is represented by the number of pixels with fluorescence signal in the red-excited fluorescence scan. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Determination coefficients (r^2) presented for individual sites and species.

Expected Relationship Between Epiphyte Accumulation and Seagrass Leaf Biomass

There is an expected relationship between epiphyte accumulation and seagrass leaf area (or biomass as a proxy) (Dunton *et al*. 2007), so the efficacy of the fluorescence-based method can be assessed by comparing this expected relationship for purely fluorescence-based measures (Epiphyte Load correlation with Scanned Leaf Area) with that observed for purely biomass-based measures (Epiphyte Biomass correlation with Leaf Biomass). These relationships are explored in Appendix F. Figure 20 to Appendix F. Figure 24.

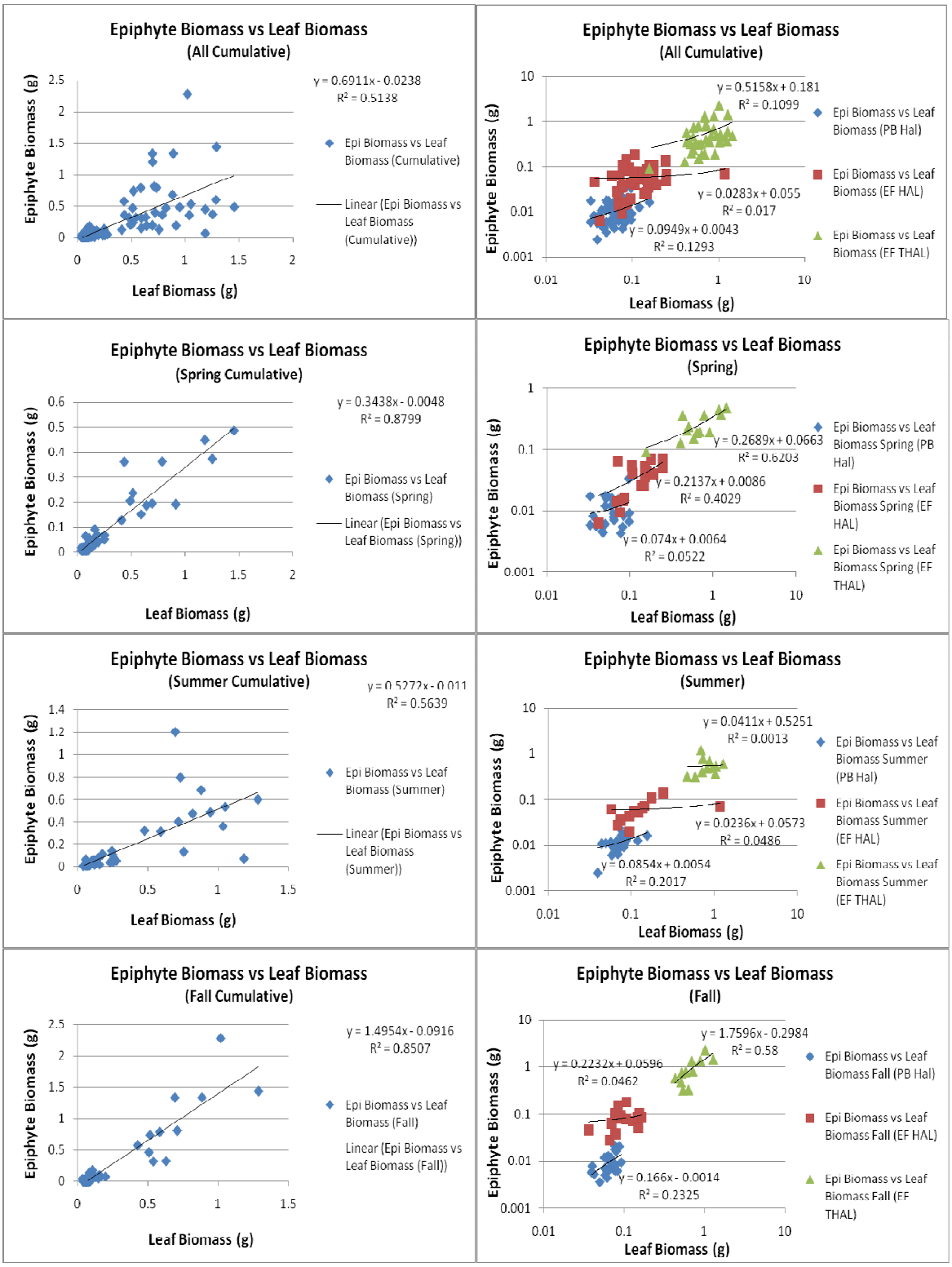
The biomass-based measures (Appendix F. Figure 20) had good correlations (0.51 to 0.88) for mixed species and sites over all or individual seasons. Again, distinct clustering by site and species was observed, with poorest correlations in the summer seasonal data. For each individual season, the cumulative data had better correlation than observed for any of the individual species or sites.

The clustering phenomenon is generally reproduced in the fluorescence-based measures, but the overall correlation is poor (Appendix F. Figure 21). Broken out by species/site/season, correlations varied from 0 to 0.73. This may be due in large part to the problems with the Scanned Leaf Area measure, discussed above.

As an alternative, a mixed fluorescence and biomass measure was explored, replacing the Scanned Leaf Area with Leaf Biomass (Appendix F. Figure 22). Epiphyte Load by green-excited fluorescence correlated with Leaf Biomass (0.44 to 0.8) for all or individual season cumulative data. The typical clustering pattern and poorer summer correlations observed for biomass-based measures were also found in this mixed measure. Broken out by season, site and species, correlations varied from 0.05 to 0.8.

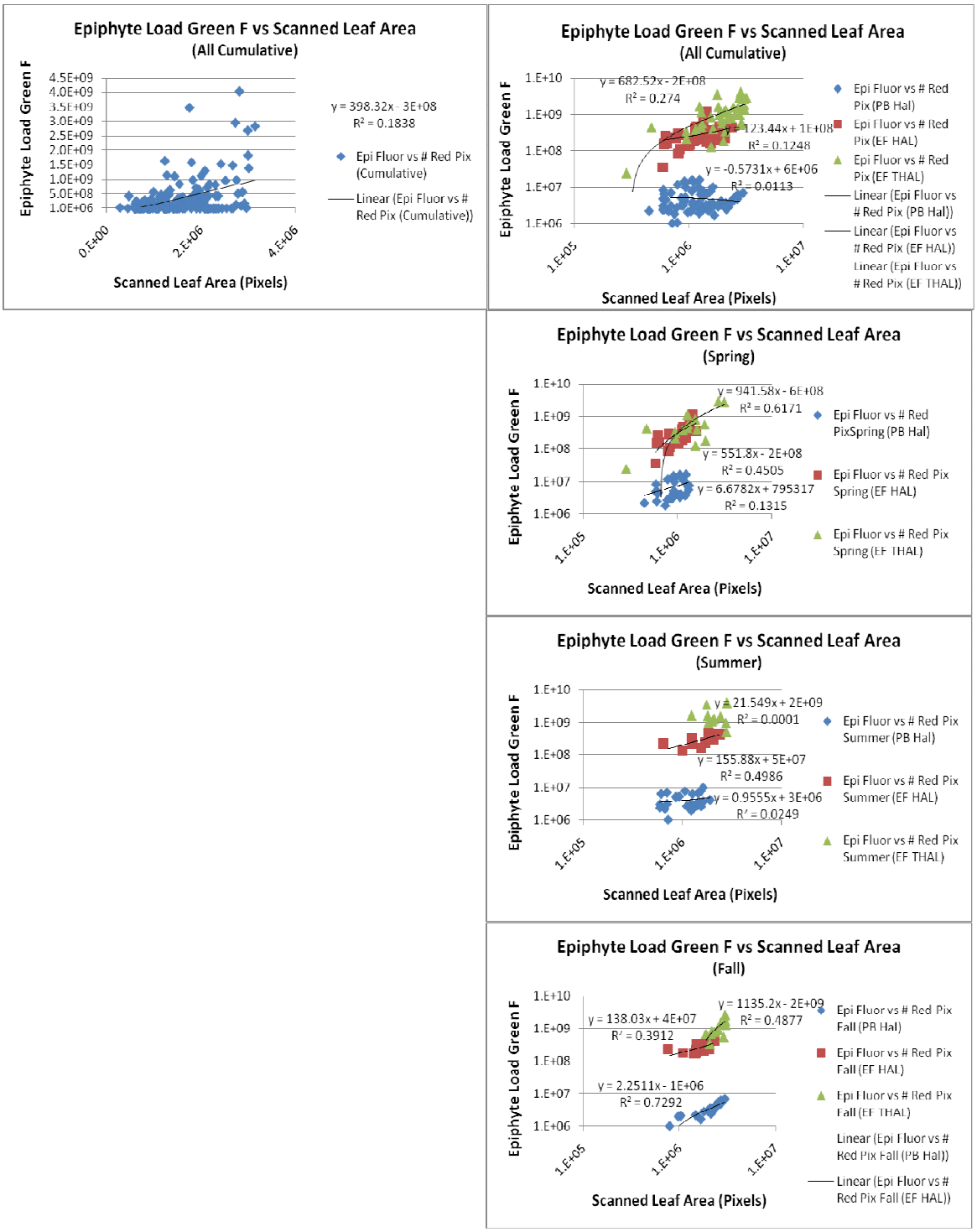
The epiphyte accumulation metric most useful for comparative studies between sites or conditions is the epiphyte measure which is normalized for differences in leaf size (or biomass). Plots of fluorescence-based normalized epiphyte accumulation (Epiphyte Load-Green F/Scanned Leaf Area) vs. the biomass-based normalized measure (Epiphyte Biomass/Leaf Biomass) reveal weak, none, or even negative correlations, with only one exception (Appendix F. Figure 23).

Comparison of the mixed measure normalized epiphyte metric (Epiphyte Load (Green F)/Leaf Biomass) to the biomass-based normalized epiphyte measure (Appendix F. Figure 24) reveals weak correlation for total cumulative data (0.29) or correlations ranging from 0.03 to 0.48 for seasonal cumulative data and a range of -0.03 to +0.73 when broken out by season/site/species. The good correlation of Green F with Epiphyte Biomass in the fall (0.94, Appendix F. Figure 18) did not hold when data were normalized to Leaf Biomass (0.48, Appendix F. Figure 24). However, normalization of Green F to Leaf Biomass data brought the values for East Flats *Halodule* and *Thalassia* to near superimposition. The Port Bay *Halodule* values remained distinctly clustered and dissimilar to the East Flats *Halodule* samples. This was also observed in the normalized fluorescence data (Appendix F. Figure 23). Potential explanations of these results are discussed below.



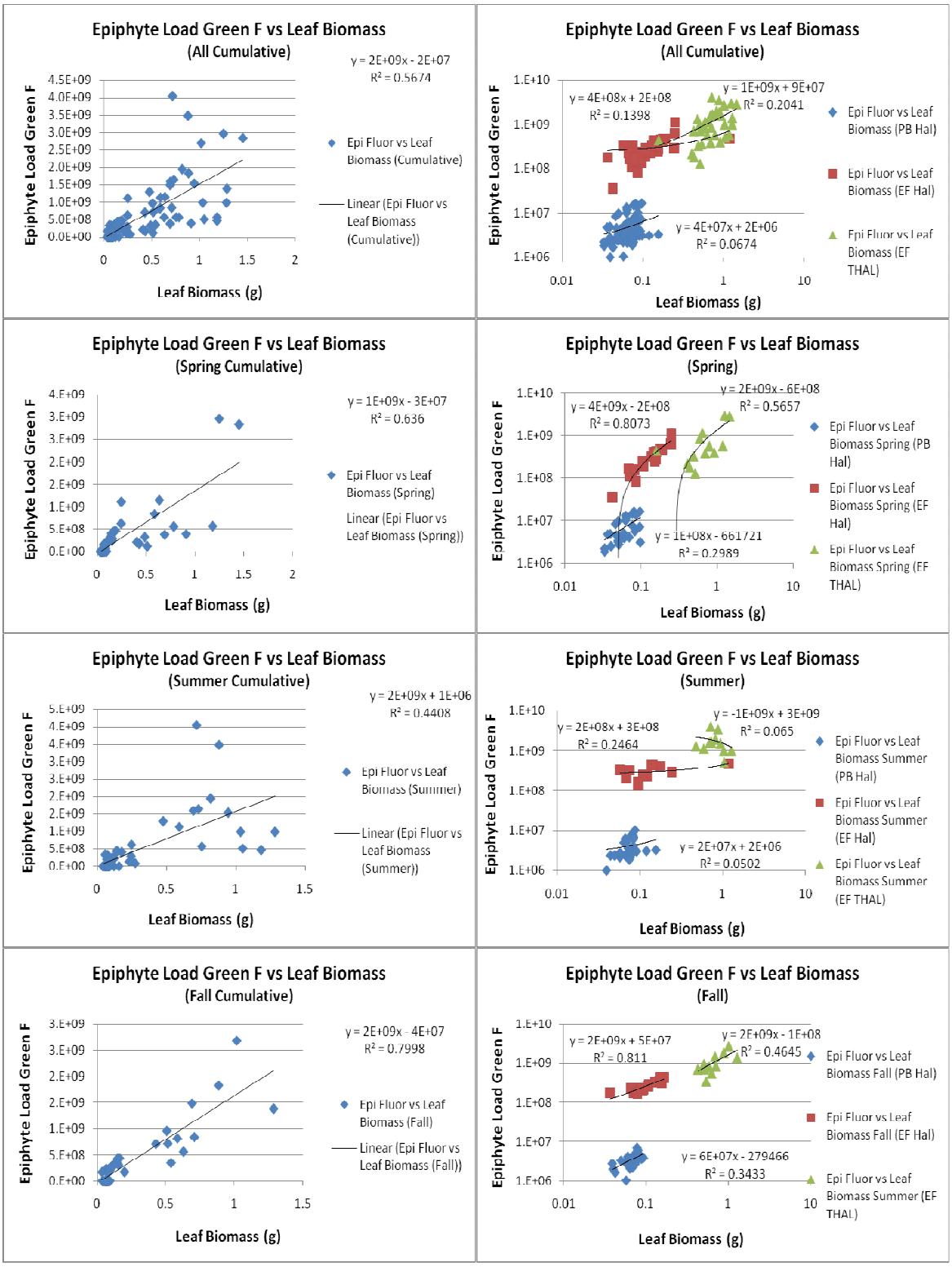
Appendix F. Figure 20. Correlation of biomass-based measures: Epiphyte Biomass vs. Leaf Biomass.

Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species.



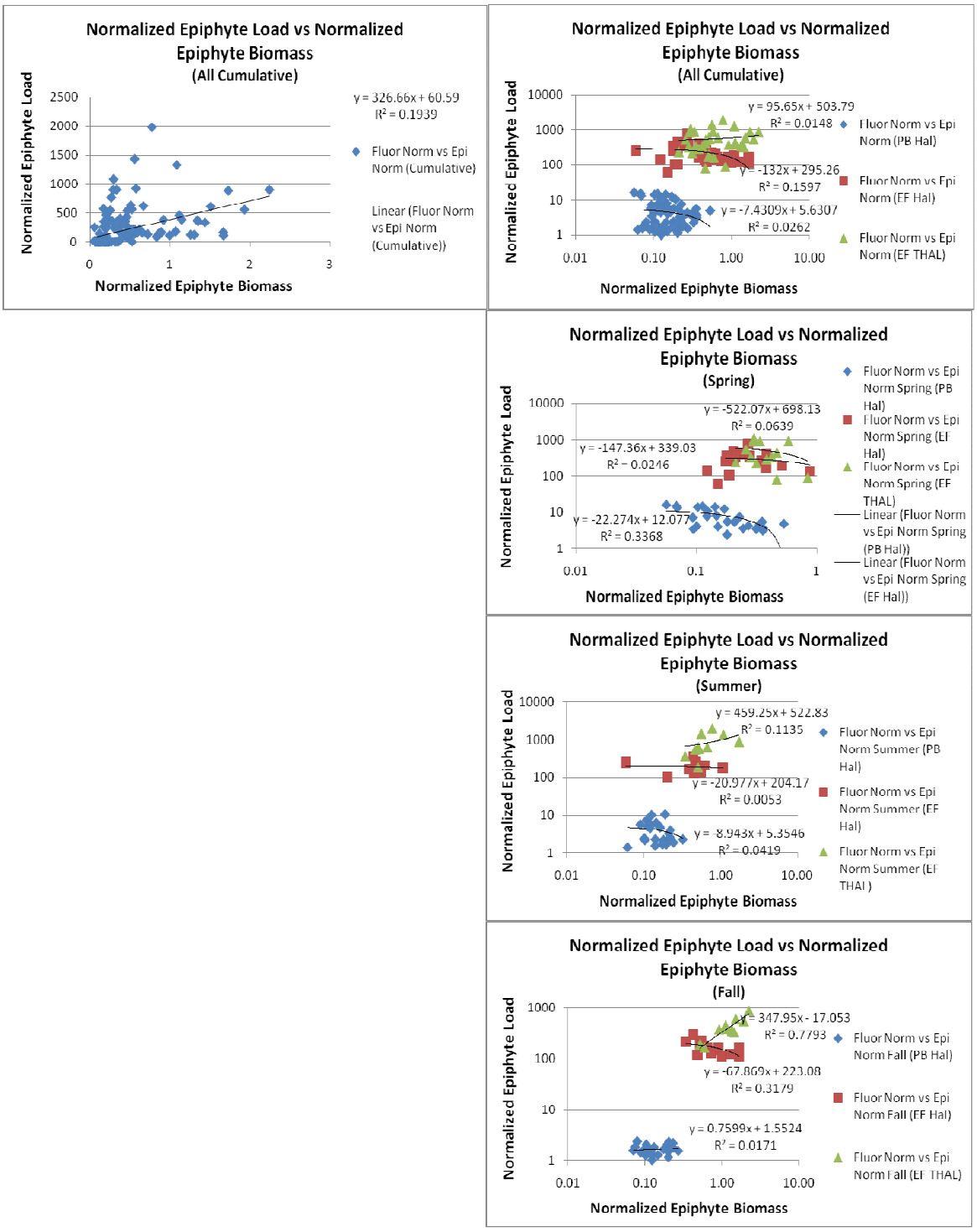
Appendix F. Figure 21. Correlation of fluorescence-based measures: Epiphyte Load (Green F) vs. Scanned Leaf Area.

Epiphyte Load is the intensity of green-excited fluorescence. Scanned leaf cross-sectional area is represented by the number of pixels with fluorescence signal in the red-excited fluorescence scan. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Determination coefficients (r^2) presented for individual sites and species.



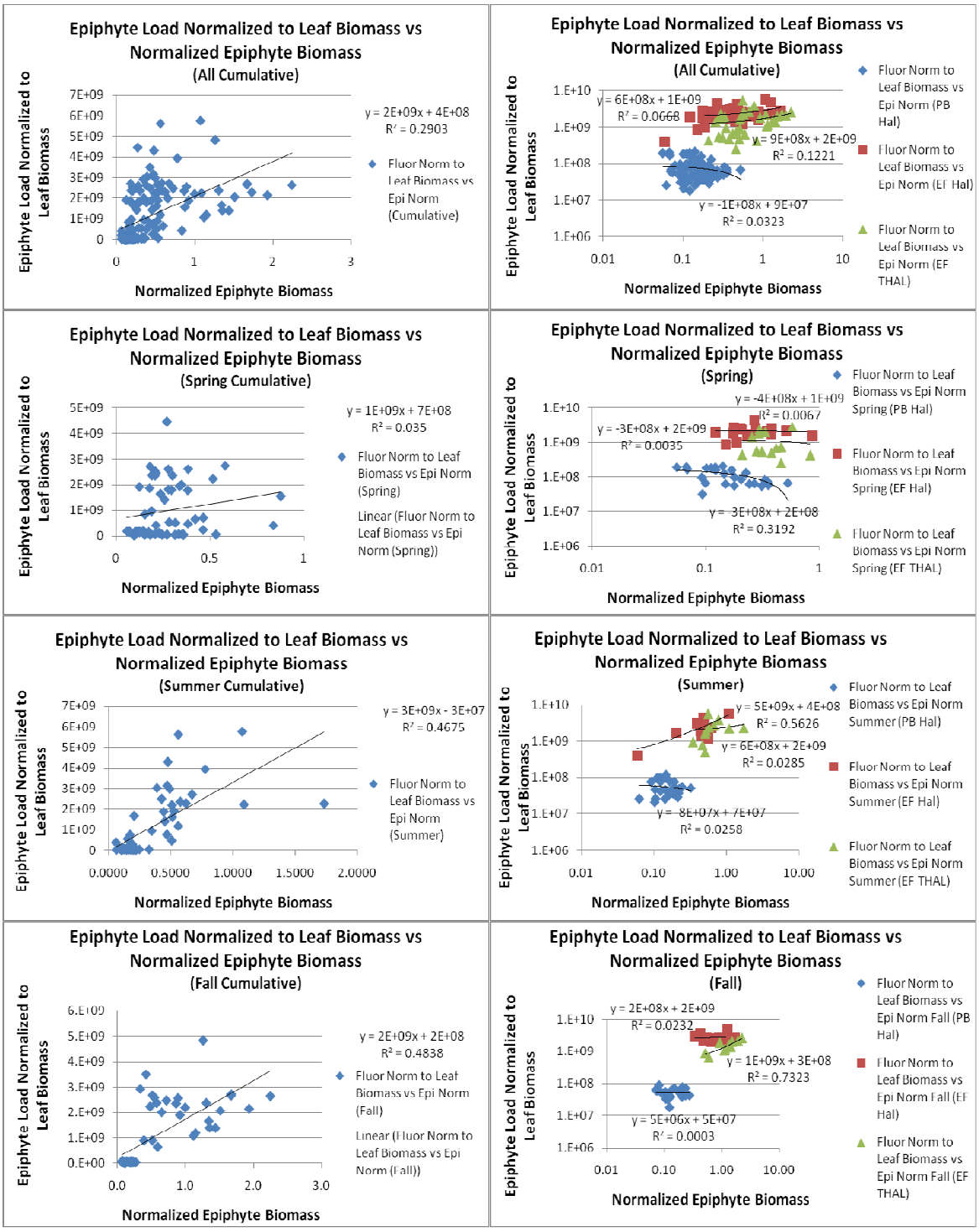
Appendix F. Figure 22. Correlation of mixed measures: Epiphyte Load (Green F) vs. Leaf Biomass.

Epiphyte Load is the intensity of green-excited fluorescence. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species.



Appendix F. Figure 23. Correlation of normalized epiphyte measures: Normalized Epiphyte Load vs. Normalized Epiphyte Biomass.

Normalized Epiphyte Load is the Epiphyte Load (Green F) divided by the Scanned Leaf Area (pixels of Red F). Normalized Epiphyte Biomass is Epiphyte Biomass divided by Leaf Biomass (dry). Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Determination coefficients (r^2) presented for individual sites and species.



Appendix F. Figure 24. Correlation of *mixed measure* Normalized Epiphyte Load with Normalized Epiphyte Biomass: Green epiphyte fluorescence normalized to Leaf Biomass vs. normalized Epiphyte Biomass.

***Mixed measure* Normalized Epiphyte Load is Epiphyte Load (Green F) divided by Leaf Biomass (dry). Normalized Epiphyte Biomass is Epiphyte Biomass divided by Leaf Biomass (dry). Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species.**

Fluorescence Measures for Epiphytes Removed from Seagrass Leaves (Plate Assays)

Scanning intact seagrass leaves for epiphyte fluorescence relies on green-excited fluorescence, but this measure only detects fluorescence from the types of algal epiphytes that contain phycoerythrin, fucoxanthin or peridinin pigments (Appendix F. Table 1). Thus, green algal epiphytes, which can be a major constituent, are not detected with appreciable efficiency. This could in part explain some of the results described above.

A potential solution is to remove epiphytes from the blades by scraping, and then measuring fluorescence of the removed epiphytes. In this case, there is no potential fluorescence interference from the seagrass leaf so, in addition to green-excited fluorescence, red-excited fluorescence can be used to measure epiphytes. The red-excited fluorescence *would* detect green algal epiphytes. In addition, *change* in the relative fluorescence levels from green- vs. red-excitation is proposed to be an indicator of large shifts in algal epiphyte composition from one class to another.

Green-excited plate fluorescence correlated with epiphyte biomass (0.54 to 0.83) for total cumulative data or cumulative data by season, with clustering by site and species (Appendix F. Figure 25). Broken out by season, site and species, correlations ranged from -0.15 to 0.7. These results and the higher correlations for cumulative data compared to individually analyzed data are reasonably consistent with results from epiphyte fluorescence on the seagrass leaves (Appendix F. Figure 18). Plots of Epiphyte Load (Green F, Plate Assay) vs. Scanned Leaf Area, or normalized to Scanned Leaf Area and plotted vs. Normalized Epiphyte Biomass were also consistent with measures taken from scanned seagrass leaves (data not shown).

In contrast to the previous results however, normalization of the Plate Assay Epiphyte Load (Green F, Plate Assay) to Leaf Biomass did in fact correlate with Normalized Epiphyte Biomass (0.66) for cumulative data (Appendix F. Figure 25), and exhibited the same superimposition of EF *Halodule* and *Thalassia* data seen in Appendix F. Figure 24, as well as the same distinct clustering of the Port Bay *Halodule* data.

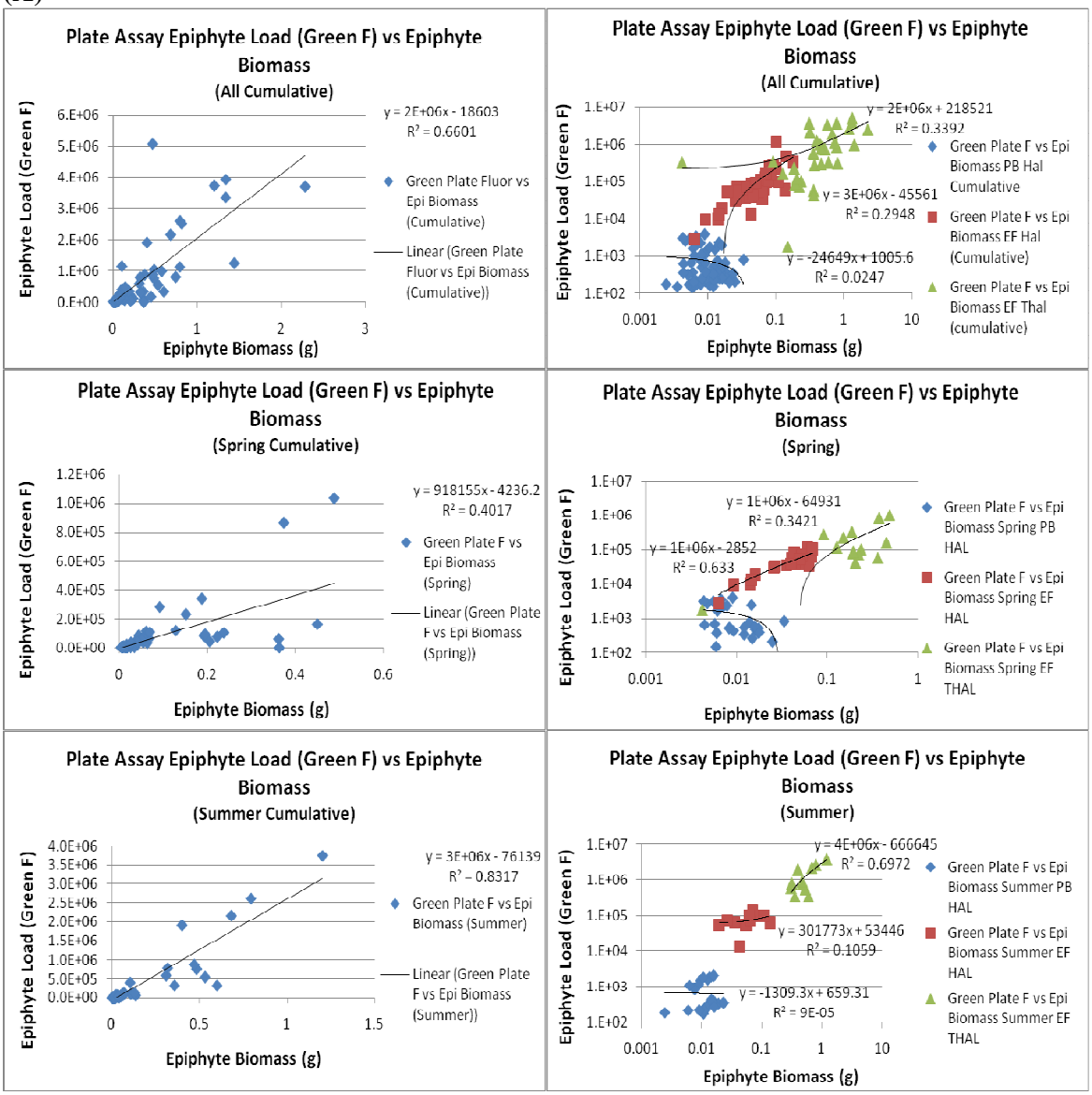
Similar comparisons made for the red-excited fluorescence of removed epiphytes (Epiphyte Load-Red F, Plate Assay) yielded similar results, but with even stronger correlations (Appendix F. Figure 26). The Plate Assay Epiphyte Load (Red F, Plate Assay) correlated with Epiphyte Biomass (0.75 to 0.92) for total cumulative data or cumulative data by season, with clustering by site and species (Appendix F. Figure 26). Broken out by season, site and species, correlations ranged from 0 to 0.81. These results and the higher correlations for cumulative data compared to individually analyzed data are consistent with results for Green F from both the Plate Assays (Appendix F. Figure 25) and scans of the seagrass leaves (Appendix F. Figure 18). Plots of Epiphyte Load (Red F, Plate Assay) vs. Scanned Leaf Area, or normalized to Scanned Leaf Area and plotted vs. Normalized Epiphyte Biomass were also consistent with measures taken from scanned seagrass leaves (data not shown).

Again, similar to comparisons made for Epiphyte Load (Green F, Plate Assay), normalization of the Epiphyte Load (Red F, Plate Assay) to Leaf Biomass correlated with Normalized Epiphyte

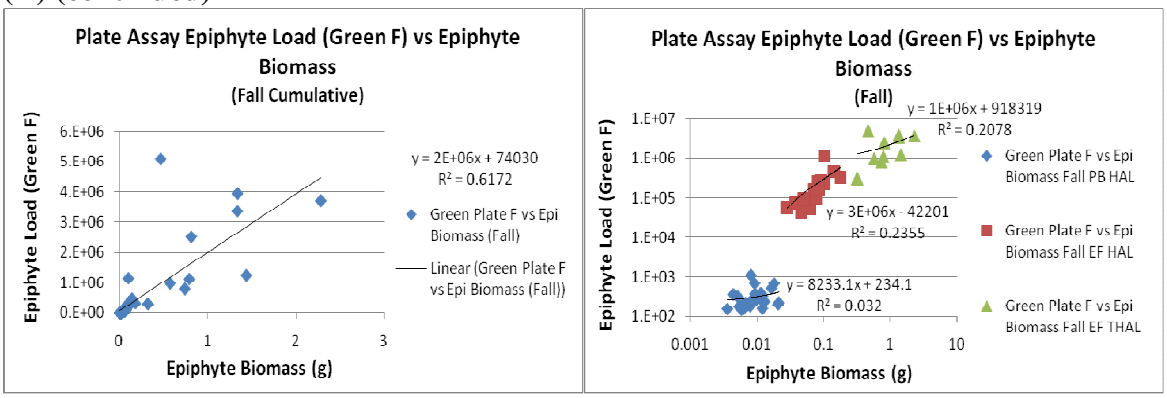
Biomass (0.56) for cumulative data, and exhibited the same superimposition of EF *Halodule* and *Thalassia* data. However, the distinct clustering of the Port Bay *Halodule* data seen above was barely noticeable here and the grouping of Port Bay data overlapped some with that of East Flats. Although there was still a lack of correlation within the cumulative Port Bay *Halodule* data alone, the coalescence of normalized data suggests that this may be a useful measure.

One interpretation of this result is that Port Bay seagrasses have an epiphyte community that is rich in green algae (possibly other classes as well) and unique from the algal composition found at East Flats (probably rich in red algae). The high resolution images (Appendix F. Figure 13 to Appendix F. Figure 15) support such a difference.

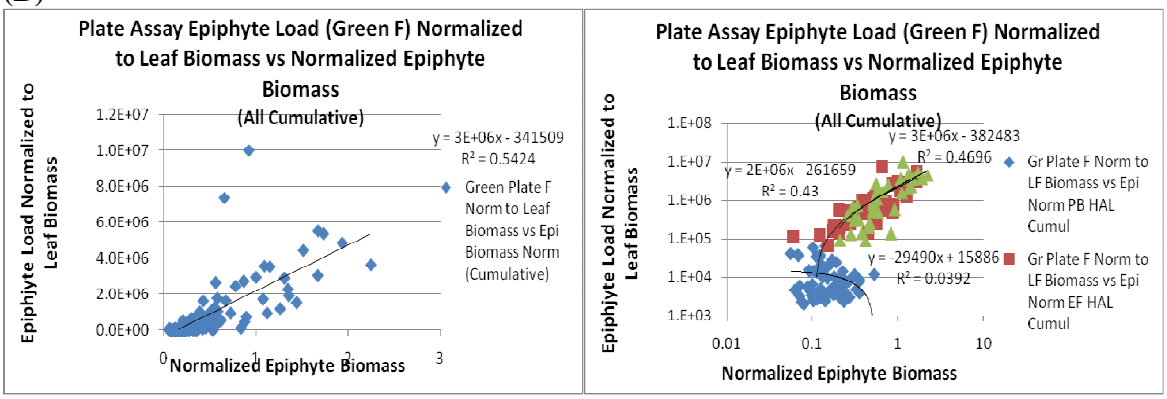
(A)



(A) (continued)



(B)



Appendix F. Figure 25. Correlation of Green F of removed epiphytes with Epiphyte Biomass.

(A) Plate Assay Epiphyte Load (Green F, Plate Assay) vs. Epiphyte Biomass. Green-excited fluorescence of removed epiphytes from Plate Assay. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species. (B) Normalized Plate Assay Epiphyte Load (Green F normalized to Leaf Biomass, Plate Assay) vs. Normalized Epiphyte Biomass. Green-excited fluorescence of removed epiphytes normalized to dry Leaf Biomass. Epiphyte Biomass normalized to dry Leaf Biomass. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species.

(A)

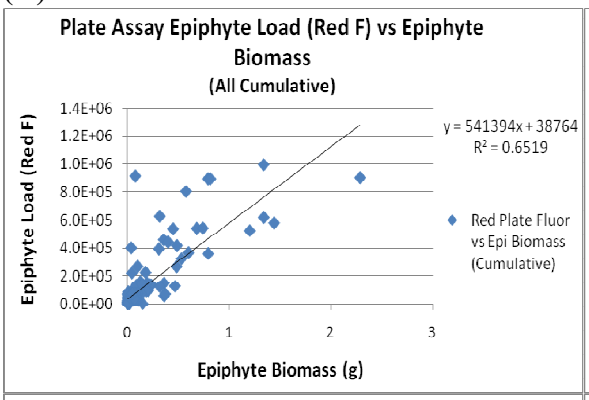


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(All Cumulative)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 541394x + 38764
R² = 0.6519
Red Plate Fluor vs Epi Biomass (Cumulative)

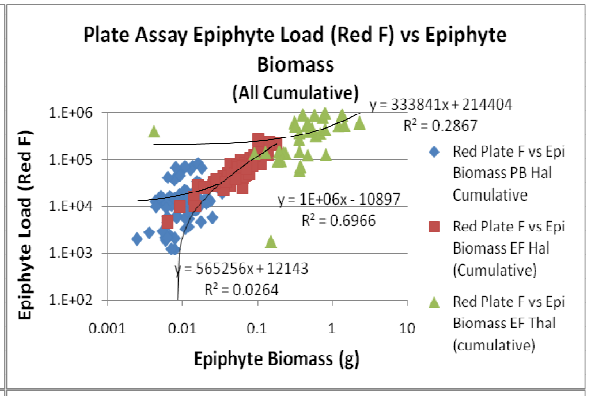


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(All Cumulative)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 333841x + 214404
R² = 0.2867
y = 1E+06x - 10897
R² = 0.6966
y = 565256x + 12143
R² = 0.0264
Red Plate F vs Epi Biomass PB Hal Cumulative
Red Plate F vs Epi Biomass EF Hal (Cumulative)
Red Plate F vs Epi Biomass EF Thal (cumulative)

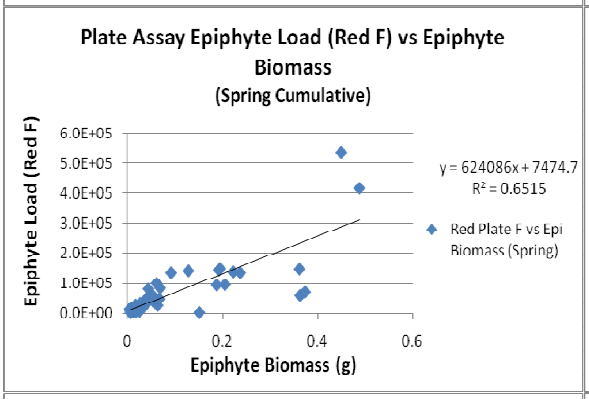


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Spring Cumulative)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 624086x + 7474.7
R² = 0.6515
Red Plate F vs Epi Biomass (Spring)

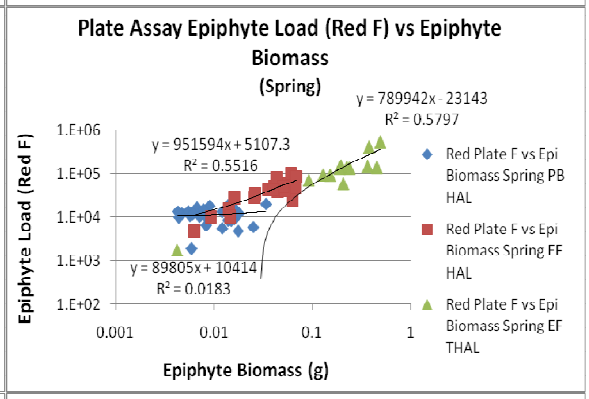


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Spring)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 789942x - 23143
R² = 0.5797
y = 951594x + 5107.3
R² = 0.5516
y = 89805x + 10414
R² = 0.0183
Red Plate F vs Epi Biomass Spring PB HAL
Red Plate F vs Epi Biomass Spring EF HAL
Red Plate F vs Epi Biomass Spring EF THAL

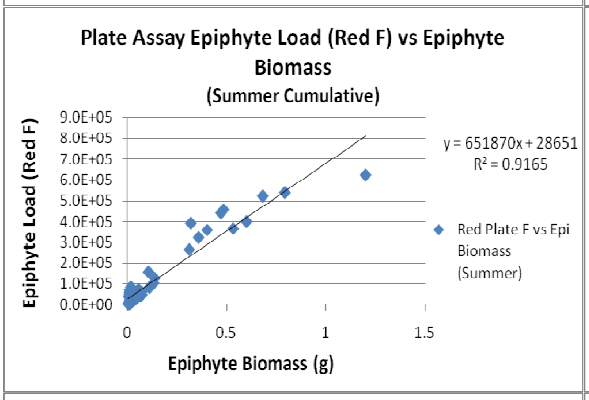


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Summer Cumulative)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 651870x + 28651
R² = 0.9165
Red Plate F vs Epi Biomass (Summer)

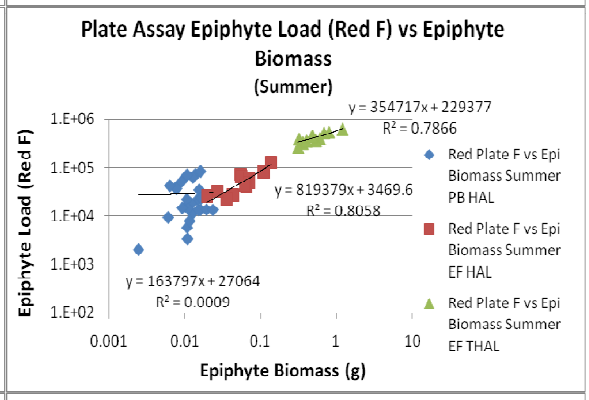


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Summer)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 354717x + 229377
R² = 0.7866
y = 819379x + 3469.6
R² = 0.8058
y = 163797x + 27064
R² = 0.0009
Red Plate F vs Epi Biomass Summer PB HAL
Red Plate F vs Epi Biomass Summer EF HAL
Red Plate F vs Epi Biomass Summer EF THAL

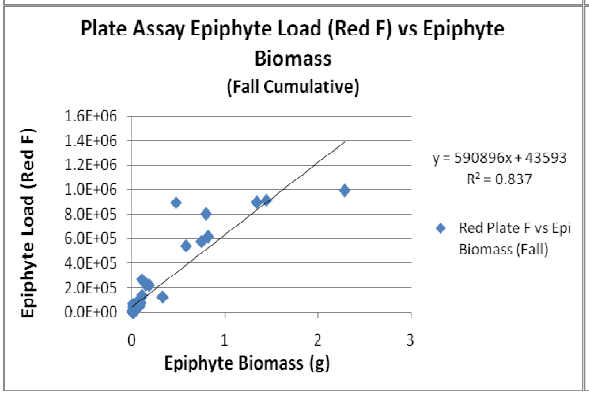


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Fall Cumulative)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 590896x + 43593
R² = 0.837
Red Plate F vs Epi Biomass (Fall)

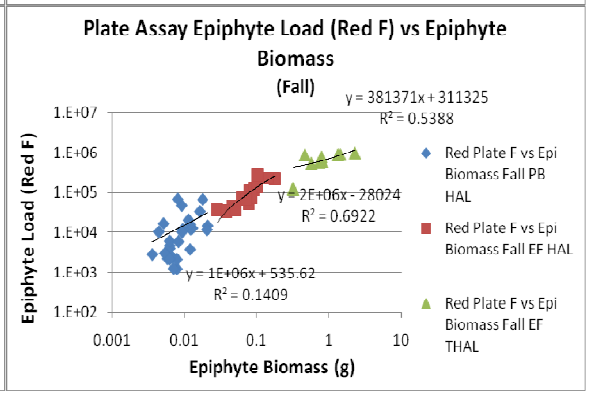
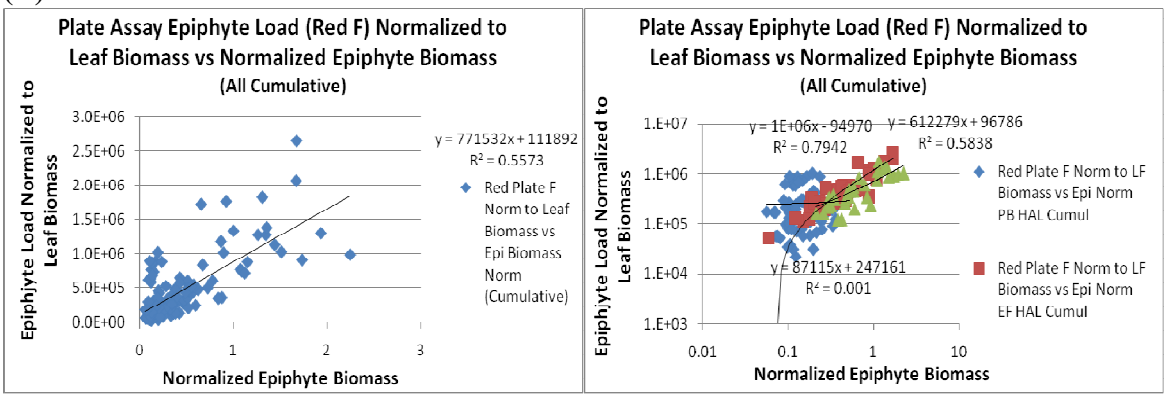


Plate Assay Epiphyte Load (Red F) vs Epiphyte Biomass
(Fall)
Epiphyte Load (Red F)
Epiphyte Biomass (g)
y = 381371x + 311325
R² = 0.5388
y = 2E+06x - 28024
R² = 0.6922
y = 1E+06x + 535.62
R² = 0.1409
Red Plate F vs Epi Biomass Fall PB HAL
Red Plate F vs Epi Biomass Fall EF HAL
Red Plate F vs Epi Biomass Fall EF THAL

(B)



Appendix F. Figure 26. Correlation of Red F of removed epiphytes with Epiphye Biomass.

(A) Plate Assay Epiphyte Load (Red F, Plate Assay) vs. Epiphyte Biomass. Red-excited fluorescence of removed epiphytes from Plate Assay. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. Seasonal cumulative data for all sites and all species. Determination coefficients (r^2) presented for individual sites and species. (B) Normalized Plate Assay Epiphyte Load (Red F normalized to Leaf Biomass, Plate Assay) vs. Normalized Epiphyte Biomass. Red-excited fluorescence of removed epiphytes normalized to dry Leaf Biomass. Epiphyte Biomass normalized to dry Leaf Biomass. Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species.

Plate Fluorescence Ratios

Change in the relative fluorescence levels from red- vs. green-excitation of removed epiphytes is proposed to be an indicator of large shifts in algal epiphyte composition from one class to another. Ratios of red-excited to green-excited fluorescence in the Plate Assay (R/G; Plate Assay Fluorescence Ratio) plotted vs. Epiphyte Biomass are extremely different for Port Bay vs. East Flats samples (Appendix F. Figure 27). This ratio was many-fold higher at Port Bay (and exhibited a wider range relative to epiphyte biomass changes) when compared to East Flats samples. Both East Flats *Halodule* and *Thalassia* samples had low ratios (generally less than 2) over a very wide range of epiphyte biomass. This is consistent with the interpretation that Port Bay seagrasses have an epiphyte community that is rich in green algae whereas the algal composition found at East Flats is probably rich in red algae.

There was no correlation of the Plate Assay Fluorescence Ratio (R/G) with Epiphyte Biomass when data were considered cumulatively over all seasons. However, when broken down by season, correlation of the Plate Assay Fluorescence Ratio with Epiphyte Biomass for Port Bay *Halodule* was 0.28, 0.02 and 0.48 respectively for spring, summer and fall. This suggests that composition may change somewhat as the epiphyte load increases at Port Bay. This may be an important factor contributing to the general lack of correlations for Port Bay data, especially when presented cumulatively over multiple seasons.

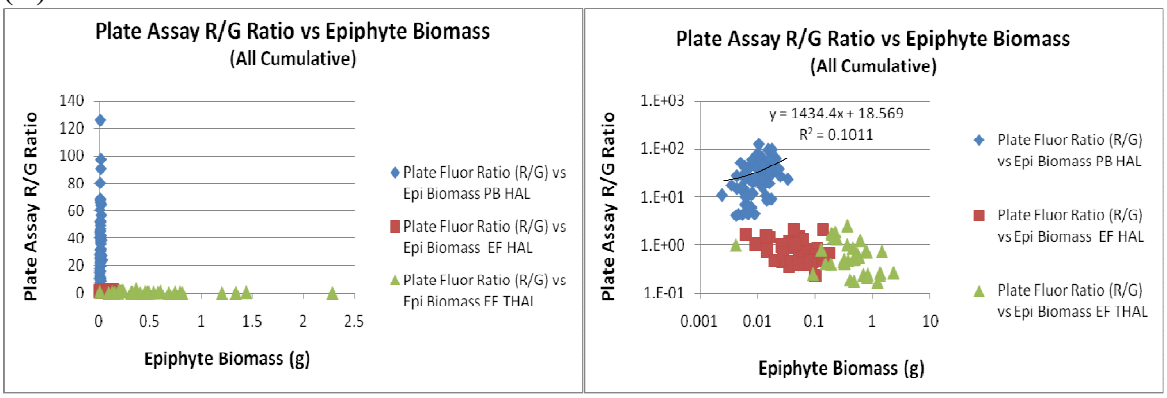
Appendix F. Figure 27C presents the plate fluorescence ratios by season, transect and depth. Several trends were apparent at Port Bay: 1) the R/G ratio was generally lower in the spring compared to summer and fall; 2) the R/G ratio increased with increasing depth in the spring, but

generally decreased with increasing depth in the summer and fall; and 3) there were different values bucking these "trends" for transects 3 and 1 in spring and summer, respectively. There was no clear trend in a comparison of one transect to another.

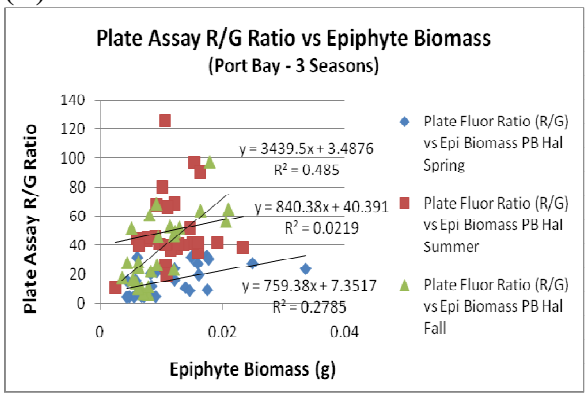
For East Flats, R/G ratios for *Halodule* + *Thalassia* (collectively) are shown because not all species were present at all depths of all transects. With exceptions, several trends were also apparent at East Flats: 1) R/G ratio generally decreased over the progression through the seasons spring → summer → fall; 2) R/G ratio generally decreased with increasing depth; and 3) R/G ratios were generally lower at transect 2 compared to the other transects.

The R/G ratio, if an indicator of the *relative* abundances of green vs. red algae, is expected to decrease with increasing depth, because more light attenuation at deeper depths is usually accompanied by enrichment of green wavelengths of sunlight relative to red, and this favors red algae or other epiphyte classes which produce green light-absorbing accessory pigments such as phycoerythrin and fucoxanthin (Kirk 1983; Raven *et al.* 2005). This trend was generally observed at both Port Bay and East Flats, except for the spring data and Transect 1 in the fall at Port Bay, and Transect 1 at East Flats in the fall, where the trend was clearly reversed. It should be noted that the depth gradient from shallow to deep at Port Bay was very small (maximum difference of 0.12 m) compared to East Flats.

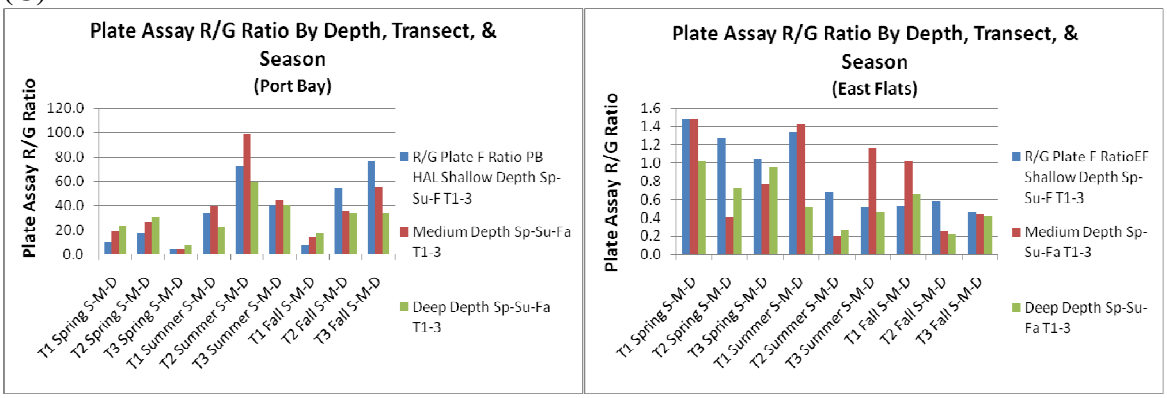
(A)



(B)



(C)



Appendix F. Figure 27. Correlation and comparison of R/G PlateAssay fluorescence ratios for removed epiphytes.

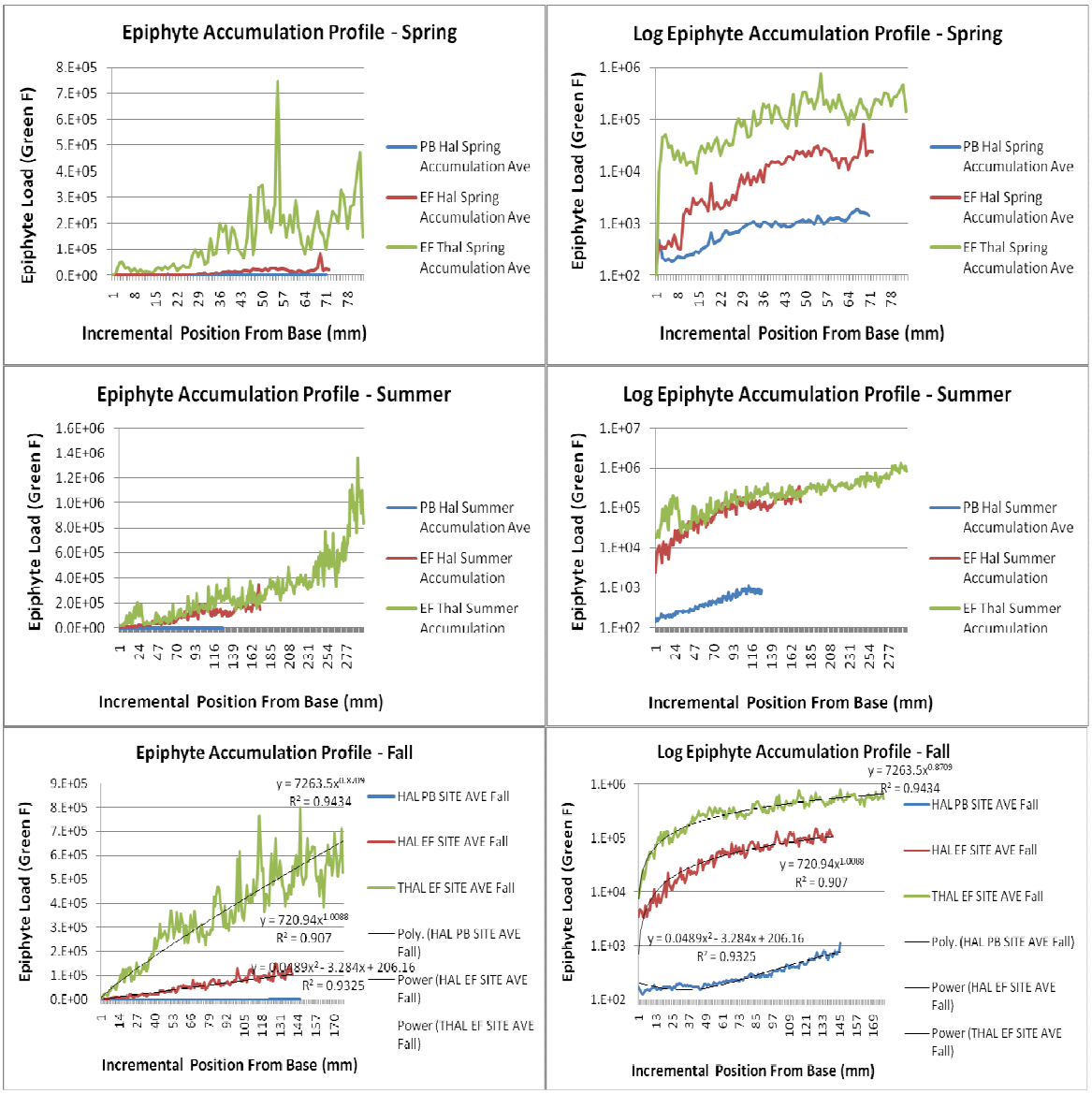
(A) R/G Plate Assay fluorescence ratio vs. Epiphyte Biomass. R/G Plate Assay fluorescence ratio is red-excited fluorescence divided by green-excited fluorescence, for removed epiphytes (from Plate Assays). Cumulative results in the left column and delineated by site and seagrass species using log-log plots in the right column. Total cumulative data for all seasons, all sites and all species. (B) Correlation of R/G ratio with Epiphyte Biomass for PB *Halodule* over three seasons. Determination coefficients (r^2) presented for individual seasons. (C) R/G ratios compared by season, transect and depth. Each measure is the average of three replicates at that depth/transect/season. Port Bay *Halodule* on left; East Flats *Halodule* + *Thalassia* on right. For East Flats, various distributions of different species necessitated averaging data for the two species.

Epiphyte Accumulation Profiles

A unique feature of the fluorescence-based epiphyte characterization is the spatial resolution that can be obtained for epiphyte accumulation. The Epiphyte Biomass measures often use a collection of multiple whole shoots, comprising varying numbers of blades of varying length and age, all of which are expected to have varying epiphyte loads. The Epiphyte Biomass comprises an average for the site if a representative sample is taken. Biomass measurement can achieve resolution to the level of blade for *Halodule* or perhaps segments of blades for the larger *Thalassia* leaves, but the biomass weights are very small and can be difficult to weigh. In contrast, epiphyte fluorescence image analysis (Epiphyte Load-Green F) allows the image to be analyzed by virtual "slicing" at 1 mm intervals and quantification of each slice (Appendix F. Figure 28). This allows every blade of every shoot, young or old, to be analyzed along the age gradient of the leaf (base to tip), providing a temporal view of the epiphyte recruitment and growth *relative* to the growth of the seagrass leaf. This unprecedented resolution is proposed to provide insight into any changes which may occur to the *relative* growth of epiphytes and seagrass blades, which is a possible outcome of eutrophication.

In this study, a single blade from each depth of each transect was analyzed for its epiphyte accumulation profile. There was an element of subjectivity in choosing the blade to be analyzed, but the selection criterion was as long a blade as possible that showed a large gradient of accumulation (light at the base and heavier towards the tip) and that was not obviously twisted. For Port Bay, 9 *Halodule* blade profiles were averaged for each season, and for East Flats, typically 6 *Halodule* and 5 *Thalassia* blade profiles were separately averaged (not all species were present at all depths of each transect at East Flats). The archived images can be revisited in the future and analyzed more extensively or randomly if desired.

These "average" profiles (Appendix F. Figure 28) reveal distinct accumulation kinetics for Port Bay *Halodule* vs. either *Halodule* or *Thalassia* at East Flats. Replicate blades for a site or species generally showed similar profile shapes. In fitting a regression line to the data, the East flats samples (of either species) were best fit with a power relationship, while the Port Bay samples were fit better by a polynomial equation. There was typically a 10 to 100-fold difference in epiphyte fluorescence comparing Port Bay to East Flats, consistent with differences observed in other parts of this study. The data are consistent with a higher rate of recruitment and/or growth of epiphytes at East Flats vs. Port Bay. It should be noted however, that if the Port Bay samples have a higher proportion of green algal epiphytes, as the Plate Assay R/G ratio data suggests, then these profiles obtained for *scanned leaves* are missing an important component of the epiphyte load. The altered kinetics at Port Bay could also potentially reflect red algal components being out-competed by green algal epiphytes, whereas the reverse might be true at East Flats. Alternatively, successional changes or sequential layers of epiphyte colonization (Corlett and Jones 2007) with blade age (length) could account for differences in levels of Green F. It seems unlikely that grazing differences could account for these observations of accumulation patterns.



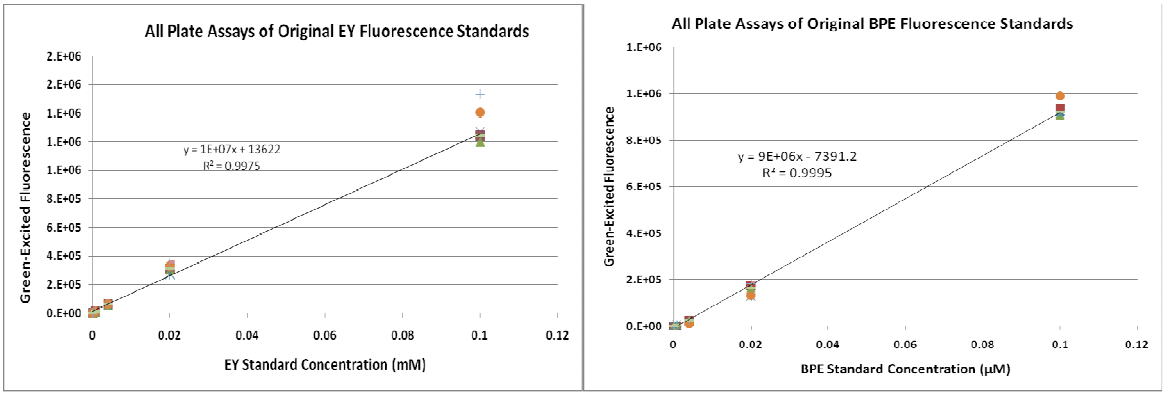
Appendix F. Figure 28. Epiphyte accumulation profiles by season, site and species.

Epiphyte Load (Green F) vs. incremental position from base of blade. Green-excited epiphyte fluorescence images from a single blade of each species from each depth of each transect were analyzed. Blades were selected based on gradient of epiphyte accumulation. Images were divided into 1 mm segments and quantified. All images from a season for each species were averaged based on length from the leaf base. Typically, 9 blades averaged for PB *Halodule*, and 5-6 blades averaged for EF *Halodule* or *Thalassia.* Plotted length represents minimum length common to all blades of a sample averaged. Left column plots use linear axis for fluorescence; fluorescence for right column plots expressed on a logarithmic scale.

Fluorescence Measurement Sensitivity and Quality Control

Quality control measures, as specified in the QAPP (Radloff 2010), were implemented as described. Successive fluorescence measurements of a sample generally gave results well within the specified range of ± 15%, and were usually within 5% (see Appendix F. Table 4). The fluorescence images comprise archival type data that could potentially be used in future comparisons. Of particular concern is any change, short term or long term, in instrument sensitivity. Lasers used for fluorescence excitation tend to decrease in power over time. Therefore, documentation of Green F instrument sensitivity was established by seasonal measurement of fluorescence from a dilution series of two fluorophores serving as reference standards. Eosin Y (EY) is a chemical fluorophore, and B-Type Phycoerythrin (BPE) is a protein and pigment-based fluorophore which is the major epiphyte pigment detected in this assay.

The EY and BPE dilution series from the original standard stock solutions were run for every project sampling event and also used to prepare new serial dilutions which were also run for every project sampling event. The individual results of all of the runs of these original standard solutions (Appendix F. Figure 29) show linearity and reveal no large changes in instrument sensitivity for both of the standards tested. This information is potentially useful for any future studies which may require comparison to this data.



Appendix F. Figure 29. Reproducibility and sensitivity of green-excited fluorescence reference standards.

Five-fold dilution series of stock solutions of fluorescence reference standards (0.1 mM eosin Y – EY; 0.1 µM B-type phycoerythrin – BPE) were analyzed in the Plate Assay for every sampling event. Green-excited fluorescence plotted vs. concentration.

Appendix F. Table 3. Summary of determination coefficients (r^2) between measured parameters.

			Determination Coefficients (r^2)												
			All Seasons	Spring				Summer				Fall			
Y -Parameter	X -Parameter	Figure	All Cumul	Spring Cumul	PB Hal	EF Hal	EF Thal	Summer Cumul	PB Hal	EF Hal	EF Thal	Fall Cumul	PB Hal	EF Hal	EF Thal
Epiphyte Load	Epiphyte Biomass	18	0.48	0.51	0.16	0.34	0.36	0.54	0.00	0.24	0.00	0.94	0.08	0.01	0.91
Scanned Leaf Area	Leaf Biomass	19	0.21	0.66	0.55	0.53	0.72	0.26	0.06	0.04	0.05	0.26	0.24	0.48	0.60
Epiphyte Biomass	Leaf Biomass	20	0.51	0.88	0.05	0.40	0.64	0.56	0.20	0.05	0.00	0.85	0.23	0.05	0.58
Epiphyte Load	Scanned Leaf Area	21	0.18	0.60	0.13	0.45	0.62	0.19	0.02	0.50	0.00	0.18	0.73	0.39	0.49
Epiphyte Load	Leaf Biomass	22	0.57	0.64	0.30	0.81	0.57	0.44	0.05	0.25	0.07	0.80	0.34	0.81	0.46
Normalized Epiphyte Load (Scanned Leaf Area)	Normalized Epiphyte Biomass	23	0.19	0.06	0.34	0.02	0.06	0.41	0.04	0.01	0.11	0.68	0.02	0.32	0.78
Normalized Epiphyte Load (Leaf Biomass)	Normalized Epiphyte Biomass	24	0.29	0.03	0.32	0.00	0.01	0.47	0.03	0.56	0.03	0.48	0.00	0.02	0.73
Plate Assay Epiphyte Load (Green F)Epiphyte	Epiphyte Biomass	25	0.67	0.54	0.30	0.63	0.34	0.83	0.00	0.11	0.70	0.62	0.03	0.24	0.21
Plate Assay Epiphyte Load (Green F) Epiphyte	Scanned Leaf Area	25	0.15	0.55	0.02	0.38	0.57	0.07	0.01	0.30	0.25	0.15	0.10	0.10	0.16
Plate Assay Epiphyte Load (Green F) Epiphyte	Leaf Biomass	25	0.29	0.66	0.15	0.42	0.49	0.29	0.03	0.38	0.15	0.54	0.10	0.15	0.04
Normalized Plate Assay Epiphyte Load- Epiphyte Green F (Leaf Biomass)	Normalized Epiphyte Biomass	25	0.54	0.18	0.26	0.41	0.03	0.81	0.04	0.50	0.87	0.45	0.00	0.20	0.05
Plate Assay Epiphyte Load (Red F)Epiphyte	Epiphyte Biomass	26	0.85	0.75	0.02	0.55	0.58	0.92	0.00	0.81	0.79	0.84	0.14	0.69	0.54
Plate Assay Epiphyte Load (Red F) Epiphyte	Scanned Leaf Area	26	0.24	0.69	0.01	0.36	0.81	0.17	0.01	0.08	0.36	0.17	0.17	0.09	0.19
Plate Assay Epiphyte Load (Red F) Epiphyte	Leaf Biomass	26	0.53	0.81	0.15	0.38	0.67	0.63	0.03	0.05	0.01	0.80	0.14	0.04	0.26
Normalized Plate Assay Epiphyte Load-Red F Epiphyte Red F (Leaf Biomass)	Normalized Epiphyte Biomass	26	0.56	0.23	0.06	0.31	0.25	0.21	0.02	0.66	0.67	0.63	0.06	0.79	0.23
Plate Assay R/G fluorescence Ratio	Epiphyte Biomass	27	0.08	0.13	0.28	0.33	0.01	0.18	0.02	0.18	0.14	0.12	0.48	0.03	0.02

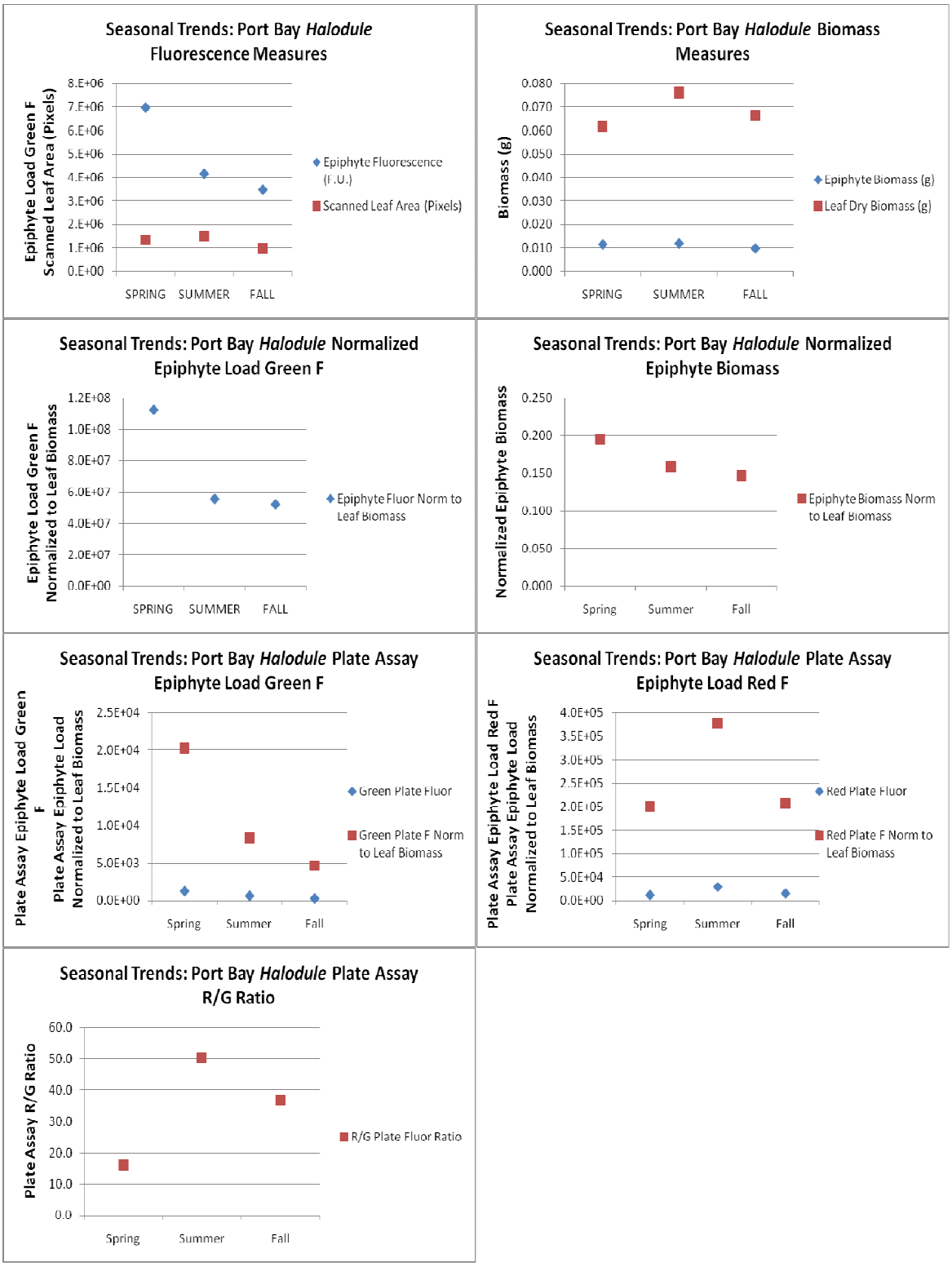
Comparison of Seasonal Trends in Epiphyte Measures

Comparison of seasonal trends obtained for the different epiphyte measures reveals how each performs in detecting this natural variation (where it exists). Alternative measures like the fluorescence method would be expected to exhibit trends similar to those of biomass-based measures if indeed they are measuring the same thing. Comparison of mean values of the various epiphyte measures for the two different sites and seagrass species, averaged over the three depths at the three transects for each, shows how each measure performed (Appendix F. Figure 30 to Appendix F. Figure 32).

*Halodul*e at Port Bay showed seasonally progressing declines in Normalized Epiphyte Biomass, Normalized Epiphyte Load (Green F) and Plate Assay Normalized Epiphyte Load (Green F or Red F) of removed epiphytes (Appendix F. Figure 30). The decline was largely driven by declining values for the epiphyte measures (as opposed to increases in the seagrass dry Leaf Biomass or Scanned Area). Deviating somewhat from this trend, leaf dry biomass and normalized red-excited fluorescence of removed epiphytes spiked higher in the summer and then returned to near spring levels in the fall. The R/G fluorescence plate ratio also increased from spring through fall, but spiked higher in the summer, explaining the spike in the Red F for removed epiphytes. This suggests a change in composition of the algal epiphyte classes present at Port Bay, but overall, epiphyte fluorescence measures (including the Scanned Leaf Area) showed seasonal patterns similar to the biomass-based measures.

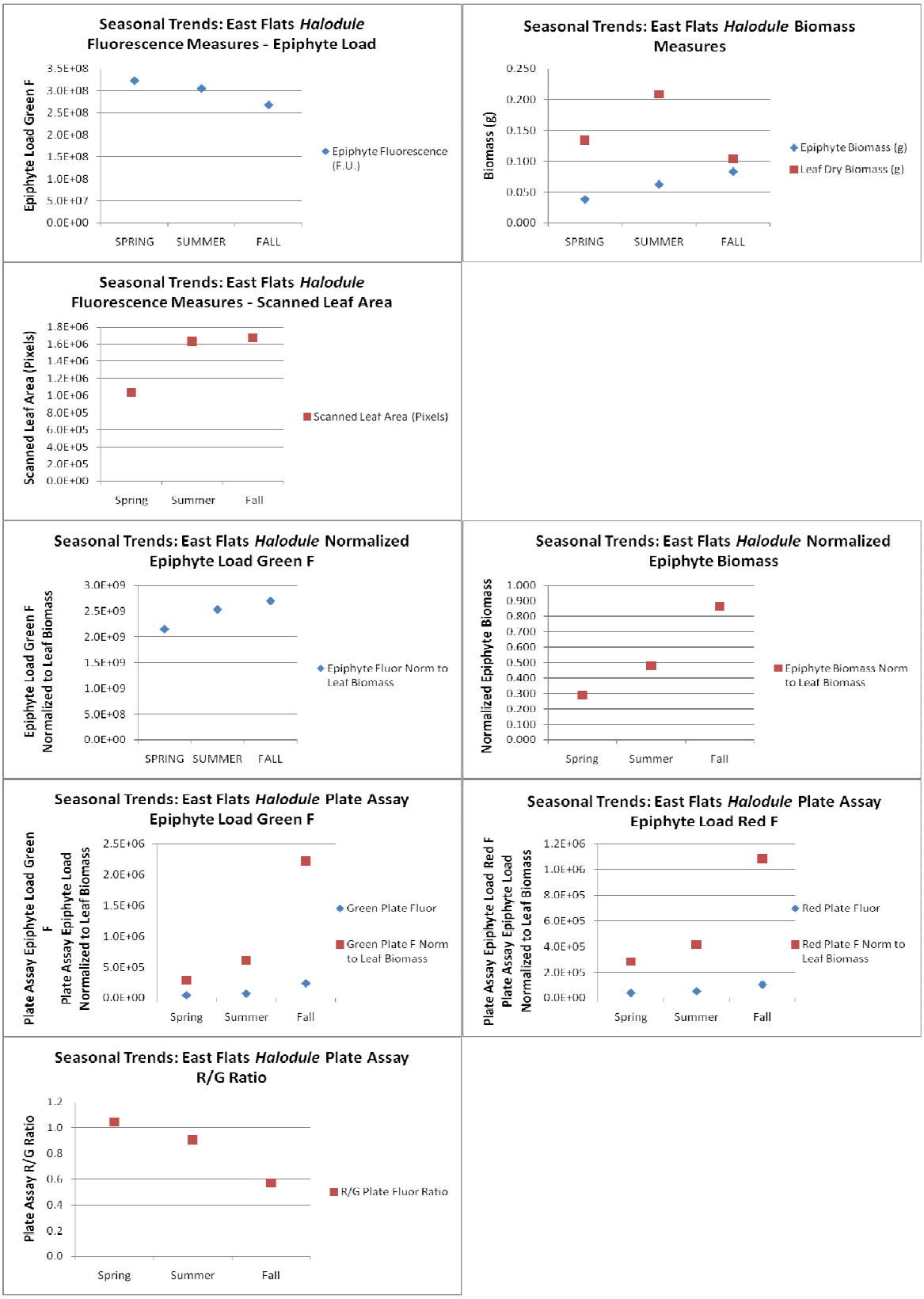
*Halodul*e at East Flats showed seasonally progressing increases in Normalized Epiphyte Biomass, Normalized Epiphyte Load and Plate Assay Normalized Epiphyte Load (Green F or Red F) of removed epiphytes (Appendix F. Figure 31). The large increases in the fall values were driven in part by a decline in the seagrass dry Leaf Biomass. The R/G fluorescence ratio progressively declined from spring through fall, suggesting some change in composition of the algal epiphyte classes present at East Flats. Overall, normalized epiphyte fluorescence measures showed seasonal patterns similar to the biomass-based measures. However, Scanned Leaf Area and un-normalized Epiphyte Load (Green F) from scanned leaves deviated from their biomass-based counterparts.

Thalassia at East Flats showed seasonally progressing increases in Normalized Epiphyte Biomass, and Plate Assay Normalized Epiphyte Load (Green F or Red F) of removed epiphytes (Appendix F. Figure 32). The dry Leaf Biomass was mostly flat across seasons, so the increases were largely driven by the epiphyte measures. However, normalized and un-normalized epiphyte fluorescence from scanned leaves increased from spring to summer and then declined in the fall. The R/G fluorescence ratio decreased progressively from spring through fall.



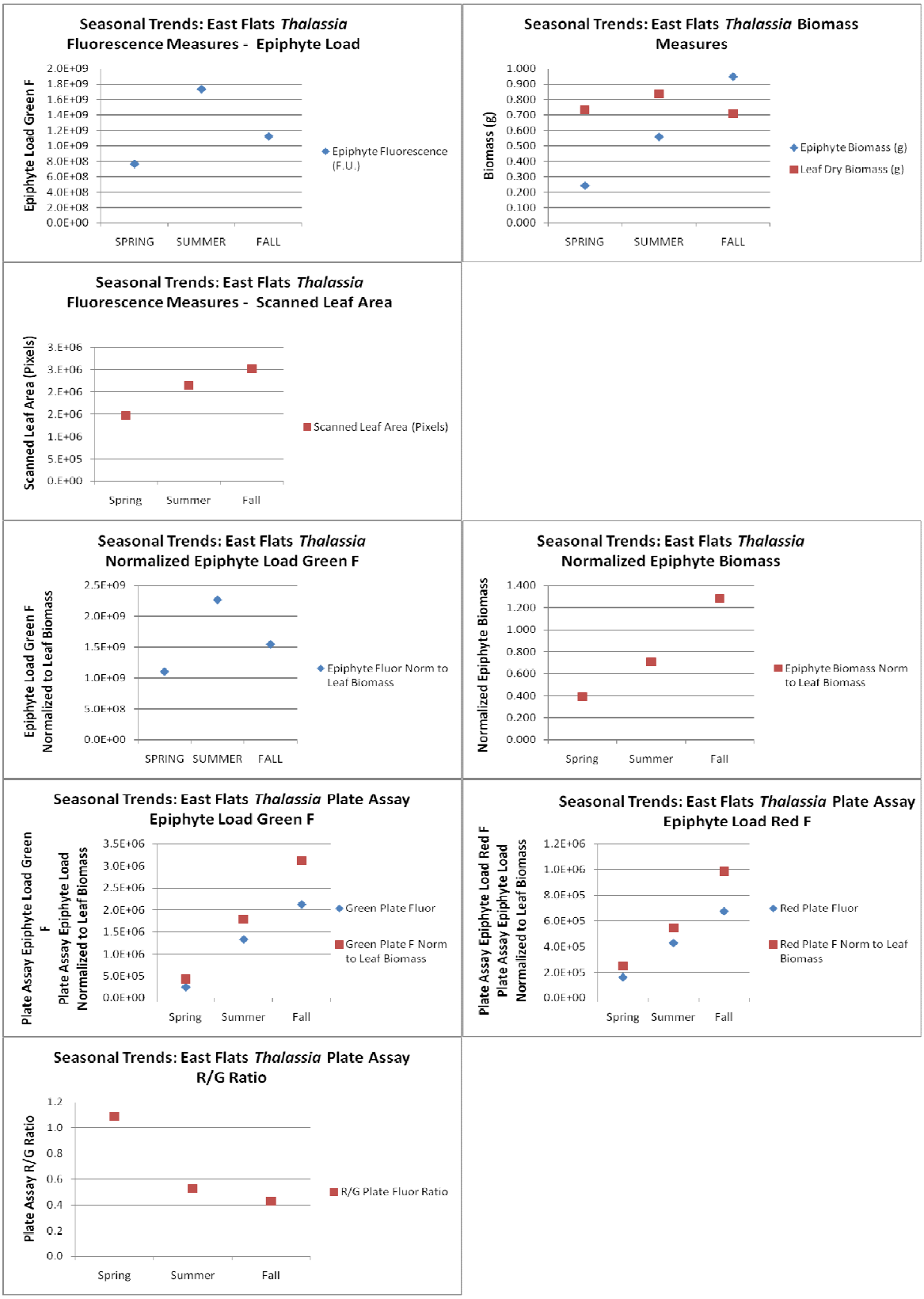
Appendix F. Figure 30. Seasonal trends for Port Bay *Halodule* epiphyte and seagrass parameters.

Note that un-normalized measures of Epiphyte Load (Green F), Plate Assay Epiphyte Load (Green F or Red F), Scanned Leaf Area, Leaf Biomass and Epiphyte Biomass pertain to the whole shoot samples analyzed, typically 10 – 15 whole shoots.



Appendix F. Figure 31. Seasonal trends for East Flats *Halodule* epiphyte and seagrass parameters.

Note that un-normalized measures of Epiphyte Load (Green F), Plate Assay Epiphyte Load (Green F or Red F), Scanned Leaf Area, Leaf Biomass and Epiphyte Biomass pertain to the whole shoot samples analyzed, typically 10 – 15 whole shoots.



Appendix F. Figure 32. Seasonal trends for East Flats *Thalassia* epiphyte and seagrass parameters.

Note that un-normalized measures of Epiphyte Load (Green F), Plate Assay Epiphyte Load (Green F or Red F), Scanned Leaf Area, Leaf Biomass and Epiphyte Biomass pertain to the whole shoot samples analyzed, typically 10 – 15 whole shoots.

Summary and Discussion

Appendix F. Table 3 summarizes the observed correlations between measured and calculated parameters.

There were large, approximately order of magnitude, differences in epiphyte accumulations, by any measure, between different sites and seagrass species. Differences were larger between Port Bay and East Flats *Halodule* samples than between East Flats *Halodule* and *Thalassia* samples. By contrast, differences in epiphyte accumulations between different seasons (for any site or species) were smaller. Epiphyte fluorescence measurements were correlated with epiphyte biomass measurements, but the fluorescence characterization distinguished the Port Bay samples from the East Flats samples to a greater extent (nearly two orders of magnitude).

In contrast, the fluorescence-based proxy for leaf cross-sectional area (Scanned Leaf Area) was not consistently correlated with Leaf Biomass. Strong correlations were observed for the spring sampling, both cumulatively and individually for site and species. The lack of correlation in summer and fall samples may be attributed *in part* to an overestimation of leaf cross-sectional area when there are large accumulations of filamentous epiphytes extending out from the leaf surface (particularly summer and fall).

An expected correlation between Epiphyte Biomass and Leaf Biomass was strong for cumulative data, but inconsistent for individual intra-site comparisons. Epiphyte Load (Green F) showed a nearly identical pattern of correlations with Leaf Biomass across cumulative and individual comparisons, but for either measurement, only about half of the variability in the overall cumulative data is accounted for. Epiphyte fluorescence correlations with Scanned Leaf Area (instead of Leaf Biomass) were consistently strong only for spring samples, the same period for which Scanned Leaf Area correlated with Leaf Biomass. Leaf Biomass is suggested to be more useful than Scanned Leaf Area for normalization of Epiphyte Load across multiple seasons and, probably, across multiple studies. Leaf area from morphometrics of scanned images may provide an alternative solution for this purpose, but was not specifically calculated here.

The epiphyte accumulation metric considered most useful for comparisons between sites, conditions or studies, is the epiphyte measure which is normalized for differences in leaf size (or biomass). Normalized Epiphyte Load (Green F), normalized to either Scanned Leaf Area or Leaf Biomass, correlated with the Normalized Epiphyte Biomass in cumulative summer and fall data, but not in the spring or overall cumulative data. For the fluorescence-based epiphyte measures, correlations were generally stronger when the epiphytes were removed from the leaf. Normalization of both fluorescence and biomass data brought the values for East Flats *Halodule* and *Thalassia* to near superimposition, while Port Bay *Halodule* values remained distinctly clustered and dissimilar to the East Flats *Halodule* samples. Coalescence of the data is expected for normalization, so there are inherent differences between Port Bay and East Flats that are not accounted for by either Epiphyte Biomass or Epiphyte Load (Green F) measurements.

In general, the moderate to strong correlation of epiphyte fluorescence measures with Epiphyte Biomass became less consistent upon normalization. In actuality, because the “un-normalized” data were taken from the same seagrass shoots, they are *de facto* normalized to number of whole

shoots. But this method of comparison is less useful for comparing different studies, but perhaps still useful when monitoring a given location over time. However, in the comparison of seasonal trends, *normalized* fluorescence patterns mimicked the biomass-based measures, and this was most especially true for epiphytes removed from the leaves (Appendix F. Figure 30 to Appendix F. Figure 32). This difference may be related to how well a mean value represents a transect.

A prominent feature of the data is the unexpected inconsistency in linearity at different scales (different sites, different species) for both the biomass- and fluorescence-based measurements, manifest as "clustering" of data by site and seagrass species. Likewise, inconsistencies in patterns of correlations were also observed at different scales for both types of measures. In some cases, good correlation of overall cumulative data (all seasons/sites/species) arose from data that was not correlated at all at the level of individual season/site/species. In other cases, data that was correlated at the level of individual season/site/species, yielded only poor correlations overall. These inconsistencies are suggested to reflect different ecophysiologies (*e.g.* seagrass growth, turnover and colonizing epiphyte species or characteristics) for the unique conditions at Port Bay vs. East Flats.

One possible explanation for the clustering is that data from individual sites and species reflect a combination of biological and measurement variation resulting from a single "condition" with typically little true intra-site variation. Each cluster is representative of a single point that can be distinguished from other conditions (sites) on a larger scale. In this view, inter-site rather than intra-site comparisons and correlations are most biologically informative. The cumulative comparisons, overall and by season, would be most appropriate. In this respect, the fluorescence- and biomass-based epiphyte measurements are correlated and clearly able to distinguish the different epiphyte accumulation patterns at the sampling sites.

An alternative explanation, not necessarily at odds with that above, is that different factors are responsible for the different patterns of intra- vs. inter-site epiphyte accumulation. In this case, the often times nearly flat intra-site response of Epiphyte Load (Green F) to changing Epiphyte Biomass may hold clues to the underlying causes of variation in epiphyte accumulation by the different measures.

The fluorescence "Plate Assays" for removed epiphytes provided unique insight into differences between Port Bay and East Flats epiphyte accumulations. There were generally stronger correlations of epiphyte fluorescence with epiphyte biomass, compared to fluorescence measured by scanning the intact leaf. Of course, this assay requires the additional work of leaf scraping, but because of the sensitivity of the measurement, very little sample material is required (less than a single blade), so it does offer efficiency over the amount of scraping required for biomass measurements.

However the most significant insight came from the *ratio* of Red F to Green F made possible by the Plate Assay. This R/G ratio was consistently one to two orders of magnitude higher for Port Bay vs. East Flats removed epiphytes, regardless of seagrass species. There was weak correlation of this R/G ratio with epiphyte biomass in some cases, most notably at Port Bay, but not consistently. This suggests very different epiphyte community compositions which could be interpreted as enrichment of green algal epiphytes at Port Bay relative to enrichment at East Flats

of red algal epiphytes (or other classes preferentially absorbing green wavelengths of light; see Appendix F. Table 1).

This interpretation is supported by observation of the archived high resolution Green F epiphyte images. Whereas Port Bay *Halodule* epiphytes were predominantly small, hard-to-visualize colonial or crustose forms and a few filamentous types, East Flats *Halodule* had an abundance of larger colonial or crustose forms on young blades and remarkable complete coverage by filamentous forms on the older blade. In a few cases, epiphyte biomass actually exceeded leaf biomass. East Flats *Thalassia* exhibited nearly complete coverage by crustose forms, as well as some filamentous forms on the oldest blades.

The different predominance of epiphyte morphotypes observed on young vs. old blades at East Flats is indicative of the successional colonization and growth patterns observed previously (Corlett and Jones 2007; see also Armitage *et al.* 2005). Eutrophication and/or changes in grazing pressure would be expected to influence these patterns, which can be documented and quantified to some degree by monitoring "accumulation profiles" in which the incremental epiphyte Green F is plotted as a function of distance from the base of the leaf. As an example, very different "kinetics" (plot shape) are observed for epiphyte accumulation at Port Bay vs. East Flats. These profiles are interpreted as a higher rate of epiphyte colonization/growth, relative to growth rate of the seagrass leaf, at East Flats. Similar profile shapes on both *Halodule* and *Thalassia* suggest that *site conditions* are primarily responsible for this difference. This metric should be tested and developed further.

Recommendations

The fluorescence-based epiphyte characterization methods provide valuable insight into the dynamics of epiphyte accumulation on seagrasses, insights not obtainable from biomass measurements. However, the fluorescence methods do not, at this level of development, appear to be an entirely suitable *replacement* for epiphyte biomass measurements. Given that most existing studies have used biomass measures, abandonment of that might limit comparability of studies going forward. Nonetheless, there was moderate to strong correlation of epiphyte fluorescence and biomass on various scales, generally similar seasonal trends, and similar internal inconsistencies for *both* measures when compared across different scales (sites/species/seasons). The best correlation values were obtained for data that was "unnormalized" (actually normalized to whole shoots), whereas normalization to Leaf Biomass or Scanned Leaf Area reduced correlations to varying degrees. Normalization to Leaf Biomass was clearly the better approach of the two, but normalization to leaf area from morphometric measurements should be explored as well. The normalized fluorescence data, especially for removed epiphytes, did reproduce the seasonal trends observed for the biomass-based measures.

The accumulation profiles, high resolution scans and Plate Assay-generated R/G ratios can provide beneficial information about how the epiphytes may be responding to environmental conditions and, given that all three could be accomplished with very small sample sizes (one to several shoots), it is recommended that these measures be considered for use in future studies and long term monitoring. Any future studies involving the fluorescence scanning of seagrass leaves should incorporate the suggested improvements to reduce drying or twisting artifacts during the scanning process.

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Appendix – Data Compilation

Appendix F. Table 4. Project data organized by season, site, and species.

SPRING

Port Bay	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3018	h	S	1	7627156	1337724	0.014	0.077	5.70	99053974	0.18	784	8474	10.8
3019	h	S	1	5543519	1318526	0.008	0.083	4.20	66789386	0.10	545	6538	12
3020	h	S	1	4293409	919028	0.018	0.053	4.67	81007717	0.33	516	4772	9.3
3021	h	M	1	3075759	884290	0.009	0.098	3.48	31385296	0.09	431	9374	21.8
3022	h	M	1	3869557	896607	0.016	0.060	4.32	64492617	0.27	469	9210	19.6
3023	h	M	1	3122875	856156	0.012	0.050	3.65	62457500	0.24	339	5503	16.2
3024	h	D	1	1831968	747463	0.006	0.033	2.45	55514182	0.18	151	1902	12.6
3025	h	D	1	3751299	1211984	0.025	MV	3.10	MV	MV	212	5870	27.7
3026	h	D	1	4016754	1079226	0.015	0.070	3.72	57382200	0.36	263	8397	32
3027	h	S	2	4868556	595717	0.006	0.039	8.17	124834769	0.15	690	10933	15.8
3028	h	S	2	4560840	629268	0.004	0.047	7.25	97039149	0.09	650	9923	15.3
3029	h	S	2	4706891	622290	0.008	0.036	7.56	130746972	0.23	505	10339	20.5
3030	h	M	2	7167734	1283476	0.034	0.096	5.58	74663896	0.35	833	19931	23.9
3031	h	M	2	2498345	607864	0.006	0.040	4.11	62458625	0.15	374	11850	31.7
3032	h	M	2	5099860	920219	0.012	0.059	5.54	86438305	0.21	545	13170	24.2
3033	h	D	2	2210611	449998	0.017	0.033	4.91	66988212	0.53	385	12462	32.4
3034	h	D	2	3370667	1067512	0.018	0.050	3.16	67413340	0.36	389	12000	30.8
3035	h	D	2	2754357	783854	0.016	0.051	3.51	54007000	0.31	590	16255	27.6
3036	h	S	3	8380221	597491	0.005	0.046	14.03	182178717	0.10	2668	12046	4.5
3037	h	S	3	16123722	1067021	0.006	0.081	15.11	199058296	0.07	3085	13506	4.4
3038	h	S	3	13150700	928390	0.009	0.063	14.17	208741270	0.14	3992	18113	4.5
3039	h	M	3	14828868	914732	0.004	0.077	16.21	192582701	0.06	3146	13228	4.2
3040	h	M	3	11831214	794580	0.007	0.064	14.89	184862719	0.11	2202	10416	4.7
3041	h	M	3	16357241	1237521	0.007	0.097	13.22	168631351	0.07	3508	16555	4.7
3042	h	D	3	9673096	1214651	0.006	0.050	7.96	193461920	0.12	1653	11888	7.2
3043	h	D	3	13640459	1098782	0.015	0.086	12.41	158609988	0.17	2424	21365	8.8
3044	h	D	3	10053299	932861	0.008	0.063	10.78	159576175	0.12	2337	14952	6.4

SPRING

East Flats	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3045	h	S	1	190960399	1130876	0.041	0.107	168.86	1784676626	0.38	55546	51370	0.92
3046	h	S	1	234724036	1229102	0.054	0.105	190.97	2235467010	0.51	38498	57227	1.49
3047	h	S	1	354815046	1578299	0.060	MV	224.81	MV	MV	113145	100242	0.89
3048	h	M	1	35611937	589738	0.006	0.042	60.39	847903262	0.15	2782	4736	1.7
3049	h	M	1	112224403	831269	0.063	0.072	135.00	1558672264	0.87	33514	25273	0.75
3050	h	M	1	83665684	804751	0.016	0.086	103.96	972856791	0.19	19280	28074	1.46
3054	h	D	2	1110846498	1439501	0.067	0.249	771.69	4461230916	0.27	62294	48204	0.77
3056	h	D	2	145861378	1014167	0.009	0.076	143.82	1919228652	0.12	9528	10037	1.05
3060	h	S	2	283787041	807594	0.043	0.156	351.40	1819147699	0.28	82535	80836	0.98
3061	h	S	2	163026875	640147	0.014	0.069	254.67	2362708333	0.21	9980	15557	1.56
3064(a)	h	S	3	250033062	625893	0.035	0.152	399.48	1644954355	0.23	35705	42364	1.19
3064(b)	h	S	3	325027556	1131592	0.026	0.137	287.23	2372463912	0.19	30289	28419	0.94
3065	h	S	3	414154563	1132757	0.044	0.159	365.62	2604745679	0.28	39932	39798	1
3066	h	M	3	304192208	1145303	0.053	0.152	265.60	2001264526	0.35	69042	40629	0.59
3067	h	M	3	628487540	1340297	0.050	0.247	468.92	2544483968	0.20	53004	49803	0.94
3068	h	M	3	155084458	602746	0.015	0.083	257.30	1868487446	0.18	13181	10258	0.78
3069	h	D	3	467484918	1153369	0.068	0.179	405.32	2611647587	0.38	106253	83809	0.79
3070	h	D	3	469664282	1320288	0.040	0.191	355.73	2458975298	0.21	39410	42397	1.08
3071	h	D	3	399256375	1124358	0.026	0.147	355.10	2716029762	0.18	33055	33072	1
3045	t	S	1	126628735	1552090	0.237	0.513	81.59	246839639	0.46	102445	135522	1.32
3046	t	S	1	181344290	1989828	0.361	0.433	91.14	418808984	0.84	58574	147664	2.52
3047	t	S	1	391343426	1615649	0.191	0.912	242.22	429104634	0.21	81694	144532	1.77
3048	t	M	1	450170390	1273822	0.222	MV	353.40	MV	MV	74534	138345	1.86
3049	t	M	1	380031977	1138870	0.194	0.692	333.69	549179158	0.28	89406	148672	1.66
3050	t	M	1	564899245	1297938	0.363	0.785	435.23	719616873	0.46	MV	MV	MV
3051	t	D	1	325154012	942675	0.205	0.488	344.93	666299205	0.42	42863	59372	1.39
3052	t	D	1	215940931	947494	0.128	0.408	227.91	529266988	0.31	118504	95126	0.8
3053	t	D	1	572005552	1937696	0.449	1.183	295.20	483521177	0.38	162530	141809	0.87
3058(a)	t	M	2	2835168062	3147175	0.487	1.453	900.86	1951251247	0.34	1037821	537532	0.52
3058(b)	t	M	2	2957950061	2725833	0.373	1.251	1085.15	2364468474	0.30	866451	417294	0.48
3059	t	M	2	433105751	469847	0.091	0.158	921.80	2741175639	0.58	281837	70970	0.25
3054	t	D	2	1147000633	1277568	0.187	0.638	897.80	1797806635	0.29	340572	135309	0.4
3055	t	D	2	840126521	1542428	0.151	0.589	544.68	1426360817	0.26	230840	93833	0.41
3056	t	D	2	24065824	284813	0.004	MV	84.50	MV	MV	1789	1767	0.99

SUMMER

PORT BAY	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3120	H	S	1	3453644	1502677	0.011	0.075	2.30	46171710	0.14	222	5836	26.34
3121	H	S	1	3103918	1411469	0.013	0.120	2.20	25909166	0.10	296	11249	38.06
3122	H	S	1	2742150	1423838	0.012	0.057	1.93	48447871	0.20	217	7936	36.52
3123	H	M	1	3289050	1408727	0.016	0.157	2.33	20989470	0.10	381	13580	35.66
3124	H	M	1	4179359	1901114	0.015	0.083	2.20	50353723	0.18	322	13426	41.65
3125	H	M	1	3182331	1233800	0.019	0.087	2.58	36747473	0.22	326	13639	41.82
3126	H	D	1	2399606	1259789	0.011	0.044	1.90	54785536	0.25	169	3349	19.87
3127	H	D	1	3681720	1610107	0.023	0.072	2.29	51135000	0.32	348	13433	38.61
3128	H	D	1	1009133	716194	0.002	0.039	1.41	25677682	0.06	182	2017	11.08
3129	H	S	2	3041553	591795	0.015	0.091	5.14	33497277	0.16	426	22047	51.71
3130	H	S	2	2422912	1242480	0.012	0.067	1.95	36271142	0.18	265	18191	68.57
3131	H	S	2	2609228	1541720	0.015	0.078	1.69	33408813	0.20	358	34844	97.3
3132	H	M	2	2178392	666991	0.016	0.076	3.27	28587827	0.21	263	23808	90.49
3133	H	M	2	2185117	1281937	0.010	0.057	1.70	38268246	0.18	197	15844	80.3
3134	H	M	2	1914907	1226737	0.011	0.074	1.56	25737994	0.14	177	22258	125.9
3135	H	D	2	2712848	1108164	0.006	0.058	2.45	46452871	0.10	211	9376	44.49
3136	H	D	2	2400405	591302	0.011	0.049	4.06	48788712	0.22	217	14408	66.45
3137	H	D	2	3113095	697641	0.009	0.075	4.46	41563353	0.12	217	14679	67.77
3138	H	S	3	6667885	1439549	0.014	0.083	4.63	80239288	0.16	1824	73102	40.08
3139	H	S	3	7804262	1541135	0.010	0.080	5.06	98166823	0.12	1484	60375	40.68
3140	H	S	3	10205595	1618859	0.013	0.087	6.30	117305690	0.15	1551	63426	40.89
3141	H	M	3	6336433	1265185	0.008	0.067	5.01	95141632	0.11	896	38547	43.01
3142	H	M	3	7872757	1061490	0.009	0.082	7.42	96479871	0.11	1126	51317	45.57
3143	H	M	3	5115412	858371	0.008	0.064	5.96	79803624	0.12	819	36998	45.19
3144	H	D	3	5294095	928601	0.006	0.069	5.70	76504262	0.09	1063	42448	39.91
3145	H	D	3	7036918	697630	0.011	0.084	10.09	84173662	0.13	1800	72093	40.05
3146	H	D	3	6412825	610314	0.016	0.084	10.51	76525352	0.19	2024	85011	41.99

SUMMER

EAST FLATS	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3147	H	S	1	291164275	2052301	0.136	0.244	141.87	1193785467	0.56	62087	128803	2.075
3148	H	S	1	230616270	643444	0.054	0.122	358.41	1885660424	0.44	53136	72758	1.369
3151	H	M	1	135642746	1000162	0.043	0.095	135.62	1424818764	0.45	13334	27299	2.047
3152	H	M	1	241025094	1697600	0.055	0.109	141.98	2217342170	0.51	51880	64997	1.253
3157	H	S	2	406204550	1937844	0.110	0.176	209.62	2301442209	0.62	98494	82377	0.836
3162	H	D	2	321009513	1246397	0.035	0.074	257.55	4314643991	0.48	63936	23129	0.362
3165	H	S	3	465179045	1841113	0.070	1.183	252.66	393086906	0.06	131660	61303	0.466
3166	H	S	3	431918755	2155909	0.071	0.144	200.34	2993199966	0.49	98214	49431	0.503
3167	H	S	3	433850482	2363924	0.065	0.137	183.53	3162175528	0.47	69960	41687	0.596
3171	H	D	3	209000345	1267225	0.027	0.069	164.93	3042217538	0.39	71530	32402	0.453
3172	H	D	3	159697321	1548011	0.019	0.095	103.16	1679256795	0.20	52832	25678	0.486
3173	H	D	3	332659259	1849110	0.062	0.058	179.90	5775334357	1.07	99730	43889	0.44
3156	S	S	2	568454273	993264	0.130	0.755	572.31	753019305	0.17	125965	103018	0.818
3158	S	S	2	620615085	1965908	0.105	0.247	315.69	2511594841	0.43	403825	158840	0.393
3168	S	M	3	138514909	1906846	0.036	0.231	72.64	600411396	0.16	30796	34092	1.107
3169	S	M	3	85211488	2850464	0.052	0.274	29.89	311331707	0.19	47114	44090	0.936
3170	S	M	3	136851470	2743994	0.039	0.252	49.87	543061389	0.16	25787	37138	1.44
3148	T	S	1	983451412	1827687	0.600	1.283	538.09	766763926	0.47	324136	401552	1.239
3149	T	S	1	509363903	2780534	0.532	1.050	183.19	485293353	0.51	546630	367176	0.672
3150	T	M	1	984632240	2751369	0.358	1.035	357.87	951703306	0.35	324886	323970	0.997
3153	T	D	1	1533439455	2421021	0.484	0.945	633.39	1622858985	0.51	757337	458293	0.605
3154	T	D	1	1129331908	2005172	0.311	0.592	563.21	1909267807	0.53	591419	266713	0.451
3155	T	D	1	1294897251	2083137	0.319	0.476	621.61	2722087978	0.67	780834	394124	0.505
3159	T	M	2	1589195713	1798050	1.202	0.694	883.84	2288588296	1.73	3738643	626524	0.168
3160	T	M	2	3472751300	1752564	0.682	0.879	1981.53	3950797838	0.78	2162372	524326	0.242
3161	T	M	2	1641230261	1234462	0.794	0.731	1329.51	2246106830	1.09	2611673	540803	0.207
3163	T	D	2	4037369654	2813533	0.401	0.717	1434.98	5633277039	0.56	1907623	360713	0.189
3164	T	D	2	1942298656	MV	0.471	0.818	MV	2373867827	0.58	877011	442231	0.504

FALL

PORT BAY	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3268	h	S	1	2040425	976336	0.008	0.040	2.09	50883421	0.20	200	1225	6.111
3269	h	S	1	2132435	1036523	0.007	0.064	2.06	33215495	0.10	222	2394	10.78
3270	h	S	1	1051557	792014	0.007	0.058	1.33	18256193	0.12	191	1248	6.531
3271	h	M	1	2261241	1475498	0.008	0.069	1.53	32866880	0.11	182	2089	11.48
3272	h	M	1	2806897	2203184	0.006	0.039	1.27	71787645	0.15	149	2184	14.61
3273	h	M	1	3362256	2154253	0.004	0.050	1.56	67379872	0.07	156	2778	17.81
3274	h	D	1	7000597	2961080	0.006	0.078	2.36	89981968	0.08	157	3960	25.25
3275	h	D	1	1691288	1661396	0.005	0.043	1.02	39795004	0.12	179	2956	16.54
3276	h	D	1	3269639	2165179	0.006	0.063	1.51	51653062	0.10	211	2147	10.17
3277	h	S	2	3949386	2286204	0.009	0.093	1.73	42466519	0.10	260	11976	46.07
3278	h	S	2	2451691	2123131	0.013	0.064	1.15	38367627	0.20	243	12697	52.33
3279	h	S	2	3286048	2136473	0.021	0.077	1.54	42620601	0.27	227	14746	65.09
3280	h	M	2	3723631	2093227	0.021	0.088	1.78	42362122	0.23	204	11553	56.6
3281	h	M	2	3153055	1975414	0.012	0.057	1.60	55707690	0.21	159	3741	23.51
3282	h	M	2	4900472	2638257	0.006	0.083	1.86	59041826	0.07	187	4677	25
3283	h	D	2	2603826	2085131	0.006	0.060	1.25	43109700	0.10	211	6052	28.69
3284	h	D	2	3458434	2071300	0.008	0.076	1.67	45565669	0.11	262	5891	22.46
3285	h	D	2	4232450	2366582	0.012	0.060	1.79	70073676	0.20	241	11956	49.54
3286	h	S	3	6165771	2677654	0.016	0.080	2.30	77168594	0.21	518	33222	64.13
3287	h	S	3	5586766	2542622	0.018	0.077	2.20	72555397	0.23	689	67268	97.7
3288	h	S	3	4362534	2371205	0.009	0.069	1.84	62860716	0.13	688	47084	68.43
3289	h	M	3	2984786	2078154	0.005	0.058	1.44	51639903	0.09	323	16764	51.86
3290	h	M	3	4398884	2444034	0.008	0.078	1.80	56468348	0.10	1131	68821	60.82
3291	h	M	3	2916563	1793063	0.011	0.060	1.63	48528510	0.19	389	20627	53.05
3292	h	D	3	3739685	2257589	0.012	0.069	1.66	54435002	0.18	336	15848	47.2
3293	h	D	3	2757339	2072155	0.009	0.074	1.33	37211049	0.13	377	10093	26.78
3294	h	D	3	3702272	2321807	0.004	0.062	1.59	60199540	0.07	365	10251	28.12

FALL

East Flats

	Species	Depth	transect	Epiphyte Load (F.U.)	Scanned Leaf Area	Epiphyte Biomass (g)	Leaf Biomass (g)	Norm Epiphyte Load (Fluor)	Norm Epiphyte Load (F per g Dry Leaf Biomass)	Norm Epiphyte Load (Biomass)	Plate Epiphyte Load (Green F)	Plate Epiphyte Load (Red F)	Plate Fluor Ratio (Red F: Green F)
3296	h	S	1	218284140	1500536	0.080	0.093	145.47	2354737216	0.86	253780	108653	0.428
3297	h	S	1	201553978	1787404	0.091	0.092	112.76	2197971407	1.00	270557	121792	0.45
3298	h	M	1	180547112	1097348	0.063	0.071	164.53	2557324537	0.89	53648	71211	1.327
3299	h	M	1	231683576	764231	0.028	0.066	303.16	3494473243	0.42	55853	37915	0.679
3300	h	M	1	176499931	1427952	0.046	0.037	123.60	4835614542	1.26	44216	46319	1.048
3304	h	S	2	285406062	1727109	0.179	0.107	165.25	2659888741	1.67	326696	221460	0.678
3305	h	S	2	231687632	2065906	0.143	0.085	112.15	2716150439	1.67	472191	225911	0.478
3306	h	S	2	183935647	1500928	0.101	0.078	122.55	2373363191	1.31	225183	141180	0.627
3310	h	D	2	312855417	1880476	0.102	0.156	166.37	2008057875	0.65	1147121	267943	0.234
3313	h	S	3	440213194	2311396	0.085	0.165	190.45	2672818421	0.52	181076	75605	0.418
3314	h	S	3	264541821	2006211	0.077	0.107	131.86	2472353472	0.72	103849	52446	0.505
3315	h	S	3	348487936	2023204	0.085	0.147	172.25	2377134624	0.58	158606	74418	0.469
3319	h	D	3	175040244	1469641	0.038	0.078	119.10	2232656171	0.48	72405	33504	0.463
3320	h	D	3	334992308	1511471	0.071	0.130	221.63	2582824272	0.55	164798	66249	0.402
3321	h	D	3	436629382	2011163	0.051	0.149	217.10	2926470386	0.34	93831	38027	0.405
3318	syr	M	3	179527983	2919515	0.079	0.200	61.49	898538455	0.39	145176	73568	0.507
3295	t	S	1	1385179216	3011540	1.441	1.287	459.96	1076034503	1.12	1249051	916223	0.734
3301	t	D	1	716099849	2131833	0.742	0.516	335.91	1388328517	1.44	796863	579643	0.727
3302	t	D	1	710465805	1896896	0.576	0.428	374.54	1658416913	1.34	984262	543470	0.552
3303	t	D	1	817893219	2303595	0.792	0.585	355.05	1398347101	1.35	1124015	804583	0.716
3307	t	M	2	1487188711	2670524	1.338	0.692	556.89	2148185340	1.93	3365233	896446	0.266
3308	t	M	2	2686839745	2988117	2.284	1.018	899.17	2639850408	2.24	3710591	995756	0.268
3309	t	M	2	1829741638	2995545	1.339	0.886	610.82	2064938086	1.51	3939182	904822	0.23
3311	t	D	2	843384644	2178080	0.815	0.709	387.21	1189037986	1.15	2513891	618308	0.246
3312	t	D	2	963821467	2517270	0.468	0.509	382.88	1894675578	0.92	5091022	896607	0.176
3316	t	M	3	349805667	2078294	0.320	0.539	168.31	648990106	0.59	308972	127044	0.411
3317	t	M	3	566004619	2879044	0.326	0.630	196.59	898705333	0.52	297880	123577	0.415