FINAL REPORT

LANDSCAPE MONITORING AND BIOLOGICAL INDICATORS FOR SEAGRASS CONSERVATION IN TEXAS COASTAL WATERS

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for the

Table of Contents

Chapters

I. Plant and Water Quality Indicators of Seagrass Condition

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Executive Summary

Given the threats to coastal resources, implementation of a seagrass monitoring program in Texas is a top priority; however, to achieve maximum effectiveness, the program design should both detect changes in seagrass distribution, abundance, and condition as well as identify causative factors that drive those changes. Therefore, monitored habitat quality or stressor indicators should be strongly related to seagrass characteristics so that the seagrass condition at a site may be adequately characterized based on values of stressor indicators. We examined numerous abiotic and biotic variables at 40 sites in seagrass beds of Redfish Bay and East Flats to determine the strength of their relationship with seagrass biomass, density, cover and community composition. Strong relationships would suggest possible stressors as well as identify potential indicators of current and future seagrass condition. Both univariate and multivariate statistical analyses were used to assess these relationships and identify candidate variables for inclusion in a monitoring program. All variables except N:P of *Thalassia testudinum* leaves exhibited significant site x sampling date interaction terms, indicating both spatial and temporal variability in Redfish Bay and East Flats. Parametric and nonparametric analyses, however, revealed only modest associations between both abiotic and biotic variables and seagrass measurements. As expected, Spearman correlations demonstrated strong relationships among various measures of seagrass condition such as *T. testudinum* shoot density, cover, and biomass. On the other hand, associations between abiotic variables and seagrass condition indicators were less robust. Silt and sand content of the sediments were positively correlated with *T. testudinum* shoot density ($r_s = 0.57l$) and *Syringodium filiforme* cover ($r_s = 0.45$), respectively, and NH_4^+ was positively correlated with *T. testudinum* root:shoot ratios ($r_s = 0.56$) and negatively correlated with aboveground biomass ($r_x = -0.62$). Simple multiple regression models (3rd order or less) explained a fraction of the variance in *T. testudinum* biomass ($R^2 < 0.34$), while the best model (i.e. lowest AIC) contained 10 variables ($R^2 = 0.58$; AIC = 704). Similarly, non-metric multi-dimensional scaling showed that the measured variables were weakly related to patterns in community structure and density of seagrasses. A model containing drift algal biomass and depth had the highest rank correlation ($r_s = 0.42$). For both parametric and

non-parametric analyses, metrics of light and nutrients were important variables; however, the large within site variability of seagrass biomass and community structure suggests that factors varying across small spatial-scales are also important. Based on the results of these analyses, we recommend a monitoring program that captures the inherent variability of the seagrass system across both spatial and temporal scales through random as well as fixed point monitoring. Continuous measurements of DO, salinity, temperature, and light at representative deep and shallow sites would provide a detailed account of tidal and diel variation in these parameters and permit an integrated assessment of the total exposure of representative sites to conditions that exceed established light and oxygen thresholds for sustaining seagrass growth. Monthly sampling of all water quality parameters, including water column nutrients, TSS, and chlorophyll *a*, would provide the temporal resolution to adequately characterize seasonal and interannual variation as well as correlate changing water quality to seagrass condition determined by semiannual measurements of biomass, cover, density, and tissue nutrient content. Although this sampling protocol is finance and labor intensive, more frequent and long-term measurements are necessary to effectively track seagrass changes and identify causative factors so that appropriate management actions may be taken to maintain the integrity of seagrass systems in Texas.

Introduction

In 1999, Texas Parks and Wildlife drafted a Seagrass Conservation Plan that proposed a Seagrass Habitat Monitoring program (TPWD, 1999). One of the main recommendations of this plan was to develop a monitoring program that could detect changes in seagrass ecosystems prior to actual seagrass mortality. To achieve this objective it is necessary to identify both the environmental parameters that elicit a seagrass stress response as well as the physiological or morphological variables that best reflect the impact of these environmental stressors.

Numerous researchers have related seagrass health to environmental stressors; however, these studies did not arrive at a consensus regarding the most effective habitat quality and seagrass condition indicators. Kirkman (1996) recommended biomass, productivity, and density for monitoring seagrass whereas other researchers focused on changes in seagrass distribution as a function of environmental stressors (Dennison et al. 1993; Livingston et al. 1998; Koch 2001; Fourqurean et al. 2003a). The most important environmental variables affecting seagrass also varied among these studies. Salinity, depth, light, nutrients, sediment characteristics, and temperature were among the variables identified as contributing to patterns in the measured seagrass response variable. The relative influence of these various environmental variables is likely a function of the seagrass species in question, geographic location of the study, hydrography, methodology and other factors specific to the individual studies. Because no generalized approach can be extracted from previous research, careful analysis of local seagrass ecosystems is necessary to develop an effective monitoring program for Texas.

Traditional broad-scale monitoring efforts are often costly and labor intensive. Field-based sampling of plant condition indicators and environmental variables involves processing a large volume of samples collected over broadly distributed sampling sites. Additionally, extrapolating point measurements to larger spatial scales is problematic. Concurrent analysis of high-resolution photography may minimize these limitations. Landscape patterns in biotic and abiotic variables that are apparent in photography should also reflect changes in environmental stressors, human impacts, or other disturbances. If

point measurements of habitat quality and seagrass condition indicators are correlated with these landscape features and seagrass bed characteristics, the extent of seagrass impacts could be extrapolated over large areas. In this way, aerial photography could be a cost effective tool for monitoring the response of seagrasses to human or natural stressors (Dobson et al. 1995; Robbins 1997).

Unfortunately, identifying factors that drive seagrass dynamics can be difficult. At both micro- and landscape scales, inferences on stressors and response must be made with caution. Environmental stressors can influence seagrass condition directly, eliciting a positive or negative effect, or they may act indirectly through interaction with other variables. Consequently, identifying causative factors requires deciphering complex interactions at both point and landscape scales. Combining remote sensing and field sampling into one monitoring program would permit extrapolation of plant level responses across seagrass landscapes.

We used a multi-scale approach to identify unique seagrass indicators for a seagrass monitoring program for the state of Texas. Intensive field sampling of environmental variables and seagrass physiology and morphometrics was combined with analysis of aerial photography to generate a suite of indicators that are most relevant for successful maintenance and growth of seagrass habitat. With this approach, we addressed the following objectives:

a) identify important habitat quality and seagrass condition indicators from extensive field sampling.

b) evaluate East Flats as a reference site for Redfish Bay.

c) use aerial photography to identify landscape features and classify seagrass landscape indicators.

d) relate habitat quality and seagrass condition indicators to seagrass landscape indicators to identify key parameters for long-term monitoring.

e) address the question of scale in the interpretation of aerial imagery.

Methods

Study Areas

Redfish Bay and East Flats are in the coastal bend area of southern Texas (W 97˚7'; N 27˚53' and W 97º12'; N 27º80'; Figure I.1). Redfish Bay is bounded by mainland Texas to the west and numerous dredge-spoil islands to the east. The Gulf Intracoastal Waterway runs along the western edge of the bay, and a causeway bisects Redfish Bay through the center along an east-west axis. East Flats is located in the eastern portion of Corpus Christi Bay between the Point of Mustang and the main axis of Mustang Island. Both Redfish Bay and East Flats are shallow embayments (maximum depth < 3.5 m) that contain five seagrass species (*Thalassia testudinum* Banks ex König, *Halodule wrightii* Ascherson, *Syringodium filiforme* Kützing, *Halophila engelmanni* Ascherson, and *Ruppia maritima* Linnaeus).

Forty sites were selected in Redfish Bay (30 sites) and East Flats (10 sites), with the number in each region scaled to the area of the region (Figures I.2 and I.3). To ensure even, yet random selection of sampling sites, we used the stratified-random method of hexagonal tessellation developed by the USEPA EMAP program (http://www.epa.gov/emap). Study regions were divided into 0.66 km^2 hexagonal subunits. ArcGIS v. 9.1 was used to overlay a shapefile containing the subunits onto a basemap of the study areas developed using digital geographic data obtained from the USGS National Hydrography Dataset (http://nhd.usgs.gov/data.html). Sampling points were then randomly selected from within hexagonal subunits. Only one sampling point was chosen for a given hexagon, and not all hexagons contained sampling points. The likelihood of selection for an individual hexagon was a function of the extent of water coverage in the hexagonal area. All data points were in the North American Datum (NAD) 1927 geographic coordinate system and were projected in Transverse Mercator (UTM Zone 14N). Selected points were located in the field using a Global Positioning System (GPS; Garmin GPSMAP76, ± 5 m accuracy) and permanently marked with a PVC pole. Deep sites (>1.75 m) could not be marked with PVC poles and were located using the GPS unit.
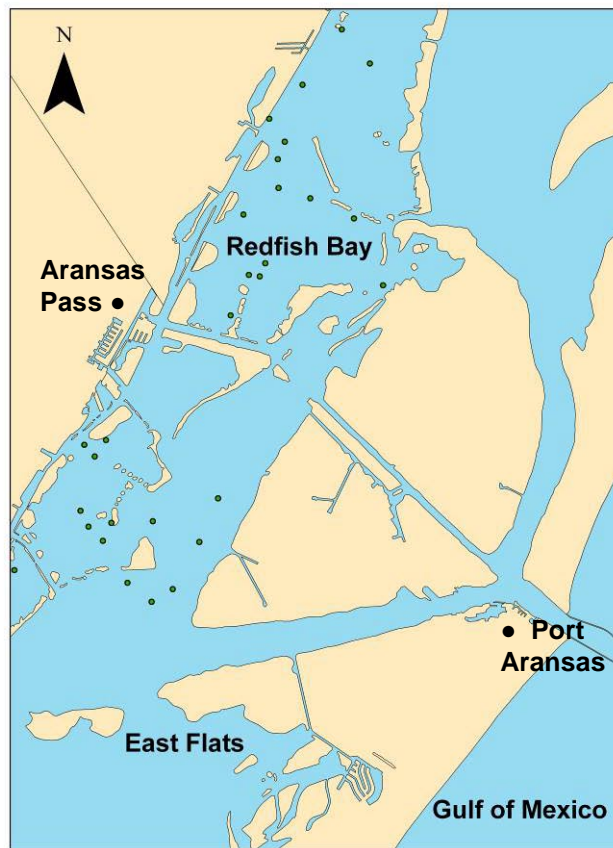
Figure I.1 - Map showing the location of Redfish Bay and East Flats in the Coastal Bend of Texas.
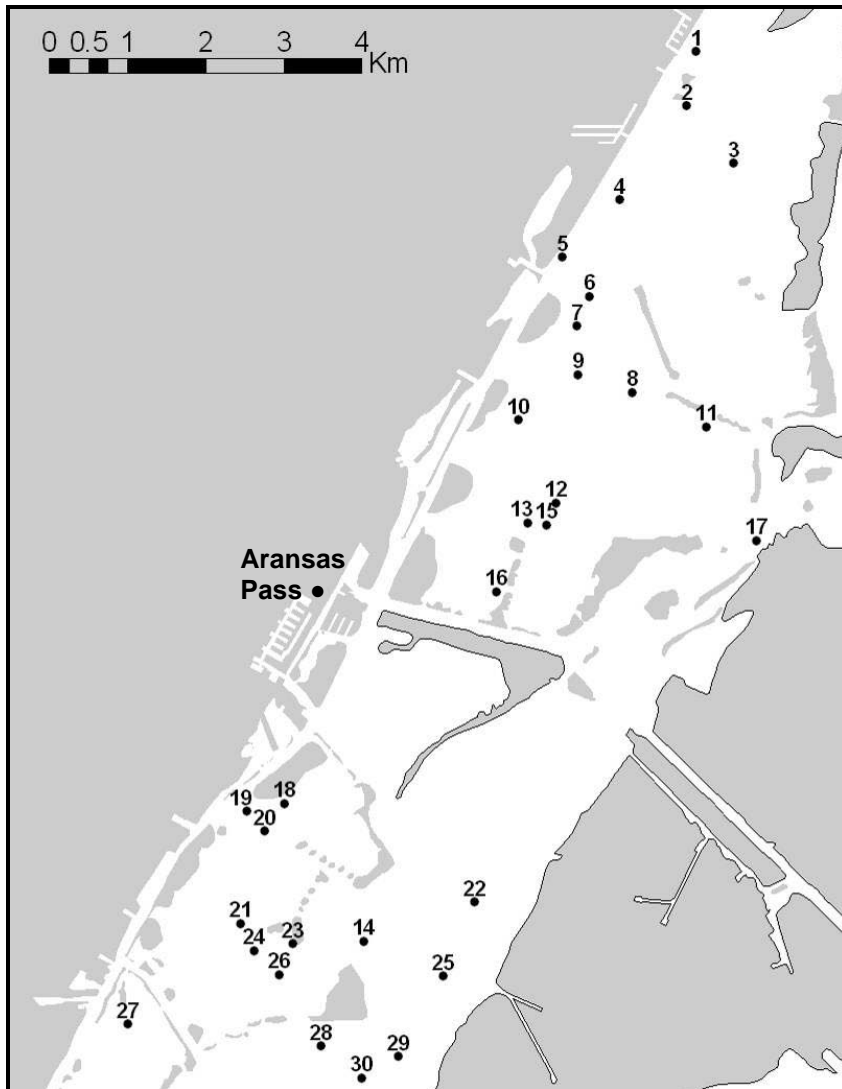
Figure I.2 - Location of sampling sites in Redfish Bay based on hexagonal tessellation procedures.

Figure I.3 - Location of sampling sites in the East Flats area based on hexagonal tessellation procedures.

Transect Sampling Protocol

At each site, a temporary 50-m transect was extended in a southerly direction from the marker pole. Ten 0.25 m^2 quadrats were placed along each transect to measure macroalgal biomass and percent cover of seagrass. Quadrat locations were selected randomly prior to each sampling period, and the same set of locations was used at all 40 sites. A different set of ten locations, however, was used for each sampling period. Each quadrat was examined while snorkeling. All seagrass species occurring in the quadrat were listed, and the raw cover value of each species was recorded. Cover was defined as

6

the fraction of the total quadrat area obscured by a particular species when viewed from directly above. Additionally, raw cover values were scored in accordance with Braun-Blanquet methodology (Braun-Blanquet 1972). The Braun-Blanquet scores were used to calculate density, abundance, and frequency for each species as outlined in Fourqurean et al. (2001).

In addition to percent cover, macroalgal biomass was determined at each of the ten quadrat locations. A 0.0625 m^2 quadrat was placed at each quadrat location, and all unattached macroalgae within the quadrat were collected and placed in a plastic bag. Samples were stored on ice and returned to the lab where they were refrigerated until processed. Algae were separated by species, and dried to constant mass at 60ºC. Total algal biomass was determined by combining the dry mass of all algal species from a sample.

Water Quality Analysis and Light Measurements

At each site, water samples were collected in acid-washed, polyethylene bottles. Three replicates were taken for each of the following measurements: inorganic nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) and phosphorus (PO_4^{-3}), total suspended solids (TSS) and chlorophyll a. All samples were placed immediately on ice. Water samples for inorganic N and P analysis were frozen until they were analyzed on a Lachat Quikchem 8000® (Loveland, CO). Samples were filtered prior to analysis. TSS samples were filtered onto dried and weighed 47 mm glass fiber filters. Filters with filtrate were dried in an oven at 60ºC to constant mass.

Upon return to the laboratory, chlorophyll a extractions were performed immediately. Phytoplankton was collected on 0.45 μm cellulose-nitrate membrane filters, and chlorophyll was extracted overnight with 5 ml of 90% acetone. Between 12 and 24 hours after extraction commenced, chlorophyll a content was determined with a spectrophotometer according to methods outlined in Parsons et al. (1984).

Dissolved oxygen, conductivity, salinity, and temperature were measured in the field using the YSI 600XLM-Sonde (YSI Incorporated, Yellow Springs, Ohio, USA). Three

measurements of each parameter were made immediately upon arrival at each site. The percent surface irradiance (% SI) and the diffuse light attenuation coefficient (K_d) were calculated from measurements of surface and underwater irradiance. Measurements of photosynthetically active radiation (PAR = ca. 400 to 700 nm wavelength) were collected using an LI-192SA quantum-sensor that provides input to a LI-1000 datalogger (LI-COR Inc., Lincoln, Nebraska, USA). At each site, three instantaneous measurements were recorded both at the water surface and at the height of the seagrass canopy. At sites lacking seagrass, PAR was measured at the sediment surface. Light attenuation was 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} s^{-1}) at depth z (m) and at the surface, respectively. Percent surface irradiance available at the seagrass canopy was calculated as follows:

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

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

Sediment Analysis

Samples for sediment grain size, total organic carbon and pore water NH_4^+ were collected with a plastic corer and put into separate, sterile Whirlpak bags. All samples were then placed on ice until they could be stored in the freezer. Thawed sediments for grain size analysis were oxidized with 3% hydrogen peroxide to remove organic matter. Dried and cleaned sediment samples were then separated into size classes using a combination of sieving and size-dependent settlement rates in sediment slurries (Folk, 1974). The 4 resulting size classes were rubble (> 250 μm), sand (62 – 250 μm), silt (3.9 – 61 μm), and clay (< 3.9 μm).

Total organic carbon was determined as percent loss on ignition (Heiri et al. 2001). Individual samples were homogenized, dried at 105°C to constant weight (12-24 h) and

combusted at 550°C for 4 hours in a muffle furnace. A final dry weight was obtained, and loss on ignition (LOI) was obtained with the following equation:

$$LOI = [(DW_{105} - DW_{550}) / DW_{105}] \times 100$$

where DW_{105} and DW_{550} are the dry weights following heating to 105°C and 550°C, respectively.

For determination of porewater NH_4^+ concentration, sediment samples were thawed and homogenized. Sediments were put into centrifuge tubes and spun at 10,000 rpm for 20 minutes. A known volume of supernatant was removed from the tube and the NH_4^+ concentration was determined colorimetrically as outlined in Parsons et al. (1984).

Seagrass and Epiphyte Biomass

Three replicate biomass cores were used to estimate above- and below-ground biomass, root:shoot ratio, blade length and width, and shoot density. A 15 cm diameter corer was used to sample *Thalassia,* and a 9 cm diameter corer was used to sample *Halodule, Syringodium, Ruppia,* and *Halophila*. Samples of each species present were collected at each site. Species presence (i.e. seagrass species composition) was determined by visual *in situ* analysis of plants observed within a 25 m radius of each site. Cores were sieved in the field to remove sediment from the roots and rhizomes. Seagrass samples were then placed in pre-labeled plastic bags and immediately placed on ice. Biomass samples were refrigerated until they were processed. Processing of all biomass samples was completed within 30 days of their collection.

In the lab, biomass cores were sorted by species, and the number of shoots per core of each species was counted. Additionally, the length and width of the longest leaf of 5 haphazardly selected shoots of each species was determined. The aboveground portions of the shoots were separated from belowground portions and dried to estimate seagrass biomass (g m^{-2}).

Estimates of algal epiphyte biomass on *Thalassia* leaves were made from separate leaf samples of entire shoots taken directly adjacent to the biomass cores. Shoots were placed

in plastic bags and refrigerated until processing occurred. Triplicate 10 cm segments of *Thalassia* leaves were collected from 3 different shoots. Segments were taken from the middle portion of the leaf to minimize differences in epiphyte biomass due to leaf age. The segments were scraped with a razor blade to remove the epiphytes, and the epiphytes were transferred to a pre-weighed glass fiber filter. Epiphyte samples were dried to constant weight and the final weight was used to calculate the epiphyte biomass per unit area of *Thalassia* leaf (mg cm^{-2}).

Scraped seagrass tissue (i.e. epiphytes removed) was dried and ground to a fine powder using a Wig-L-Bug (DENTSPLY Rinn Corp., Elgin, Illinois, USA). Carbon and nitrogen content of seagrass tissue was determined with an automatic elemental analyzer (model NC 2500, Fison Instruments, Rodano-Milan, Italy). Phosphorus content was determined using the method outlined by Fourqurean et al. (1992).

Statistical Analysis

Correlation analysis, linear regression, and hypothesis testing was performed with SAS v. 9.1 (SAS Institute Inc., Cary, NC USA). Spearman correlation coefficients were calculated between all pair-wise combinations of sediment, water quality, and plant variables to identify strong associations between variables. Stepwise mulitple least squares linear regression was performed for selected variables to examine the ability of a subset of variables to predict responses in plant condition variables. It is important to note that significance testing on parameters of the fitted regression models was not performed due to violations of the assumptions of normality. Coefficients of multiple determination (r^2) were calculated and are valid representations of the fit of the models. Analyses of variance (ANOVAs) were used to compare sample means for differences in these variables among sampling date and sites. The residuals of fitted models were analyzed for departures from normality. Shapiro-Wilk tests of normality indicated significant departures from normality in all cases ($p < 0.05$). Because of violations of the normality assumption, only Friedman's non-parametric ANOVAs on ranked data were used.

Non-metric multidimensional scaling (NMDS) and cluster analysis was used to investigate multivariate differences between bays, sites, and seasons and to relate environmental conditions to plant condition variables. Primer v. 6 (Primer-E Ltd., Plymouth, UK) was used to transform all data and perform all NMDS and cluster analyses. Sediment and water quality data were always normalized prior to analysis. Similarly, plant abundance and percent cover metrics were $\log(x + 1)$ transformed to down-weight highly abundant species. Cluster analysis was based on Euclidean Distance and was performed using the group average cluster mode. NMDS ordination was based on Bray-Curtis dissimilarity matrices of plant community structure or normalized Euclidean Distance matrices of environmental variables at the 40 sites. Relation of environmental variables to plant community structure was done following Clarke and Ainsworth (1993) using the Biota and Environment Matching procedure in Primer v. 6.

Results

Temporal and Spatial Variability in Physicochemical Variables

Site depths ranged from 0.3 m at site 23 in south Redfish Bay to 3.0 m at site 29 in southeast Redfish Bay (Figure I.4). Of the 30 sites, six (14, 22, 25, 28, 29 and 30) were at depths 1.7 m or greater and had no seagrass at any time over sampling. The depths at the remaining 24 sites were 1 m or less. All relatively shallow sites had seagrass at some point during sampling. In East Flats, four sites were deeper than 2.2 m (31, 36, 39, and 40; Figure I.5). No seagrasses were found at deep sites. The remaining six sites were 1.2 m deep or less. Based on these depth differences, sites were classified as deep (>1.7 m) or shallow (<1.7 m). ANOVA on %SI as a function of depth indicated that depth has a significant effect on light availability (n = 200; df = 1; F = 240.15; p < 0.0001). Average %SI at deep sites was $13.3 \pm 15.5\%$; %SI at shallow sites was $59.5 \pm 18.0\%$.
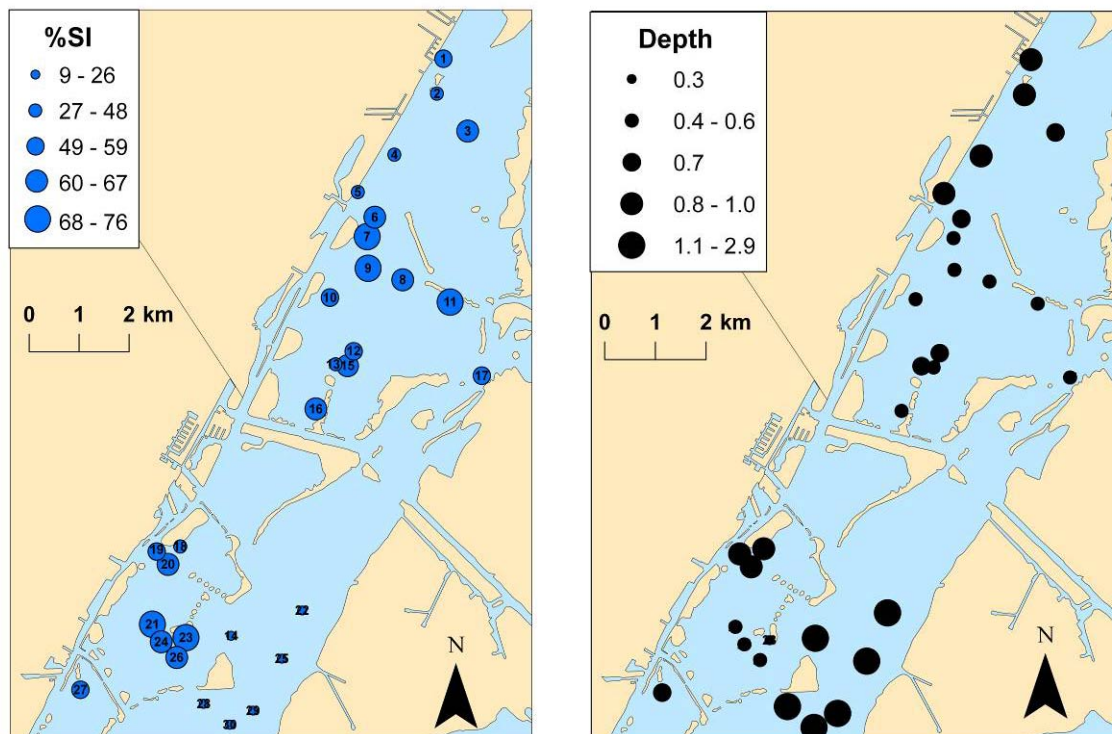

Figure I.4 - Maps of Redfish Bay showing water depth (m; left panel) and % SI (right panel) at the 30 sites.
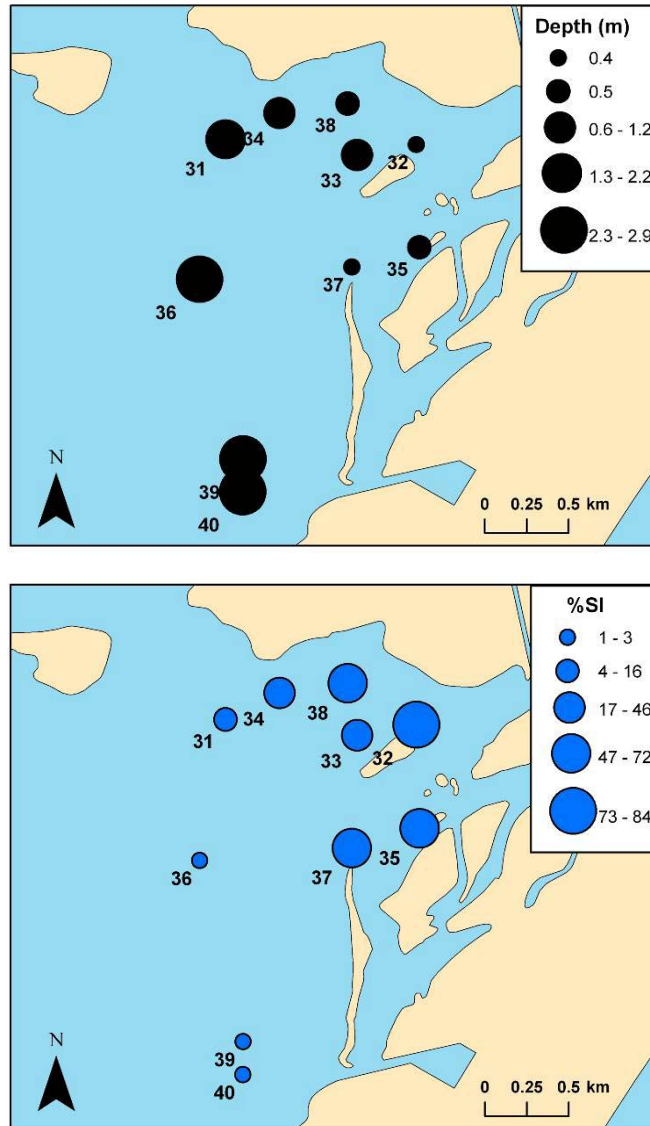
Figure I.5 - Map of East Flats showing depth (m; top panel) and %SI (bottom panel) at the 10 sites.

Strong temporal and spatial variability characterized the environmental variables in East Flats and Redfish Bay. Friedman's ANOVAs indicated significant sampling date x site interaction terms for all measured water column (Table 1) and sediment (Table 2) parameters except light attenuation and %SI (no replication exists for these variables at the sampling date x site level). Average salinity ranged from 12.1 – 39.4 psu throughout the study. Highest values were recorded in the deepest sites of south Redfish Bay and East Flats where the Corpus Christi Bay is most influential (Figures I.6 and I.7). Strong seasonal differences in salinity were also evident with an average summer salinity of 31.4

13

psu compared to 22.1 psu in the winter. Temperature values also exhibited a strong seasonal pattern with average temperature in the summer (30.8 °C) approximately twice the average temperature in winter (15.9 °C).

Table I.1 - Results of Friedman's non-parametric ANOVA of ranked water column variables.

Response variable	Factor	df	F	p
chorophyll a	Sampling	5	292	<0.0001*
	Site	39	18	<0.0001*
	sampling*site	155	9.5	<0.0001*
Total suspended solids	Sampling	5	267	<0.0001*
	Site	39	13	<0.0001*
	sampling*site	155	11	<0.0001*
Salinity	Sampling	5	123956	<0.0001*
	Site	39	6828	<0.0001*
	sampling*site	155	959	<0.0001*
Temperature	Sampling	5	2760936	<0.0001*
	Site	39	18514	<0.0001*
	sampling*site	155	22356	<0.0001*
Dissolved oxygen	Sampling	5	2662	<0.0001*
	Site	39	311	<0.0001*
	sampling*site	154	211	<0.0001*
NH_4^+	Sampling	5	228	<0.0001*
	Site	34	13	<0.0001*
	sampling*site	98	7.2	<0.0001*
NO_3^-	Sampling	5	264	<0.0001*
	Site	34	7.9	<0.0001*
	sampling*site	98	13	<0.0001*
PO_4^-	Sampling	5	101	<0.0001*
	Site	34	8.7	<0.0001*
	sampling*site	98	9.7	<0.0001*

* significant at $\alpha = 0.05$

Table I.2 - Results of Friedman's nonparametric ANOVA of ranked sediment characteristics.

| Response variable	Factor	df	F	p
Rubble	sampling	3	1832	<0.0001*
	site	39	42	<0.0001*
	sampling*site	93	2.3	<0.0001*
Sand	sampling	3	3588	<0.0001*
	site	39	92	<0.0001*
	sampling*site	93	1.9	<0.0001*
Silt	sampling	3	1052	<0.0001*
	site	39	24	<0.0001*
	sampling*site	93	2.7	<0.0001*
Clay	sampling	3	1591	<0.0001*
	site	39	36	<0.0001*
	sampling*site	93	2.8	<0.0001*
Total organic carbon	sampling	5	50	<0.0001*
	site	39	67	<0.0001*
	sampling*site	151	3.2	<0.0001*
Porewater NH_4^+	sampling	5	82	<0.0001*
	site	39	5.2	<0.0001*
	sampling*site	151	3.1	<0.0001*

* significant at $\alpha = 0.05$
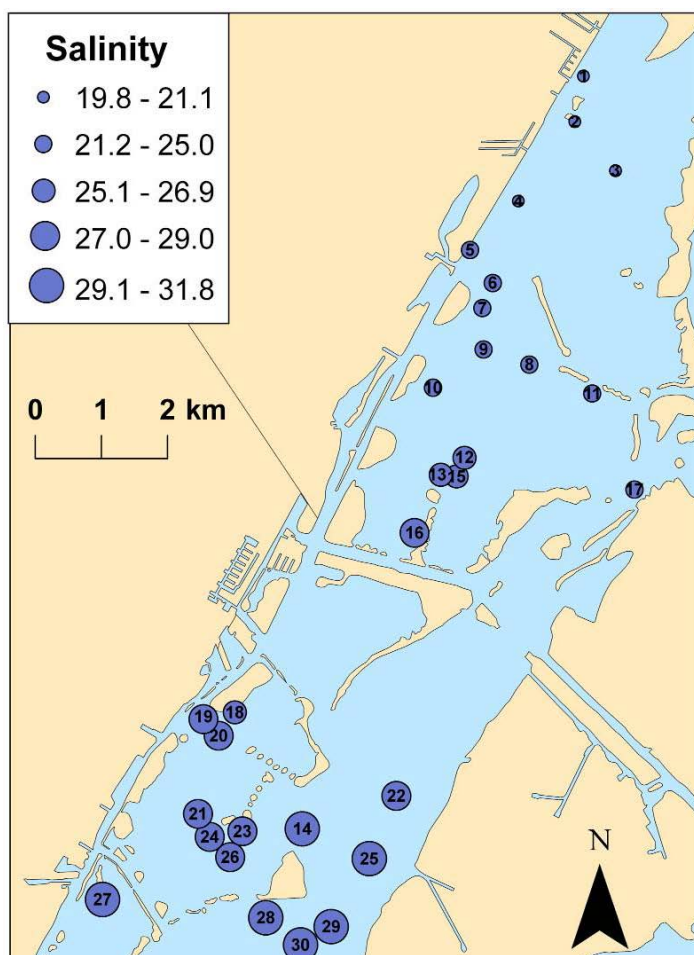
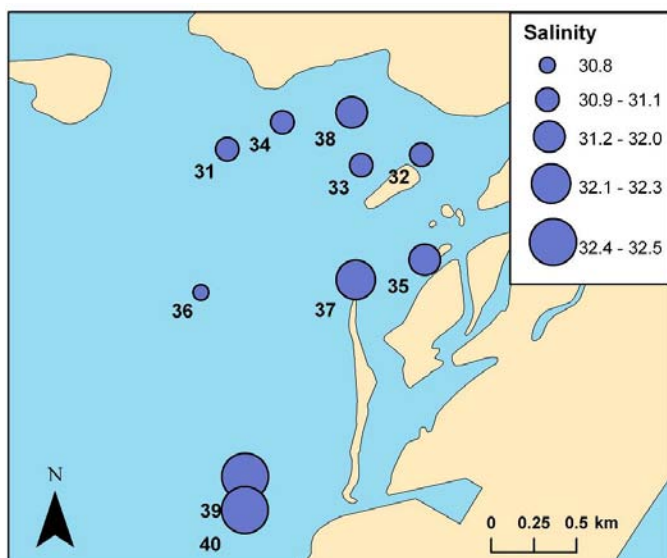Figure I.6 - Map of Redfish Bay showing average salinity (psu) at the 30 sites.

Figure I.7 - Map of East Flats showing average salinity (psu) at the 10 sites.

Chl *a* concentrations ranged from the detection limit of the method (0.2 μg L^{-1}) to 20.7 ±
4.1 μg L^{-1} (mean ± s.d.) with higher values occurring in winter 2003, winter 2005 and
summer 2005 (Figures I.8 and I.9). Average TSS values ranged from 0.6 ± 0.6 to 90.7 ±
9.9 mg L^{-1}.   Highest TSS values occurred during the summers of 2003 and 2005 and
winter 2004. Total suspended solids were not strongly correlated with depth (r_s = -0.24;
n = 156). Chl *a* and TSS were not strongly related to light attenuation or the amount of
light available for seagrass photosynthesis. Although both chl *a* and TSS were positively
correlated with light attenuation, the Spearman correlation coefficients were only 0.34
and 0.31, respectively. Mean light attenuation coefficients ranged from 0.6 ± 0.5 m^{-1} for
site 32 in East Flats to 2.5 ± 1.6 m^{-1} for site 23 in Redfish Bay. Additionally, Spearman
correlation coefficients between %SI and chl *a* and TSS were -0.26 and -0.13 (n = 156),
respectively, indicating a weak negative association between these variables. A stronger
relationship between %SI and depth (r_s = -0.51; n = 156) suggests that depth is the main
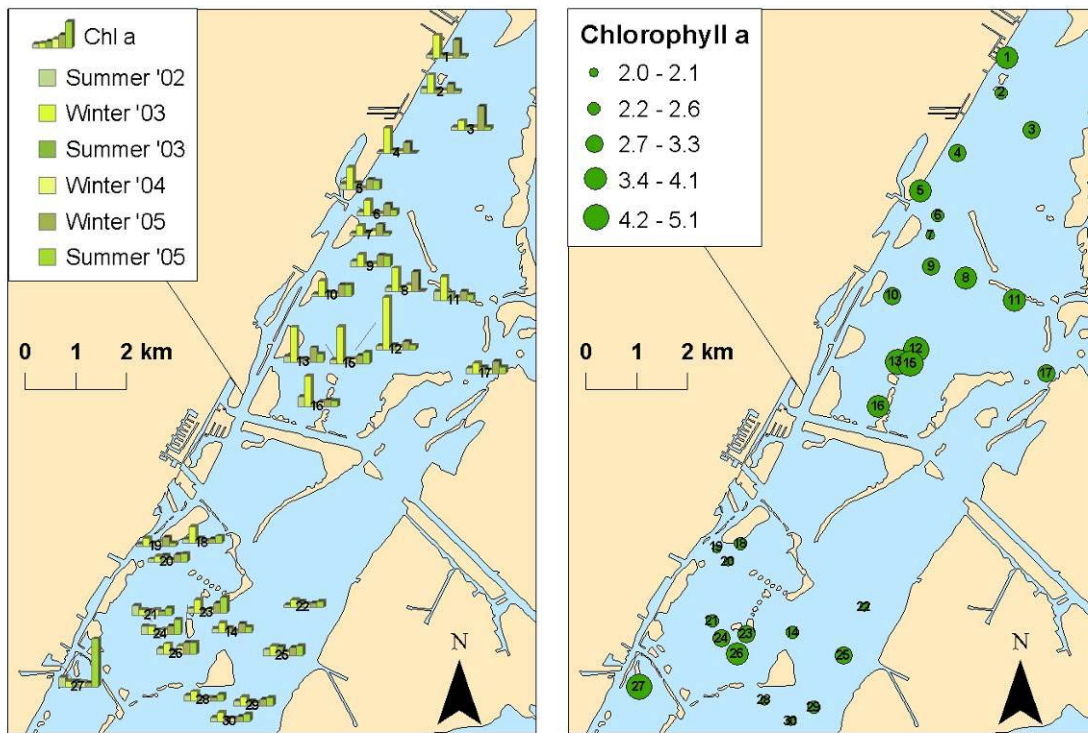factor influencing light regime in Redfish Bay and East Flats.

Figure I.8 - Map of Redfish Bay showing mean seasonal chl *a* values (left panel; bar
height represents chl *a* concentration) and mean overall chl *a* (right panel; μg L^{-1}).
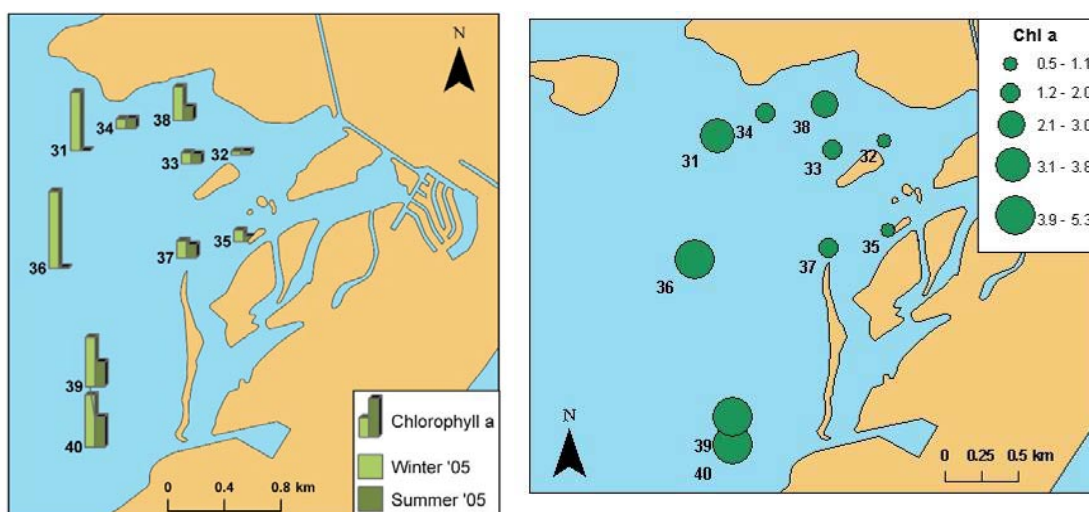
Figure I.9 - Maps of East Flats showing mean seasonal chl *a* values (top panel; bar height represents chl *a* concentration) and mean overall chl *a* (bottom panel; $\mu g\ L^{-1}$).

Water column nutrient concentrations were generally low throughout the study. Average NO_3^- values only exceeded 1 μM at five sites during winter 2002 (sites 4, 8, 17, 24, and 25; Figures I.10 and I.11). At all other sites, NO_3^- concentration was less than 1 μM on each sampling date. PO_4^- concentrations exhibited a temporal trend with the highest PO_4^- concentrations occurring in the first three sampling periods from summer 2002 to summer 2003. Although there was a significant sampling x site interaction term (p < 0.0001, Table 1), the range of PO_4^- values was small and no clear spatial pattern emerged (Figures I.12 and I.13). The maximum average PO_4^- concentration was 1.35 ± 0.01 μM while the minimum was 0.05 ± 0.02 μM. Water column concentrations of NH_4^+ also varied temporally (Figures I.12 and I.13). The overall average during winter sampling dates was 1.6 ± 0.8 μM compared to 0.8 ± 0.5 μM during summers. Maximum NH_4^+ concentration was 5.1 ± 0.8 μM, and the minimum was 0.02 ± 0.03 μM. Water column nutrient concentrations were not strongly correlated with one another. Both PO_4^- and NH_4^+ were negatively correlated with NO_3^- (r_s = -0.29 and –0.14, respectively), but they were positively correlated with one another (r_s = 0.19). Water column nutrients also were not significantly correlated to chl *a* (p > 0.06 in each case).

18
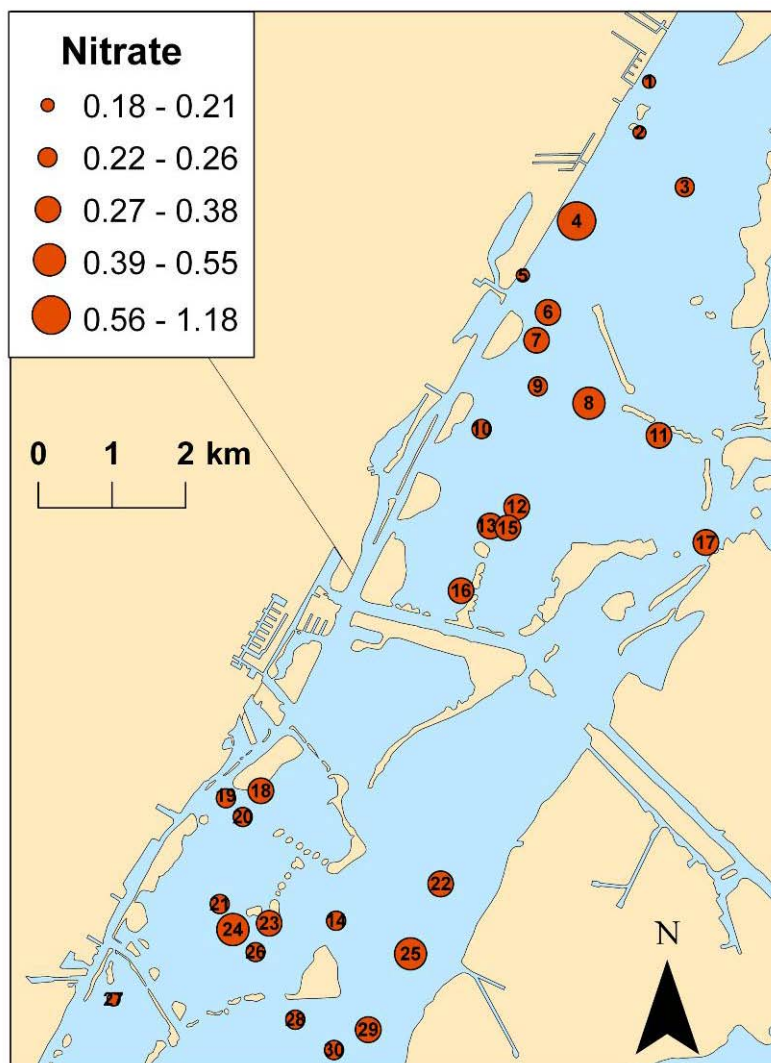
Figure I.10 - Map of Redfish Bay showing average NO_3^- concentration (μM).
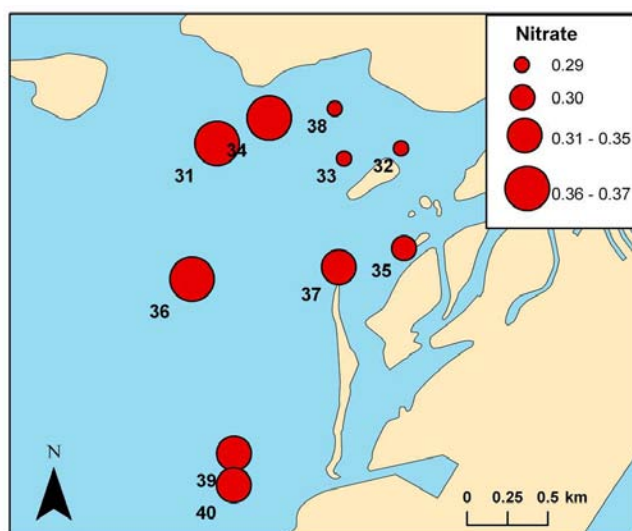
Figure I.11 - Map of East Flats showing average NO_3^- concentration (μM).
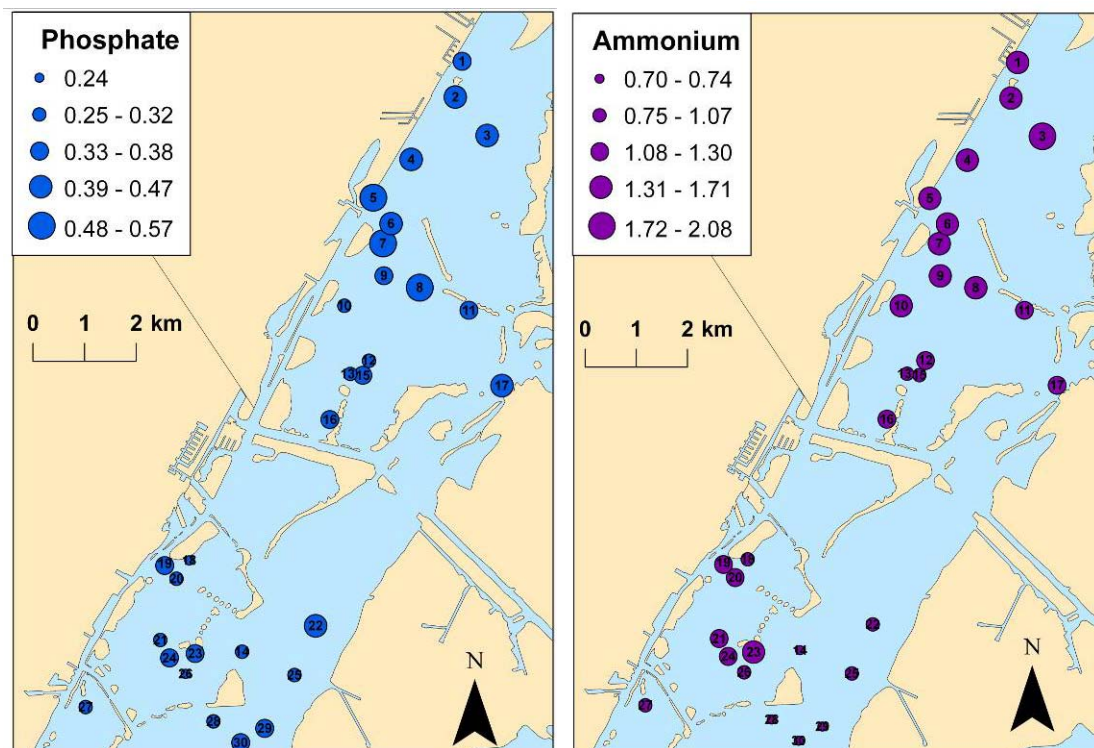
Figure I.12 - Maps of Redfish Bay showing average PO_4^{3-} (left panel) and NH_4^+ (right panel) concentrations (μM).
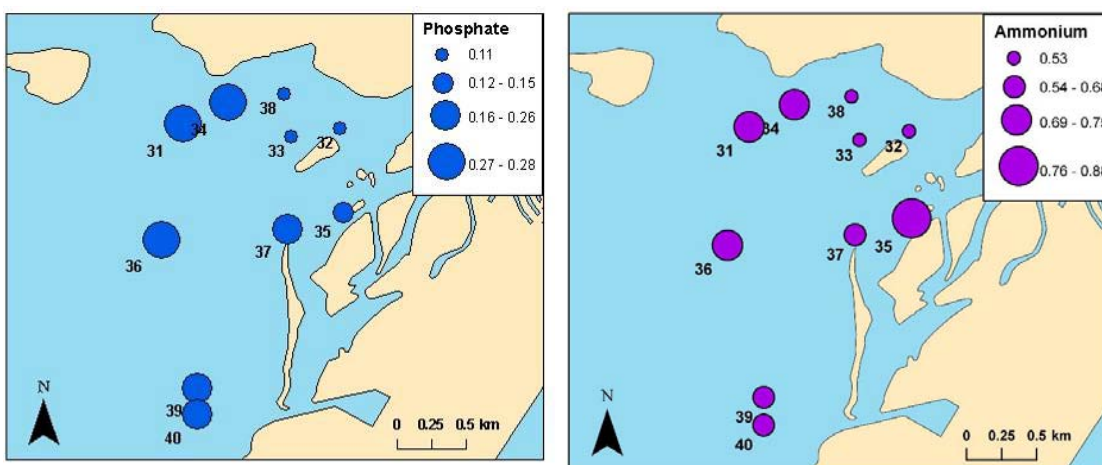
Figure I.13 - Maps of East Flats showing average PO_4^{3-} (top panel) and NH_4^+ (bottom panel) concentrations (μM).

Analysis of dissolved oxygen concentrations also indicated a significant sampling date x site interaction (p < 0.0001, Table 1). Average dissolved oxygen concentrations ranged from 2.4 ± 0.12 at site 35 in East Flats to 16.8 ± 0.07 mg L^{-1} at site 15 in Redfish Bay

during summer 2005. Interpretation of these values is difficult considering the known variation due to the time of measurement.

Sediment composition varied significantly by sampling date and site (p < 0.0001, Table 2). In Redfish Bay, the average proportion of sand in sediments was highly variable. At sites 1, 11, 17, and 23, sand contributed greater than 70% of the total sediment mass. Sand constituted between 50% and 70% of total sediment mass at an additional 6 sites. In most cases, rubble was the next most important component by weight in Redfish Bay sediments. Average contribution by rubble ranged from 1.3 ± 0.7% to 53.4 ± 9.9%, while the contribution from clay was 2.4 ± 1.0% to 42.7 ± 13.4%. The maximum contribution by silt was 29.9 ± 13.2%.

Sand dominated the sediment composition at most sites in East Flats. Average sand constituted at least 70 ± 5.2% of the mass at 8 of the 10 sites with the remainder composed of relatively small portions of rubble, silt, and clay. Although the largest portion of sediments at sites 39 and 40 (both bare sites greater than 2 m deep) was composed of sand (33 ± 5.1% and 39 ± 6.2%, respectively), rubble and clay each contributed between 25% and 30% to the total mass.

The organic content of sediments also differed significantly by sampling date and site (p < 0.0001, Table 2). Average TOC for sampling date x site combinations ranged from 0.6 ± 0.1% LOI to 3.8 ± 0.4% LOI. Organic carbon exhibited strong correlations with sediment composition. TOC was negatively correlated with the percent contribution of sand (r_s = -0.70). Conversely, TOC was positively associated with silt, clay, and rubble (r_s = 0.53, r_s = 0.47, and r_s = 0.44, respectively).

Porewater NH_4^+ concentrations varied tremendously throughout the study. ANOVA indicated a significant sampling date x site interaction. Average porewater NH_4^+ calculated for sampling date x site combinations ranged from 19.9 ± 16.7 µM to 552.5 ± 193.3 µM (Figures I.14 and I.15). In addition to the large range in values across sites and time, there was substantial within site and sampling date variation. Within site 23 in

summer 2005 alone, porewater NH_4^+ measurements ranged from 137.9 to 611.9 μM. Porewater NH_4^+ was not strongly correlated with other sediment characteristics.

Figure I.14 - Map of Redfish Bay showing mean porewater NH_4^+ concentrations (μM).
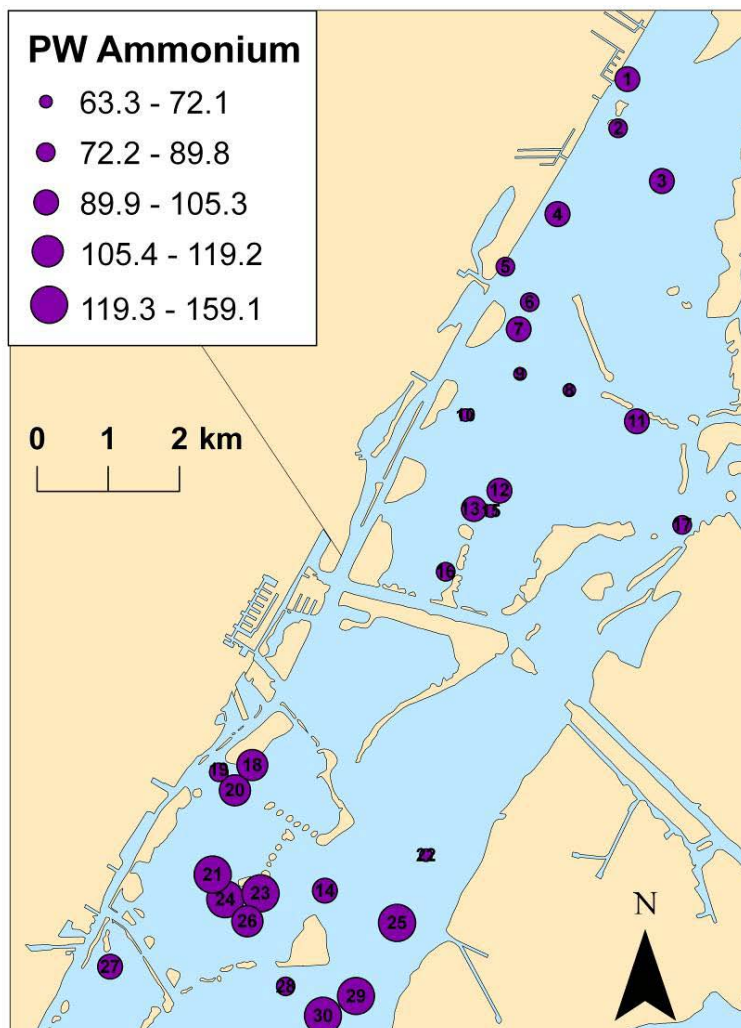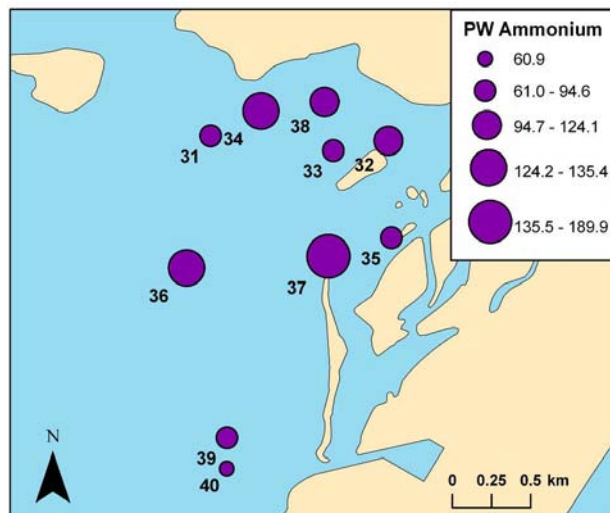
Figure I.15 - Map of East Flats showing mean porewater NH_4^+ concentrations (μM).

Temporal and Spatial Variability in Plant Variables

Thalassia testudinum was the most prevalent species in Redfish Bay and East Flats, consistently occurring at the majority of sites where seagrass was present. *Halodule wrightii* was also common in both bays; however, it was absent at many sites throughout the study. *Syringodium filiforme*, *Ruppia maritima*, and *Halophila engelmannii* were only present sporadically. Because of the infrequent appearance of 4 of the 5 seagrass species, the majority of analyses were performed on *T. testudinum*. Less common species were, however, included in multivariate analyses of community structure and its relationship with physical and chemical variables.

Thalassia testudinum characteristics varied extensively over space and time. Similar to patterns in physical and chemical variables, analyses of plant characteristics revealed significant sampling date x site interaction terms (Table 3). *T. testudinum* blade length differed significantly by sampling date and site. Average blade length, calculated for each sampling date x site combination, ranged from 4.1 ± 7.1 cm to 43.5 ± 1.9 cm (excluding sites without *T. testudinum*). Blade length exhibited seasonal variation with winter blade lengths ranging from 4.1 ± 7.1 cm to 23.3 ± 3.7 cm. In contrast, average blade lengths during summer ranged from 16.4 ± 6.7 cm to 43.5 ± 1.9 cm at vegetated sites. The longest average blade length occurred at site 27 in south Redfish Bay (28.6 ±

10.8 cm), while site 3 in north Redfish Bay had the shortest average blade length (8.7 ± 13.9 cm).

ANOVA on blade width also produced a significant interaction between sampling date and site (Table 3). Average blade width for sampling date by site combinations ranged from 2.5 ± 4.4 mm to 8.2 ± 0.8 mm. Sites 27 and 21 in Redfish Bay had the highest average blade width (7.3 ± 0.6 mm and 7.0 ± 0.9 mm, respectively), and site 3 had the narrowest blades (1.9 ± 2.9 mm). Seasonal variation in blade width was less apparent than variation in blade length. Average winter blade width was 4.1 ± 2.8 mm, and average summer blade width was 4.5 ± 2.9 mm. Blade width and length were positively correlated with one another, although the strength of the relationship was moderate (r_s = 0.55).

Epiphyte biomass on *Thalassia testudinum* blades ranged from 0.02 ± 0.04 mg cm^{-2} to 18.4 ± 3.2 mg cm^{-2} for sampling date by site combinations, yielding a significant sampling date x site interaction term ($p < 0.0001$; Table 3). The highest epiphyte biomass generally occurred during winter sampling in 2004 and 2005 at sites 10, 11, 12, 13, 15 and 17 in Redfish Bay and sites 33 and 37 in East Flats (Figures I.16 and I.17). Epiphyte biomass was not strongly correlated with other plant or physicochemical variables.

Table I.3 - Results of Friedman's non-parametric ANOVA of ranked data for *Thalassia testudinum*.

Response variable	Factor	df	F	p
Thalassia shoot density	sampling	5	90.6	<0.0001*
	site	39	52.7	<0.0001*
	sampling*site	155	7.3	<0.0001*
Epiphyte biomass	sampling	5	103	<0.0001*
	site	39	51	<0.0001*
	sampling*site	121	7.5	<0.0001*
Blade length	sampling	5	398.1	<0.0001*
	site	39	126.6	<0.0001*
	sampling*site	155	10	<0.0001*
Blade width	sampling	5	17.8	<0.0001*
	site	39	78.9	<0.0001*
	sampling*site	155	4.9	<0.0001*
Aboveground biomass	sampling	5	135.3	<0.0001*
	site	39	75.7	<0.0001*
	sampling*site	155	5.2	<0.0001*
Belowground biomass	sampling	5	35.8	<0.0001*
	site	39	65.4	<0.0001*
	sampling*site	155	3.5	<0.0001*
Root:shoot	sampling	5	82	<0.0001*
	site	39	62.5	<0.0001*
	sampling*site	155	6	<0.0001*
Total biomass	sampling	5	53.5	<0.0001*
	site	39	65.1	<0.0001*
	sampling*site	155	3.6	<0.0001*

Thalassia testudinum shoot density varied substantially throughout time and space (Table 3; Figures I.18 and I.19). Among sites with vegetation, sites 32 and 35 in East Flats lacked *T. testudinum* on each sampling date. *Halodule wrightii* and *Ruppia maritima* were common at these shallow sites. Average *T. testudinum* densities were less than 500

shoots m^{-2} at sites 1, 2, 3, and 6 in north Redfish Bay and site 34 in East Flats. On the other hand, *T. testudinum* shoot densities were greater than 1000 shoots m^{-2} for 26 sampling date x site combinations. Sites 7, 10, 12, 37, and 38 each averaged greater than 1000 shoots m^{-2} throughout the study. Shoot density did not vary greatly between seasons, but average shoot density by sampling date ranged from 426 ± 450 shoots m^{-2} in winter 2005 to 733 ± 508 shoots m^{-2} in summer 2002.
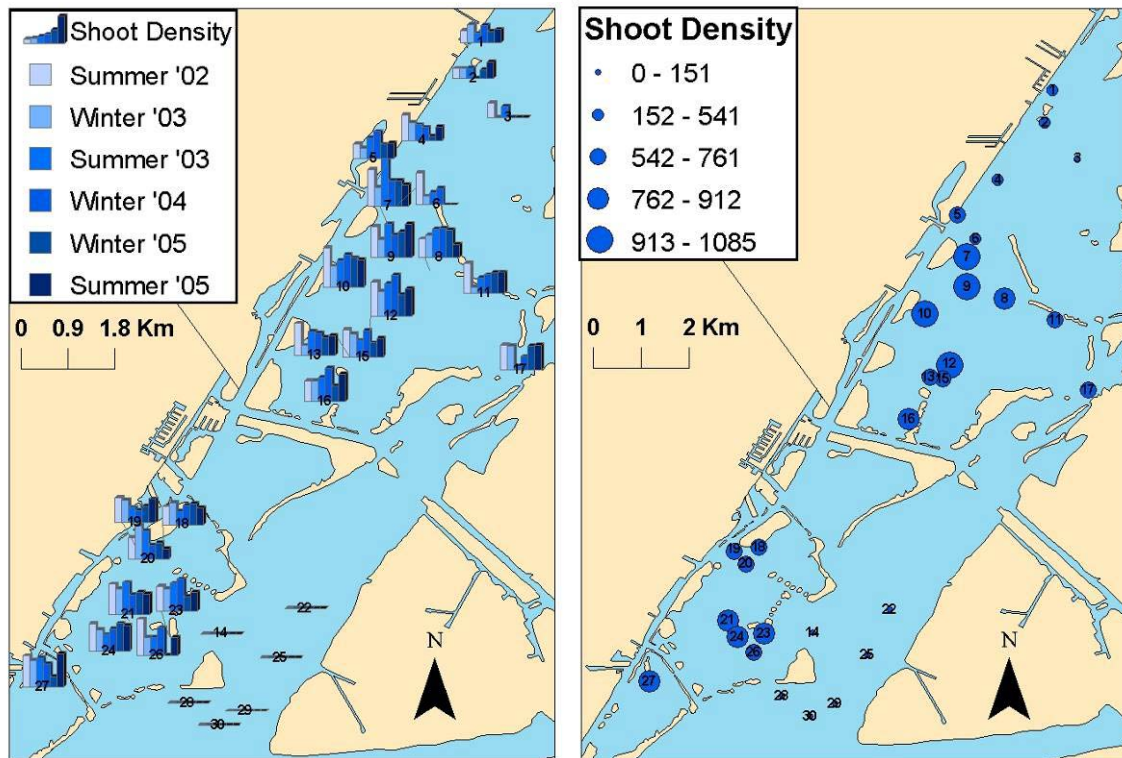
Figure I.18 - Maps of Redfish Bay showing seasonal (left panel) and overall (right panel) mean *Thalassia* shoot density (# m^{-2}).
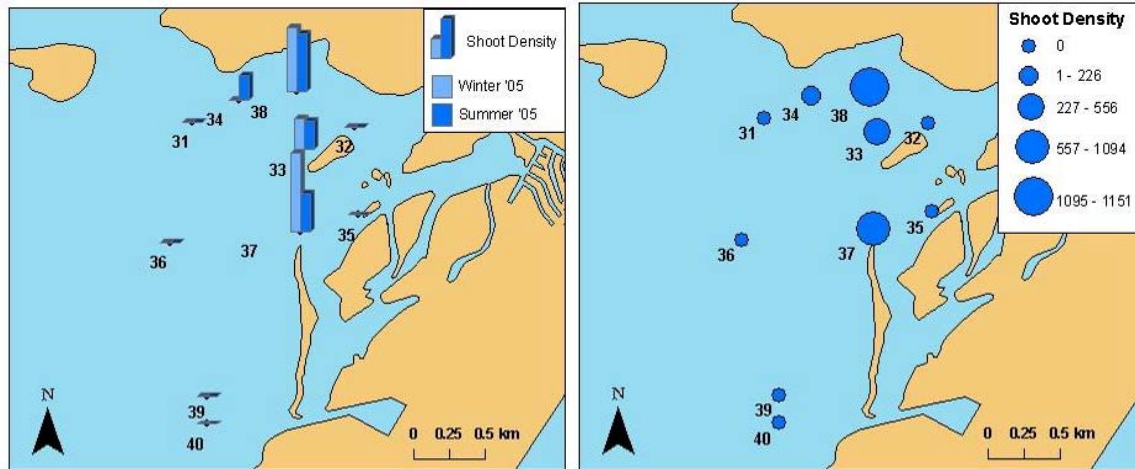
Figure I.19 - Maps of East Flats showing seasonal (top panel) and overall (bottom panel) mean *Thalassia* shoot density (# m^{-2}).

Strong seasonal and site effects were also evident in analysis of aboveground *Thalassia testudinum* biomass (Table I.3; Figures I.20 and I.21). Perusal of average aboveground biomass indicated that 46 of the 50 sampling date x site combinations with greatest aboveground biomass occurred during summer sampling dates. Overall average *T. testudinum* aboveground biomass was 55 ± 58 g m^{-2} in winter and 137 ± 127 g m^{-2} in summer. Sites 21, 23, 27, 38, and 37 had the greatest average aboveground biomass (177 ± 97 g m^{-2} to 241 ± 136 g m^{-2}). The significant sampling date x site interaction term (p <0.0001; Table 3) largely resulted from seasonal differences among sites. Aboveground biomass was positively correlated with shoot density, water temperature, and salinity ($r_s = 0.62$, $r_s = 0.56$ and $r_s = 0.49$, respectively).

Figure I.20 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) *Thalassia* aboveground biomass (g m^{-2}).

Figure I.21 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) *Thalassia* aboveground biomass (g m^{-2}).

Although the sampling date x site interaction term was significant (p <0.0001; Table 3), the seasonal nature of effects on belowground biomass of *Thalassia testudinum* were less evident than for aboveground biomass. In contrast to aboveground biomass, only 32 of 50 sampling date x site combinations with the greatest aboveground biomass occurred during summer sampling dates (Figures I.22 and I.23). Sites with greatest belowground biomass were similar to those with greatest aboveground biomass (23, 27, 38, 37, and 21 in increasing order). The range of biomass values on individual sampling dates for sites with *T. testudinum* was 55 ± 14 g m^{-2} to 793 ± 31 g m^{-2}. *T. testudinum* belowground biomass was strongly correlated with shoot density ($r_s = 0.75$).

Not surprisingly, *Thalassia testudinum* root:shoot values reflected the importance of belowground biomass. In only one instance was aboveground biomass equal to belowground biomass (1 ± 1 g above g below^{-1}; site 33 in summer 2005). The range of values for sampling date x site combinations in which *T. testudinum* was present was 1 ± 1 to 25 ± 8 g above g below^{-1}. Greatest root:shoot ratios occurred during the winter sampling periods, reflecting the influence of seasonal effects on aboveground biomass.
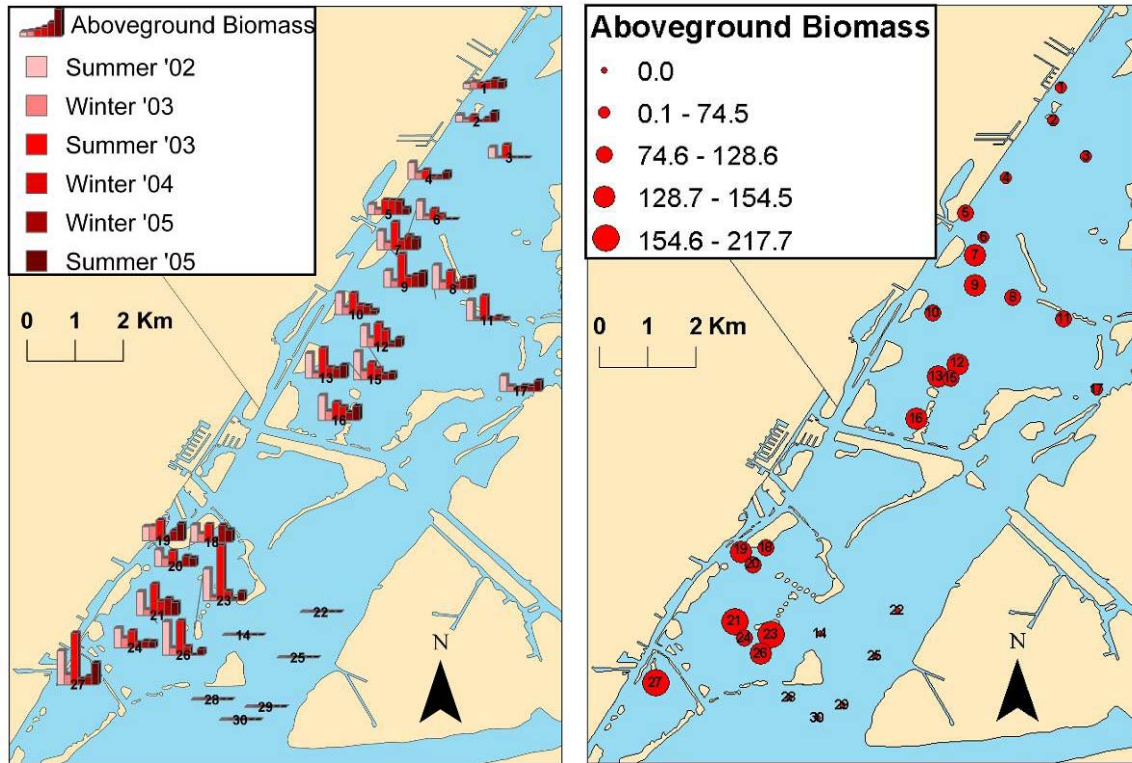
Figure I.22 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) *Thalassia* belowground biomass (g m^{-2}).
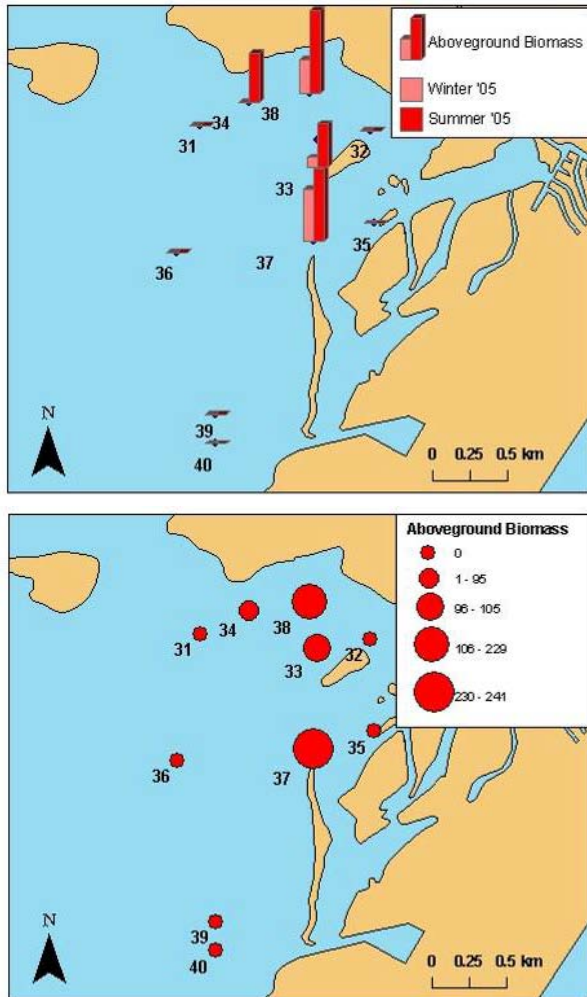
Figure I.23 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) *Thalassia* belowground biomass (g m^{-2}).

Analysis of total *Thalassia testudinum* biomass revealed a significant sampling date x site interaction term (p <0.0001, Table 3). Total *T. testudinum* biomass ranged from 86 ± 8 g m^{-2} to 1326 ± 118 g m^{-2} for sampling date x site combinations (Figures I.24 and I.25). The highest values tended to occur during summer months, reflecting the influence of greater aboveground biomass during summer sampling dates. Sites 12, 21, 23, 27, 37, and 38 frequently had high total *T. testudinum* biomass values. Spearman correlation coefficients indicated strong associations between total *T. testudinum* biomass and shoot density ($r_s = 0.76$), aboveground biomass ($r_s = 0.81$), and belowground biomass ($r_s = 0.97$).
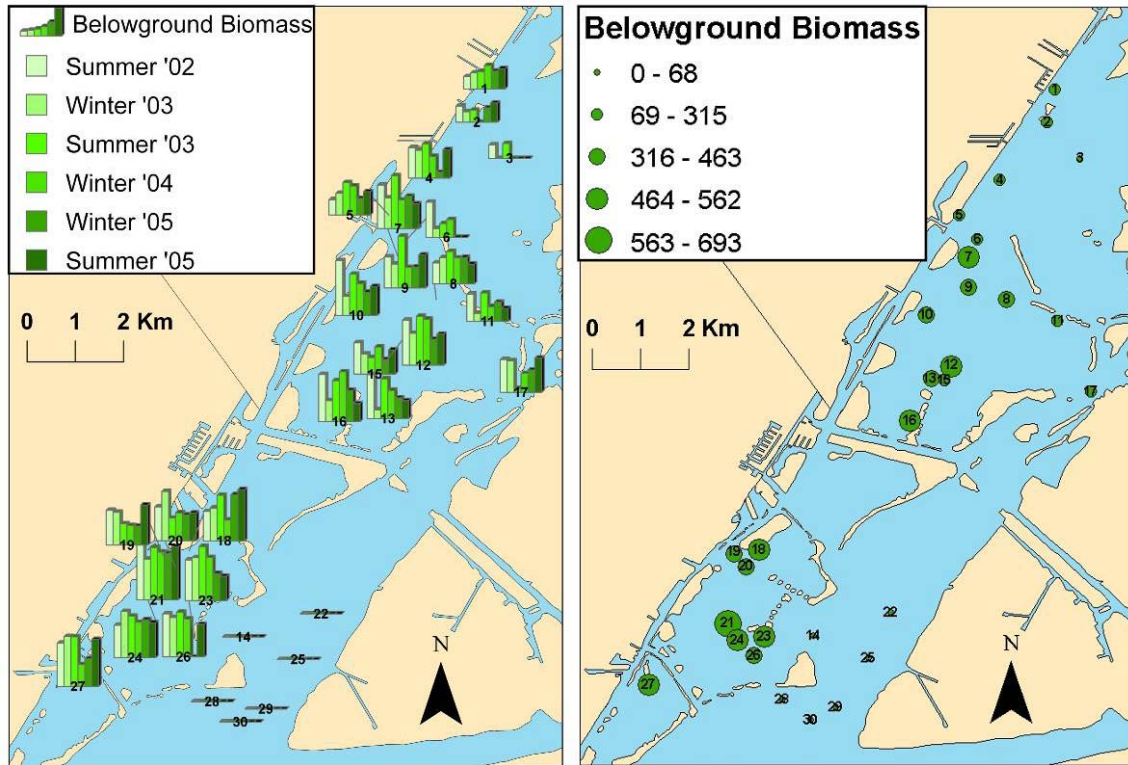

Figure I.24 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) total *Thalassia* biomass (g m^{-2}).

Figure I.25 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) total *Thalassia* biomass (g m^{-2}).

Drift algal biomass was highly variable throughout the study (Figures I.26 and I.27). Results of non-parametric ANOVA indicated that sampling date and site interacted to affect drift algal biomass ($p < 0.0001$, Table 4). Algal biomass was greatest during the winter 2005 sampling at site 24 (612 ± 224 g m^{-2}), equaling the total *Thalassia testudinum* biomass (613 ± 41g m^{-2}). In many cases, however, no drift algae were present. Sites 24 and 20 had the highest average algal biomass throughout the study (211 ± 263 g m^{-2} and 166 ± 279 g m^{-2}, respectively). Drift algal biomass was generally lower at sites in East Flats (0–28 ± 29 gm^{-2}).

34

Table I.4 - Results of Friedman's non-parametric ANOVA of ranked transect data.

Response variable	Factor	df	F	p
Drift algal biomass	sampling	5	90.6	<0.0001*
	site	39	52.7	<0.0001*
	sampling*site	155	7.3	<0.0001*
Thalassia cover	sampling	5	13.1	<0.0001*
	site	39	42.7	<0.0001*
	sampling*site	155	3.4	<0.0001*
Total seagrass cover	sampling	5	73.3	<0.0001*
	site	39	81	<0.0001*
	sampling*site	155	5.5	<0.0001*

* significant at $\alpha = 0.05$

Figure I.26 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) drift algal biomass (g m^{-2}).
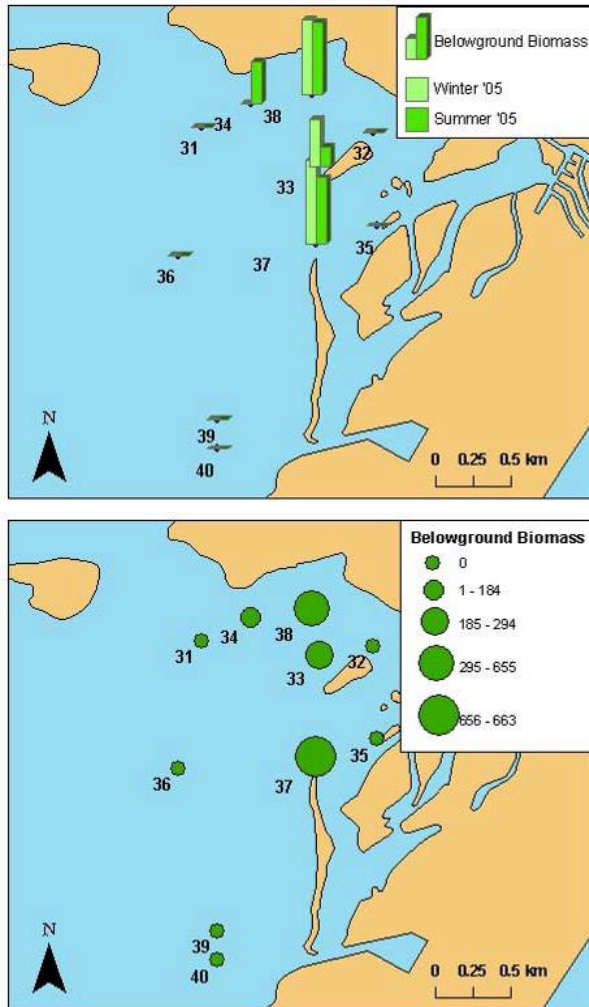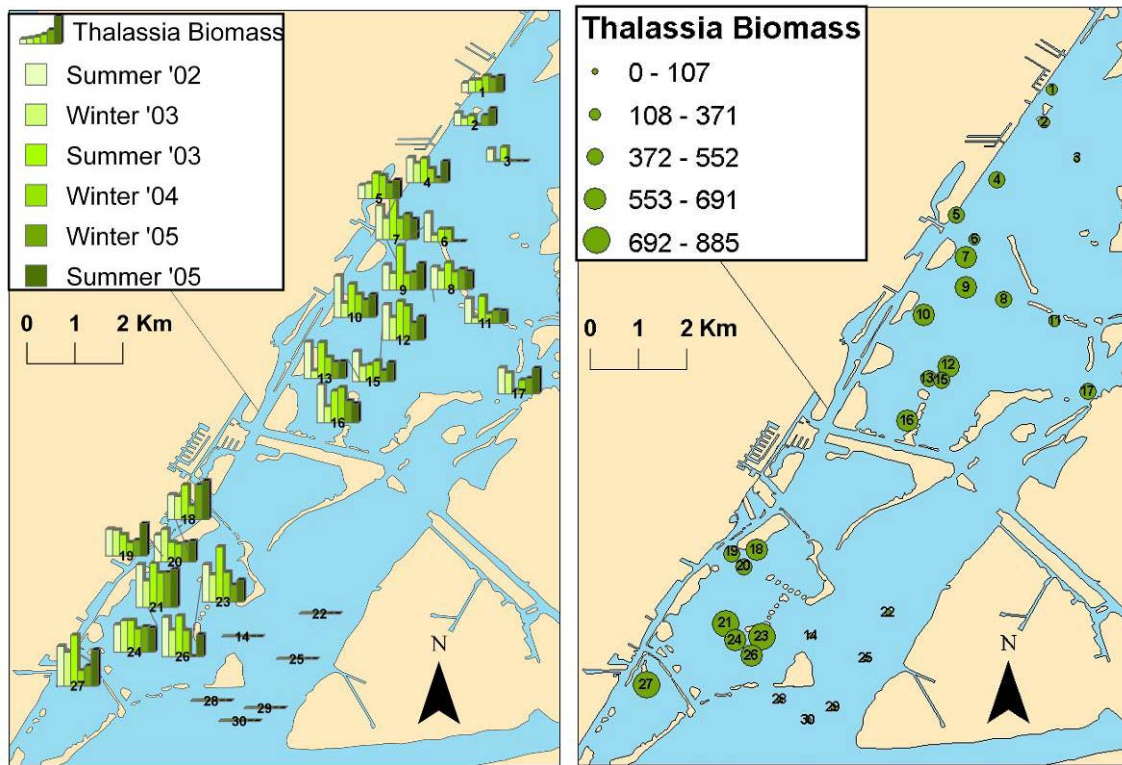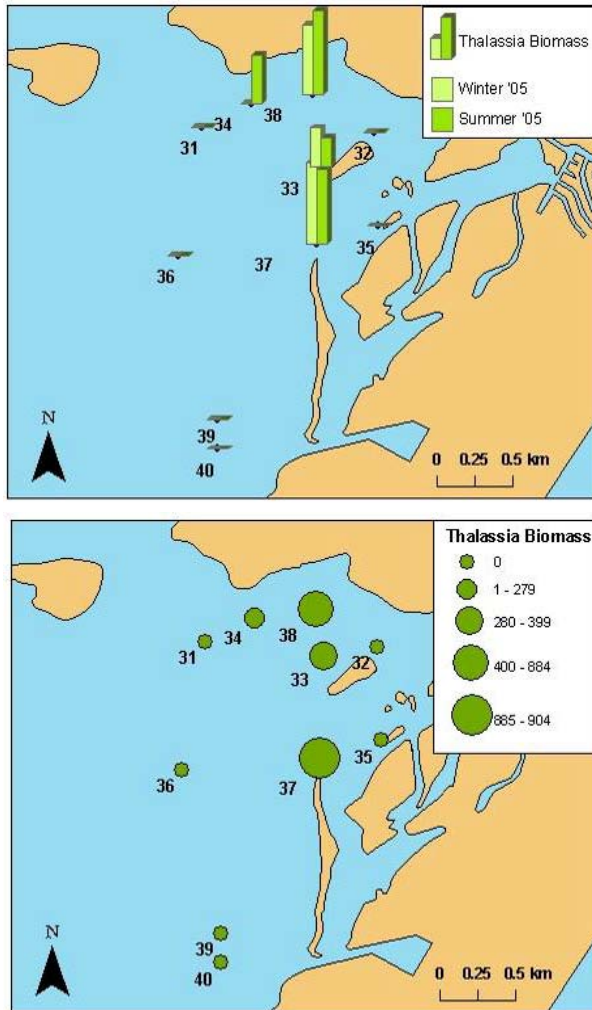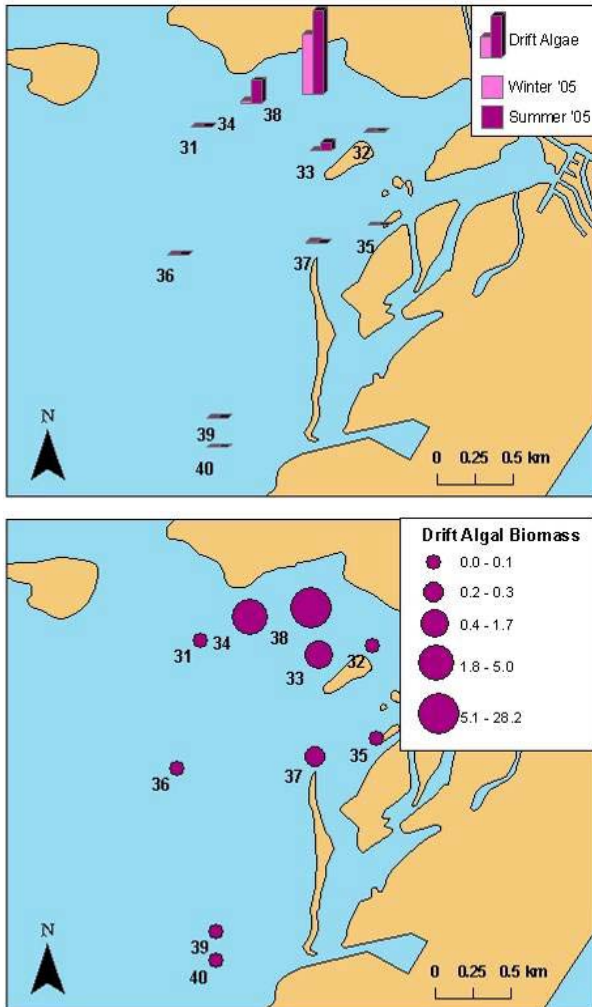
Figure I.27 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) drift algal biomass (g m^{-2}).

Total seagrass cover differed significantly among sampling date x site combinations (p < 0.0001, Table 4). In addition to sites deeper than 1.7 m, site 3 had 0% cover during winter 2005 (Figure I.28). For all other sampling date x site combinations, average seagrass cover ranged from $0.2 \pm 0.6\%$ to $100 \pm 0\%$ during the study (Figures I.28 and I.29). Notably, *post hoc* Tukey tests indicated that total seagrass cover declined at several sites (p < 0.05; n = 5) from summer 2002 to summer 2005. Seagrass cover at site 2 declined from $97 \pm 8\%$ to $20 \pm 34\%$ as a result of reduced cover of *Halodule wrightii* and *Syringodium filiforme* ($47 \pm 48\%$ to $13 \pm 32\%$ and $39 \pm 48\%$ to $6 \pm 18\%$, respectively). At site 3, seagrass cover declined by about 85% largely due to a decrease in cover of *Ruppia maritima* ($62 \pm 29\%$ to $0 \pm 1\%$). Site 6 also experienced a decline in

seagrass cover as *H. wrightii* cover declined from 81 ± 12% to 29 ± 34%. Declines in *H. wrightii* (41 ± 33% to 11 ± 22%) and *R. maritima* (41 ± 38% to 0 ± 0%) cover also resulted in reduced seagrass cover at site 16 (97 ± 4% to 46 ± 39%).

Figure I.28 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) percent seagrass cover.
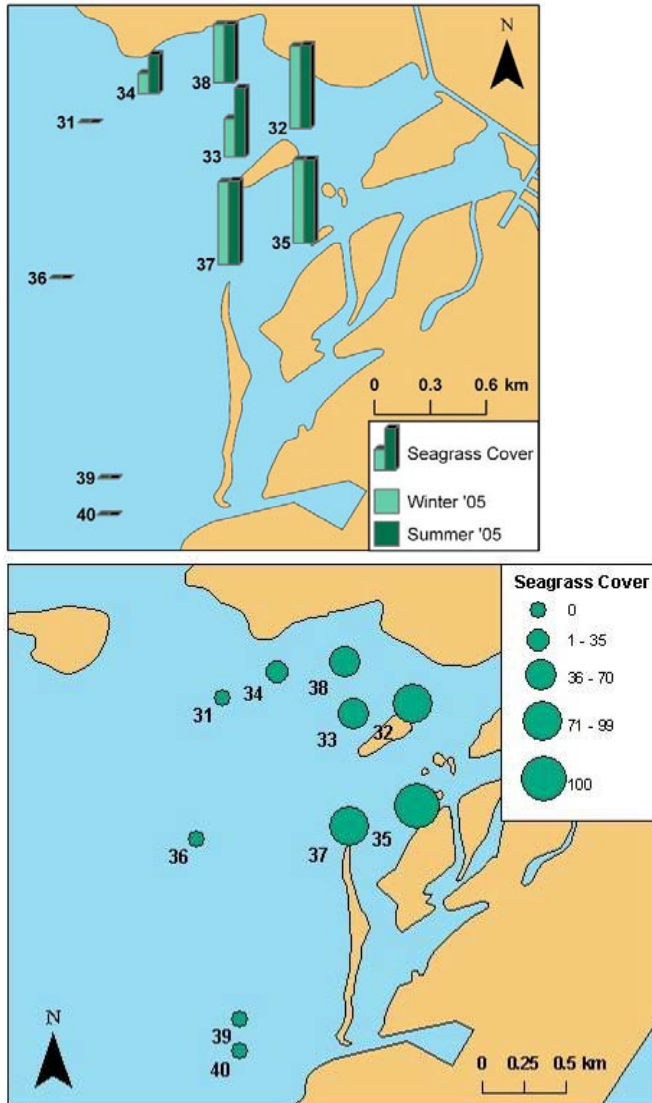
Figure I.29 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) percent seagrass cover.

Several sites, most notably in south Redfish Bay, exhibited significant declines ($p < 0.05$; n = 10) in total seagrass cover as a result of a decrease in *Thalassia testudinum* cover (Figures I.30 and I.31). Total seagrass cover at site 26 declined from $100 \pm 0\%$ in summer 2002 to $1 \pm 4\%$ in summer 2005. In this case, the decline resulted entirely from a reduction in cover of *T. testudinum*. Seagrass cover also declined significantly at sites 23 and 27. At site 23, total seagrass cover dropped from $93 \pm 19\%$ to $25 \pm 41\%$ due to loss of *T. testudinum* ($39 \pm 38\%$ to $0 \pm 0\%$). Additionally, losses of *T. testudinum* ($69 \pm 48\%$ to $33 \pm 45\%$) at site 27 were largely responsible for reductions in total seagrass

cover (87 ± 20% to 40 ± 48%). Sites 21 and 24 appeared to undergo similar declines; however, losses were not statistically different due to high within transect variability.
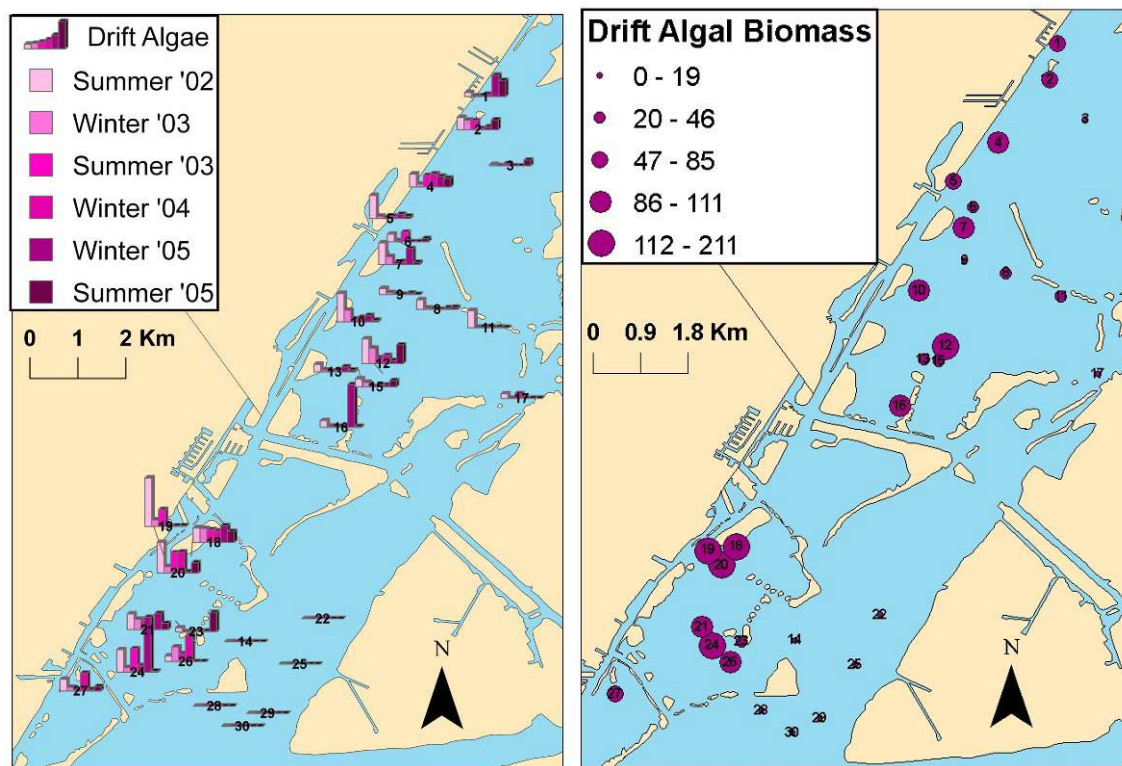

Figure I.30 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) percent *Thalassia* cover.
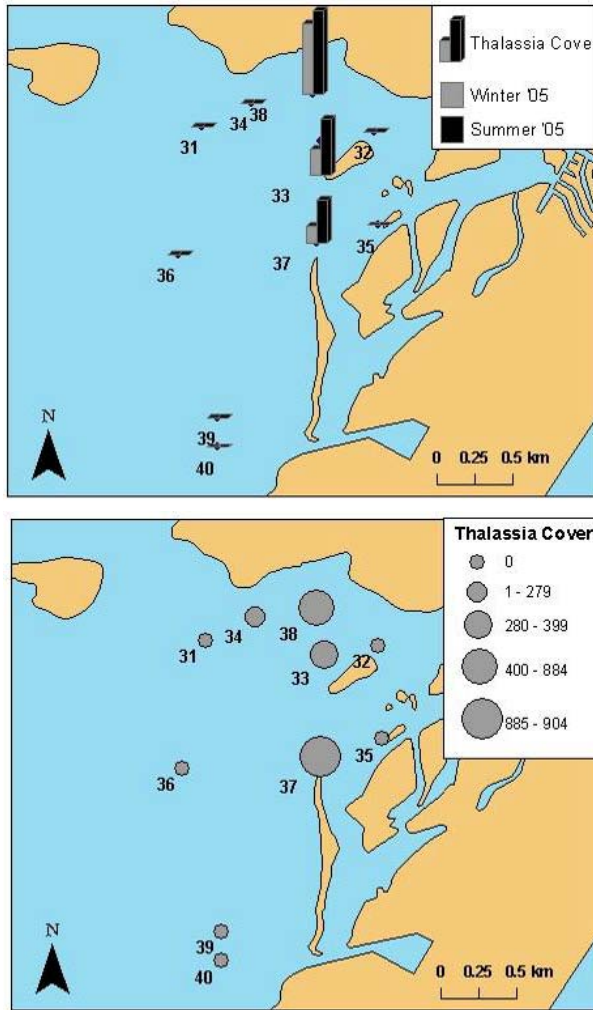
Figure I.31 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) percent *Thalassia* cover.

Analysis of *Thalassia testudinum* tissue C:N ratios yielded a significant interaction term for sampling date and site ($p = 0.01$, Table 5). C:N values ranged from 10 ± 0.8 to 29 ± 1.6 for sampling date x site combinations (Figures I.32 and I.33). All average C:N values greater than 20 occurred during summer sampling periods (16 total sampling date x site combinations). Conversely, all average values less than 14 occurred in the winter (13 combinations). Sites 12, 26, and 34 had averaged values greater than 20, while sites 7, 9, 11, 18, 20, 23, and 24 had C:N values less than 16.
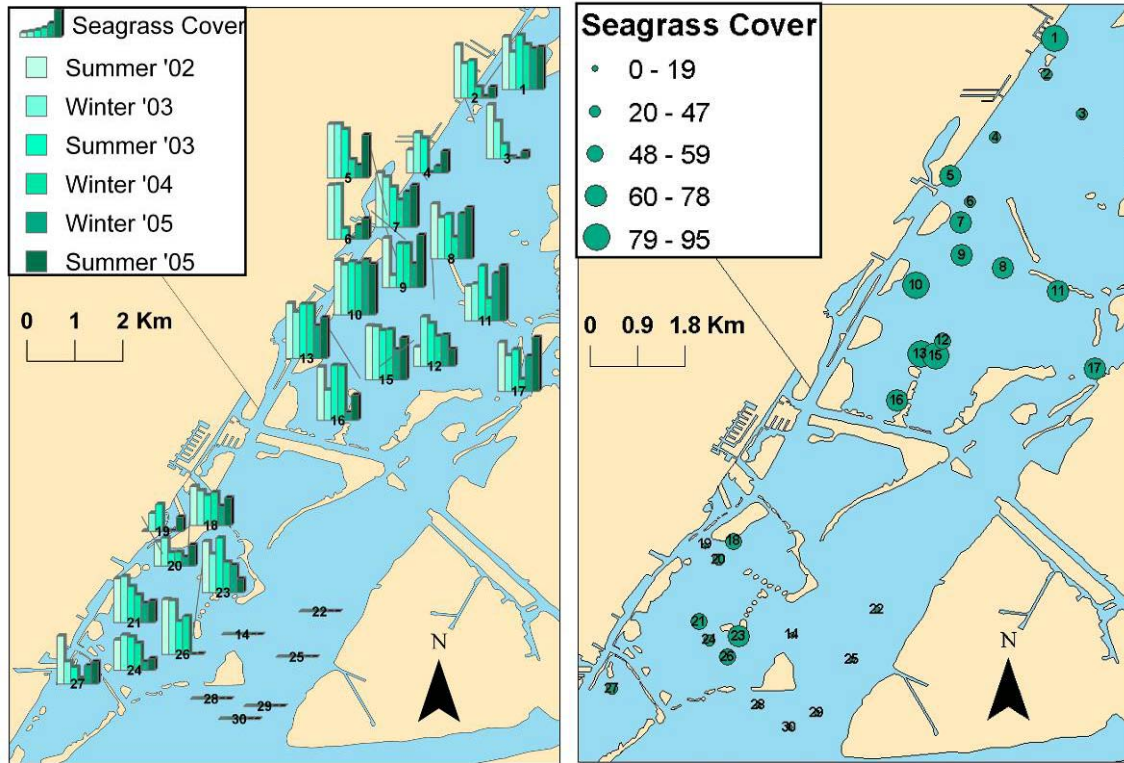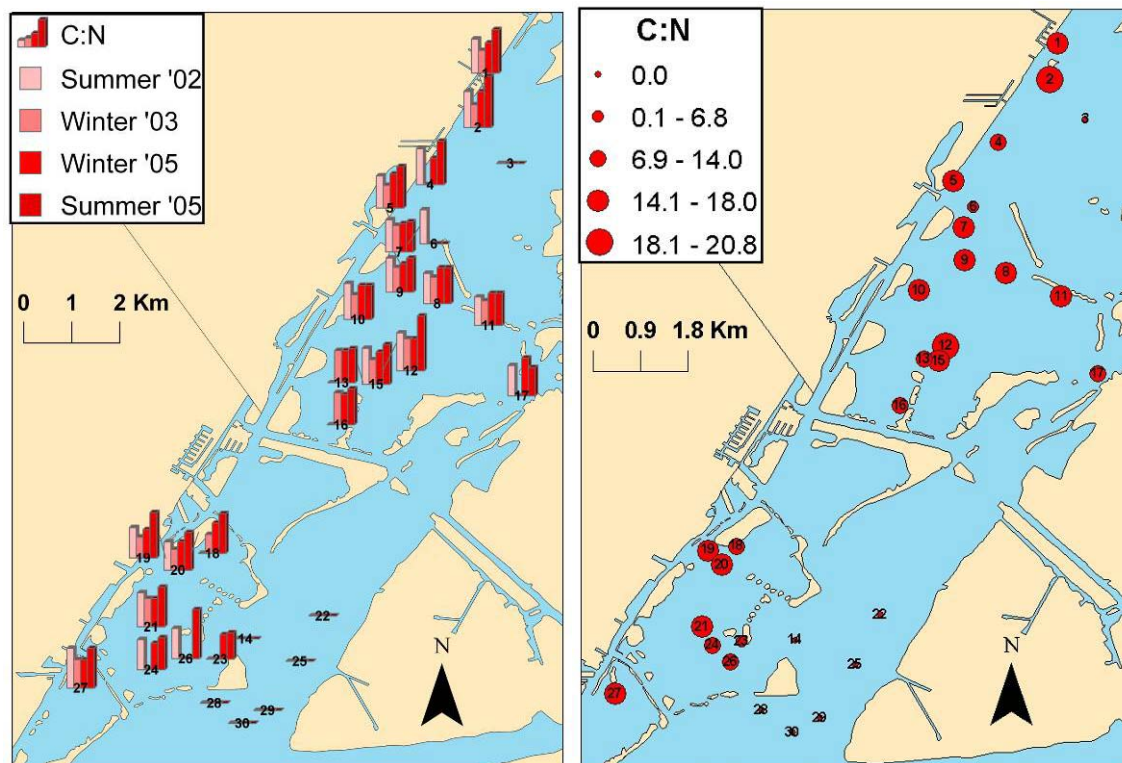
Figure I.32 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) tissue C:N.
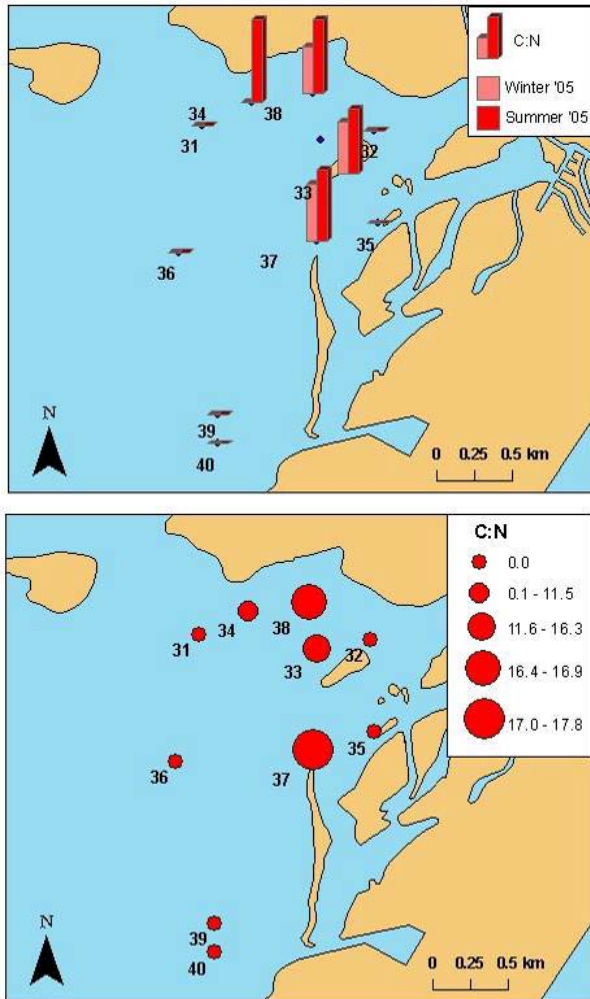
Figure I.33 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) tissue C:N.

Table I.5 - Results of Friedman's non-parametric ANOVA of ranked tissue nutrient data for *Thalassia testudinum*.

Response variable	Factor	df	F	p
C:N	sampling	3	60.8	<0.0001*
	site	26	2.8	0.0002*
	sampling*site	56	1.7	0.01*
N:P	sampling	3	37.5	<0.0001*
	site	26	2	0.009*
	sampling*site	56	1.3	0.12
C:P	sampling	3	50.7	<0.0001*
	site	26	2.4	0.001*
	sampling*site	56	2.4	0.0001*

* significant at $\alpha = 0.05$

Thalassia testudinum N:P was the only plant characteristic that did not exhibit a significant sampling date x site interaction term (p = 0.12, Table 5). Analysis did reveal, however, significant main effects of site (p = 0.009) and sampling date (p < 0.0001). *Post hoc* Tukey tests revealed that the only differences among sites were differences between site 6 (45.6 ± 0.1) and sites 16 (26.7 ± 6.3), 18 (24.5 ± 5.6), and 19 (27.4 ± 6.8). Tissue N:P values were significantly higher during summer 2002 (46 ±14) than winter 2003 (29 ± 7), winter 2005 (32 ± 9), and summer 2005 (26 ± 8; Figures I.34 and I.35). In addition, N:P values in summer 2005 were significantly lower than values in winter 2005.
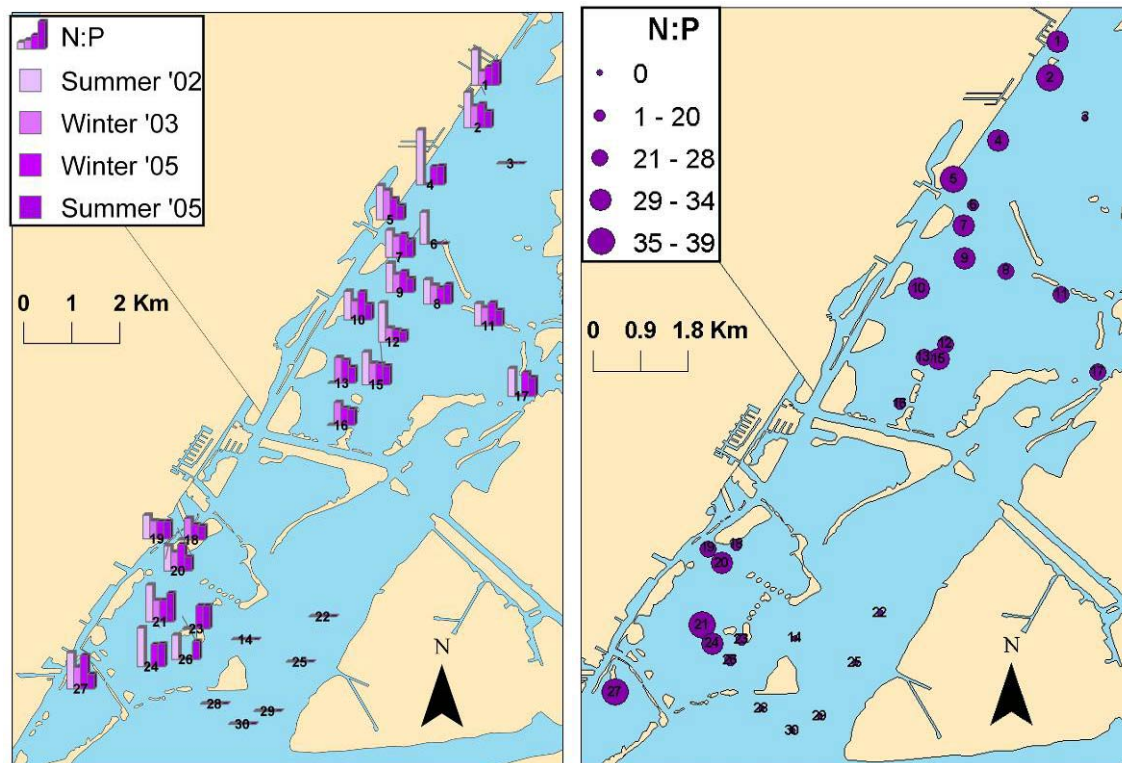
Figure I.34 - Maps of Redfish Bay showing mean seasonal (left panel) and mean overall (right panel) tissue N:P.

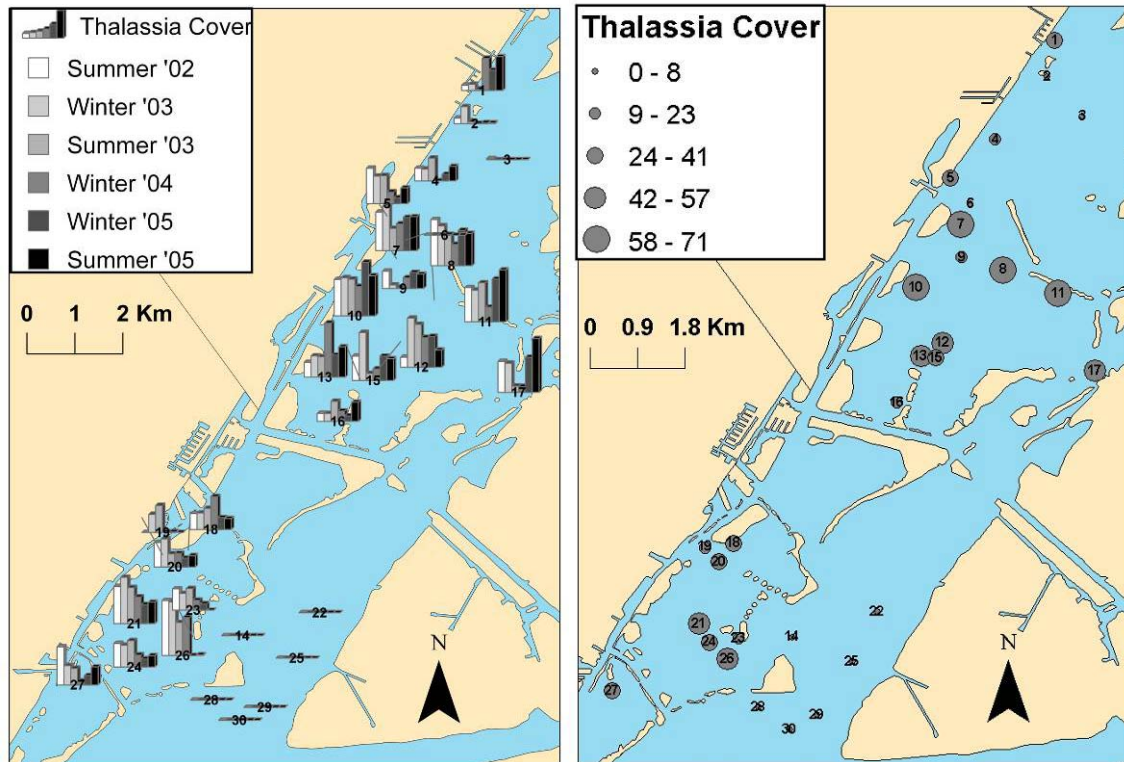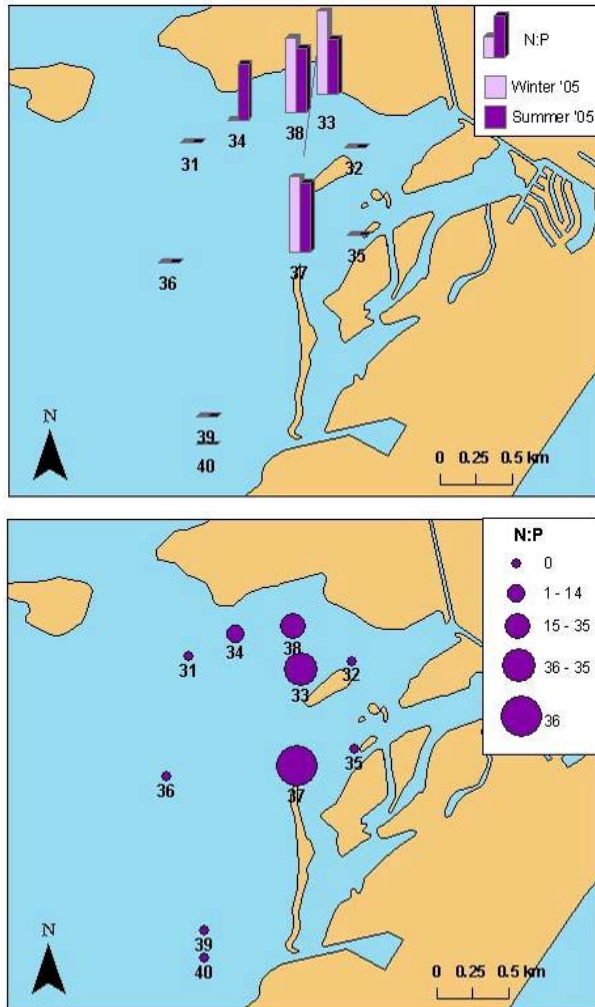Figure I.35 - Maps of East Flats showing mean seasonal (top panel) and mean overall (bottom panel) tissue N:P.

Like C:N, C:P also differed significantly by sampling date x site combination (p = 0.0001, Table 5). Values ranged from 234 ± 1 at site 1 in winter 2003 to 1484 ± 471 at site 4 in summer 2002. Highest values of C:P occurred during summer sampling dates, particularly in 2002.

Multivariate Analyses for Bay and Temporal Comparisons

Separate multivariate analyses were performed on the physical and chemical parameters and community composition data to assess differences between Redfish Bay and East Flats. Cluster analyses generated from the suite of environmental data identified several statistically distinct clusters ($\alpha = 0.05$; darkened branches); however, samples within

45

clusters were not grouped by bay (Figure I.36). Samples collected from East Flats were distributed throughout the dendrogram and, in many cases, were most closely linked to samples from Redfish Bay.

Figure I.36 - Dendrogram based on Euclidean distance among environmental conditions for each sampling date by site combination. Solid lines indicate significantly distinct clusters ($\alpha = 0.05$). ● = East Flats, ■ = Redfish Bay

The pattern of sample distribution obtained by non-metric multidimensional scaling (nMDS) mirrored the results of cluster analysis (Figure I.37). East Flats samples did not form aggregations in the ordination plot and overlapped with samples collected from Redfish Bay. The stress (0.22) for the 2-dimensional plot was relatively high and suggested caution in interpreting details; however, the lack of pattern in sample

distributions by bay was clearly apparent. Ordination reinforced the notion that environmental conditions are not different at the bay scale.
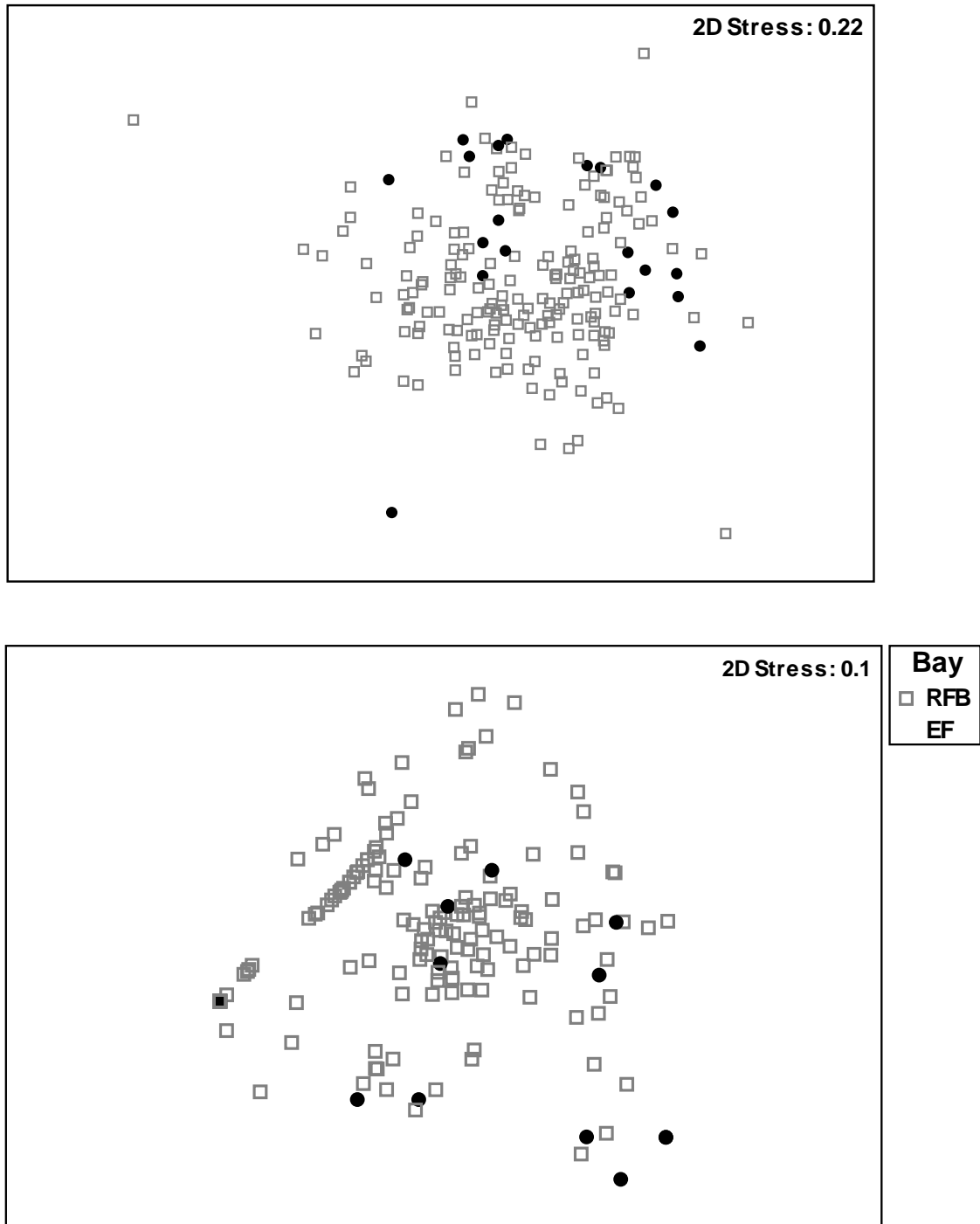
Figure I.37 - NMDS plots based on Euclidean distance (top panel) among environmental conditions and similarity of seagrass community structure (bottom panel) for each sampling date by site combination.

Cluster analysis and nMDS were repeated using the community composition data based on seagrass density as calculated from Braun-Blanquet scores of percent cover data. Results of the cluster analysis on community composition were similar to results from the environmental data (Figure I.38). Statistically distinct clusters ($\alpha = 0.05$) contained samples from both Redfish Bay and East Flats, and samples from East Flats were distributed throughout the dendrogram. The nMDS ordination plot showed a similar relationship among samples from the two bays (Figure I.37). Moderate stress (0.12) indicated that the general pattern among bays was reliable. Both the cluster analysis and nMDS clearly demonstrated that seagrass community composition did not differ as a function of bay.
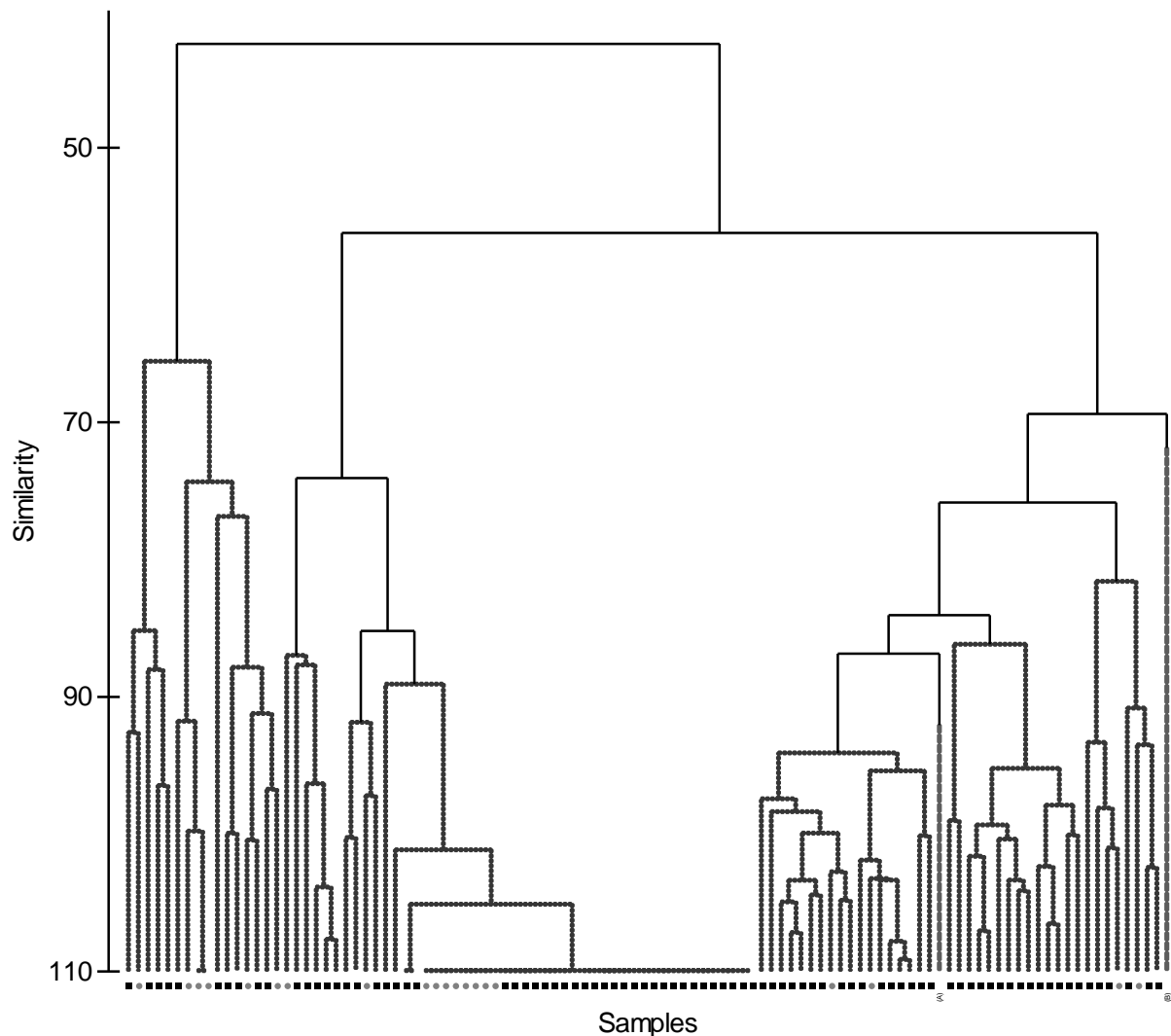
Figure I.38 - Dendrogram based on similarity among seagrass community structure for each sampling date by site combination. Solid lines designate distinct clusters (α = 0.05). ● = East Flats, ■ = Redfish Bay

The same multivariate approach was used to assess differences in environmental conditions during each of the sampling periods. Cluster analysis clearly identified distinct groups based on season and sampling period (Figure I.39). The majority of samples from summer sampling periods formed 2 clusters that were significantly different from 2 other clusters containing the majority of samples from winter (α = 0.05). Differences between clusters within a season were largely based on depth. Deep sites (> 1.7 m) formed a distinct cluster from shallower sites (α = 0.05), which was itself divided into two clusters based on seasonal differences in environmental conditions. The same

49

general pattern is readily apparent in the ordination plot of environmental variables (Figure I.40).
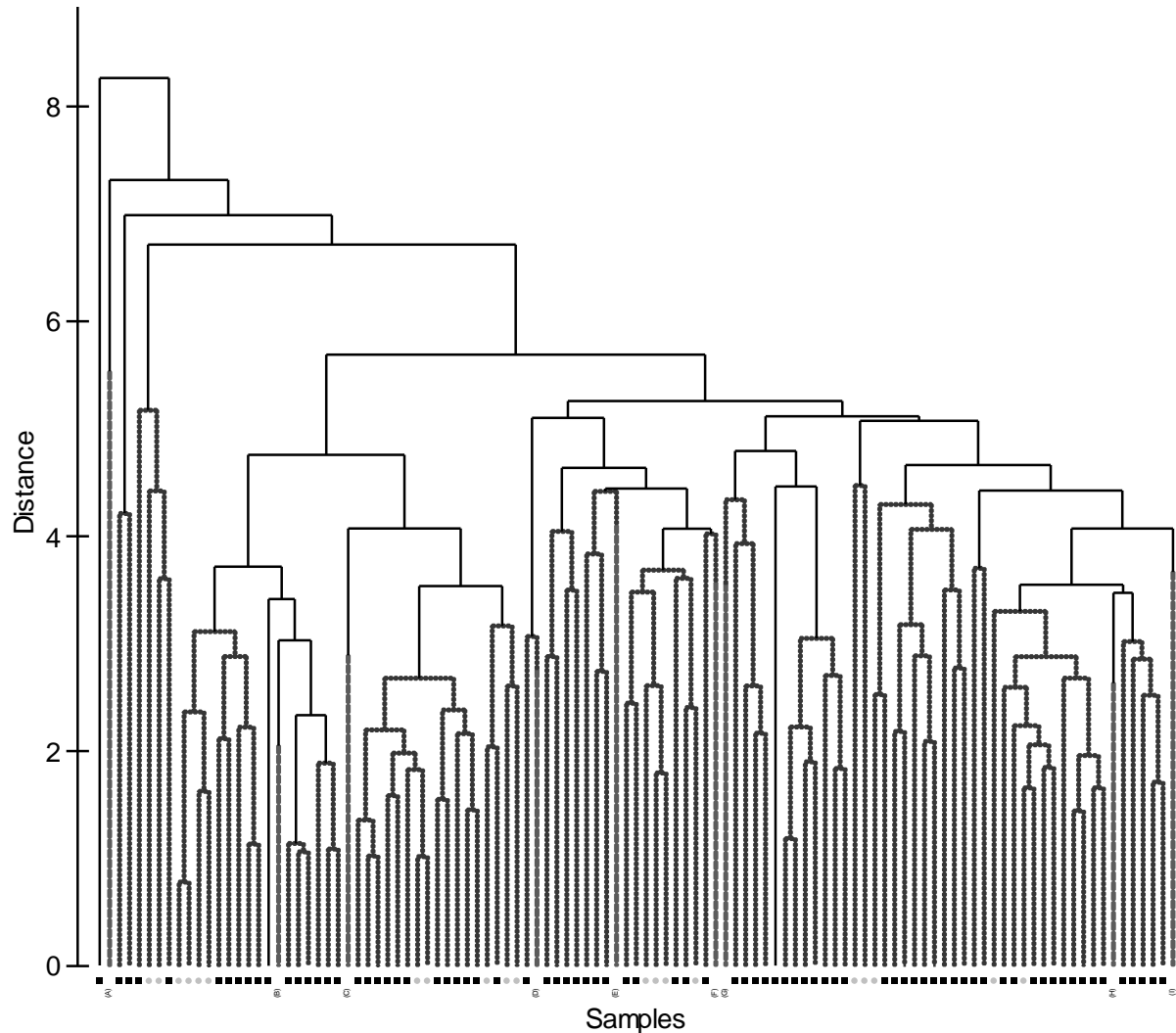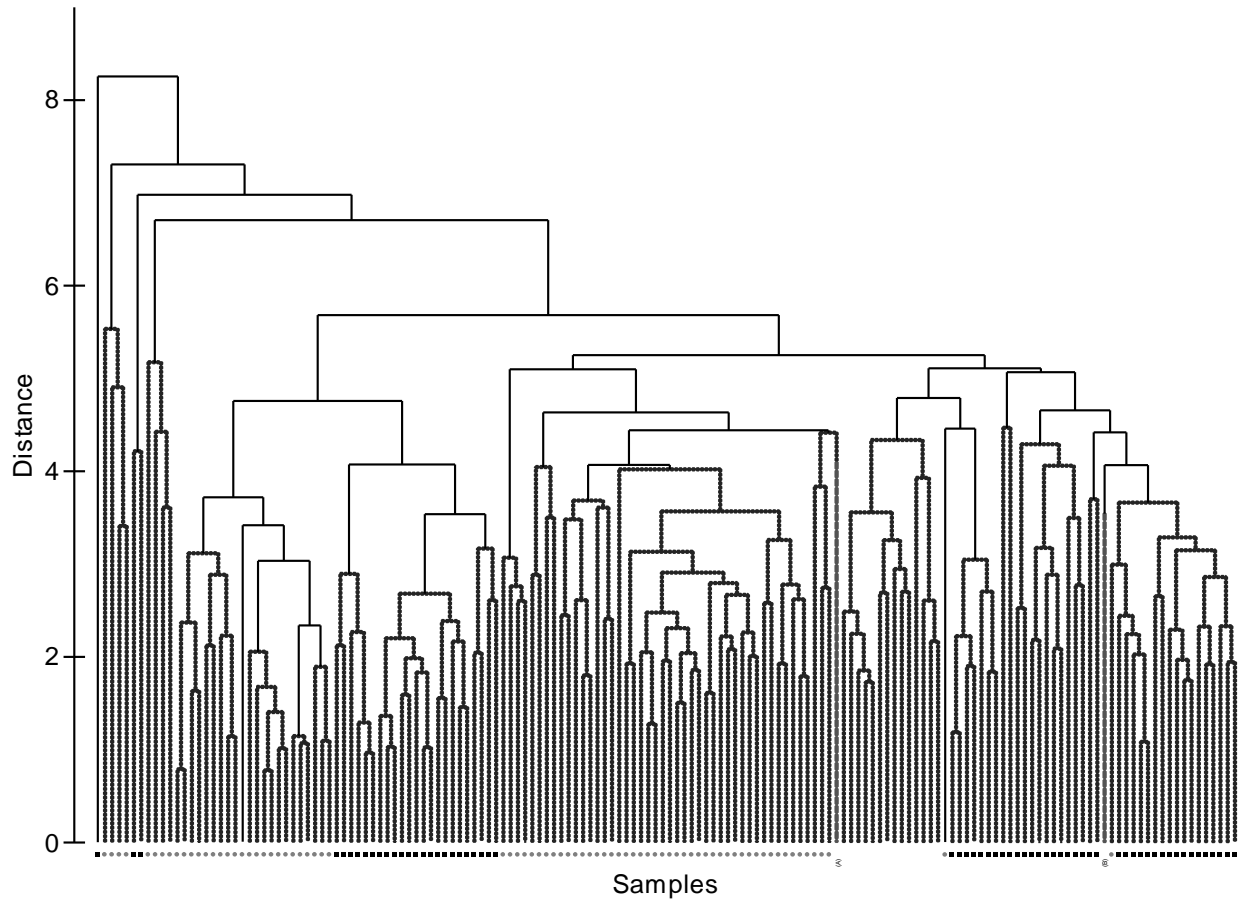

Figure I.39 - Dendrogram based on Euclidean distance among environmental conditions for each sampling date by site combination. Solid lines indicate significantly distinct clusters (α = 0.05). ● = Winter, ■ = Summer
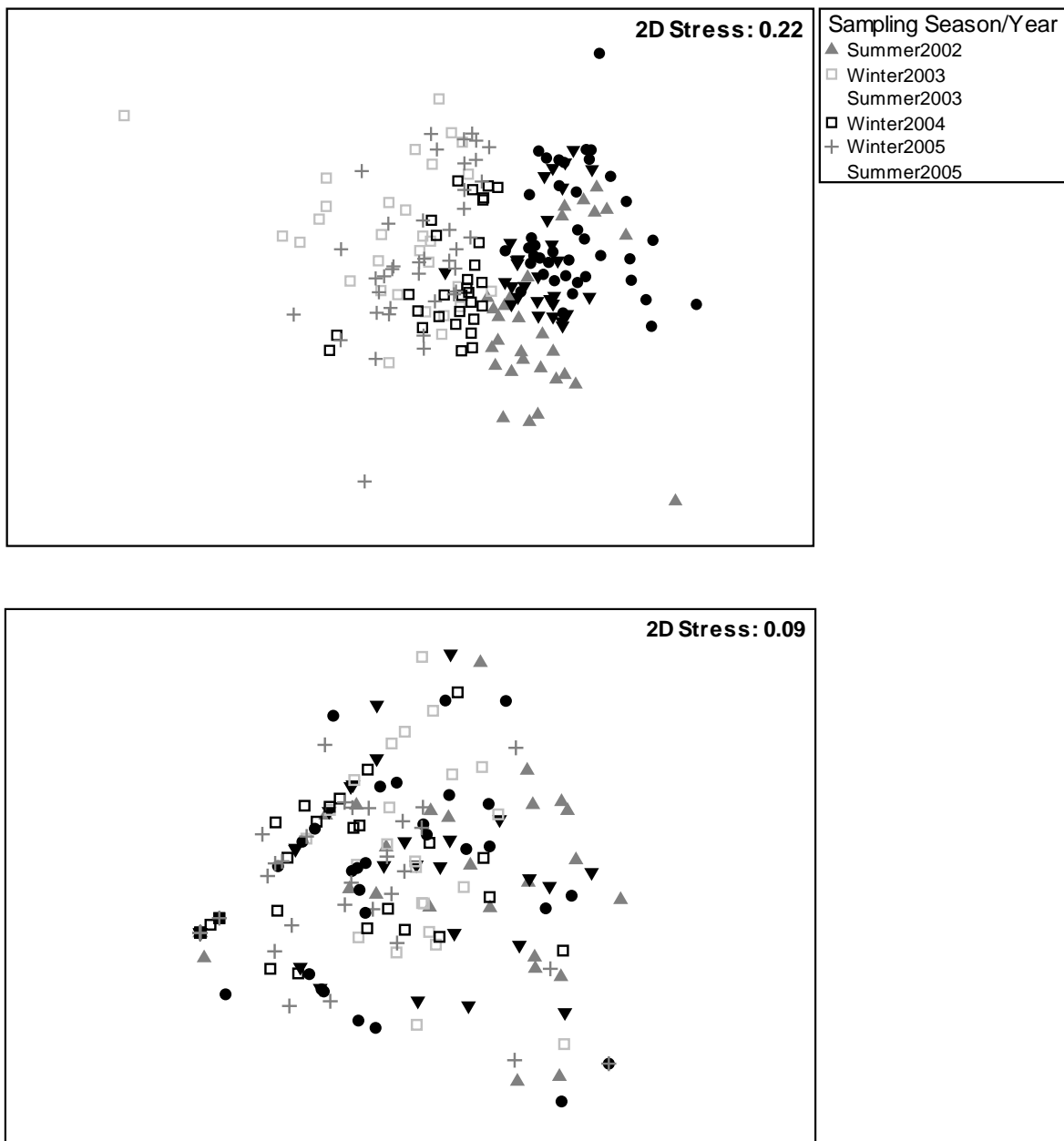
Figure I.40 - NMDS plots based on Euclidean distance (top panel) among environmental conditions and similarity of seagrass community structure (bottom panel) for each sampling date by site combination.

Distinct seasonality was not apparent from cluster analysis based on the community composition data (Figure I.41). Samples collected during both summer and winter sampling periods were represented in most clusters. The lack of seasonality and aggregation of samples within a sampling period was visible in the ordination plot (Figure I.40). In general, samples from individual sampling periods were widely

distributed across the plot and overlapped other sampling periods. Together, these multivariate analyses indicated that community composition does not vary greatly seasonally or interannually.
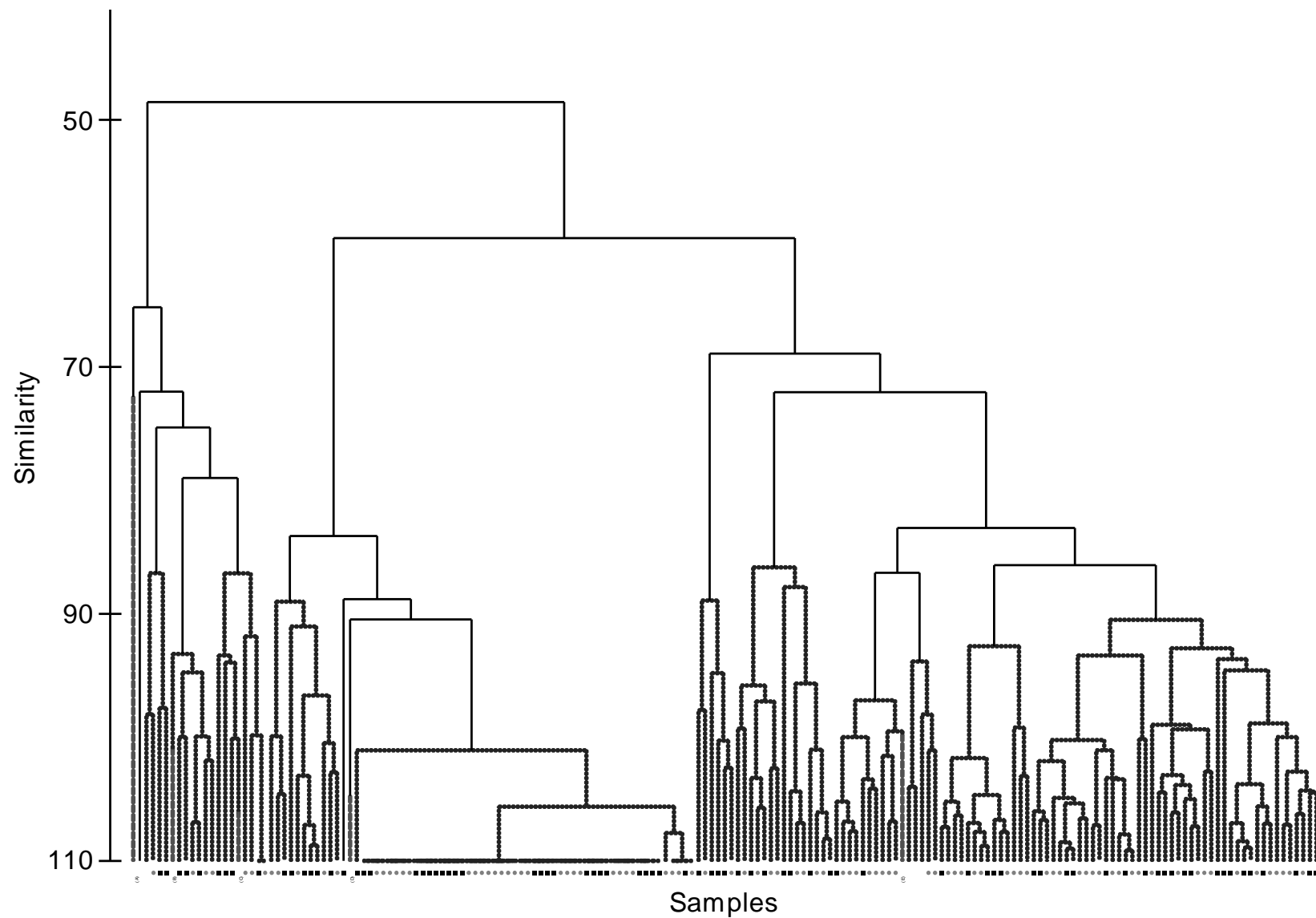
Figure I.41 - Dendrogram based on similarity of seagrass community structure for each sampling date by site combination. Solid lines indicate significantly distinct clusters (α = 0.05). ● = Winter, ■ = Summer

Univariate Assessment of Indicators

Spearman correlation coefficients were calculated between environmental variables and plant characteristics to evaluate their potential relationship with seagrass condition (Table 6). Multiple variables were correlated with seagrass percent cover. Percent sand content of the sediments was positively correlated with *S. filiforme* cover ($r_s = 0.45$; $p < 0.0006$) while percent silt was negatively correlated with cover ($r_s = -0.45$; $p < 0.0005$). *Halophila engelmannii* cover was positively correlated with water column PO_4^{3-} concentration ($r_s = 0.48$; $p < 0.0002$) and N:P of *Thalassia testudinum* leaves (0.61; $p < 0.0003$).

Table I.6 - Significant correlations between seagrass and environmental variables. $\alpha = 0.05$.

	Correlate	
Variable	Positive	Negative
Thalassia cover	shoot density	
Syringodium cover	% sand	% silt
Halophila cover	PO_4^{3-}, N:P	
Thalassia shoot density	% silt, *Thalassia* cover above-, belowground, total biomass	% sand
Thalassia N:P	% silt, PO_4^{3-}	TSS, epiphytes
Thalassia blade length	blade width, above-, belowground, total biomass	NH_4^+
Thalassia blade width	blade length, above-, belowground, total biomass	PO_4^{3-}
Root:shoot	NH_4^+	
Thalassia aboveground Biomass	blade length, width, shoot density, belowground & total biomass, C:N, N:P	NH_4^+
Thalassia belowground Biomass	blade length, width, shoot density, aboveground & total biomass	

Thalassia total biomass	blade length, width, shoot density, above-, belowground biomass

Sediment characteristics were also strongly correlated with *Thalassia testudinum* shoot density. Percent sand in the sediments was negatively correlated with shoot density (r_s = 0.51; $p < 0.0001$). Similarly, shoot densities and percent silt were significantly correlated (r_s = 0.57l $p < 0.0001$); however, the association was positive. The relationship between shoot density and sediment grain size probably reflected the baffling effect of *T. testudinum* leaves promoting the settling of fine particles from the water column.

Few environmental parameters were significantly correlated with the tissue nutrient content of *Thalassia testudinum*. Total suspended solids and epiphytes were negatively correlated with N:P (r_s = -0.48 and r_s = -.49, respectively; $p < 0.007$), but percent silt and PO_4^{3-} were positively correlated with N:P (r_s = -0.61 in both cases; $p < 0.0003$). On the other hand, C:N was not strongly correlated with any environmental parameters.

Water column nutrient concentrations also exhibited significant correlations with *Thalassia testudinum* blade characteristics and biomass. NH_4^+ was negatively correlated with blade length (r_s = -0.71; $p < 0.0001$) and its covariate, aboveground biomass (r_x = -0.62; $p < 0.0001$). Consequently, NH_4^+ was positively correlated with root:shoot ratio (r_s = 0.56; $p < 0.0001$). In addition, blade width and PO_4^{3-} were negatively correlated (r_s = -0.46; $p < 0.0004$).

Seagrass characteristics were highly correlated. Blade length and width were positively associated with one another (r_s = 0.55; $p < 0.0001$) as well as *Thalassia testudinum* biomass. Blade length was more strongly correlated with aboveground biomass (r_s = 0.83; $p < 0.0001$) than either belowground (r_s = 0.46; $p < 0.0001$) or total *T. testudinum* biomass (r_s = 0.59; $p < 0.0001$). Correlations with the biomass parameters and blade width were similar but of slightly lesser magnitude.

Significant positive associations were found between *Thalassia testudinum* shoot density and *T. testudinum* cover and biomass measurements. The Spearman correlation coefficient between shoot density and percent cover was 0.49 ($p < 0.0001$). Stronger correlations existed

between shoot density and aboveground ($r_s = 0.62$; $p < 0.0001$), belowground ($r_s = 0.75$; $p < 0.0001$) and total biomass ($r_s = 0.76$; $p < 0.0001$). Correlations between shoot density and other seagrass characteristics were not strong ($r_s < 0.4$).

Linear regression revealed a moderately strong model for shoot density and total biomass (Figure I.42). Using all data, the modeled relationship was:

$$\delta = 1.053\tau + 104.8$$

where δ = shoot density and τ = total biomass. This equation explained 68% ($r^2 = 0.68$) of the variance in *Thalassia testudinum* shoot density. Separation of the data into seasons yielded slightly better models. Regression of summer values generated:

$$\delta = 1.067\tau + 77.96$$

with $r^2 = 0.77$. The fitted model for winter data explained 84% of the variation ($r^2 = 0.84$) in that seasonal data subset. The least squares regression model was:

$$\delta = 1.366\tau + 56.495$$

These equations indicated that measurement of shoot density, particularly in winter months, yielded relatively little unique information in addition to that provided by a total biomass estimate.
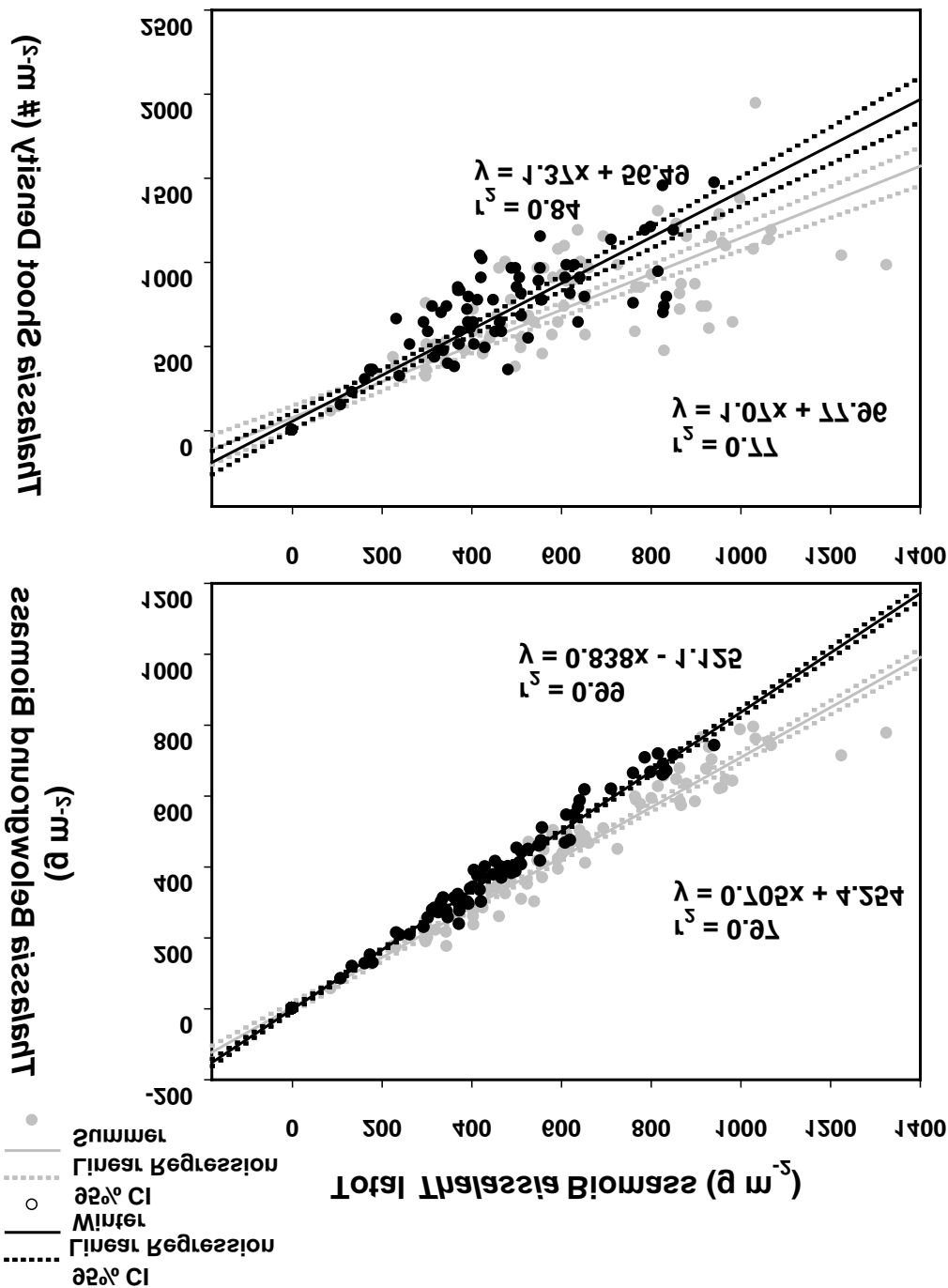
Figure I.42 - Linear regression of total *Thalassia* biomass with *Thalassia* shoot density (top panel) and *Thalassia* belowground biomass (bottom panel).

In addition to positive correlations with shoot density, blade length and blade width, aboveground *Thalassia testudinum* biomass was strongly correlated with other seagrass characteristics. As expected, aboveground biomass is strongly related to belowground

biomass ($r_s = 0.67$; $p < 0.0001$), and by definition, it is correlated with total *T. testudinum* biomass ($r_s = 0.81$; $p < 0.0001$). Surprisingly, correlation between aboveground biomass and root:shoot ratio was relatively weak ($r_s = -0.32$). Stronger correlations were found between tissue nutrients and aboveground biomass. Correlation coefficients between aboveground biomass and both C:N and N:P were 0.56 and 0.60, respectively ($p < 0.0001$).

The correlation coefficient between belowground biomass and total *Thalassia testudinum* biomass was among the largest of the comparisons made. Spearman correlation between the two autocorrelated variables was 0.97 ($p < 0.0001$). The strength of this relationship indicated both the dominance of belowground biomass relative to aboveground biomass and the independence of belowground biomass from seasonal effects. Total biomass was not strongly correlated with percent cover, root:shoot ratio, or tissue nutrients. ($r_s < 0.4$).

Linear regressions were performed on the relationship between total *Thalassia testudinum* biomass and belowground biomass. For all data, the relationship between total biomass and belowground biomass was:

$$\beta = 0.79(\tau) - 12.949$$

where β = belowground biomass and τ = total biomass ($r^2 = 0.98$). Slight seasonality was apparent (Figure I.42), although the relationship was not very different. For winter, the equation was:

$$\beta = 0.84(\tau) - 1.13$$

Linear regression explained 99% of the total variance in the winter biomass data ($r^2 = 0.99$). Regression of summer biomass data yielded the equation:

$$\beta = 0.705(\tau) + 4.254$$

The equation explained 97% of the variation in the data ($r^2 = 0.97$). These equations indicated that measurement of total biomass was a reliable predictor of belowground biomass regardless of the time of sampling.

Tissue nutrients were not correlated with percent cover. Spearman correlation coefficients for percent cover with both C:N and N:P were -0.12 and 0.14, respectively (p > .46). Nutrient ratios were not strongly correlated with each other (r_s = -0.06; p = 0.73). Raw percent cover scores were compared with three abundance estimates derived from Braun-Blanquet classification. For each species, density calculated from Braun-Blanquet scores had the highest correlation with raw percent cover score. Spearman correlation coefficients between the raw percent cover and the corresponding density were at least 0.99 (p < 0.0001). Comparison of correlation coefficients among the various environmental and seagrass variables and the four percent cover statistics indicated that there was little difference among correlations for the percent cover estimates. For example, Spearman correlation coefficients for the percent cover estimates and chl *a* were 0.17, 0.16, 0.20, and 0.15 for percent cover, density, abundance, and frequency of *Thalassia testudinum*, respectively. Although the magnitudes were slightly different, the interpretation was identical. The same pattern arose for correlations with all other variables.

Based on the correlations, total biomass appeared to be the best measure of seagrass condition. Although aboveground biomass was more strongly correlated with blade characteristics, total biomass was less seasonally variable. Samples collected at any time of the year were easily interpreted. Additionally, total biomass was more strongly correlated with shoot density than either aboveground or belowground biomass, permitting reasonable inferences to be made regarding shoot density. The tight correlation between total biomass and belowground biomass indicated that regression equations for calculating belowground biomass from total biomass could be used without separating seagrass tissues. The strong associations between total biomass and other seagrass variables, reduction of seasonal influence and ease of sample processing suggest that total biomass is the most informative, interpretable, and economical measure of seagrass condition.

Although tissue nutrient contents were poorly correlated with most variables, monitoring C:N ratios is recommended due to its potential to illustrate changes in nutrient availability. Strong seasonality in C:N values was characterized in this study. That characterization provides the background for future comparisons. C:N values that fall outside the range measured for sites

during this study may indicate changes in nutrient availability that can threaten seagrass condition.

Multivariate Assessment of Indicators

Multiple linear regression and nMDS were used to evaluate the combined ability of environmental and seagrass variables to explain variability in seagrass indicators. Because total biomass represented the best seagrass condition indicator, it was the only response variable used in regression analysis. Below- and aboveground biomass, root:shoot, and shoot density measures were excluded from consideration as possible independent variables. Univariate analyses showed that density and biomass were strongly correlated and, therefore, were problematic with regard to multicollinearity. Furthermore, all three biomass estimates and root:shoot were calculated from the same data so assessing the ability of one biomass measure to account for variability in another provided little unique information.

Multiple regression on total *Thalassia testudinum* biomass revealed low coefficients of multiple determination. The greatest reduction in total variation associated with two regressors was 30% ($R^2 = 0.30$) by %SI and salinity (Table 7). Third and fourth order models with the lowest Akaike's Information Criterion (AIC) accounted for little further variation in the dependent variable. The third order model with %SI, salinity, and blade width as independent variables had an R^2 of 0.34. In the four parameter model with percent cover of *Syringodium filiforme*, %SI, C:N, and blade width, R^2 was 0.40. Addition of further parameters in the model yielded modest increases in explained variance that likely resulted simply from increasing the number of parameters in the model. The model with the lowest AIC had 10 independent variables and accounted for 58 percent of the variance in total biomass (Table 7).

Table I.7 - Regression models for the response variable total *Thalassia testudinum* biomass.

Model type	Independent variables	AIC	R^2
1st order	%SI	733.07	0.17
	Blade length	734.24	0.16
	Salinity	734.74	0.15
2nd order	%SI, salinity	723.65	0.3
	%SI, blade length	724.41	0.29
	%SI, blade width	725.35	0.28
3rd order	%SI, salinity, blade width	721.07	0.34
	%SI, NH_4^+, blade width	721.26	0.34
	% sand, %SI, salinity	721.37	0.34
4th order	cover *Syringodium*, %SI, C:N, blade width	717.26	0.4
	cover *Syringodium*, %SI, NH_4^+, blade width	717.53	0.39
	% sand, %SI, salinity blade width	717.55	0.39
Lowest AIC	cover *Syringodium*, cover Bare, % silt, salinity, temperature, PO_4^{3-}, C:N, N:P, drift algae, blade width	704	0.58

Ordination of seagrass cover data revealed a triangular spread of sampling points with a three way gradient in seagrass cover (Figure I.43). Points in the upper right portion of the plot were sites characterized by high cover of *Thalassia testudinum* (Figure I.44). Moving to the lower right corner, cover of *T. testudinum* decreased, while cover of *Halodule wrightii* increased (Figure I.44). The third corner of the triangle represented sites with little or no seagrass cover (Figure I.44).
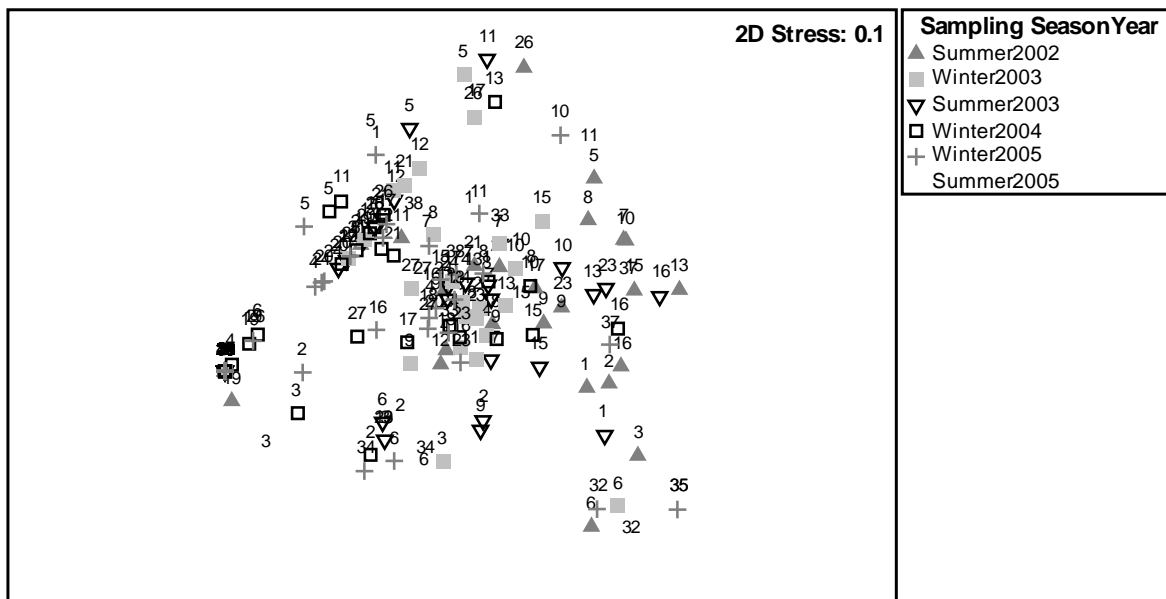
Figure I.43 - NMDS plot based similarity of seagrass community structure (bottom panel) for each sampling date by site combination.
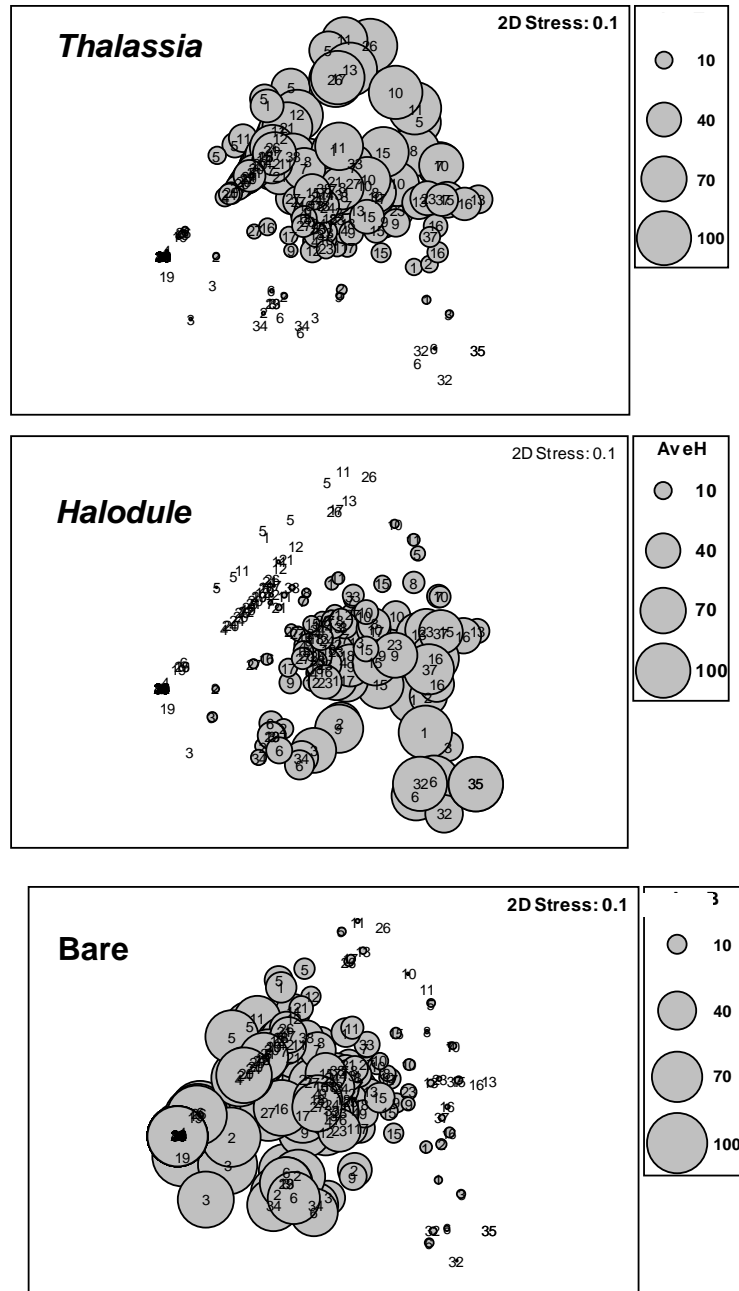
Figure I.44 - NMDS plots based on similarity of seagrass community structure for each sampling date by site combination (as in Figure I.43) with percent cover overlays. *Thalassia* – top panel; *Halodule* – middle panel; Bare – bottom panel.

Initial analysis of environmental data required removal of sediment grain size and tissue nutrient data due to missing values for 2 entire sampling dates. Ordination of the environmental data generated a large, relatively non-descript cluster (Figure I.45). The cluster was slightly bifurcated as samples from summer and winter collections formed loose associations. Several points were distant from the main cluster; however, these positions

appeared to result from an atypical value for one environmental variable at a given site and sampling date. For example, site 4 in winter 2003 had an unusually high value of NO_3^- (6.01 $\pm$ 0.05 μM). It is important to note that the stress value was reasonably high (stress = 0.22), making interpretation of the 2-dimensional plot tenuous.
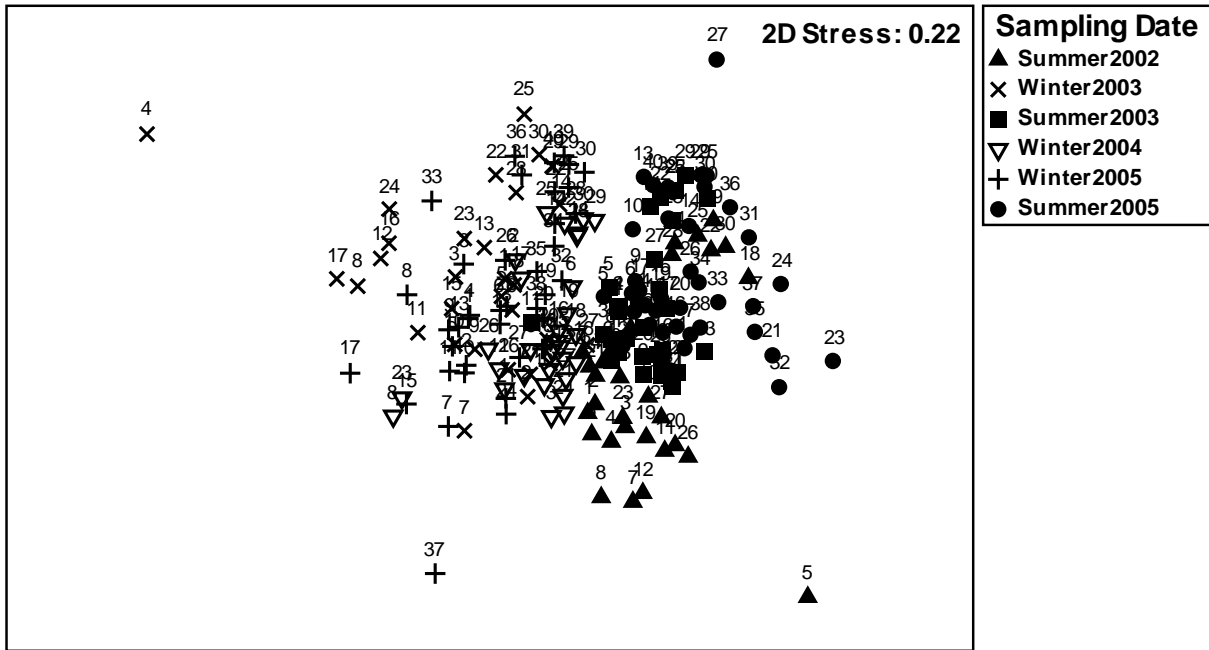
Figure I.45 - NMDS plots based on Euclidean distance among environmental conditions for each sampling date by site combination

Relation of environmental data to seagrass cover data identified depth and drift algal biomass as the combination of variables that produced the largest rank correlations between the environmental and seagrass cover sample similarities (r_s = 0.42). The strength of relationship between these variables and seagrass cover can be assessed by performing NMDS using only these variables and comparing the resulting ordination with that derived from the seagrass cover data. Depth and drift algal biomass was the best combination for grouping sites in a manner that was similar to those produced in ordination of the seagrass cover data (Figure I.43), although the magnitude of the correlation coefficient was not large. Ordination of sites based solely with drift algal biomass and depth as variables yielded a pattern somewhat similar to seagrass cover (Figure I.46).

The second best combination for relating the environmental variables to the seagrass community structure involved four variables: %SI, dissolved oxygen, depth, and drift algal biomass. The correlation for this model was slightly weaker ($r_s = 0.41$) than for the 2-variable model. Like the seagrass cover ordination, the 4-variable combination generated 2 distinct groups based on depth (Figures I.47 and I.48). Sites greater than 1.7 m deep were grouped separately from shallow sites. The addition of %SI and dissolved oxygen, however, did not distinguish any further groupings.

Figure I.46 - NMDS plots based solely on Euclidean distance among depth and drift algal biomass data for site by sampling date combinations. no overlay – top panel; depth overlay – middle panel; drift algal biomass overlay – bottom panel.
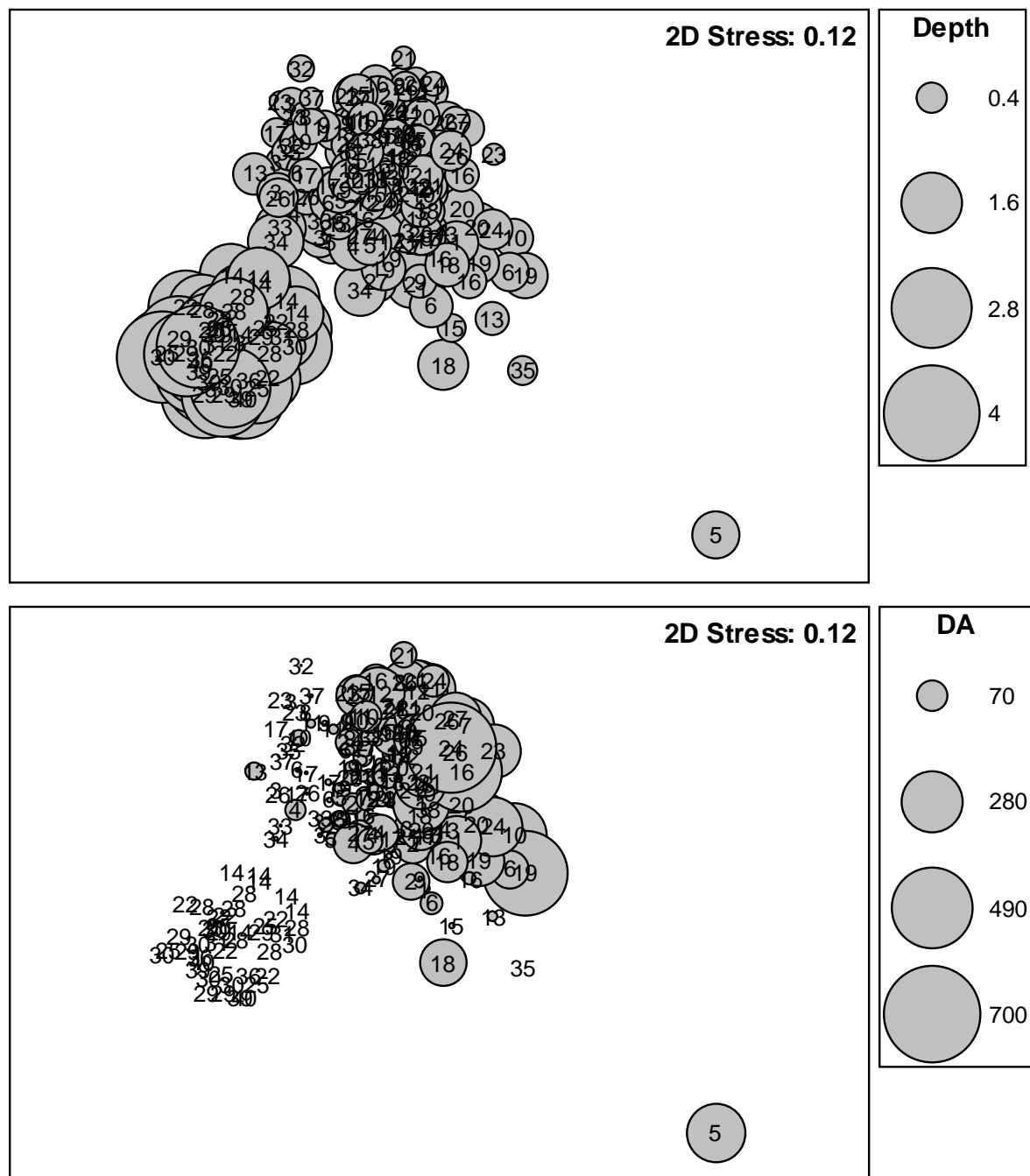
Figure I.47 - NMDS plots based solely on Euclidean distance among depth, drift algal biomass, %SI, and dissolved oxygen data for site by sampling date combinations. depth overlay - top panel; drift algal biomass overlay - bottom panel.
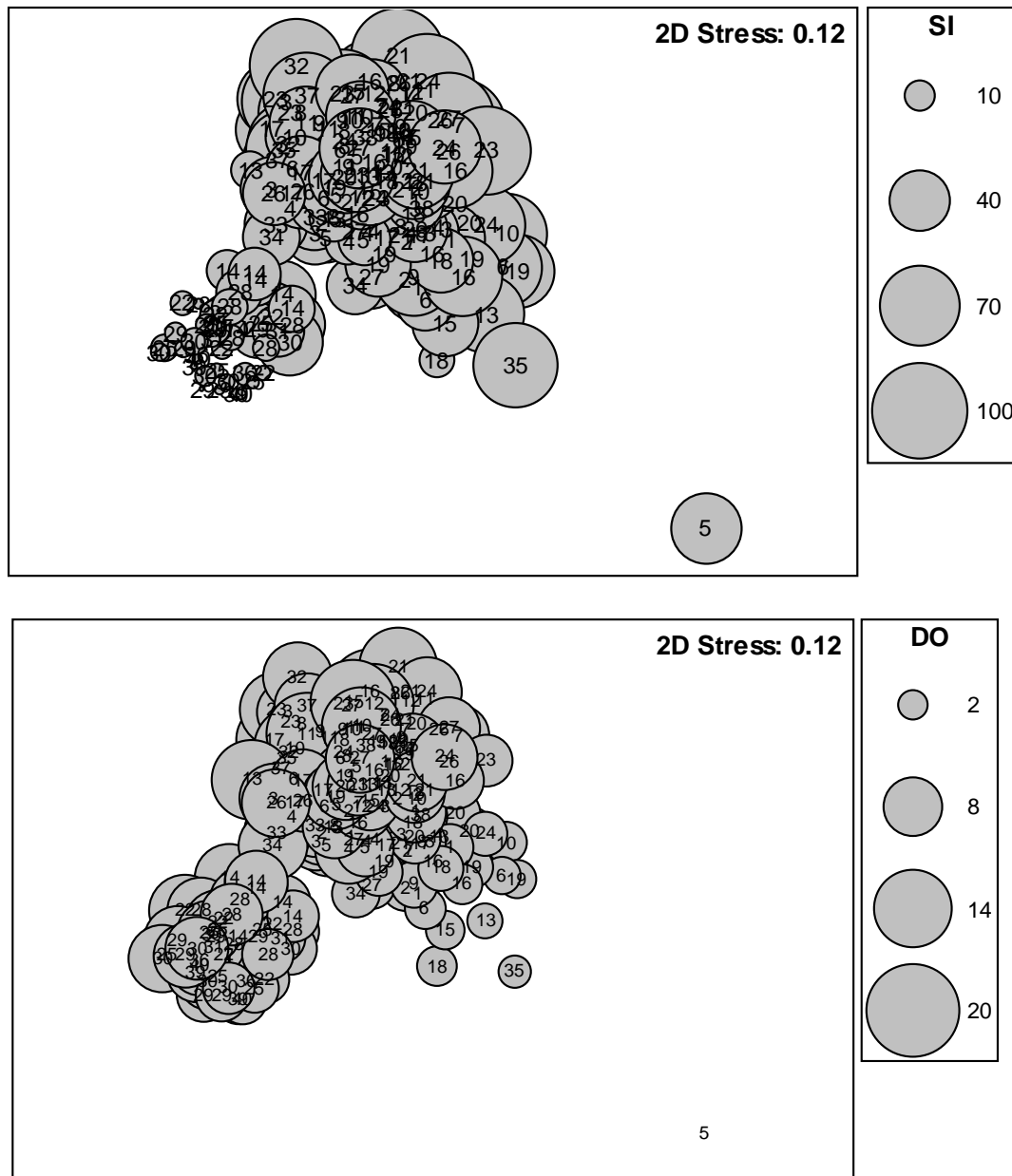
Figure I.48 - NMDS plots based solely on Euclidean distance among depth, drift algal biomass, %SI, and dissolved oxygen data for site by sampling date combinations. %SI overlay - top panel; dissolved oxygen - bottom panel.

Because depth dominated the ordination and deep sites never had seagrass, sites greater than 1.7 m deep were removed from analysis to examine their influence on nMDS. Removal of deep sites did not greatly alter the ordination of environmental data (Figure I.49). Comparisons with the ordination from seagrass cover, however, revealed TSS and PO_4^- as the combination that produced the greatest matching coefficient ($r_s = 0.11$; Figure I.50). The low

matching coefficient suggested that none of the variable combinations provided a good match between the seagrass cover and environmental data.
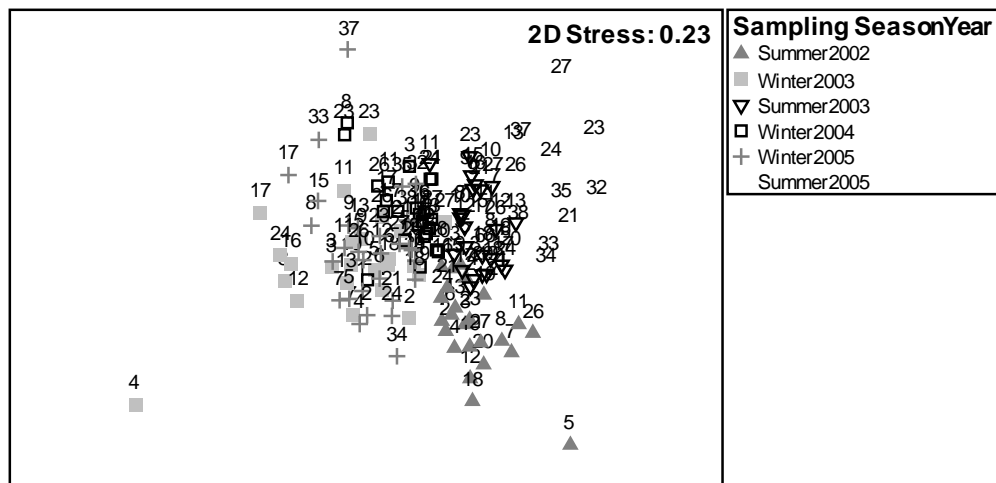
Figure I.49 - NMDS plot based on Euclidean distance among environmental variables for all sites < 1.7 m deep (top panel).
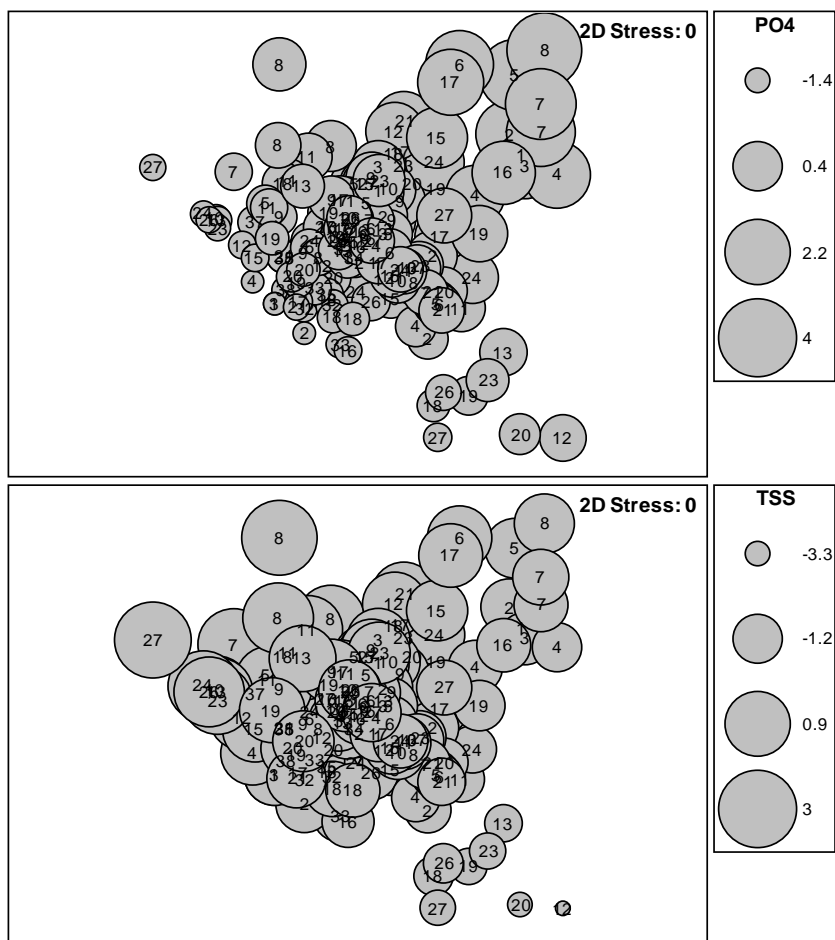
Figure I.50 - NMDS plots based solely on Euclidean distance among PO_4^{3-} and total suspended solids data for site by sampling date combinations. PO_4^{3-} overlay - top panel; total suspended solids - bottom panel.

To investigate the potential of tissue nutrient contents as an important indicator of seagrass cover, the summer 2003 and winter 2004 sampling dates were removed from the analysis. Ordination of the environmental data yielded a large cluster subdivided into portions by sampling dates (Figure I.51). Matching between the seagrass and environmental data ordinations identified a 4-variable combination with the strongest matching coefficient (r_s = 0.24). The 5 variables were similar to variables that were selected in the previous analyses. The combination included dissolved oxygen, depth, PO_4^-, and drift algal biomass. As a result, tissue nutrient content did not contribute greatly in distinguishing among the seagrass community structure at the sites.
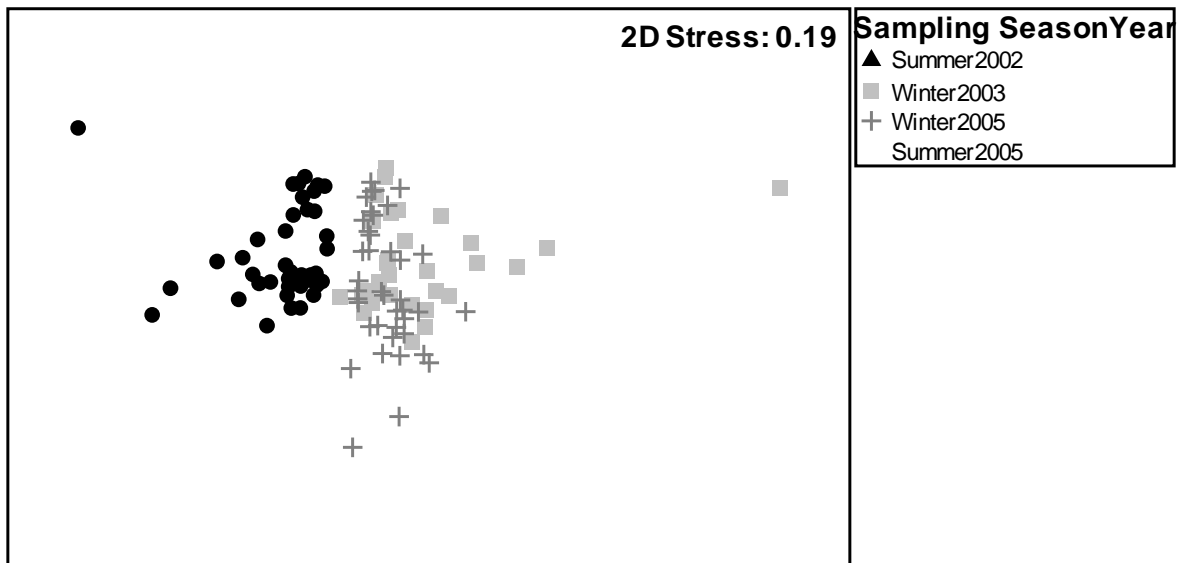
Figure I.51 - NMDS plot based on Euclidean distance among environmental variables for site by sampling date combinations (excluding Summer 2003 and Winter 2004).

Discussion

Identification and development of ecological indicators is of primary importance in efforts to monitor and manage natural resources of societal value. The objective is to find reliable signals that identify the current status of natural resources and permit prediction of their future state. Useful ecological indicators characterize the ecosystem and its state as well as identify possible sources of stress in the system (Jackson et al. 2000).

The EPA has identified a four step process in the evaluation of ecological indicators for incorporation into monitoring programs. The steps involve determining the conceptual relevance, feasibility of use, the response variability, and the utility and ease of interpretation (Jackson et al. 2000). Each of these steps has been considered in our search for effective indicators to be used in a monitoring program for seagrass habitats in Texas.

Parameters that have been evaluated as possible indicators were classified as either stressor or condition indicators (R-EMAP publication). Stressor indicators are those that quantify environmental variables that may elicit changes in ecological resources (e.g. nutrient concentrations), whereas condition indicators measure the state of an ecological resource that provides valued ecosystem services (e.g. cover of seagrasses) (REMAP publication). The following discussion evaluates the suitability of the various stressor and condition indicators examined in the course of this study for their relevance, feasibility, variability, and utility as ecological indicators for a seagrass monitoring program.

Stressor indicators

<u>Water Quality</u>

Light

Clearly light is one of the most important resources for submerged aquatic vegetation (SAV), and light reduction is implicated in seagrass loss around the globe. As a result, the relationship between light and seagrass performance has been extensively studied along with the factors affecting its availability for seagrass (Orth and Moore 1983; Gallegos 1994, 2001;

Onuf, 1994; Batiuk et al. 2000; Burd and Dunton 2001). Indeed numerous models have been developed to predict the maximum depth of SAV based on light measurements (Gallegos 1994, 2001; Burd and Dunton 2001; Kemp et al. 2004). Two commonly used measures of the light conditions in seagrass habitat are light attenuation (K_d) and %SI. Both of these parameters quantify the loss of light energy available to seagrass due to absorption and scatter by the water itself, total suspended solids, colored dissolved organic matter (CDOM), and phytoplankton, and they therefore provide an indication of the available PAR for seagrass photosynthesis.

Light attenuation ranged from 0.3-2.5 m^{-1} in East Flats, 0.003-5.5 m^{-1} in Redfish Bay, and 0.5-14.5 m^{-1} in lower Laguna Madre, while %SI ranged from 95-0.1% in East Flats, 99-0.2%, and 80-4% in lower Laguna Madre. As mentioned previously, seagrasses were absent at sites in Redfish Bay and East Flats >1.7m deep. These sites all had average %SI values less than the 18-20% threshold established previously for the area (Onuf, 1994; Dunton, 1996). However, site 14 in Redfish Bay (1.7 m) also lacked seagrass, but the average %SI was 26±15%. Based on %SI, it appears that enough light is present at site 14 in Redfish Bay to support seagrass growth; however, no seagrass was present. Therefore, it is possible to conclude that some factor other than light prevents seagrass colonization at the site. Alternatively, relatively few instantaneous light measurements may not accurately reflect the light availability experienced at the site over time. Continuous measurement at the site might reveal extended exposure to light levels below the minimum threshold for seagrass. Therefore, extrapolation of the light regime experienced at a site from a few instantaneous measurements is somewhat tenuous. Additionally, both estimates of light availability were weakly correlated with seagrass condition indicators. At sites where light permits seagrass growth, light availability was not a strong predictor of seagrass condition. The most effective use of light attenuation and %SI appears to be as a delimiter of the distribution limits at which seagrass ceases to occur. The correlation between %SI and depth suggests that tracking the light availability and depth of the deepest edge of seagrass beds may be an effective means of assessing light-mediated changes in seagrass distributions within the three bays.

Problems associated with light measurements also arise when assessing the spatial variability of these parameters. Estimates of light attenuation and %SI are dependent upon the amount of suspended solids and phytoplankton. These variables may fluctuate over short time scales due to differences in wind speed, wind direction, and nutrient availability. Because only 3-7 sites could be sampled in a day, measurements of light availability may differ substantially between sites based on differences in weather and nutrient loading. Continuous monitoring or sampling all sites in the shortest time possible would minimize daily variability in light measurements.

Despite the complexities involved in relating light to seagrass status, the conceptual relevance and feasibility of light measurements make their inclusion in a seagrass monitoring program appealing. Both light attenuation and %SI are readily determined from routine depth and light measurements. Long-term changes in light availability are also easily quantified with continuous measurements recorded by in situ quantum sensors and data-loggers that are relatively easy to maintain. Several Li-Cor light sensors with associated data loggers could be deployed at several locations throughout each bay. Alternatively, more affordable HOBO Light Intensity Loggers may be deployed at each site to concurrently monitor light at all locations. While this equipment costs several thousand dollars, it could provide valuable information regarding changing light regimes in Texas seagrass beds, and the light data that is obtained can be used to verify the effectiveness of existing models to make geographic comparisons between systems.

Dissolved oxygen

Monitoring dissolved oxygen concentration is desirable for assessing the onset of eutrophication-induced periods of anoxia that can have devastating effects on benthic communities. In this study, dissolved oxygen was highly variable and poorly related to any of the sea grass condition indicators. Major problems arise in interpreting dissolved oxygen concentrations as hourly and daily variation is high due to effects of time of day and daily irradiance levels. Measurements taken at different sites on the same day are likely to vary substantially (as much as 2-3-fold) from the morning to the afternoon. Also, anoxia is likely to occur during the night when respiration rates are highest and photosynthesis stops. As a

result, a firm understanding of spatial and temporal differences in dissolved oxygen concentrations cannot be obtained without measuring diel variation and appropriate corrections for the time of sampling. Because of the strong temporal dependence of dissolved oxygen concentrations, it is not feasible to measure DO at multiple sites in a given day. It would be more effective to have one to several data sondes making measurements at key locations within a bay. Although spatial resolution would be lost, critical nighttime concentrations and diel variation could be readily observed to identify any periods of low oxygen concentrations that may threaten seagrass communities.

Conductivity, salinity and temperature

Salinity and temperature are easily measured parameters that require little instrumentation and no sample processing. Salinity is typically measured with a refractometer or is calculated from conductivity measurements. Most data sondes are equipped with a conductivity probe and provide both conductivity and salinity measurements in their output. Because of the ease of measurement, conductivity, salinity, and temperature are cost-effective parameters to monitor. Despite their ease of sampling, their potential to stress seagrass communities is relatively low except at extreme values. All 5 seagrass species in Texas are euryhaline and easily tolerate the range of salinities found in lower Laguna Madre, East Flats, and Redfish Bay. Additionally, temperature stress is only likely during persistent cold or extreme high temperatures occur (Zieman 1975; McMillan 1979). In shallow areas, *Thalassia* may experience temperature stress and desiccation during low tide; however, *Halodule* appears to tolerate these conditions without major physiological consequences. The main benefit to measuring these parameters is the ability to detect local climatological influences from precipitation and temperature changes. Monitoring salinity and temperature at a deep and a shallow site would allow for the characterization of the relative influences of fresh and sea waters as well as the range of temperature variation among the sites. Shallow sites are likely to be characterized by greater freshwater influence and greater temperature ranges while deep sites are likely to be more marine influenced with little temperature variation.

Total suspended solids

Total suspended solids are tightly linked to light attenuation and availability. The quantity of suspended solids in the water column absorbs and scatters light as it travels through the water column. As a result of this effect on light transmittance, measurement of TSS has clear relevance to seagrass condition. In this study, TSS was positively correlated with light attenuation, but the strength of association was weak ($r_s = 0.31$). Despite the relevance of TSS to light conditions, TSS is highly variable in both space and time, and as a result, values may be difficult to interpret. The quantity of TSS may change quickly as a function of wind speed and direction, sediment grain size, and depth of the site. Shallow sites with a longer fetch are more likely to experience sediment resuspension and increased TSS. Although the prevailing wind direction is generally predictable for a given season (generally NW in the summer and SE in the winter), wind speed varies daily, making comparison of TSS samples separated by several weeks is tenuous. As was the case with light attenuation and availability, high daily variability suggests that instantaneous measures of TSS may not accurately reflect the cumulative effect of suspended particles on seagrass communities at a given site. Rather than collecting TSS samples at various sites on different days, a more effective approach may be to collect samples at strategic locations (e.g. shallow sites with long fetches relative to prevailing winds) in one day. Sampling in temporal proximity may minimize the variability associated with changing wind speeds and direction. Alternatively, continuous measurements with a turbidity meter deployed on a multiparameter data sonde would permit estimation of the duration of turbid conditions associated with high TSS. Coupling these measurements with light recordings at the seagrass canopy would allow estimation of the effect of high TSS values on the light reaching the seagrass.

Water column nutrient concentrations

The effects of eutrophication on seagrass communities are of great concern as elevated nutrients contribute to seagrass declines (Cambridge and McComb 1984; Orth and Moore 1984; Short and Wyllie-Echeverria 1996). The impact of nutrient enrichment is generally mediated indirectly through increases in phytoplankton, epiphyte, and/or drift algal growth that decreases light available to seagrass. Because excess nutrients are typically delivered as

dissolved inorganic forms, measurement of water column nutrient concentrations is an intuitively appealing parameter to measure in any seagrass monitoring program. In this study, NO_3^- values tended to be relatively low overall (<1µM in most cases) and exhibited relatively little spatial and temporal variability. PO_4^{3-} and NH_4^+ concentrations showed a slight gradient of decreasing values from north Redfish Bay to south Redfish Bay. PO_4^{3-} concentrations were <1µM in all cases, but NH_4^+ concentrations occasionally reached 5 µM in north Redfish Bay.

Unlike many of the other water quality variables, PO_4^{3-} and NH_4^+ were correlated with several seagrass parameters. PO_4^{3-} concentration was positively correlated with cover of *Halophila* and the tissue N:P ratios of *Thalassia* leaves, and it was negatively correlated with *Thalassia* blade width. NH_4^+ was negatively correlated with blade length and aboveground biomass. These correlations suggest the potential exists for these variables to serve as possible indicators of seagrass condition.

One logistical consideration for using water nutrient concentrations as indicators is the feasibility of large scale sampling efforts. Water samples are relatively easy to take, and automated analyses are common practice; however, analysis may cost $10-20 per sample. If water quality is monitored at many sites at frequent intervals, the costs may become prohibitive. Additionally, there is reason for caution in interpreting instantaneous measurements of water column nutrients. Instantaneous measurements may not accurately reflect actual loading rates at a given site (Tomasko et al. 1996) and may miss episodic events that greatly alter nutrient concentrations on a short-term basis (e.g. high river inflow associated with severe rain events). Nutrient uptake by producers may occur rapidly (Thomas et al. 2000; Cornelison and Thomas 2002), lowering residual concentrations in the water column. In light of these limitations, it may be more effective to determine actual nutrient loading rates to a particular location or measure parameters that integrate nutrient conditions at a site over time. Tissue nutrient concentrations, epiphyte biomass, and drift algal biomass have all been suggested as potential indicators of nutrient loading. While the conceptual relevance of measuring nutrient concentrations is high, the feasibility and ease of interpretation must be strongly considered when incorporating such measurements into a seagrass monitoring program.

Chlorophyll a

Chlorophyll *a* is commonly used as an estimate of the standing stock of phytoplankton in the water column. In the classic eutrophication paradigm, increases in phytoplankton can decrease light reaching benthic communities and contribute to anoxia associated with nutrient enrichment. Although the functional link between chlorophyll *a* concentrations and seagrass condition is well established, chlorophyll *a* in this study was not strongly correlated with nutrient concentrations, light attenuation, %SI, or seagrass condition indicators. Despite the lack of relationship with other indicators, previous work, particularly in Laguna Madre has demonstrated the effect that phytoplankton blooms can have on the light regime and consequently the seagrass community (Onuf 1996). Chlorophyll *a* samples are easy to collect and analyze, and they are relatively cost effective. Regular monitoring of chlorophyll would provide valuable information regarding the possible changes in light regime that is less sensitive to daily variation in conditions as TSS measurements.

Sediment Quality

Sediment grain size

Analysis of sediment grain size revealed correlations between the total organic carbon content of the sediments as well as the percent cover and shoot density of various seagrass species. Sandy sediments were negatively correlated with total organic carbon and shoot density of *Thalassia*. On the other hand, percent sand was positively correlated with *Syringodium* cover, while percent silt was negatively correlated with *Syringodium* cover. The value of these relationships and estimates of sediment grains size for a seagrass monitoring program is ambiguous because the presence of seagrass modifies the sedimentation rate and sediment composition. Seagrasses reduce water velocity in the bed; thereby, increasing particle retention within the beds (Gacia et al. 1999). In particular, lower water velocities permit retention of smaller grain sizes which therefore alters the sediment composition and organic content (Kenworthy et al. 1982). As a result, correlations between sediment composition and seagrass condition indicators may not reflect the influence of sediment grain size on seagrass condition but, rather, reflect the effects of seagrass abundance

and composition on the sediments. Indeed, the two dominant seagrasses, *Thalassia* and *Halodule*, could be found in a broad range of sediment types.

From a logistical standpoint, the feasibility of performing sediment grain size analysis as part of a monitoring program must be considered. Although the analysis is straightforward and requires few expendables, the process is time consuming. Sediment samples are easily collected and stored until analysis commences, but processing takes several weeks of dedicated labor. Processing 120 samples per sampling period required 3-4 weeks to complete. Considering the time investment and difficulty in interpreting relationships with condition indicators, we recommend elimination or reduced sampling frequency of sediment composition.

Total organic carbon

The organic carbon content of sediments reflects both the sedimentation of fine organic particles from the water column as well as the accumulation of detritus from producers and consumers. TOC of the sediments was positively correlated with the relative proportion of silt, clay, and rubble, but it was negatively correlated with the amount of sand. TOC was not strongly correlated to any seagrass condition indicators despite the expected association with detrital input from seagrasses. Although TOC analysis is cost-effective and straightforward, organic carbon content of the sediments contributed little to our understanding of seagrass dynamics in this study.

Sediment porewater ammonium concentration

Of the various sediment quality parameters measured, porewater NH_4^+ has the greatest potential to provide information regarding the status of seagrass in Texas. Not only does ammonium in the sediments affect the carbon and nitrogen dynamics of seagrass (Lee and Dunton 1999), but porewater NH_4^+ concentrations can reach toxic levels (Peralta et al. 2003). Additionally, these high concentrations may occur under thick accumulations of drift algae that are believed to be symptoms of eutrophication that may contribute to seagrass mortality. Although porewater NH_4^+ was not correlated with drift algal accumulations or seagrass condition indicators, the lack of relationship may not be indicative of no effect. High

porewater NH_4^+ concentrations associated with drift algae are likely to be localized to areas under the accumulations. Such an association may have contributed to the high variability among samples collected within a site. Because collection of sediments for analysis was haphazard and not designed to explicitly characterize conditions associated with different benthic features, any association between drift algal abundance and porewater NH_4^+ was likely missed.

Although porewater NH_4^+ concentration is clearly relevant to seagrass monitoring, the high degree of spatial variability suggests that routine analysis may produce results that are difficult to interpret. Porewater NH_4^+ did not contribute significantly to our understanding of seagrass dynamics in Redfish Bay and East Flats, and it is unlikely that continued monitoring via random sampling at sites would alleviate problems associated with the high variability. The most effective use of porewater NH_4^+ analysis may be in targeted studies examining the possible role of porewater NH_4^+ concentration in changing seagrass condition.

<u>Nutrient Response Indicators</u>

Epiphyte biomass

Epiphyte communities consist of a variety of bacteria, algae, and invertebrates that may intercept light and compete for nutrients with seagrass. Because algae have higher nutrient uptake rates, grow on seagrass leaf surfaces, and have rapid growth rates, they have the potential to respond rapidly to elevated water column nutrient concentrations in the absence of intense grazing (Silberstein et al. 1986; Wear et al. 1999). Monitoring epiphyte biomass may provide an indication of changing nutrient availability in the water column and light reaching the seagrass leaf surface. Despite the connection between epiphyte biomass and nutrient availability, epiphytes in this study were not strongly correlated with water column nutrient concentrations. Epiphyte values tended to be greatest in winter, perhaps due to lower grazing rates and reduced leaf elongation rates of the seagrass. Epiphyte biomass was not related strongly to seagrass condition indicators.

Epiphyte sample processing is a simple procedure; however, it is a meticulous process that requires much care in handling the grass blades. In addition, standardization of the area

sampled and age of the seagrass leaf is required. Although it is not difficult to standardize the area to be scraped, ensuring the age of the leaf is more problematic. Relative position on the leaf must be standardized as well as the age of the leaf. The relative age of seagrass leaves can be determined readily, but it is impossible to know how leaf age compares between shoots. Disparities in leaf ages do introduce some uncertainty and likely some variability into epiphyte biomass interpretation and estimation. Because of these challenges and the meticulous nature of sample processing, epiphyte sampling is not recommended for a long term monitoring project.

Drift algal biomass

Drift algal accumulations are associated with elevated nutrient inputs into estuarine systems and have been implicated in seagrass declines (Lapointe et al. 1994; Valiela et al. 1997; Hauxwell et al. 2001; McGlathery 2001). Because of this association, drift algal biomass may be an effective indicator of elevated nutrient loading. In this study, drift algal biomass was highly variable ranging from 0 to >600 g m^{-2}. Drift algal accumulations tend to be patchy in distribution, but appeared frequently in south Redfish Bay and Terminal Flats. Although drift algal biomass was not strongly correlated with other stressor or condition indicators, site by site comparisons suggest dense accumulations occurred in patchy seagrass beds (Figure I.52). Within these accumulations, ammonium concentrations may be high, dissolved oxygen may be low, and sulfides may increase to toxic levels. It is not clear from our data whether the drift algae created underlying bare patches by shading light or altering the water chemistry, causing seagrass mortality. Alternatively, the patches may have previously existed and the drift algae simply collected in bare patches as a result of hydrodynamic processes. Distinguishing between these scenarios would require careful experimentation to determine the factors contributing to drift algal accumulation, the residence time of the accumulations, and their potential for causing seagrass mortality.

Given the possible links between drift algal accumulations, elevated nutrients, and seagrass mortality, drift algal biomass estimations should be included in a monitoring program. Extensive algal accumulations are know to occur throughout Redfish Bay and East Flats and may represent a response to changing nutrient regimes. Drift algae may integrate nutrient

conditions at a site over time and may therefore be better indicators of actual loading rates than instantaneous measurements of water column nutrients. In addition, collecting, drying, and weighing drift algae require little equipment and relatively short processing times. Wet weight determinations may be sufficient, but proper comparison to dry weights should be made to ensure a strong relationship between wet weight and actual biomass. Unless information regarding the community composition is considered important (e.g. to assess the relative amounts of green algae present), estimates of total drift algal biomass are probably sufficient for seagrass monitoring purposes.
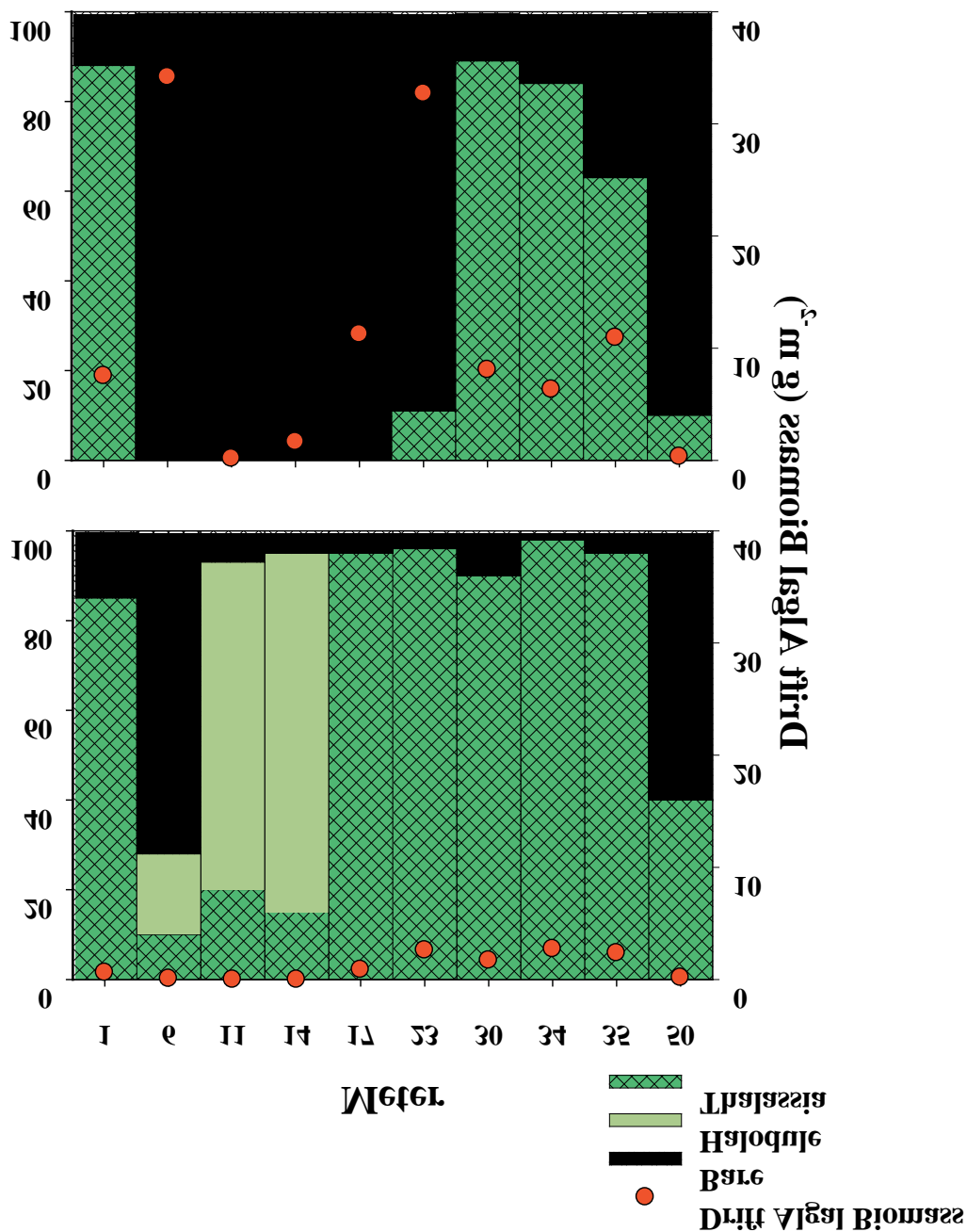
Figure I.52 - Percent cover of seagrass and drift algal biomass at each meter mark sampled at sites 21 (top panel) and 8 (bottom panel) in Redfish Bay.

Tissue Nutrient Content

Interest in measuring tissue nutrient content stems from the concept that the elemental composition of seagrass leaf represents an integration of nutrient availability and growth over the life span of the leaf. While instantaneous measures of water column nutrients provide a

snap shot of the nutrient conditions experienced by seagrass, it is believed that tissue nutrient content is a function of the actual nutrient loading into the seagrass system. According to this premise, C:N and N:P ratios should decline with increasing nitrogen and phosphorus availability, respectively. During the course of this study, CNP ratios varied between sampling dates and sites. Low CN values (<12) were found at several sites in south Redfish Bay in winter (January and February) of 2003 and high values (>23) were found at several sites throughout Redfish Bay in summer 2005. Although it is difficult at this point to determine how anomalous these data points are, repeated measurements at the extremes of the range may suggest changes in nutrient loading. The widespread use of CNP ratios as indicators of nutrient availability, the ease of sample collection, and the stability of samples over time arguer for inclusion of tissue nutrient content in a monitoring program. However, determinations of P content are somewhat labor intensive (requiring several days to process a batch of 40 samples), involve the use of hazardous chemicals, and are sensitive to contamination. To minimize contamination, dedicated glassware and frequent acid washing are required. Another drawback to tissue nutrient content is the cost associated with analysis. P analysis requires chemicals and expendables, while CN analysis requires oxidation in an elemental analyzer (~$6 per sample). While the data obtained during this analysis is likely to be informative, if cost is a critical issue, CNP content of seagrass tissue may be unfeasible.

Condition Indicators

Seagrass Response Indicators

Leaf morphometrics

Leaf length and width are commonly measured parameters of seagrass response to environmental quality. Leaf characteristics may change in response to salinity (Dawes et al. 1985), nutrients (Lee and Dunton 2000a), light (McMillan and Phillips 1979), and turbidity (Lee and Dunton 1997). Hackney and Durako (2004) effectively demonstrated the utility of leaf morphometrics as indicators of seagrass condition. In their study, size-frequency patterns of leaf characteristics differed inter-annually and spatially as a function of environmental variability. In our study, *Thalassia* leaf morphometrics also varied temporally and spatially. Analysis revealed an interactive effect of both sampling date and site. This result suggests

that changes in leaf length and width may occur relatively quickly in response to changing environmental conditions and are sensitive to subtle differences in environmental parameters among sites. Blade length exhibited greater seasonal variability than blade width, indicating that being aware of the seasonal variation in length is critical to interpreting seagrass response to environmental conditions (Table 8). The variations in length and width were negatively correlated with water column NH_4^+ and PO_4^{3-} concentrations, suggesting some sensitivity in these parameters to nutrient loading. Because of the differences among sites, the correlations with water quality parameters, and the demonstrated value as an indicator in the literature, leaf morphometrics appear to be potentially valuable indicators in a seagrass monitoring plan. In addition, the measurements are easily measured on blades taken in seagrass core samples and can be processed quickly during processing the cores. Finally, there is virtually no cost associated with measuring leaf morphometrics.

Table I.8 - Summary statistics for condition indicators at East Flats, Lower Laguna Madre, and Redfish Bay.

Location	Season	Species	Blades/shoot						Blade Width (mm)						Blade Length (cm)					
			mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile	mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile	mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile
East Flats																				
	S	Halodule	2.9±0.7	3	2	2	3	4	0.9±0.2	0.9	0.7	0.8	1	1.2	16.8±6.3	16	9	11.1	22.3	25.5
		Halophila																		
		Ruppia																		
		Syringodium	2.0±0.4	2	1.9	2	2	2	1.5±0.3	1.4	1.1	1.2	1.5	2	31.1±8.2	29.9	22.2	26	33.9	40.4
		Thalassia	3.2±0.8	3	3	3	3	4	7.0±1.1	7	5.9	6.4	7.9	8	35.3±8.3	35.3	26	29.7	40.5	43.9
	W	Halodule	1.8±0.7	2	1	1	2	3	0.7±0.3	0.5	0.5	0.5	1	1	7.1±4.2	5.9	2.8	3.8	9.2	14
		Halophila																		
		Ruppia																		
		Syringodium	1.3±0.4	1	1	1	1.3	2	1±0.2	1	0.8	0.9	1.1	1.2	9.6±5.7	7.9	3.1	5.4	13.5	18
		Thalassia	2±0.5	2	1.4	2	2	3	6.4±1.2	6.8	4.9	5.2	7.1	8	13.9±3.8	13.9	9.1	11	16.5	17.9
Lower Laguna Madre																				
	S	Halodule	2.1±0.9	2	1	1.75	2.3	3	1±0.1	1	1	1	1	1.1	9.8±2.4	8.9	7.2	8.6	10.5	13.2
		Halophila																		
		Ruppia																		
		Syringodium	1.7±0.6	2	1	1	2	2	1.3±0.3	1.2	1	1.1	1.5	1.7	26.4±10.1	25	15	19.9	31.5	39.8
		Thalassia	2.7±0.7	3	2	2	3	4	6±1	6	4.9	5.2	6.8	7.1	26.6±8.3	26	16.5	21.2	31.6	37.4
	W	Halodule	2.3±0.8	2	1	2	3	3	1±0.1	1	0.9	1	1	1.1	11.3±2.6	10.5	8.7	9.6	13.8	14.9
		Halophila																		
		Ruppia																		
		Syringodium	1.2±0.4	1	1	1	1	2	1±0.1	1	0.9	1	1.1	1.2	15.3±6	14.9	7.2	11.1	19.2	22.8
		Thalassia	2.1±0.6	2	1	2	2	3	5.8±1.1	6	4.3	5.1	6.4	7	16.1±5.5	15.8	9.2	12.2	19.8	23
Redfish Bay																				
	S	Halodule	2.7±0.7	3	2	2	3	3	0.9±0.1	1	0.7	0.8	1	1	21.2±7.5	20.4	12.2	15.9	26	31
		Halophila	5.5±0.9	6	4	5	6	6	4.4±1.1	4.8	3	4	5	5.9	2.2±1.2	2.1	1.5	1.7	2.5	2.8
		Ruppia	2.6±0.9	3	1.6	2	3	4	0.9±0.2	0.9	0.6	0.8	1	1	15.4±8.3	15.4	5.1	8.3	21.4	26
		Syringodium	1.8±0.5	2	1	2	2	2	1.2±0.2	1.1	1	1	1.2	1.5	31.5±11.5	31.5	16.7	23.6	39	47.1
		Thalassia	3.3±0.8	3	2	3	4	4	6.1±1.4	6	4.4	5	7	8	30.2±9.5	29.9	19.4	25	35.7	42.5
	W	Halodule	1.9±0.6	2	1	2	2	3	0.7±0.4	0.7	0.5	0.6	0.8	0.9	8.5±3.5	8.3	4.1	5.9	10.8	13.3
		Halophila	4.8±1.7	5	3	4	5.3	6	3.9±0.7	4	3	3.5	4.2	4.8	1.6±0.4	1.5	1.1	1.2	1.9	2.01
		Ruppia	2±0.6	2	1	2	2	3	0.7±0.2	0.7	0.5	0.6	0.8	1	9.8±3.8	10.1	5	7.1	12.9	14.5
		Syringodium	1.3±0.5	1	1	1	2	2	1±0.1	1	0.9	0.9	1	1.1	14.8±5.6	15	7.1	10.8	18.5	22
		Thalassia	2.1±0.6	2	1	2	2	3	5.9±1.3	6	4.1	5	6.9	7.5	14.9±5.3	14.6	8	10.9	18.5	22

Table I.8 - continued.

Location	Season	Species	Aboveground Biomass (g m^{-2})						Belowground Biomass (g m^{-2})						Total Biomass (g m^{-2})					
			mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile	mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile	mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile
East Flats																				
	S	Halodule	98±49	88.26	50	68	121	174	211±146	163	75	91	336	393	309±155	275	141	175	410	523
		Halophila																		
		Ruppia																		
		Syringodium	40±21	34.97	23	32	41	61	30±9	29	22	26	32	38	70±30	63	47	60	72	99
		Thalassia	241±83	237.90	162	177	277	320	443±225	460	241	281	609	717	684±286	692	412	486	877	1007
	W	Halodule	21±16	17.76	4	7	34	42	123±110	82	20	39	179	290	144±113	125	29	49	204	305
		Halophila																		
		Ruppia																		
		Syringodium	9±6	7.92	4	5	11	16	31±8	32	22	25	37	38	40±13	39	27	31	48	54
		Thalassia	125±126	81.71	39	52	139	242	607±301	705	248	463	767	930	606±379	787	279	506	1105	1157
Lower Laguna Madre																				
	S	Halodule	6±3	6.92	4	5	8	8	20±9	20	13	16	25	28	27±11	29	18	22	33	35
		Halophila																		
		Ruppia																		
		Syringodium	62±48	47.66	15	26	78	128	93±59	86	30	46	122	172	154±97	138	50	81	205	286
		Thalassia	142±141	109.56	46	77	164	256	1109±971	824	235	377	1584	2363	1250±1048	953	291	458	1785	2586
	W	Halodule	23±5	23.89	19	21	26	27	39±7	35	35	35	41	45	62±9	63	55	58	67	70
		Halophila																		
		Ruppia																		
		Syringodium	33±30	22.81	6	12	47	71	69±49	58	16	30	98	140	101±74	88	22	43	143	203
		Thalassia	68±42	56.41	24	42	83	125	859±745	631	220	342	1109	1841	927±775	691	249	378	1183	2046
Redfish Bay																				
	S	Halodule	99±105	75.18	25	45	120	175	125±108	100	47	70	153	214	224±209	190	76	113	262	363
		Halophila	39±122	7.07	2	5	19	36	35±119	8	3	5	20	28	75±240	20	6	8	37	63
		Ruppia	122±142	78.60	19	34	158	218	70±129	24	2	8	67	145	192±249	119	33	66	215	355
		Syringodium	179±167	134.48	26	57	260	345	105±83	89	22	39	143	205	283±240	210	52	106	393	517
		Thalassia	182±114	160.70	51	112	226	351	460±223	450	178	310	618	761	642±311	627	272	416	855	1080
	W	Halodule	16±19	9.14	1	4	26	38	65±51	54	11	21	89	136	81±61	73	15	33	110	166
		Halophila	12±7	10.59	5	7	15	19	9±5	9	5	6	11	14	20±12	17	12	12	26	33
		Ruppia	37±28	27.77	6	14	50	79	93±53	90	20	67	118	157	130±73	131	29	84	181	224
		Syringodium	48±39	37.21	9	17	64	116	84±90	59	19	26	102	149	132±114	108	28	50	174	256
		Thalassia	76±48	68.13	22	40	107	144	386±200	366	153	241	500	658	462±232	435	169	300	583	788

Table I.8 - continued.

Location	Season	Species	CN mean ± s.d.	CN median	CN 10th %ile	CN 25th %ile	CN 75th %ile	CN 90th %ile	CP mean ± s.d.	CP median	CP 10th %ile	CP 25th %ile	CP 75th %ile	CP 90th %ile	NP mean ± s.d.	NP median	NP 10th %ile	NP 25th %ile	NP 75th %ile	NP 90th %ile
East Flats																				
	S '05	Halodule	20±2	19	19	19	20	22	812±282	738	606	656	931	1046	46±11	44	37	40	51	55
		Thalassia	20±3	21	17	18	23	23	626±118	626	511	571	721	747	36±7	37	28	30	41	45
	W '05	Halodule	14±1	14	13	13	14	14	226±19	226	215	219	232	236	19±0.4	19	19	19	19	19
		Thalassia	14±2	15	12	14	15	16	568±111	583	449	514	605	672	46±6	44	42	42	46	51
Lower Laguna Madre																				
	S '02	Thalassia	18±2	18	16	17	18	19	662±115	674	531	563	761	801	38±9	39	26	33	42	47
	W '03	Thalassia	15±3	15	11	13	17	18	537±150	504	407	456	600	668	35±5	34	31	32	37	41
Redfish Bay																				
	S '02	Thalassia	17±2	17	15	16	19	20	816±252	825	538	612	921	1119	54±13	53	40	44	60	65
	W '03	Thalassia	14±3	13	11	11	15	17	404±140	375	274	309	471	582	34±8	34	25	30	38	41
	S '05	Halodule	17±1	17	16	16	18	19	315±80	298	251	266	348	393	21±4	20	18	19	22	25
		Thalassia	21±4	20	15	17	23	25	506±170	467	354	416	557	730	29±9	29	18	22	36	39
	W '05	Thalassia	16±3	16	13	15	18	19	489±138	468	347	379	574	665	36±11	35	24	28	42	47

Table I.8 - continued.

Location	Season	Species	Percent Cover					
			mean ± s.d.	median	10th %ile	25th %ile	75th %ile	90th %ile
East Flats								
	S	Halodule	44±42	30	0	0	98	100
		Halophila	0±0	0	0	0	0	0
		Ruppia	9±24	0	0	0	0	22
		Syringodium	5±11	0	0	0	2	22
		Thalassia	25±39	0	0	0	52	92
		Total Seagrass	83±32	100	30	89	100	100
	W	Halodule	53±47	56	0	0	100	100
		Halophila	0±0	0	0	0	0	0
		Ruppia	0.03±0.3	0	0	0	0	0
		Syringodium	5±15	0	0	0	0	11
		Thalassia	16±31	0	0	0	0	72
		Total Seagrass	73±39	97	0	55	100	100
Lower Laguna Madre								
	S	Halodule	0.05±1	0	0	0	0	0
		Halophila	0±0	0	0	0	0	0
		Ruppia	0±0	0	0	0	0	0
		Syringodium	19±26	5	0	0	30	64
		Thalassia	68±32	80	15	45	95	100
		Total Seagrass	87±26	100	46	90	100	100
	W	Halodule	0.01±0.1	0	0	0	0	0
		Halophila	0±0	0	0	0	0	0
		Ruppia	0±0	0	0	0	0	0
		Syringodium	16±25	0	0	0	25	56
		Thalassia	59±37	66	5	25	98	100
		Total Seagrass	75±34	95	10	54	100	100
Redfish Bay								
	S	Halodule	23±36	0	0	0	40	94
		Halophila	0.7±3	0	0	0	0	0
		Ruppia	4±15	0	0	0	0	5
		Syringodium	3±17	0	0	0	0	0
		Thalassia	37±43	8	0	0	91	100
		Total Seagrass	68±42	96	0	20	100	100
	W	Halodule	15±31	0	0	0	7	84
		Halophila	0.04±1	0	0	0	0	0
		Ruppia	0.01±0.3	0	0	0	0	0
		Syringodium	2±13	0	0	0	0	0
		Thalassia	35±42	7	0	0	85	100
		Total Seagrass	52±42	60	0	2	96	100

Biomass and root:shoot ratios

Biomass measurements have a long history as indicators of resource condition. The response of both aboveground and belowground biomass to numerous environmental variables has been studied in seagrass beds throughout the country as well as locally in Laguna Madre and Corpus Christi Bay (Orth and Moore 1983, Quammen and Onuf 1993; Lee and Dunton 2000b). Belowground biomass to aboveground biomass ratios of seagrasses are typically >1, reflecting the importance of belowground tissues for nutrient uptake, anchoring in the sediments, and carbohydrate storage. Changes in this ratio therefore may reflect performance of these basic functions as well as the photosynthetic potential of the plant based on light and nutrient availability. Biomass is generally used as an estimate of primary production and reflects the contribution of seagrass to ecosystem function. Inferences on the contribution of seagrass productivity to higher trophic levels and detrital pathways can be obtained from understanding changes in seagrass biomass.

Aboveground biomass tends to vary seasonally in Texas (Table 8) as leaves may be sloughed and growth and elongation slow dramatically. In both winter and summer, the proportion of belowground biomass can be effectively calculated from total *Thalassia* biomass (Figure I.42). Total, above-, and belowground biomass were strongly correlated with leaf morphometrics and shoot density; however, they were not strongly correlated with many environmental parameters. Aboveground biomass was negatively correlated with water column NH_4^+ concentration while root:shoot was negatively correlated with NH_4^+ concentration. Although multiple regression models explained little variation in total *Thalassia* biomass, it appears that salinity, light, water column nutrients, and drift algae influence total biomass (Table I.7).

Seagrass biomass is easily determined from core samples and requires little financial or time investment. Cores can be taken while snorkeling or from the side of a boat (Onuf et al. 1996). They are easily sieved in the field and sorted in the laboratory. During sorting, above- and belowground contributions to total biomass can be readily separated, while shoot density and leaf morphometrics can be easily estimated. Consideration should be given to objectives of biomass sampling prior to developing the sampling protocol. In this study,

biomass samples were collected from beds containing the target species and sorted into species-specific contributions to the biomass in the core. Therefore, all cores were taken from within developed seagrass beds and may reflect the optimal condition of the plants at the site. Random sampling of seagrass cores at each site would likely provide a better estimate of average conditions at a site; however, because of the patchy nature of seagrass beds, within site variability would be high. As a result, a larger sample size at each site would be necessary to effectively characterize seagrass biomass at the site and understand the variability within the site. Overall, the conceptual relevance, feasibility, and ease of interpretation suggest that biomass measurements should be an integral part of any seagrass monitoring program despite the destructive nature of biomass sampling.

Shoot Density

In addition to biomass, shoot density has been used extensively as an indicator of seagrass condition (Orth and Moore 1983, Quammen and Onuf 1993 and Kaldy and Dunton 2000). Generally, higher shoot densities are indicative of favorable conditions for seagrass growth while low shoot densities imply a population that is declining or stressed. At the same time, differences in shoot density provide inferences regarding the function of seagrass as habitat in the ecosystem. Higher shoot densities provide greater substrate for epiphytic algal communities that are important in trophic dynamics and may represent more effective refugia for fauna (Bologna and Heck 1999; Boström and Mattila 1999). Therefore, shoot density is an indicator not only of seagrass condition but also the condition of the ecosystem as a whole.

Thalassia shoot density varied in Redfish Bay and East flats as a function of both sampling date and site. Both between and within site variability was high (Figures I.18-I.19; Table I.8) in both bays during winter and summer. As expected, shoot density was strongly correlated to the various biomass components and percent cover estimates (Figure I.42; Table I.6); however, no strong relationship existed between shoot density and the stressor indicators. The lack of strong relationships with other parameters is likely a function of the high within site variability in shoot density that arises from the patchy nature of the seagrass beds. Despite this high variability, the ease of sampling, importance for ecosystem services, and the

strong presence in the literature suggest that inclusion of shoot density estimates in seagrass monitoring is warranted.

Community composition and percent cover

Community structure and the relative cover of seagrass is a valuable mechanism for assessing changes in seagrass systems under changing environmental conditions. Fourqurean et al (2003a) related decades of water quality data in Florida Bay to changes in seagrass community structure as determined by Braun-Blanquet scoring of percent cover estimates. By monitoring these changes, the studies detected shifts in the distribution and abundance of individual species that could have significant effects on the function of the seagrass ecosystem. For example, increases in the abundance of *Halodule* relative to *Thalassia* may result from changing nutrient availability (*Halodule* requires relatively greater nutrients than *Thalassia*). These changes may greatly affect the resident fauna because faunal densities tend to be higher in *Halodule* beds than in dense *Thalassia* beds. Such changes in community composition, therefore, may lead to changes in the ecosystem services provided by the seagrass community.

In this study, obvious changes in the community composition of seagrasses changed over time. Although *Halophila* is relatively rare, its abundance declined in Redfish Bay after the summer of 2002. The decline was correlated with PO_4^{3-} concentrations and may be indicative of changes in the nutrient regime within the bay. Also, *Thalassia* cover declined at several sites and, in some cases, was replaced by *Halodule*. Such changes are unlikely to be observed in biomass estimates or aerial photography. Overall, *Thalassia* cover declined throughout Redfish Bay from 2002 to 2005 (Figure I.53).

Although tracking the community structure is of critical importance, significant drawbacks to acquiring percent cover data exist. Percent cover estimates are frequently subject to observer bias. To ensure consistency between estimates careful training is required, and statistical analysis for observer bias may be necessary. Consistency in percent cover estimates is of particular concern in the turbid waters that may characterize the bays of the Texas coast.

A second drawback of percent cover estimation is the difficulty of sampling. Samplers must enter the water at the sites and submerge themselves to determine the cover of each species in a given quadrat. This task becomes extremely challenging when visibility prevents visual estimation and requires estimation by feel. On average, sampling 10, 0.25 m^2 quadrats at a site requires 45-60 minutes. This rate of sampling greatly limits the number of sites that can be sampled within a day. Ideally, a photographic system of estimation could be developed to facilitate this process; however, the issue of low visibility will be difficult to solve.
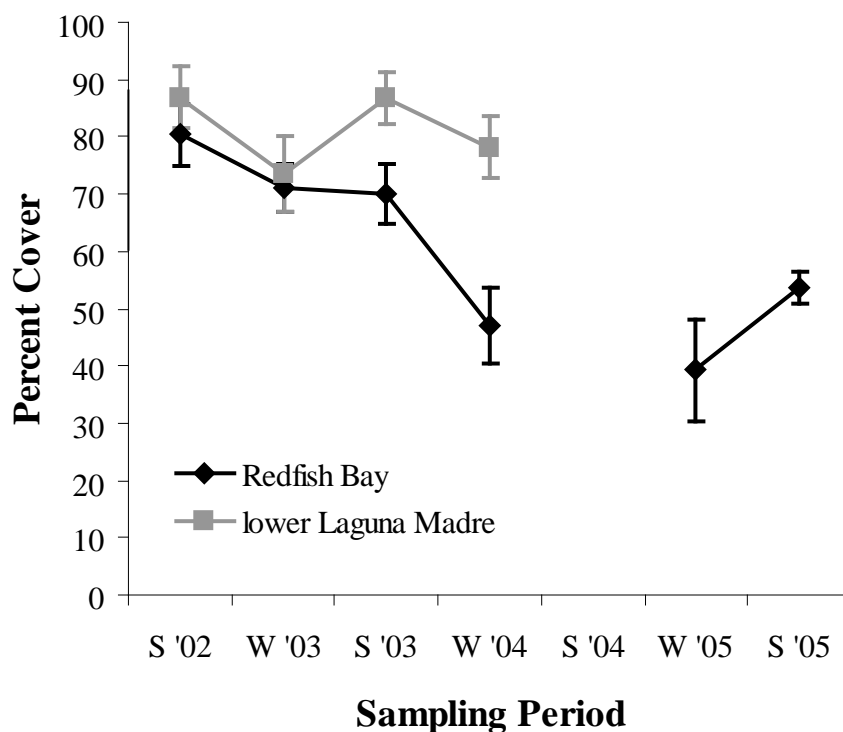

Figure I.53 - Percent cover of seagrass in Redfish Bay and lower Laguna Madre through the duration of monitoring.

Finally, percent cover sampling occurred at 10 randomly determined quadrat locations (along a 50 meter transect) that were chosen prior to each sampling date. Therefore, most of the sampling locations differed between sampling dates. As a result, community composition estimates are derived from different plots within the seagrass beds. Given the high within site variability at most sites (0-100% cover), it is difficult to determine with any confidence the extent to which temporal differences in community structure reflect changes in seagrass condition or changes in the location of sampling. While the random sampling approach

satisfies critical statistical assumptions, it may take several years of sampling at each site to get an accurate estimate of the community composition.

Despite these numerous weaknesses in acquiring community structure estimates, the value of collecting this data is critical to seagrass monitoring. It is unlikely that trends in the relative abundance of the various species can be effectively monitored by other indicators. In our estimation, these estimates are critical to any monitoring program.

Additional Considerations

Age-specific population demographics

In addition to water quality monitoring and assessments of seagrass biomass and density, population demographic models can be used to examine changes in seagrass population size, recruitment, and mortality (e.g. Duarte et al. 1994, Peterson and Fourqurean 2001). These reconstructive techniques rely on a plastochron interval determined from the number of leaf scars on individual short shoots. (Erickson and Michelini, 1957). These intervals provide estimates of the age-frequency distribution of the population of seagrass that can be used to calculate recruitment and mortality rates as well as predict population growth using basic population models (Duarte et al. 1994). One of the advantages of applying demographic models is that population trends can be assessed relatively quickly from data collected over relatively short time periods (single samplings to several years), and comparison of spatial and temporal differences in population growth dynamics may reveal locations of specific concern. Relating population growth estimates with water quality assessments and other potential stressors may reveal the mechanisms driving the population trends.

Although this approach has strong conceptual appeal, its application is not universally accepted. Two assumptions that are required for application of the model are that 1) mortality and recruitment rates are constant and 2) the plastochron interval is constant over time and space. Several papers have called the validity of these assumptions into question (Jensen et al. 1996; Kaldy et al. 1999). Peterson and Fourqurean (2001) examined these assumptions and clearly demonstrated that the plastochron interval varies spatially. The authors also suggest that the assumptions of the technique be tested explicitly. At the same

93

time, there is further disagreement about whether age structure of a population can be used to estimate survival and population growth rates (Ebert et al. 2002). Because the debate regarding the use of age distributions to predict population dynamics continues (Ebert and Williams 2003; Fourqurean et al. 2003b), we cannot recommend using this technique in a seagrass monitoring program unless these concerns can be resolved.

Geostatistical analysis

One of the limitations of water quality and habitat monitoring are the trade-offs that govern the number and allocation of samples to be collected. Frequently cost and the logistics of sample processing are limiting factors that constrain the frequency of sampling, the number of locations sampled, and the number of samples collected at each location. Determining the proper balance between these competing interests is difficult. One approach that has been employed to minimize the constraints on the number of sampling locations is to use geostatistical analysis to develop prediction surfaces for measured parameters so that values can be generated in areas that have not been sampled. The predicted values are then related to data collected at additional sites that are beyond the scope of the usual monitoring program. For example, Fourqurean et al. (2003a) used point kriging to develop prediction surfaces from water quality parameters measured at 28 sampling sites. Those surfaces were used to generate values for the water quality parameters at an additional 649 sites where measurements of seagrass community structure had been obtained. Together these data were used to develop a discriminant function model that classified the community structure at a site based on the predicted water quality characteristics of the site. The authors' model was reasonably successful in classifying the seagrass community, and the authors suggest this technique could provide a valuable management tool (Fourqurean et al. 2003a).

Unfortunately, the study by Fourqurean et al. (2003a) did not assess the accuracy of the kriging algorithm in generating water quality estimates. The validity of the approach clearly hinges on the degree to which predicted values reflect actual water quality conditions at a given site. In ArcMap (ArcGIS v. 9), cross validation permits comparison of predicted estimates with the actual measured value collected at the monitored sites. For each site at which monitoring data is collected, the model generates a prediction based on a model

constructed using all data except the value at the given site. The predicted value is then compared to the actual measurement to determine the fit of the model. Additional error estimates (e.g mean error, root mean square error, etc.) are also generated. The standard error of the predictions can easily be generated to examine the spatial variability in prediction accuracy.

In this study, we used geostatistical analysis (ordinary kriging) to generate prediction surfaces and examined their accuracy. The suitability of the prediction surfaces varied depending on the variable. For example, predictions of salinity (Figure I.54) were relatively similar to actual measurements. The same was also true for depth, %SI, and NH_4^+ (Figures I.55-I.57). For these parameters, the cross-validation suggests that the prediction surfaces generated with kriging are reasonable.
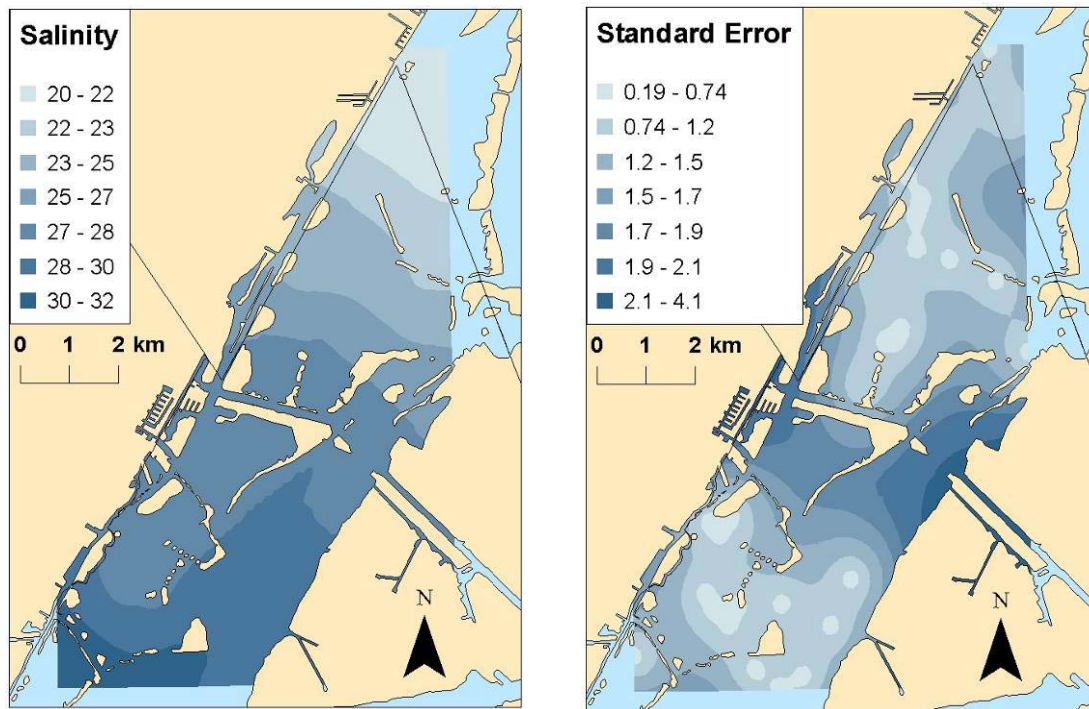

Figure I.54 - Prediction map (left panel) and prediction standard error map (right panel) of average salinity values (psu) in Redfish Bay from 2002-2005. Generated by ordinary kriging.
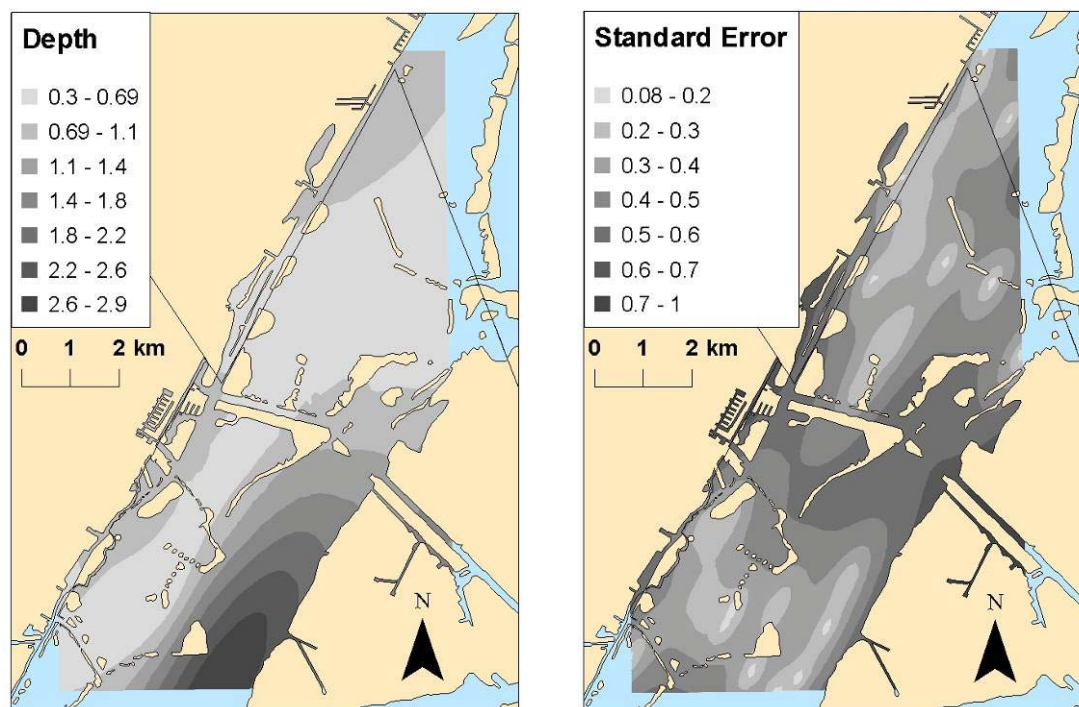
Figure I.55 - Prediction map (left panel) and prediction standard error map (right panel) of average depth values (m) in Redfish Bay from 2002-2005. Generated by ordinary kriging.
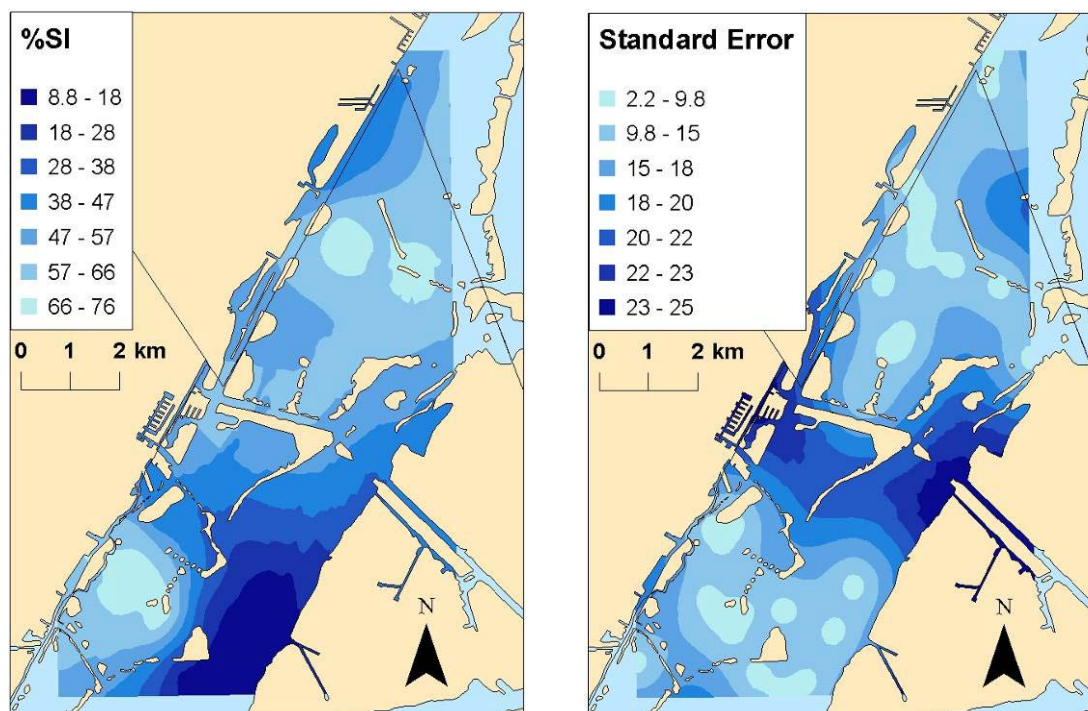

Figure I.56 - Prediction map (left panel) and prediction standard error map (right panel) of average %SI values in Redfish Bay from 2002-2005. Generated by ordinary kriging.
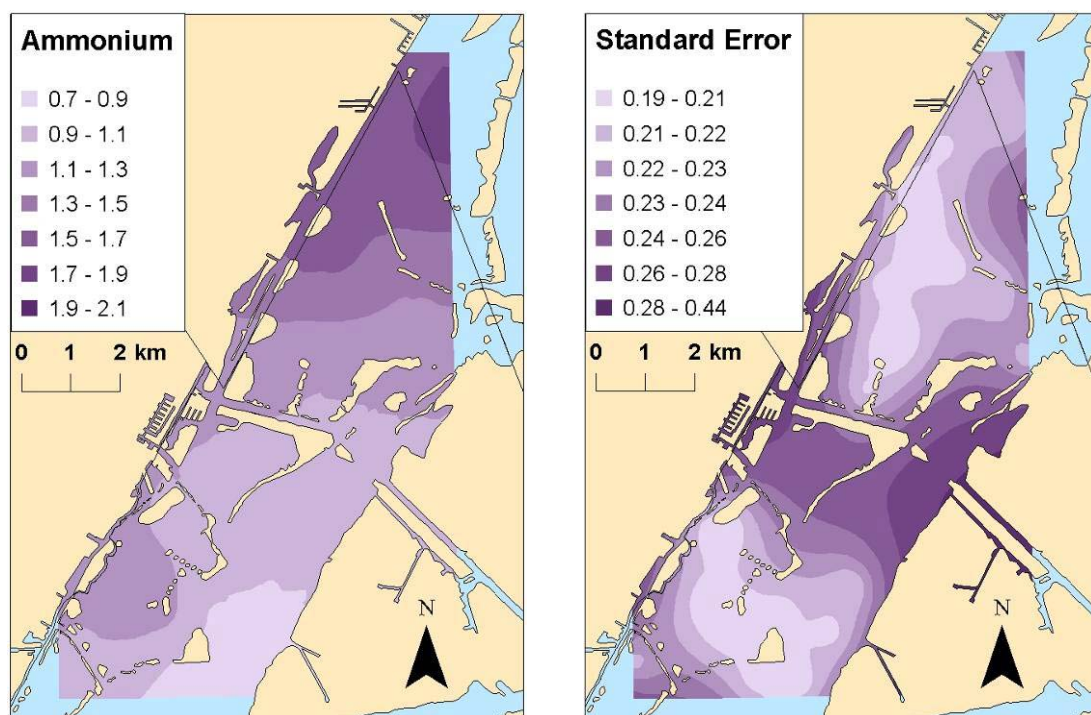
Figure I.57 - Prediction map (left panel) and prediction standard error map (right panel) of average NH_4^+ concentration (µM) in Redfish Bay from 2002-2005. Generated by ordinary kriging.

Prediction surfaces for the remaining parameters were less accurate, particularly for patchy cover types (Figures I.58-I.61). Interpretation of the standard errors indicates that we can be 95% confident that the true value lies within ± 2 SE if the data are normally distributed (Johnston et al. 2003). Examination of the predicted values from the cross validation indicates that the relative ranking of the predicted measurements for the sites may not reflect the relative rankings of the actual measurements among the sites. For example, average drift algal biomass at site 12 in Redfish Bay was 140 g/m^{-2}, but the predicted value was 48. The disparity between values represents a change in the relative ranking of drift algal biomass at site 12 from 4th out of 30 to 24th. Similar errors were encountered when predicting percent cover of *Thalassia*, percent cover of *Halodule*, NO_3^-, and chl *a*. The semivariograms for variables with poor predictive power suggests that spatial autocorrelation (a requirement for effective kriging) is lacking among sites for certain parameters. For instance, drift algal biomass at one site may be independent of drift algae at all neighboring sites. Therefore, values generated by the kriging algorithm based on the values at neighboring sites may be inaccurate. The poor predictive power of the models in our study suggest that prediction surfaces for most parameters would misrepresent conditions at unsampled sites and lead to

97

inaccurate characterization of the relationships between seagrass condition indicators and water quality data. Indeed, the spatial representations themselves may be misleading.
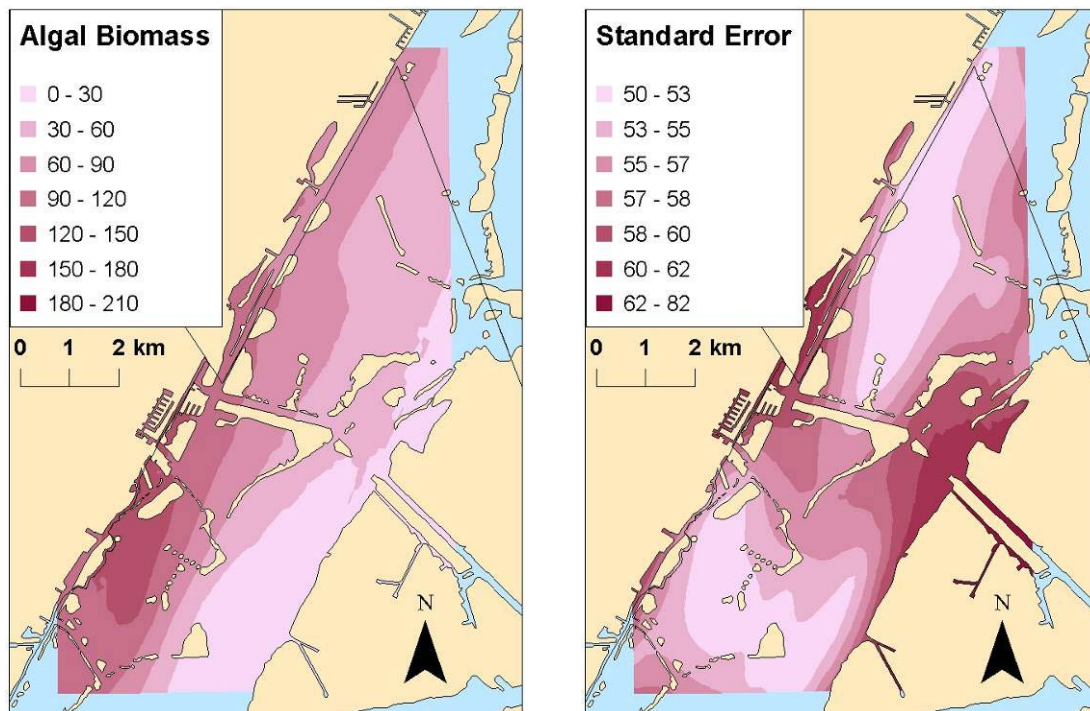

Figure I.58 - Prediction map (left panel) and prediction standard error map (right panel) of average drift algal biomass (g m^{-2}) in Redfish Bay from 2002-2005. Generated by ordinary kriging.
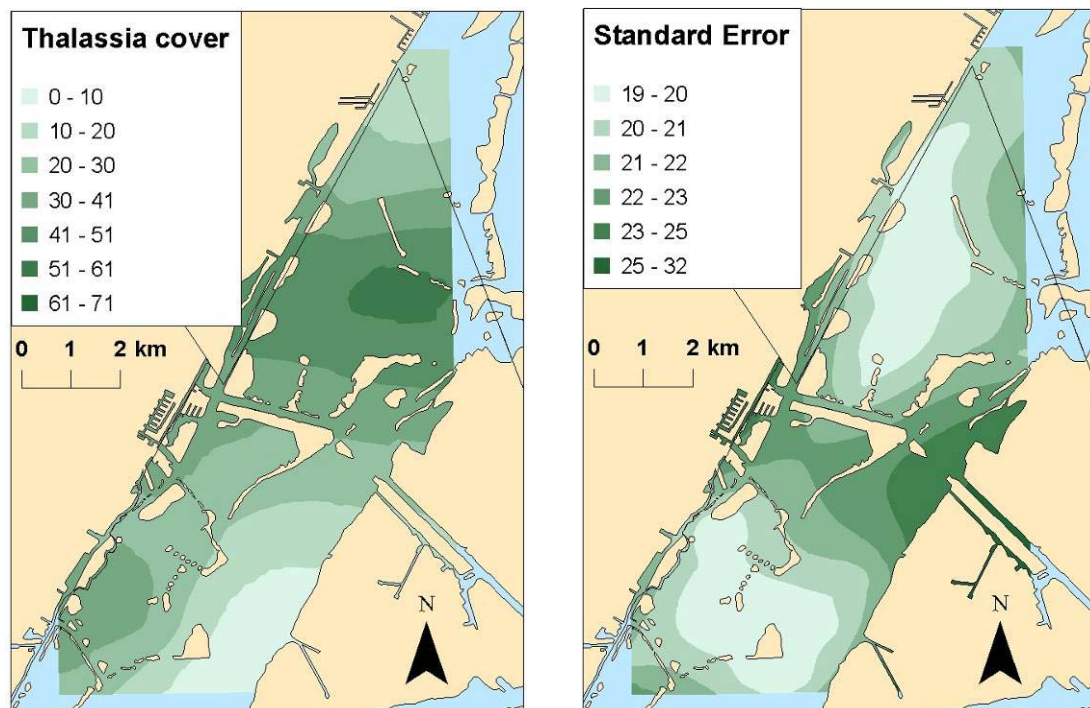
Figure I.59 - Prediction map (left panel) and prediction standard error map (right panel) of average percent cover of *Thalassia* in Redfish Bay from 2002-2005. Generated by ordinary kriging.
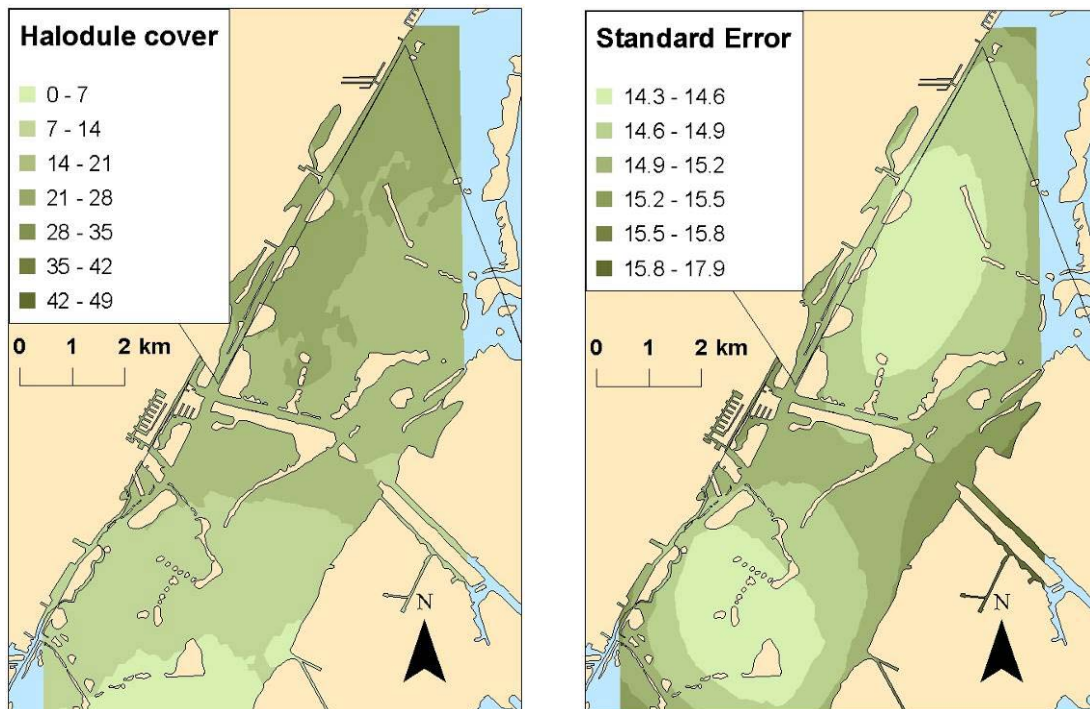

Figure I.60 - Prediction map (left panel) and prediction standard error map (right panel) of average percent cover of *Halodule* in Redfish Bay from 2002-2005. Generated by ordinary kriging.
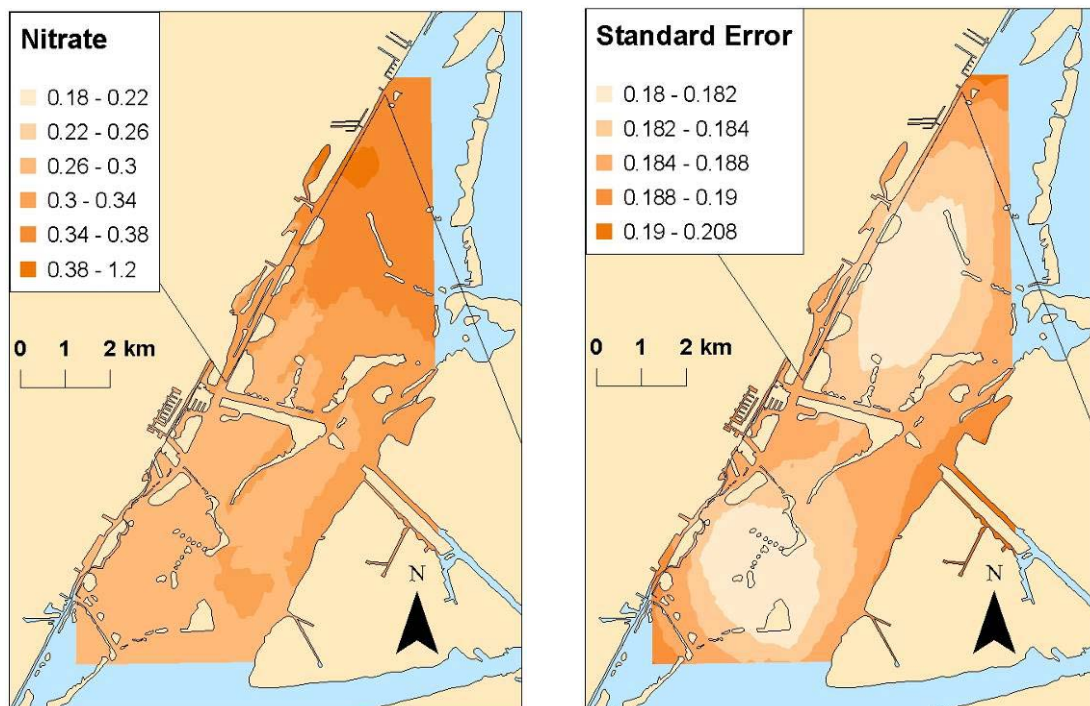
Figure I.61 - Prediction map (left panel) and prediction standard error map (right panel) of average NO_3^- concentration (µM) in Redfish Bay from 2002-2005. Generated by ordinary kriging.

These results lead to several conclusions regarding the use of geostatistical analysis to provide predicted values for further analysis. First, the fit of the models should be analyzed to assess their accuracy. Second, for relatively small scale studies such as ours (58 km^2; compared to 17000 km^2 for Florida Bay in Fourqurean et al. 2003a), water quality measurements should be made at each of the sites at which the condition indicators are measured to eliminate the need to use geospatial analysis to generate prediction surfaces. If all measurements are taken at all sites during sampling, parameters can be related directly with correlation analysis or multivariate approaches.

Proposed Indicators for Seagrass Monitoring

Our evaluation of potential indicators for a seagrass monitoring program was modeled on the EPA R-EMAP framework in which variables are classified as either stressor indicators or condition indicators (EPA 1993). Under this classification scheme, stressors are variables that may change the state of valuable resources, while condition indicators provide information regarding the status of the resource of interest. Relationships between these indicators were then examined using both univariate and mulitivariate statistics to identify stressor indicators that were particularly influential on condition indicators, and conversely, condition indicators that were sensitive to stressors. Frequently, these relationships are examined by comparing condition indicators at sites exposed to different environmental conditions. Comparisons of this nature permit quantification of the effects of different levels of the stressors on condition indicators so that changes in the resource of interest are expressed as a function of the changes in stressors between locations. For example, Boyer et al. (1997) characterized regions of Florida Bay based on differences in water quality and called them zones of similar influence (ZSI). This water quality data was used to construct a discriminant function model that predicts the benthic habitat type (Fourqurean et al. 2003a).

At the outset of this study, East Flats was regarded as relatively pristine compared to Redfish Bay, and disparate environmental conditions were expected between the two locations. Therefore, differences in the condition indicators detected along the range of conditions

within these two locations could be statistically related to differences in stressor indicators. Combinations of stressor and condition indicators that exhibit strong relationships would reveal indicators that are most effective for seagrass monitoring. Statistical analysis, however, revealed very few strong relationships between stressor and condition indicators. In fact, there was no discernible difference between East Flats and Redfish Bay based on their environmental conditions or seagrass community structure (see results section). The only distinct ZSI identified by the multivariate analysis consisted of all sites >1.7 m deep; however, these sites never contained seagrass and likely do not represent seagrass habitat because minimum light requirements are not met. The analysis clearly demonstrates that East Flats is not a pristine location and cannot be used as a reference site to contrast with Redfish Bay.

Further examination of indicators revealed relatively weak associations between stressor and condition indicators as identified previously. Several factors may be responsible for this lack of association between the 2 types of indicators. First, the response of seagrass within Redfish Bay and East Flats may not be in equilibrium with environmental conditions; therefore, a lag may exist between changes in stressors and detectable changes in the seagrass population. Various aspects of the biology of seagrass suggest that this may be the case (Fourqurean et al. 2003a). Seagrass plants are perennial and may persist for several years and possibly decades (Peterson and Fourqurean 2001). Much of the biomass is located within the sediments and may permit persistence of the plant through unfavorable water column conditions. Colonization of new areas may occur slowly due to infrequent sexual reproduction and slow vegetative growth, and stochastic processes affecting recruitment and mortality may mask responses to changing environmental conditions.

Second, scaling issues may make detection of clear relationships between indicators difficult. In our study, seagrass cover, density, and biomass vary at different spatial scales than do many of the water quality parameters. For instance, within a given site, the percent cover of seagrass may range from 0 to 100 along an individual transect. At the same time, salinity may vary by only 5 ppt across several kilometers. With such disparities in the spatial scale of variability, it is entirely possible to encounter the entire range of seagrass community types under similar environmental conditions, making correlation between the two difficult.

Additionally, variation in condition indicators may be strongly affected by stochastic events and unmeasured processes. While links between seagrass and water quality have been empirically established, bioturbation (Townsend and Fonseca 1998), disease (Entel and Hamilton 1999), seed dispersal, physical disturbance (Patriquin 1975), and hydrodynamics (Koch et al. 2006) may all affect seagrass abundance and distribution. If variation in seagrass dynamics due to these processes exceeds that attributable to water and sediment quality parameters, correlations between the measured stressor and condition indicators will be weak.

Finally, the relatively short duration of the study may mean that relationships between environmental conditions and seagrass may result from a lack of statistical power. Only 1 year of data (1 winter and 1 summer sampling) was collected explicitly during this study in Redfish Bay and East Flats. Even with the inclusion of 4 additional sampling dates from a previous R-EMAP project in Redfish Bay, the time series only encompasses 2002-2005. In a similar effort to characterize water quality and seagrass dynamics in Florida Bay, results suggested that decades of data are necessary to accurately predict the response of seagrass (Fourqurean et al. 2003a), even when sampling occurred on a monthly basis. Long-term data sets and frequent sampling are necessary to characterize the variation in the parameters of interest to permit identification of the signal associated with human disturbance from the noise attributable to natural variation.

Based on the analysis of potential indicators and the considerations just described, we recommend a monitoring program that tracks the stressor and condition indicators summarized in Table 9. The monitoring strategy involves continuous sampling at several sites combined with monthly, and semiannual sampling at all sites. The different temporal and spatial sampling intervals should permit characterization of the variability necessary to understand the drivers of seagrass community dynamics. To the extent possible, light availability, dissolved oxygen, salinity, and temperature should be measured on a continuous basis with a few permanently deployed sensors and sondes to capture the range of conditions that are likely to affect seagrass condition. Also, all stressor indicators should be measured at all sites on all sampling dates, while condition indicators should be measured semiannually in winter and summer. A complete data set ensures that direct comparisons can be made among variables at each site, and lags between changes in stressor indicators and responses in

condition indicators can be examined. In order to detect trends in seagrass condition, it may be necessary to monitor these variables for 10 years or more to successfully relate changes in seagrass to the influence of the stressor indicators. The data collected to this point provides and excellent background data set that can be used to characterize the inherent variability within each of the bays of interest (Table 8). The measures of central tendency, standard deviations, and percentiles should serve as guides as new data is generated. Values that repeatedly fall outside the extremes suggest changes are occurring and may provide a basis for careful monitoring of particular sites or bays. In addition to measuring these indicators, the depth of the deepest edge of the seagrass distribution should also be monitored closely. Retreat of seagrass from deeper waters may signal deteriorating light conditions. Coupling these seagrass plant scale indicators with landscape indicators (see next chapter) should provide complementary data sets to permit detection of sub-lethal effects of sediment and water quality (via measurement of stressor and condition indicators) and the impacts of physical disturbance.

Table I.9 - Recommended stressor and condition indicators to be sampled at sites of interest in seagrass monitoring program.

Indicator type	Variable	Sampling Frequency	Sites	Field Method
Stressor	k, %SI	Continuous	permanent	deployed light meter
		monthly	all	light meter and Secchi
	DO	continuous	permanent	data sonde
	Salinity	continuous	permanent	data sonde
		monthly	all	data sonde
	Temperature	continuous	permanent	data sonde
		monthly	all	thermometer
	TSS	monthly	all	water collection
	NH_4^+, NO_3^-, PO_4^{3-}	monthly	all	water collection
	chl a	monthly	all	in situ fluorescence
	drift algal biomass	semiannual	all	wet weight
	algal epiphyte biomass	semiannual	all	dry weight
	maximum bed edge depth	semiannual	permanent	PDR
	CNP ratios	semiannual	all	from biomass samples
Condition				
	leaf morphometrics	semiannual	all	from biomass samples
	shoot density	semiannual	all	benthic cores
	seagrass biomass	semiannual	all	benthic cores
	root:shoot	semiannual	all	benthic cores
	seagrass community composition	semiannual	all	percent cover

Using Monitoring Data to Inform Management Decisions

Large scale seagrass losses in Chesapeake and Florida Bays stimulated extensive research into the use of water quality data to develop management tools. In Florida Bay, Fourqurean et al. (2003a) examined long-term water quality data sets and developed a discriminant function to predict the type of habitat at un-sampled locations. In doing so, the authors developed a tool for predicting seagrass resource conditions from water quality estimates that would allow resource managers to identify areas of concern and initiate management strategies to alleviate stressors in the absence of intensive on-site sampling efforts. The model also allows resource managers to identify how water quality parameters contribute to changes in seagrass growth and community structure. By relating seagrass status to these water quality parameters it is then possible to develop management strategies that establish targets for particular water quality conditions associated with desirable seagrass condition indicators.

Similarly, Dennison et al. (1993) used water quality data to establish minimum requirements for seagrasses in Chesapeake Bay. Knowing the minimum conditions necessary to support growth, it became possible to develop targets for critical stressor indicators and initiate appropriate management practices to achieve those targeted goals. In addition, Kemp et al. (2004), building on models developed by Gallegos (1994; 2001), identified a strategy predicting habitat suitability based on estimates of the minimum light requirements for submerged aquatic vegetation in Chesapeake Bay. Both studies partition light attenuation into contributions from factors such as TSS, chl *a*, water, DOC, and epiphytes. In so doing, Gallegos (2001) generated a model for predicting the light attenuation in Rhode River as a linear function of TSS and chl *a* and examined the suitability of the simple linear regression model in comparison to more realistic models based on Lambert-Beer law. Although the model did not conform to Lambert-Beer law, the covariation of TSS and chl was nearly linear and the regression could be a simple, useful model for generating water quality targets. Establishing targets for TSS and chl concentrations would also provide insight into successful management practices to achieve those targets (e.g. reduction of TSS by limiting dredging). Although the model is less effective at predicting seagrass presence at small spatial scales (~

1 hectare), it is effective at larger spatial scales and could be useful for predicting changes in seagrass distributions as a function of changing TSS, chl, and epiphyte loads. A similar model could be developed for estuaries in Texas, but would need to be calibrated to site specific conditions (Gallegos 2001; Kemp et al. 2004).

We examined the relationship between K_d as a linear function of TSS and chl measured in Redfish Bay and East Flats. Inclusion of all data yielded the following equation:

$$K_d = 0.84 + 0.098[chl] + 0.018[TSS]$$

($r^2 = 0.27$). Using only the summer sampling season, the r^2 improved to 0.39 for the following model:

$$K_d = 0.8 + 0.042[chl] + 0.023[TSS].$$

Setting this equation equal to the following equation permits prediction of the amount of light available under given changes in TSS and chl:

$$K_d = -ln(\%SI/100)/z,$$

where %SI is the percent surface irradiance and z is depth. Thus, the TSS and chl concentrations necessary to meet the minimum light (18% SI) at a given depth can be plotted and used as a guideline for monitoring water column transparency. Comparisons of field measurements with the plot can be used to develop one of several strategies for obtaining minimum light requirements (see Gallegos 2001; Kemp et al. 2004 for discussion).

In addition to reductions in light due to TSS, chl, CDOM, and water, Kemp et al. (2004) also incorporated effects of epiphytes into their model. Because epiphyte loads were not measured as part of the monitoring program in Chesapeake Bay, epiphyte loads were estimated based on a relationship with DIN. Epiphytes could be incorporated into a model for Texas estuaries, however, no strong relationship between nutrient levels and epiphytes exists in Redfish Bay and East Flats. Epiphyte loads in these systems apparently are not strongly driven by nutrient availability, but may be controlled by factors such as herbivory and leaf turnover. Alternatively, direct measurements of epiphytic biomass can be used directly to calculate their contribution to attenuation; however, the relationship between

epiphyte biomass and attenuation needs to be empirically determined for the seagrass species in Texas.

The light models developed by Gallegos (1994, 2001) and Kemp et al. (2004) and the discriminant function model developed by Fourqurean et al. (2003a) exhibit strong potential for generating effective management strategies in Texas. However, their success is dependent upon extensive, local water quality datasets that do not currently exist in Texas. Water quality data in the Chesapeake and Florida Bays are available from 1984 and 1989, respectively. These long term datasets permit a much more thorough understanding of the inherent seasonal, spatial, and interannual variation in physical, chemical, and biological parameters and offer much better estimates for use in model development. It is imperative to compile long term data sets for estuaries in Texas to improve our ability to detect stressors and their impacts on seagrass communities.

It is also important to consider the objectives of each approach when examining their applicability to Texas estuaries. Seagrass loss in Chesapeake Bay occurred in the 1960's and 1970's (Orth and Moore 1983), prior to extensive water quality sampling. As a result, the models of Gallegos (1994, 2001) and Kemp et al. (2004) are designed to establish the minimum conditions for seagrass growth and identify goals for returning water quality to historical conditions and restore seagrass habitats. Thus, the main objective is restoration of water quality and seagrass, not detecting stressors. Application of similar models may be most effective in areas such as Laguna Madre and Galveston Bay, where well establish changes in water quality and seagrass distribution have occurred and management targets may be most effective. On the other hand, it is not at all clear if light conditions in Redfish Bay and East Flats are in fact changing or limiting in most locations. The only sites to routinely experience conditions outside the minimum light levels (~18% SI) are deeper than 1.7m. At all other sites, the median %SI was 55%, well above the minimum light level. At these locations, the greatest utility of such a model is in predicting response of seagrass to changes in water quality parameters.

Similarly, the objective of the study by Fourqurean et al. (2003a) was to relate water quality to seagrass distribution and community structure. However, regular water quality monitoring

data was collected at 28 sites compared to 649 sites where seagrass community structure was determined. To link water quality and seagrass community structure, it was necessary to spatially interpolate between water quality data points to generate estimates at the seagrass sampling locations. In contrast, water, sediment and seagrass samples were collected at each site. Because of this design, all variables were spatially and temporally congruent at a given site, and direct correlations among variables could be assessed. Thus, the need for potentially inaccurate, predicted values was minimized. Long-term measurement of variables collected in this way should permit detection of relationships between stressor and condition indicators. The current lack of obvious relationships is likely a reflection of coarse temporal sampling, and potentially, the operation of stochastic processes that obscure the influence of stressor indicators on seagrass communities.

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II. Monitoring Landscape Indicators of Seagrass Health Using High Resolution Color Aerial Photography

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Executive Summary

In support of the Texas Seagrass Monitoring Program, aerial remote sensing research has been performed to evaluate automated methods for monitoring landscape changes in seagrass beds indicative of human stressors and/or natural disturbances. This report discusses the integration of high resolution aerial color film photography, color space transformation, pixel threshold models, and geographic information system (GIS) technology to detect, assess, and monitor 1-m ground feature changes and landscape disturbances within Coastal Bend seagrass beds. The application of digital photoanalysis techniques to detect and monitor landscape stress/disturbance features in seagrass beds is part of a multiyear monitoring study being conducted by Univ. of Texas Marine Science Institute (Ken Dunton) and Texas State University (Warren Pulich) for CBBEP.

The landscape analysis techniques developed and presented here involve separating vegetation from bare patch features in seagrassbed landscapes using color space image processing of aerial photoimages, and subsequent GIS analysis of bare features, as well as identification of seagrass vegetation from macroalgae. The methods entail transforming digitized, aerial color film transparencies from red, green, and blue color space to intensity (or value), hue, and saturation color space; analyzing the saturation and intensity (or value) image bands and their histograms to identify bare areas; and developing threshold models to separate bare areas from vegetated areas employing the results obtained in the previous step. Thematic maps created with this semi-automated approach have classification accuracies ranging from 75% to 96%. Geographic information system tools were used to quantify landscape feature changes occurring at two test sites, and to correlate landscape indicator patterns with major hydrodynamic and physical factors over two consecutive years.

Results indicate that 1:24,000 scale photography of seagrass beds, traditionally used for presence/absence seagrass mapping purposes, was reasonably satisfactory for determination of generic submerged vegetation distribution when compared with similar photography taken at the 1:9,600 scale. However, seagrass bed health parameters were less accurately assessed at the smaller 1:24,000 scale. Results showed that the 1:24K scale photos

distinguished *ca* 35% less macroalgae from seagrasses, while conversely overestimating total amounts of seagrass. At the 1:24K scale, delineation and quantification of fine scale features (such as prop scars) was incomplete and very subjective. Direct comparisons of 2 sites showed 25 -30% lower values in both number and size of small bare patches (< 2-3 m^2).

The overall findings indicate that this semi-automated approach using high resolution digital color photography is an efficient protocol to accurately delineate and monitor changes in landscape disturbance indicators (i.e. amounts and patterns of bare and vegetated features) within Coastal Bend, Texas, seagrass beds. Physical, geomorphological effects on seagrass beds are clearly discriminated by landscape features reflecting three major disturbance factors: water depth, hydrodynamics, or anthropogenic disturbances. Thus, periodic monitoring of broad scale seagrass landscape dynamics by the methods described would provide an initial basis for identifying the dominant type(s) of disturbance factors affecting seagrass bed health, prior to wide scale loss of seagrass.

ADDITIONAL INDEX WORDS: Seagrass monitoring; landscape feature analysis; color space transformation; physical disturbance factors; ecosystem health indicators

Note: All figures for Chapter 2 are found in Appendix A at the end of this chapter.

Introduction

Coastal seagrass beds have long been recognized as important nursery habitats for estuarine fisheries and wildlife; providing food for fish, waterfowl, and sea turtles; contributing large amounts of organic matter to estuarine and marine food webs; participating in nutrient cycling processes; and acting as stabilizing agents in coastal sedimentation and erosion processes (Dennison *et al.* 1993; Phillips 1984; Pulich and Calnan, eds. 1998; Thayer, Kenworthy, and Fonseca 1984; Zieman 1982). Both natural and anthropogenic disturbances to seagrass beds can negatively impact the ecology of coastal environments, such that resource managers now use changes in seagrass habitat as a critical indicator of estuarine ecosystem health (Dennison *et al.* 1993; Orth and Moore 1983; Short and Wyllie-Echeverria 1996).

Resource managers for Texas state agencies (TPWD, TGLO, and TCEQ), in collaboration with coastal research scientists, have recommended coast-wide monitoring to assess the status of Texas seagrass beds and to detect sublethal stress prior to actual grassbed losses (Pulich and Calnan, eds., Seagrass Conservation Plan for Texas 1998). The Texas Seagrass Monitoring Plan (TSMP 2003) recently proposed a combination of intensive field surveys (at the plant-scale) and landscape analysis with color aerial photography (at the bed- scale) to monitor and measure indicators of seagrass health conditions. Through seagrass status and trends studies, the CBBEP has actively promoted seagrass research to evaluate disturbance factors (natural or human) at both the plant level (microscale) and landscape (bedscale) level. In the mid 1990s, CBBEP initiated a long-term status and trends assessment of seagrass dynamics in the Coastal Bend region from the late 1950s through 1996 (Pulich *et al*. 1997) and supported the assessment of boat propeller scarring impacts on Coastal Bend grassbeds (Dunton and Schonberg 2002). These studies documented changes in grassbed distributions, species composition, proliferations of macroalgae, and physicomechanical impacts to grassbeds (i.e. propeller scars, channel dredging). This work also confirmed the potential for using landscape indicators to assess stress to and degradation of seagrass beds from dredging, urbanization, boating/ship traffic, or high nutrient loading.

Intensive field sampling at microscales has traditionally been used to detect and quantify effects of specific factors related to seagrass stress or growth (Neckles, ed. 1994). However, such plant scale field sampling and analysis is very labor intensive (and also expensive). At the seagrass-bed scale, landscape features and patterns would also reflect visible effects of physical disturbances on the plant ecosystem, including anthropogenic or natural hydrodynamic disturbances. Thus, high-resolution photography over broad areas would be more cost effective in identifying characteristic human or natural physical disturbances, and possibly water quality impacts (Ferguson *et al.* 1993; Dobson *et al.* 1995; Robbins 1997). If plant scale measurements from field sampling could be functionally correlated with bedscale features and landscape patterns evident in seagrass photography, it should be possible to extrapolate the extent of altered dynamics and ecosystem stressors over wide seagrass areas.

Identifying factors responsible for seagrass plant dynamics (i.e. plant stressors or growth factors) can be difficult, even from carefully measured plant-scale (microscale) indicators (*e.g.* plant biomass, root/shoot ratios, etc.). The same problem exists when landscape indicators are monitored at the bedscale (*e.g.* bed fragmentation patterns, patchiness, macroalgae, species composition, etc.). Inferring causality of landscape changes from these effects must be approached cautiously (Duarte 1999; Kirkman 1999; Fonseca 2002). Environmental conditions and ecological factors may exert positive or negative effects, either singularly or in combination. Consequently, identifying stress factors and their effects on seagrass beds involves deciphering complex interactions through both site-specific and landscape level measurements. Extrapolation from specific field site measurements over large seagrass landscape areas requires integration of both remote sensing and field sampling data through careful statistical analysis (Heggem *et al*. 1999; Fonseca 2002; Mumby and Edwards 2002).

Classification of Landscape Indicators

The Seagrass Conservation Plan for Texas (SCPT) included a proposal to establish a seagrass habitat monitoring program for Texas coastal waters (Pulich and Calnan, eds. 1999). This monitoring program would be aimed at assessing ecosystem health, not merely mapping presence or absence of seagrass. The proposal recommended monitoring of key habitat

indices, such as landscape disturbance features at the bedscale, to detect ecosystem stress occurring before actual loss of seagrasses. Using digital photoimagery and advanced image processing software (e.g. ERDAS™, ENVI™), Pulich *et al*. (2003) proposed a variety of landscape features for delineation as landscape indicators. (*e.g.* patterns in bed morphology or bare patches, deposits of macroalgae/wrack, human disturbance features, shoals and channels, species distribution, etc.). These seagrass landscape indicators were proposed based on a conceptual seagrass landscape model that distinguishes five distinct categories of seagrass landscape indicators (Pulich *et al*. 2003) as follows:

1) Seagrass bed morphology and patterns (including shape, size, density, and edge symmetry of beds). Edge shapes and patch sizes of plant beds are often a function of hydraulics (*e.g.* water currents), depth, and localized environmental disturbances. Patchy or continuous beds can reflect two types of disturbance responses: a) expanding or colonizing patches of plants, or b) localized fragmentation from physical disturbances (*e.g.* wave energy or dynamics, light regimes).

2) Non-seagrass, natural features within the bed (such as bare patches, reefs, tidal channels, wrack or drift macroalgae accumulations). Bare patches within grassbeds can result from storms, tidal currents or fetch, human activities (see below), or macroalgae and wrack deposition. Diagnostic patterns may be indicative of specific environmental fragmentation processes.

3) Human impact features. Landscape features such as propeller scars, pipeline scars, dredged channels and spoil deposits, and industrial activities (*e.g.* aquaculture sites) are readily identified examples of human impacts to grassbeds (Pulich et al. 1997; Dunton and Schonberg 2001).

4) Distributions of seagrass species and macroalgae. Species distribution patterns often reflect successional or competition processes which result from stressor impacts to the ecosystem. Species discrimination and delineation of macroalgal abundance require corroborating GPS field data to achieve satisfactory accuracy with the aerial photography. Multispectral imagery or underwater videography also enables more accurate delineation.

5) <u>Water column physicochemical factors.</u> Parameters such as hydraulic (*i.e.* flow) patterns, turbidity, chlorophyll levels, and chemical components are indicators of water quality or hydrodynamic stress. However, identification of these parameters usually cannot be done solely from interpretation of imagery, but requires ancillary field data.

Table II.1 - Seagrass Landscape Indicator Classes and Proposed Metrics

INDICATOR CLASS	LANDSCAPE METRICS
Morphology and Patterns of Seagrass Plants	Shape, size, density, & edge symmetry of beds/patches per hectare.
Patterns in Non-seagrass Natural Features	Acreage of macroalgae, bare patches, reefs, channels, sand bars & shoals per hectare.
Human Impact Features	Linear distance of propeller scars, pipelines, "industrial activities", dredged channels per landscape unit area.
Spatial Distribution of Species	Percent species coverage over landscape unit areas; depth limit of seagrass.
Water Column Constituents	Zones (polygon areas) of turbidity, chlorophyll, other water chemistry.

After identification of landscape features in the imagery of targeted sites, field surveys and sampling for hydrographic, environmental and plant-level data would be conducted, targeting the corresponding locations of landscape features and designed to achieve the spatial and temporal coverage required for geospatial analysis. This intensive field sampling of biological and physicochemical parameters should be performed with close coordination between remote sensing analysts and field monitoring researchers. In this way, geospatial relationships would be derived between classified landscape indicators and plant

indicators/ecosystem processes, in an attempt to produce landscape indices of biological integrity (health). Spatial statistics (i.e. landscape metrics) can be derived for bed feature polygons such as bare patch size and density, edge/shape ratios, species diversity, etc., using GIS software such as Geospatial Analyst™ or Landstats™; however, these GIS procedures will require considerable research and analysis to produce suitable metrics.

Aerial Remote Sensing Analysis

Photogrammetric remote sensing research is underway by various investigators to evaluate methods for documenting landscape changes in seagrass beds related to human and/or natural disturbances. Over the years, aerial remote sensing imagery has gained acceptance with the scientific community as a cost-effective tool to provide wide-area estimates of seagrass beds. Analysts have extensively employed manual photointerpretation analysis of aerial photography for quantitative seagrass mapping purposes (Orth and Moore 1983; Ferguson, Wood, and Graham 1993; Ward, Markon, and Douglas 1997; Pulich *et al.* 1997). In recent years, the trend has shifted to using electronically scanned aerial photographs (Kurz *et al.* 2000; Moore, Wilcox, and Orth 2000; Finkbeiner *et al.* 2001), airborne electronic imagery (Garono *et al.* 2004; Lanthrop, Montesano, and Haag 2006; Su *et al.* 2006) and satellite imagery (Ackleson and Klemas 1987) to study seagrass beds. Investigators have employed various techniques to analyze the raster data; and mixed results from these studies suggest that automated procedures are still needed for accurate mapping of seagrass beds from remotely-sensed data (Meehan *et al.* 2005). Integration of geographic information systems (GIS-software used to store, retrieve, and analyze various types of geographic data) and remote sensing technologies has also provided new and useful information related to seagrasses (Finkl and DaPrato 1994; Dobson *et al.* 1995; Klemas 2001; Robbins 1997).

Remotely-sensed imagery subjected to color space transformation has helped investigators to identify land cover features not readily apparent in conventional color images (Carper, Lillesand, and Kiefer 1990; Andreadis, Glavas, and Tsalides 1995). Compared with the red, green, and blue color space used to display images on computer monitors, the intensity, hue, and saturation color space corresponds more to the human visual system. This transformation results in a decorrelation of color features. Intensity represents the brightness of a color, and

its values range from 0 to 1, with 0 and 1 equaling black and white, respectively. Hue describes the major wavelength of light contributing to a color. Terms used to characterize hue include red, green, orange, blue, and magenta. Its values range from 0º to 360º, with red, green, and blue having values of 0º, 120º, and 240º, respectively. Saturation represents the purity of a color in reference to gray. Its values range from 0 to 1, with 1 representing a pure color.

Analysts often use pixel thresholding, a computer technique employed to group image pixels into classes using cutoff values, to create binary masks for extracting important or non-essential features from remotely-sensed imagery. Earlier attempts using standard classification procedures of high resolution color photography of the Redfish Bay area did not provide the accuracy needed to meet the goals of the seagrass monitoring program, leading to the application of the semi-automated technique described in this study ((McEachron *et al.* 2002). The current study was designed to test the integration of high resolution aerial color photography, color space transformation, pixel threshold models, and geographic information system technology for monitoring and assessment of landscape feature changes and bare disturbance patterns within Coastal Bend seagrass beds. Techniques in this study have been described in a preliminary report published by Pulich *et al.* (2006).

Goals and Objectives

The goal of this landscape analysis project was to integrate landscape patterns of seagrass bed features and disturbance indicators (e.g. vegetation morphology patterns, vegetative species composition, and physical disturbance features such as bare patch shapes and sizes) with field measurements of seagrass plant/habitat indicators (biomass, plant composition, water and sediment nutrients, etc.) for seagrass management purposes.

Specific objectives were to:

1. Establish 6 to 8 priority target sites in the Texas CBBEP study area for long-term seagrass landscape monitoring as recommended by the Texas Seagrass Monitoring Plan (2003); and acquire true color aerial photography at both 1:24,000 and 1:9,600 scales at these sites.

2. At two sites (Terminal Flats and East Flats), conduct intensive landscape analysis studies to measure seagrass landscape features and develop disturbance indicators from the high-resolution 1:9,600 scale photography.

3. Compare landscape disturbance indicators at the two scales of photography to determine the optimum spatial scale needed to assess seagrass ecosystem health for management and conservation purposes.

4. From spatial and statistical analyses, integrate these landscape indicator measurements with microscale plant measurements from field sampling, leading to establishment of landscape indices of ecosystem health.

Some of this work was initiated in 2000, when the Texas Parks and Wildlife Department (TPWD) began development of a management program to protect sensitive seagrass beds in the Redfish Bay area of the Coastal Bend of Texas (McEachron *et al.* 2002). As part of their program, TPWD established the Redfish Bay, Texas, Scientific Area, a "no-motor zone" where boaters and fishermen were to voluntarily restrict their use of boat gasoline engines to prevent propeller scarring of shallow grass beds. To assess motorboat impacts and subsequent recovery particularly of turtlegrass (*Thalassia testudinum*) beds in this Scientific Area, research staff at TPWD designed and instituted a landscape monitoring protocol for the Terminal Flats site based on high resolution, aerial remote sensing data (Dunton and Schonberg 2002; McEachron *et al.* 2002).

Materials and Methods

Study Area and Sampling Design

Corpus Christi Bay and Redfish Bay, Texas (Figure II.1) support extensive seagrass meadows containing the four species of true seagrasses found on the Texas Gulf coast: *Halodule wrightii*, *Thalassia testudinum*, *Syringodium filiforme*, and *Halophila engelmannii* (Pulich *et al*. 1997). The Terminal Flats site near Aransas Pass, Texas (Figure II.2) has been the focus of much seagrass monitoring and management work since 1999, because propeller scarring has caused a major reduction in seagrass acreage in this region (Dunton and Schonberg, 2002; McEachron *et al*. 2002). Seagrasses at the shallow Terminal Flats site grow less than 0.6 m below the water surface. The region also represents the largest percentage and extent of *Thalassia* occurring this far north on the Texas coast. Landscape-scale remote sensing monitoring techniques are considered critical to management and protection of these sensitive beds.

Recommended procedures for seagrass photographic analysis [compiled in Dobson *et al*. (1995) for the NOAA-CCAP Program , and recently reiterated by the NOAA-Benthic Habitat Mapping Program (Finkbeiner *et al*. 2001)] are based on photointerpretation of large format (9 in x 9 in) 1:24,000 scale photos and manual digitization to quantify seagrass coverage. These methods were employed in the earlier study for CBBEP (Pulich *et al*. 1997). However recent work described in the TSMP (2003), Dunton and Schonberg (2002), Pulich *et al*. (manuscript in prep), and Pulich *et al*. (2003) indicates that 1:9,600 scale photos, because of their higher ground resolution (< 0.3 m per pixel), produce more accurately-identified landscape feature data. A recent study by Schull and Bulthuis (2002) in Padilla Bay Washington also relied on digital photographic analysis of 1:12,000 scale aerial photography to determine status and trends of Puget Sound seagrass. Because of our interest in fine scale seagrass landscape features (*e.g*. prop scars and small, 1-2 m diam. bare patches), image processing of 1:9,600 scale photography was chosen as the data source for this study.

<u>Photography Acquisition</u>

Target sites for seagrass photography acquisition were located in the CBBEP region in consultation with the Texas Seagrass Monitoring Workgroup (see Figure II.1). Eight photographic targets sites were selected that are considered sensitive seagrass areas where future human disturbance will occur or is currently suspected to occur (e.g. channel dredging, shoreline urban development, or nonpoint source inputs).

Two priority study sites, Terminal Flats in North Redfish Bay and East Flats in Corpus Christi Bay (Figure II.1), were selected for detailed landscape indicator monitoring based on work by McEachron *et al*. (2002) and Dunton and Maidment (2001). The East Flats area was considered to be a control area (relatively undisturbed seagrass beds), as opposed to other more disturbed seagrass areas (e.g. north Redfish Bay). Six additional sites were also chosen for baseline data acquisition at both the 1:24,000 and 1:9,600 scale (Figure II.1):

1.   Two sites adjacent to Packery Channel in upper Laguna Madre, the channel separating Mustang and North Padre Islands;

2.   One site near Shamrock Island along the bayside of Mustang Island;

3.   One site in South Bay, part of north Harbor Island;

4.   One site along the west end of Kennedy Causeway over upper Laguna Madre; and

5.   One site in Estes Cove in the north Redfish Bay area.

Figure II.2 shows the relative photographic footprints covered by the large format mapping camera for the 2 scales of photography. Four (4) complete 1:9,600 scale photographs are contained within the area of one, 1:24,000 scale photo footprint. When these areas are photographed at 2 altitudes, resulting in large format 1:9,600 and 1:24,000 scale photographs (22.9 x 22.9 cm or 9 x 9 in film), the film dimensions cover a photoarea of 2.2 km x 2.2 km at 1:9,600 scale, while the area covered at the 1:24,000 scale is 5.5 km x 5.5 km.

Vertical aerial photography with a large format camera was flown on December 11, 2004, by a commercial aerial photography contractor (Krawietz Custom Aerial Photography,

Bulverde, TX) at both 1:24,000 and 1:9,600 photo scales. Large format Agfa Film (400 speed) which simulates Aerocolor Kodak 2445 color negative film, or Aerocolor Kodak 2427 film, was used in a calibrated mapping camera. Overlapping photographs at 1:9,600 scale were acquired to achieve 30% sidelap and 60% endlap. In addition, several sub areas at the two intensive study sites were flown in 2004 with a digital camera fitted with color filters (Everitt et al. 1999) by USDA-ARS-IFNRRU collaborators to acquire narrow, color band imagery at similar high resolution. Classification of spectrally-distinct vegetated features was then attempted using spectral band datasets (using color filters which cut off at 447-455 nm, 483-492nm, 555 – 565 nm, and 625-635 nm) derived from the USDA digital camera data (Everitt et al. 1999). This ancillary data was considered potentially useful to interpretation of the color film photography.

For the 1:9,600 photos, actual ground control points were derived from 1.2 m x 1.2 m (4x4 ft) square, floating, white reflective plastic targets placed in the field prior to photography being taken (10 to 12 such targets were highly visible in each photograph.), and from a few precise landmarks visible in the photoarea. Coordinates for the ground control targets were determined by DGPS accurate to < +/- 1 m. The 1:24,000 scale photographs were registered from additional field GPS points taken on highly visible landmarks and precise points (e.g. roads, houses, piers, or small islands).

In 2005, similar, end-of-year photographs were acquired of the Terminal Flats, East Flats, South Bay, Shamrock-Mustang Island, and Packery Channel sites on Dec. 11th for Year 2 comparison. These early December photographs represent end-of-growth season samples showing high biomass. Winter tides were very low on Dec. 11th of both years (as usual for this time of year), providing good light penetration into water for seagrass delineation. Photomissions occurred both years during the midmorning hours (0900 – 1130) with full sunlight, and calm, clear water conditions, a day after cold fronts passed through the Coastal Bend.

GPS Surveys

Differential GPS (DGPS) was used to precisely locate landscape features and vegetation to a spatial accuracy of <+/- 1 meter. In order to achieve this precision and accuracy, GPS

readings were acquired by averaging for 120 seconds with the GPS receiver unit at each ground point and achieving PDOP readings of 5.0 or less. This accuracy required that a 12-channel GPS unit equivalent to the GeoExplorer III (Trimble Navigation Ltd.) be used. Differential correction was performed using post-processing software (Pathfinder Office™) and corrected GPS points were converted to ArcView shape files in the UTM projection based on NAD 1983 as the datum.

GPS surveys of the Terminal Flats and East Flats sites were performed from shallow-draft boats or airboat during late winter and early spring of 2005 (and 2006) to obtain sufficient points for performing accuracy assessment, and to precisely identify features to develop landscape indicators. Ground-truthing sampling, focused, on vegetation types and bottom cover (bare bottom, seagrass species or macroalgae), as well as non-vegetated bottom features at stations where potential landscape disturbance or other features are visible in the photos. Vegetation cover was discriminated visually as either sparse (1-50% cover per m^2) or dense (51-100% cover per m^2) (Mumby and Edwards 2002; Schull and Bultuis 2002).

Film Processing and Scanning

The exposed film was sent to a commercial laboratory for processing (HAS Inc., Dayton, Ohio, formerly Precision Photo Laboratory). Positive transparencies were produced from the developed negatives and used to assess the study sites. For each year, a representative transparency of the study site was selected and digitized at 1100 dpi with a flatbed scanner and Adobe Photoshop™, Software (version 6.0). This dpi gave a ground-feature resolution of 0.206 meter per pixel (< 1 ft) for the 1:9,600 photography. During scanning, neither the brightness/contrast nor exposure time was adjusted on the scanner. The digitized files were then saved in the tagged image file format (TIFF; 8-bit radiometric resolution for each channel) for subsequent analyses.

Digital Analysis Techniques

Georegistration

Files were imported into ENVI™ software (Research Systems Inc., version 4.3) for georegistration. The digitized transparencies were georeferenced to the Universal Transverse

Mercator Coordinate System (Spheroid: GRS 1980; zone number: 14N; datum: North American Datum 1983), using the coordinates of the artificial ground control targets (GCPs) and occasionally some additional coordinates of land features located on a rectified United States Geological Survey Digital Orthophoto Quarter Quadrangle (DOQQ) of the study site and the surrounding area. The "Warp Image to Map" registration tool of ENVI was used to georeference the imagery, by linking ground control targets visible in the image to UTM coordinates obtained by DGPS. A second order polynomial and the nearest neighbor resampling technique were employed in the georeferencing process, and the imagery was resampled to a pixel resolution of 0.30 m or less (< 1 ft), meeting the specifications of the Seagrass Habitat Monitoring Program. Table II.2 lists the RMS errors for the resulting georegistered files. Figure II.3 shows the 1:24,000 scale DOQQ of N. Redfish Bay overlaid with the georeferenced Terminal Flats and South Bay 1:9,600 scale photoimages.

Table II.2 - Target site photoimages and RMS errors for georegistration.

Photoimage Files	RMSE
Terminal Flats	
2004, 1:9,600	3.73
2004, 1:24000	2.17
2005, 1:9,600	3.74
East Flats	
2004, 1:9,600	2.25
2004, 1:24000	3.62
2005, 1:9,600	3.57
Estes Flats	
2004, 1:9,600	2.30
South Bay	
2004, 1:9,600	2.96
Packery Channel	
2004, 1:24000	2.41

Image Processing

In order to simplify the image processing analyses, a subset of the 9 in. x 9 in. photoimages at the target sites was used for landscape indicator studies. This subset image, called a primary region of interest (ROI), was extracted with the 'Region of Interest' tool of the ENVI™ software. In preliminary analyses, it was also observed that deepwater channels and open bay waters could have similar spectral signatures as seagrasses, thus leading to a reduction in the overall accuracies of the thematic maps.   In this study, the primary, submerged land-cover types of interest were bare bottom (i.e., ≤ 10% vegetative cover) and vegetated bottom areas. Since seagrasses do not grow in these deepwater areas and channels, (or on emergent, terrestrial land), both deepwater and exposed land features within the ROI were masked out. In attempts to increase the classification accuracy, other researchers have also masked out emergent land and deepwater features from coastal imagery to perform automated image analysis (Ackleson and Klemas 1987; Ferguson and Korfmacher 1997; Maeder *et al.* 2002; Su *et al.* 2006; Zainal, Dalby, and Robinson 1993). Figure II.4 shows the 2004 and 2005 photographs of the Terminal Flats site with the resulting overlaid ROIs. Other examples of ROIs are shown for East Flats overlaid on the 2004 and 2005 photographs (Figure II.5) and Packery Channel in 2004 (ROI only, Figure II.6).

Separation of Bare Bottom Areas from Vegetated Areas

ERDAS™ Software

The following techniques using ERDAS™ image processing software have been described in a preliminary report published by Pulich *et al.* (2006).   This ERDAS™ procedure was used as the primary method to extract bare (= unvegetated) areas for the 2004 Terminal Flats image. An AOI (area of interest) subsample was transformed from red, green, and blue color space to Intensity, Hue, and Saturation color space with the RGB to HSI tool of ERDAS™ software. Prominent bare areas were readily separated from vegetation in this Saturation image (see Terminal Flats 2004 example in Figure II.7); they appeared in dark gray to black tones.

Prior to further analysis, the Saturation image was rescaled to 8-bit format, employing the rescale option of the software. Then, based on the difference in saturation image pixel values for bare and vegetated areas, a preliminary threshold value was determined which spectrally

separated the two areas. The inquire cursor of the ERDAS™ software was employed to position the cursor on bare areas in the Saturation image. These values were recorded. Then, the numerical data in the Saturation image histogram was assessed, and the values close to the selected cursor values were recorded as potential threshold values.

A threshold model was developed, which required the following input for execution (in order): 1) a saturation image and an AOI file, 2) the threshold equation (input file ≤ numeric value), and 3) an output name for the new file. For step two, if a value ≤ 38 was chosen as the threshold value for bare areas, then in the conditional model, image values ≤ 38 were assigned a value of one and image values > 38 were given a value of zero. For step three, the thematic output data type was selected. The output image was recoded (Recode module of ERDAS™) to display bare, vegetative, and background areas in different colors.

The thematic map and digitized aerial color photograph were then opened in the same viewer. The former was overlayed on top of the latter, resulting in the digitized aerial color photograph not being seen by the analysts. The attributes editor of the thematic image was opened, and the opacities of the bare and vegetative areas colors were changed to 0.3 and 0 respectively, causing partial transparency of the bare areas and full transparency of the vegetative areas. If the selected threshold value was too high or too low based on qualitative assessment of the thematic image and the digitized aerial photograph, then a new threshold value was selected, and the above steps were repeated until a satisfactory threshold value was obtained. The final thematic image was subjected to a 3 by 3 majority filter to remove "salt and pepper" pixels within the image. The neighborhood option of the software was used to complete this task. Figure II.8 displays the final, filtered bare mask produced for TFlats 2004 at a saturation threshold value of 38.

ENVI™ Software

Except for the previous work on 2004 TFlats imagery, all remaining classification work was done by applying the RGB to HSV tool of ENVI™ software. This procedure also transforms images from red, green, and blue color space to Hue, Saturation, and Value color space. In ENVI™, the Value (V) band is comparable to the Intensity (I) band in ERDAS™. As with the 2004 TFlats image previously processed using ERDAS, most prominent bare areas of the

2005 Terminal Flats image were readily distinguished from vegetation by dark gray to black tones in the Saturation band (same as example for 2004 in Figure II.7). However, the ENVI™ Value band [comparable to the Intensity (I) band in ERDAS™] looked promising for some minor areas, and therefore it was employed as a secondary image for extracting bare and vegetated areas. The following steps describe the process by which the Saturation band was used as the primary image to separate bare and vegetated areas for the 2005 Terminal Flats image:

1) After converting the TFlts2005_RGB image to HSV color space, the Saturation (S) band was loaded as a single, gray-scale image. (Note: With the Saturation band, higher [darker] values on the 0 – 255 DIN scale correspond to vegetation, and lower [brighter] values correspond to bare areas.). The Density Slicing tool was used to classify all pixels in the Saturation band as either bare areas or vegetation The Saturation band was sliced into 5-point increments and each resulting image visually compared to the original RGB image (TFlts2005Dec_9,600_georoi.tif) as well as to each other.

2) The following sliced images were saved as discrete files using the threshold between the vegetation/bare slice values to differentiate them from each other:

        TFlts2005_Sat-2Slice45   (0-45 bare, 46-255 veg)

        TFlts2005_Sat-2Slice50   (0-50 bare, 51-255 veg)

        TFlts2005_Sat-2Slice55   (0-55 bare, 56-255 veg)

        TFlts2005_Sat-2Slice60   (0-60 bare, 61-255 veg)

        TFlts2005_Sat-2Slice65   (0-65 bare, 66-255 veg)

Additionally, each sliced image was filtered using a 5x5 median filter to smooth out the values and create a more homogenous result which facilitated visually differentiating between the results.

3) The above five images were re-examined, and of these, TFlts2005_Sat-2Slice45 and TFlts2005_Sat-2Slice50 were deemed to have produced results sufficient for further

processing. Results included: some loss of bare area detail on the East side of the image where sunlight is reflecting blue off the water, but this area only represents about 1/20th of the entire image; across the rest of the image, the vegetation found inside bare areas is better represented; there are bare areas under the plume in the center of the image that are largely under-represented and only one area where the reverse seems to hold true (*e.g.*, there was vegetation under the plume that was classified as bare); definition of the edges of the oil channel was adequate.

4) These two images were "permanently" filtered using the <filter> <convolutions and morphology> option, choosing a median 5x5 filter with 0% addback. Results were saved as:

> TFlts2005_Sat-2Slice45-5x5median.img
>
> TFlts2005_Sat-2Slice50-5x5median.img

5) These two images were then displayed and classified in order to convert the DIN to two discrete values relating to bare and vegetated areas (e.g., converting the pixel values to only "1" and "2", which is necessary in order to eventually produce a vector file of the class). The process was to use: <classification> <unsupervised> <isodata> with settings of:

Min/Max classes: 2

iterations: 1

Change threshold: 5.0

Min # in class: 4

Max class stdv: 1

Min class Distance: 5

Max # merge pairs: 2

The results were saved as:

> TFlts2005_Sat-2Slice45-5x5median-isodata.img
>
> TFlts2005_Sat-2Slice50-5x5median-isodata.img

6) To further "homogenize" the images, the "clump" algorithm was used by accessing <classification> <post classification> <clump classes>, accepting the defaults and producing the following files:

TFlts2005_Sat-2Slice45-5x5median-isodata-clump.img

TFlts2005_Sat-2Slice50-5x5median-isodata-clump.img

The results were visually inspected and TFlts2005_Sat-2Slice50-5x5median-isodata-clump.img appeared to more accurately depict both bare and vegetated features, while largely retaining the vertical "channel" edge (there was some loss of the edge on the east side of the channel). Smaller details such as small prop scars were not well retained. Figure II.9 displays an example of a classified raster image of TFlats 2005 (although in this case bare and vegetated classes were separated using the ENVI™ value band).

7) Finally, conversion of the raster image to a vector GIS file was accomplished by using <Classification> <Post Classification> <Class to Vector> . This process took approximately 3 to 4 hours to produce vector files for the bare areas, vegetation, and the outside image area:

TFlts2005_Sat50Veg(*or Bare*)Class.evf

Once completed, it was possible to display the vectors on top of the original image for examination. The last step was to export the ENVI™ vector files to ESRI™ shape files using <file> <export active layer to shapefile>. Figure II.10 shows the final, vector file masks of bare and vegetated areas for TFlats 2005, as developed from combined saturation and value band analysis.

East Flats Image Analysis

Similar to the Terminal Flats-2005 analysis, the East Flats photoimages could not be completely separated into bare and vegetated areas using Saturation band analysis alone. Too much spectral overlap occurred, and no single saturation threshold value separating the two areas was found. However, the ENVI™ Value band [comparable to the Intensity (I) band in ERDAS™] looked most promising, and therefore it was employed as the primary image for extracting bare and vegetated areas from the East Flats 2004 and 2005 images. In the Value

image, bare areas appeared in lighter gray tones (Figure II.11); and bare areas had lower [brighter] values on the 0 – 255 DIN scale than the dark vegetation. Small details such as prop scars were also sufficiently retained, in contrast to the Saturation band analysis. For the Value image, a similar protocol employed for the Saturation image was used to find the threshold between bare and vegetated areas:

1) After converting the EstFlts2004_RGB image to HSV color space, the Value (V) band was loaded as a single, gray-scale image (Figure II.11).

2) The Density Slicing tool was used to classify most pixels in the Value band as either bare areas or vegetation. The Value band was sliced into 5-point increments and each resulting image visually compared to the original RGB image (EstFlts2004Dec_9,600_georoi.tif) as well as to each other. Sliced images were saved as discrete files using the threshold between the vegetation/bare slice values, with the following ranges being the best:

EstFlts2004_Val-2Slice170   (0-170 veg, 171-255 bare)

EstFlts2004_Val-2Slice175   (0-175veg, 176-255 bare)

EstFlts2004_Val-2Slice180   (0-180veg, 181-255 bare)

EstFlts2004_Val-2Slice185   (0-185veg, 186-255 bare)

EstFlts2004_Val-2Slice190   (0-190veg, 191-255 bare)

Additionally, these sliced images were filtered using a 5x5 median filter to smooth out the values and create a more homogenous result which facilitated visually differentiating between the results (see Figure II.12a for 'Val-2Slice 170' example).

3) The five images were re-examined, and of these EstFlts2004_Val-2Slice175 and EsTFlts2004_Val-2Slice185 (See Figure II.12 for intermediate range 'Val-2Slice 180') were considered the best files for further processing. However, the 175 and 185 thresholds each produced the most accurate results in different, specific regions of East Flats: the 175 value delineated bare areas in the deeper-water region of Corpus Christi Bay to the south, while the 185 value worked best for the shallow flats region towards the north. Overestimation of

vegetation in the deepwater part of the image resulted from bare areas with darker signatures due to bottom sediments without vegetation that appeared similar to sparse vegetation. For this reason, it was decided to divide the East Flats image into 2 parts (Shallow and Deepwater) and use a different threshold value in each to separate bare and vegetated areas. A Value band threshold of 175 was applied to the Deepwater region, while a threshold value of 185 was used for the Shallow flats region.

4) The two images were "permanently" filtered using the <filter> <convolutions and morphology> option, choosing a median 3x3 filter with 0% addback. Results were saved as:

        EstFlts2004_Val-2Slice175-3x3median.img

        EstFlts2004_Val-2Slice185-3x3median.img

5) These two images were then displayed and classified in order to convert the DIN to two discrete values relating to bare and vegetated areas. The process was to use: <classification> <unsupervised> <isodata> with settings of:

Min/Max classes:	3
# iterations:	1
Change threshold:	5.0
Min # in class:	6
Max class stdv:	1
Min class Distance:	5
Max # merge pairs:	2

The results were saved as:

        EstFlts2004_Val-2Slice175-3x3median-isodata.img

        EstFlts2004_Val-2Slice185-3x3median-isodata.img

6) To further "homogenize" the images, the "clump" algorithm was used by accessing <classification> <post classification> <clump classes>, accepting the defaults and producing

the following files:

EstFlts2004_Val-2Slice175-3x3-isodata-clump.img

EstFlts2004_Val-2Slice185-3x3-isodata-clump.img

Figure II.13 displays the "EstFlats 2004_dsr185class.tif" file, which was used for the final classified bare and vegetated areas in the shallow water region. The file "EstFlats 2004_dsr175class.tif" (not shown) was used for the final classified bare and vegetated areas in the deep- water region.

7) Finally, conversion of the Deepwater and Shallow water raster images to vector GIS files was accomplished by using <Classification> <Post Classification> <Class to Vector>. This process took approximately 6 hours per file to produce 3 vector files for the bare areas, vegetation, and the outside image area:

Shallowwater = EstFlts2004_3x3Valdsr185_vecCls1(or 2 or 3)nrth.evf

Deepwater = EstFlts2004_3x3Valdsr175_vecCls1(or 2 or 3)sth.evf

Once completed, these .evf files were displayed on top of the original image for examination.

8) The last step was to convert the ENVI™ vector files to ESRI™ shape files using <file> <export active layer to shapefile>.   Final, shape files (.shp) of EstFlats 2004 bare and vegetated areas are shown in Figure II.14, delineating the Shallow- and Deepwater regions.

GIS Analysis

ArcGIS™ or ArcView™ GIS analysis procedures were employed to assess spatial patterns as viewed in seagrass bed thematic images (i.e. vector files) of TFlats and East Flats. At the TFlats study site, a smaller landscape unit area (LUA) was subset from the larger ROI area for GIS analysis of seagrass changes occurring between years (2004 and 2005) (see Figure II.15). For the EastFlats site, landscape unit areas consisted of the Deepwater and Shallow flats subset regions (Figure II.14).

In addition to the spatial feature distributions derived from the image analyses, several

hydrographic parameters (including depth and hydrodynamics) and anthropogenic features were assessed and correlated with patterns of bare and vegetated features using GIS . The chief hydrographic parameter was bottom depth data (i.e. bathymetry), that was obtained from NOAA-National Ocean Service (2007). This consisted of approximately 1-m bottom contours compiled from NOS Estuarine Hydrographic Surveys from the last 20 years. For the TFlats site, more detailed bathymetry was available at 0.33-m intervals from the Pulich *et al.* (1997) study. Correlations between depth and seagrass patterns were expected to provide information on light-limitation effects (in deep-water) or hydraulic conditions (due to tides and fetch) on seagrass over a broad landscape scale. Figure II.16 presents a raster GRID image of the NOAA bathymetry available for Redfish Bay, Harbor Island, and East Corpus Christi Bay. Fetch effects were inferred from directional wind data which is predominantly from the S-SE over *ca* ¾ of the year (Natl. Weather Service data, UTMSI station).

Comparison of 1:9,600 vs. 1:24,000 Scale Photography

Questions have been raised about the application of 1:9,600 and 1:24,000 scales of photography for delineating seagrass landscape indicators of ecosystem health. The chief issue here pertains to the distinction between mapping seagrass bed distributions, which is routinely performed with 1:24,000 scale photos, and detecting seagrass health indicators such as bed fragmentation, bare patches and patterns, and macroalgae deposits. Because these landscape health indicators are expected to require higher resolution imagery for detection and analysis, we performed a detailed comparison of the resolution, accuracy, and effectiveness of the 2 scales of imagery from 2004 at the Terminal Flats and East Flats sites.

Resolution and accuracy were evaluated by comparing classified polygon acreages in the ROIs after manual photointerpretation of indicator classes at each scale. Because it was not practical to photointerpret the entire ROI images at this detail, a statistical subsample was evaluated for each study site, equivalent to approximately 10% of the total ROI area containing seagrass. Subsamples were selected by placing a grid over the ROI and randomly selecting 10% of the grid cells for photointerpretation. A 150m x 150m grid was applied to the 242 ha ROI at Terminal Flats, while a 100m x 100m grid was applied to the smaller (*ca* 150 ha) East Flats site. Thirteen grid cells at TFlats and twelve grid cells at East Flats site

were classified and analyzed.

Submerged landscape feature classes at the 2 sites were classified and manually digitized by "heads-up" procedures. Four classes were interpreted at Terminal Flats: Bare Bottom; Dense Seagrass; Patchy Seagrass; and Macroalgae. At East Flats, a fifth landscape class was delineated in addition, called Mixed Algae and Dense Seagrass. Shape files (*.shp) for these cover classes were first created from the 1:9,600 scale image as a reference (assuming that the resolution and spectral signatures were most accurate at the 1:9,600 scale) at a magnified scale of 200 to 500. Then the thematic layers were overlaid onto the 1:24,000 scale image and edited at the magnified scale, and new thematic layers produced.

Accuracy and effective ROI coverage were evaluated by comparing the total macroalgae accumulations in the ROI at each photoscale. Total Macroalgae in the ROI was delineated by photointerpretation at an on-screen magnification of 300 to 600 over the original photo. Macroalgae Shape files (.shp) were produced for each of the two photoscales.

Accuracy Assessment

Random ground-truth points from various survey dates were checked to determine the thematic accuracy of the map. Field locations were precisely determined by differential GPS.

Ground-truth data for the Terminal Flats 2004 photograph were acquired during boat surveys on 1 December 2004 (8 points), 4 March 2005 (22 points), 20 May 2005 (26 points), and 16 June 2005 (9 points). Ground-truth points for the East Flats 2004 photograph were acquired during boat surveys on 2 December 2004 (13 points), 21 March 2005 (21 points), 14 June 2005 (17 points). Ground-truth data were obtained for the 2005 photography on 5 December 2005 and 11 January 2006. GPS points were also collected on 13-14 July and 10 August 2006 for special studies of the Terminal Flats and East Flats sites, respectively. Error matrixes consisting of user's, producer's, and overall accuracies were used to evaluate the accuracy of the maps (Congalton and Green 1999). User's accuracy describes the reliability of the map as a predictive device. Producer's accuracy indicates the probability that the representative sample is correctly classified. Overall accuracy explains the total percentage of correctly classified land-cover types. Thematic accuracy of the classified digital photos

should be to at least 80% overall accuracy as determined by the error matrix technique. To account for the effects of registration errors on map accuracy, a 3 by 3 pixel area (ca 1m x 1m) was assessed as the sampling unit when comparing GPS reference point data to the classified thematic maps.

Quality Control

Identification of ephemeral features such as drift macroalgae, floating wrack, or sparse seagrass was most critical at the Terminal Flats and East Flats sites, for accurate classification of the 5 Landscape Classes consisting of seagrass assemblages, bare bottom, drift algae or wrack accumulations, human or natural disturbance features, and open water channels. Groundtruthing during the week before procurement of the photos in conjunction with placement of the floating targets used for GCPs helped assure that such transitory features were correctly identified and located in the study area.

Map Accuracy Standards for positional accuracy were checked for the georegistered photoimages using one GCP target as a control on each photomission date (11 December 2004 and 2005). Ground coordinates for these targets were within < 1-m (3x3 pixels) spatial accuracy of the observed values. Georegistration accuracy of the digital photoimages was also checked by overlaying GPS groundtruthing data points onto fixed landmarks and natural features (duck blinds, shoals, and mangrove bushes) visible in the georegistered photos, and four such points verified that GPS points coincided with registered photo points.

Change Analysis

Change analysis was originally designed to quantify differences in seagrass distribution between the current 2004 classified images and seagrass maps from the 1994 CBBEP study (Pulich *et al*. 1997). Change between 1994 and the new 2004 seagrass distributions was to be evaluated by thematic overlays of 1994 seagrass GRIDs onto the photoimagery data from 2004. For this analysis, comparability between the earlier mapping data (Pulich *et al,* 1997) for the Redfish Bay area and the current 2004 data was also an issue in terms of scale (or map) differences and minimum mapping unit. The earlier 1994 data were accurate at 1:24,000 scale, while current map data at 1:9,600 scale were of higher resolution. Small

seagrass patches or bare areas < 0.125 acre (*ca* 0.05 ha) in size were not delineated in the 1994 photography, since the minimum mapping unit size limit was 0.125 acre (*ca* 72 ft x 72 ft). In order to correctly compare seagrass area changes occurring between the 10 years, the assumption had been made to filter the current 1:9,600 seagrass coverage to eliminate bare areas less than 72 x 72 ft (22 x 22 m) in size (*ca* 66 x 66 pixels), prior to overlay analysis between the 2004 and 1994 datasets. In the 1994 seagrass map, such small bare areas within a seagrass bed would have been included as part of a surrounding seagrass polygon.

However, an unforeseen problem was encountered when attempting to overlay the two coverages from the different years. It was discovered that registration differences were too great between the datasets from the 2 time periods; spatial offsets were on the order of 25m to 50m. This would have introduced excessive error into the analysis, on the order of 50% or more for polygons in the 50 – 100m diameter range, making the measurement meaningless. Thus we were not able to perform an accurate overlay change analysis without re-registering or warping one dataset to another, and time did not permit this extra, very tedious analysis.

Two extra aerial color photographs were taken by the USDA-ARS, Weslaco Lab in early March of 2004, one each of the TFlats and East Flats sites. Although not technically part of this contracted study, these archived March 2004 datasets were examined and clearly demonstrate the seasonal differences in landscape features detectable by change analysis (photos available but not shown in this report). These 1:9,600 scale photographs show seagrass landscape features for each study site in the early part of the growing season when seagrass coverage is very reduced. It is particularly interesting to compare them to the same areas in the Dec. 2004 photography when seagrass coverage and macroalgae were close to maximum. Major differences are also observable in amounts of macroalgae present, as well as the grassbed morphology and distribution of bare patches.

Results and Discussion

Archived Photography of Target Sites

Georeferenced files (*.tif) of the 1:9,600 natural color photoimages have been produced for the Terminal Flats, East Flats, Estes Cove, and South Bay target sites (Figures II.3, II.4, II.5). The Packery Channel (Figure II.6) and Mustang Island sites were georegistered at 1:24,000 scale. All georegistered photoimages are now available in the GIS archive library at River Systems Institute, Texas State University-San Marcos. Due to errors in collecting ground control points, the 2004 Kennedy Causeway photograph has still not been adequately georeferenced at < 1m precision.

Terminal Flats Image and GIS Analysis

Classified vegetated and bare areas (as vector *.shp files) were compared between 2003, 2004 and 2005 for the 1:9,600 scale TFlats image as shown in Figure II.15. This GIS analysis was performed on a smaller landscape unit area (LUA) subset from the larger ROI area. ArcGIS or ArcView GIS analysis procedures were applied to assess the spatial patterns and changes occurring between years in the LUA thematic maps. Table II.3 below shows that, as total bare area in the LUA significantly increased from 2004 to 2005, the average polygon size greatly increased. The number of individual bare polygons peaked out in 2004, but then declined in 2005 to 2003 levels as much of the LUA area opened up and small bare areas became aggregated. One could hypothesize that the increase in bare polygons from 2003 to 2004 represents fragmentation occurring in the TFlats bed, prior to major loss of seagrass from 2004 to 2005. The result of this fragmentation would be the seagrass bed landscape in 2005 consisting of very large, continuous bare areas, and patchy seagrass bed morphology.

Distribution of bare areas, vegetation, and macroalgae requires further assessment as a function of depth and fetch, using the NOAA bathymetry (see Figure II.16 of NOAA data), in combination with field survey measurements at the plant scale.

Table II.3 - Comparison of bare polygon statistics for Terminal Flats LUA for 3 years.

Year	# Bare polygons	Total area (m^2)	Avg. polygon (m^2)
2003	24,489	167,219	6.83
2004	75,187	183,997	2.16
2005	18,852	547,582	104.22

GIS comparison of macroalgae accumulations (Figure II.17) revealed that Algae as percentage of the total ROI area were relatively constant in both 2004 (11.375 ha/ 241 ha = 4.72 %) and 2005 (10.554 ha/ 241 ha = 4.38 %). The location of Algae accumulations also appears similar between the 2 years, with heaviest accumulations occurring in large, open bare areas on the east side of the LUA along the 2-3 ft depth contour (between 0 to -1 m). Other algal deposits were found within the grass beds in bare depressions ('erosion blowouts') or trenches created by prop scarring or other boating activities. For both 2004 and 2005, algal accumulations are consistent with hydraulic deposition resulting from the prevailing S-SE fetch, in combination with wind-driven tides. In addition, the spoil islands and bathymetry of the western TFlats area also alter the circulation in this back part of the bay, and appear to trap drifting/floating material carried in by the fetch and tides.

East Flats Image and GIS Analysis

ArcGIS or ArcView GIS analysis procedures were employed to assess the spatial patterns in the seagrass thematic images (i.e. vector files) of East Flats during 2004. Comparison of changes in vegetated and bare areas at East Flats between 2004 and 2005 are not available. As with the TFlats study site, spatial statistics for the bare and vegetated area classes were calculated on smaller landscape unit areas (LUA), consisting of the deep-water and shallow water LUAs. However, statistics from these analyses have not been completed ; see Figure II.14.

GIS comparison of macroalgae accumulations at East Flats (Figure II.18) revealed that Algae as percentage of the ROI area was relatively similar in both 2004 (5.422 ha/ 184 ha = 2.95 %) and 2005 (6.025 ha/ 184 ha = 3.27 %)). Interestingly, this combined areal percentage of East Flats macroalgae (3.11%) is only about 68% that of the TFlats ROI (4.55%) on the bay-side of the estuary. The locations of East Flats Algae accumulations were similar between 2004 and 2005, with most deposits occurring on the north and west sides of the ROI along the -1 to 0 m depth contours. In 2004, algal deposits also appeared to be scattered more extensively over the very shallow flats to the northeast. For both years, these algal accumulations would seem to be explainable as a result of the hydraulics created by the prevailing S-SE fetch, in combination with wind-driven tides.

Distribution and patterns of bare areas, vegetation, and macroalgae can be assessed as a function of depth, using the NOAA bathmetry, as well as fetch and anthropogenic features. Correlations between depth and seagrass patterns allow for extrapolation to water quality and light effects on the seagrass landscape. By correlating landscape patterns with sufficient field GPS point data, we should be able to determine the broad scale extent of disturbances to the ecosystem. This approach will be discussed further in Conclusions section as the basis of Integrating Landscape and Plant Scale datasets.

Comparison of 1:9,600 vs. 1:24,000 Scale Photography

The resolution, accuracy, and total landscape coverage for the 1:9,600 and 1:24,000 scales of photography were compared, using macroalgae, seagrass and bare areas as landscape health indicators. Figures II.19 and II.20 present comparative overlays of 2004 macroalgae distribution delineated at 1:24,000 and 1:9,600 scales for Terminal Flats and East Flats, respectively. At both sites, less macroalgae was delineated in the ROI at the smaller scale (1:24,000) compared to the 1:9,600 scale, although the difference was much less for East Flats. TFlats decreased by 16.8% (11.38 ha vs. 9.47 ha), while East Flats decreased by only 3.7% (5.42 vs. 5.22 ha) at the smaller 1:24K scale. In addition, at East Flats, it was more difficult visually to separate seagrass from mixed algae/seagrass, leading to a decrease of 62% in the mixed algae/seagrass class. Most of these differences between the 2 photoscales were due to spectral differences from film exposure at 2 different altitudes. At 1:9,600, algae

appeared distinctly purple to reddish-brown, while at 1:24,000 many of the same algal deposits appeared greener or browner (i.e. similar to seagrass). This made algae delineation more subjective at the 1:24,000 scale.

Figures II.21 and II.22 display the classified grid polygon results for the Terminal Flats and East Flats sites, respectively. As previously stated, these grid polygons had been carefully interpreted and digitized on-screen from photography at each of the respective scales. Tables 4 and 5 present the summary statistics for the classified grid polygons in the figures. In summary, several conclusions can be drawn:

1. Overall, the difference in grid macroalgae between photoscales paralleled that observed previously for the total ROI results. When 1:9,600 was compared to 1:24,000 results, grid algae at TFLats showed a decrease of 30 % at the smaller scale, while East Flats grid algae showed a decrease of 5.8 % for pure algae and 66% for mixed algae/seagrass. This verified that it was more difficult to accurately delineate macroalgae at the smaller 1:24,000 scale than at the higher resolution 1:9,600 scale.

2. When 1:24,000 was compared to 1:9,600 photoimagery, the dense seagrass class acreage increased by 2.7% at TFlats or 7.6% at East Flats. This represents an overestimation of dense seagrass at the smaller 1:24000 scale. While this total acreage may not seem like much, it is significant to look at the change in polygon size due to increase in number of polygons. Avg. polygon size for dense seagrass shows a decrease of 74% at TFlats, and 32.6% at EFlats, between 1:9,600 and 1:24,000 scale. This represents a major increase in number of dense seagrass polygons (306% at TFlats, 59.6% at EFlats).

31

3. The main factor accounting for the decrease in dense seagrass polygon size at both sites was the large decrease in numbers of patchy and bare area polygons. Bare area and patchy seagrass area decreased slightly at both sites when 1:9,600 is compared to 1:24,000 scale, but the number of those polygons greatly decreased. For number of bare area polygons, TFlats showed a 21% decrease, and EFlats a 23.3% decrease, at the 1:24,000 scale. For number of patchy polygons, TFlats showed a 23.6% decrease, and EFlats a 20.6% decrease, at the 1:24,000 scale. This decrease in polygons was accompanied by a corresponding increase in polygon size. Bare polygon size increased by 24.8% at TFlats and 28.7% at East Flats between 1:9,600 and 1:24,000 scales. Patchy polygon size increased by 33.2% at TFlats and 25% at East Flats between 1:9,600 and 1:24,000 scales.

The major conclusion from this analysis was that bare and patchy polygons were greatly underestimated at the 1:24,000 scale compared to 1:9,600. This means that small bare patches less than 2-3 m^2 (*e.g.* prop scars, small blowouts, erosion foci, etc.) are not detectable unless the high resolution imagery is used. Such small bare patches are the main characteristic of fragmenting seagrass beds, and represent potential sublethal health indicators. Furthermore, macroalgae deposits, which may also indicate an environmental impact to seagrass beds, were also inaccurately delineated and underestimated by 5-20%. Thus, while vegetation distribution mapping (presence and absence) may be comparable between the two photoscales, landscape analysis of health indicators would require the higher resolution (1:9,600 scale) imagery for adequate detection and statistical measurement.

Table II.4 - **Terminal Flats 2004 acreage statistics** . Comparison of classified polygons in 13 grid cells (150m x 150m) at 1:9,600 and 1:24,000 photoscales.

POLYGON CLASS	1:9,600	1:24,000	Change
BARE			
# Polygons	645	511	-21 %
Area (m^2)	17,447	17,220	-1.3 %
Mean size (m^2)	27.0	33.7	+24.8%
PATCHY SG			
# Polygons	144	107	-23.6%
Area (m^2)	28,472	28,170	-1.2 %
Mean size (m^2)	197.7	263.3	+33.2%
DENSE SG			
# Polygons	60	244	+306 %
Area (m^2)	229,848	236,059	+2.7 %
Mean Size (m^2)	3,831	967.5	-74.3 %

MACROALGAE			
# Polygons	58	43	-25.9%
Area (m^2)	16,972	11,290	-33.5%
Mean Size (m^2)	292.6	262.6	-30.2%
TOTAL ACREAGE (m^2)	292,740	292,740	

Table II.5 - **East Flats 2004 acreage statistics** . Comparison of classified polygons in 12 grid cells (100m x 100m) at 1:9,600 and 1:24,000 photoscales.

POLYGON CLASS	1:9,600	1:24,000	Change
BARE			
# Polygons	567	435	-23.3%
Area (m2)	29,653	29,122	-2.1%
Mean Size (m2)	52.3	67.3	+28.7%
PATCHY SG			
# Polygons	97	77	-20.6 %
Area (m2)	20,583	20,092	-2.4 %

Mean Size (m2)	212.2	260.9	+25%

DENSE SG

# Polygons	136	217	+59.6%
Area (m2)	57,703	62,070	+7.6%
Mean Size (m2)	424.3	286.0	-32.6%

MACROALGAE

# Polygons	55	46	-16.4%
Area (m2)	8,038	7,567	- 5.8%
Mean Size (m2)	146.2	190.3	+30.2%

MIXED SG/ALGAE

# Polygons	40	23	-42.5%
Area (m^2)	4,362	1,489	-65.9%
Mean Size (m^2)	109	64.7	-40.6%
TOTAL ACREAGE (m2)	120,339	120,340	

Accuracy Assessment

Error matrix analyses of the 2004 Terminal Flats and East Flats bare and vegetative thematic maps are summarized in Tables 6 and 7, respectively. For Terminal Flats (Table 6A & 6B), overall accuracies ranged from 75.8% to 89.2%, user's accuracy ranged from 42.8% to

Table II.6. Error matrixes for the 2004 Terminal Flats thematic map.

6(A)	Reference Data- from December 2004 + (March & May 2005)				
Classified Map Data	Bare Area	Seagrass	Macro-algae	Total Points	User's Accuracy (%)
Bare Area	24	1	1	26	92.2
Seagrass	0	20	2	22	90.9
Macroalgae	2	2	3	7	42.8
Total points	26	23	6	55	
Producers Accuracy (%)	92.2	86.9	50.0		
Overall Accuracy (%)	75.8				

6(B)	Reference Data- from December 2004 + (March & May 2005)			
Classified Map Data	Bare Area	Vebetated (Seagrass + Algae)	Total Points	User's Accuracy (%)
Bare Area	24	4	28	85.7
Vegetated Area	2	25	27	92.6
Total Points	26	29	55	
Producers Accuracy (%)	92.3	86.2		
Overall Accuracy (%)	89.2			

Table II.7. Error matrixes for the 2004 East Flats thematic map.

7(A)	Reference Data- from December 2004 + March 2005				
Classified Map Data	Bare Area	Seagrass	Macro-Algae	Total Points	User's Accuracy (%)
Bare Area	13	0	1	14	92.8
Seagrass	0	16	1	17	94.1
Macroalgae	1	1	1	3	33.3
Total points	14	17	3	34	
% ProducersAccuracy	92.8	94.1	33.3		
Overall Accuracy (%)	73.4				

7(B)	Reference Data- from December 2004 + March 2005			
Classified Map Data	Bare Area	Vegetated (Seagrass + Algae)	Total Points	User's Accuracy
Bare Area	13	1	14	92.8
Vegetated Area	1	19	20	95.0
Total Points	14	20	34	
% Producers Accuracy	92.8	95.0		
Overall Accuracy (%)	93.9			

92.6%, and producer's accuracy range from 50.0% to 92.3%. Lower accuracy was related to macroalgae drifting into or out of bare areas (see Table II.6A), between the time photography was flown in December and when groundtruthing was conducted several months later. The best accuracies were achieved when the ground-truth data were obtained closer to the image acquisition date (e.g., December and March). When the accuracy assessment was performed between bare areas vs. generalized 'vegetated areas' (see Table II.6B), producer's and user's accuracies greatly improved, showing that confusion between seagrass and macroalgae was the main problem.

These results were repeated for the 2004 East Flats site. Tables II.7A and II.7B reveal that overall accuracies ranged from 73.4 % to 93.9%, while both user's accuracy and producer's accuracy for bare and seagrass areas ranged from 92.8 to 95.0 % for East Flats.

In general, most observed map errors occurred at GPS points where 1) very sparse seagrass stands were misclassified as bare, or vice versa or 2) drifting macroalgae aggregations had covered up or washed away from a bare area. Some ground-truth data for the 2004 photoimages had been collected up to four months after image acquisition. Therefore, some actual bare areas in December 2004 could have become vegetated with either seagrass or macroalgae by the time ground-truth was performed, especially by March. The opposite may have occurred for some 'vegetated' areas which later became bare, due to coverage with drifting macroalgae on the photography date. Other investigators have also postulated that substantial time between image acquisition and ground-truthing may have contributed to error obtained in their studies (Lanthrop, Montesano, and Haag 2006; Luckzkovich *et al.* 1993). To achieve the highest accuracies, Congalton and Green (1999) have recommended that ground-truth data must be obtained as close as possible to the date of image acquisition.

Correlating Seagrass Bed Patterns with Disturbance Processes

From the thematic maps of bare and vegetative areas within Terminal Flats and East Flats seagrass beds, (see Figures II.4, II.5, II.8, II.9, II.12, II.13, II.14, for example), a variety of bare area shapes are identifiable: fine-scale linear features (boat propeller scars) and small, 1-2 m^2 patches, or larger, (50-100 m^2) rounded to amoeboid, bare areas. In addition, distinctive seagrass bed morphologies are also apparent (e.g. patchy, reticulated beds; 'doughnut-

shaped' seagrass patches; and circular beds/patches mostly in deeper waters). Changes in these features over time can provide important clues about the types of disturbance processes that cause them.

Obviously, increases in continuous linear features (such as prop scars or pipeline trenches) or dredged, deepwater channels represent increased anthropogenic disturbance. The impact of propeller scarring to the central and northern sections of the TFlats seagrass bed were easily seen on all thematic maps (Figures II.4, II.8, II.10). When vegetation filled in these disturbed linear scars as well as ovoid bare patches, ground observations indicate much of this was drift macroalgae and not rooted seagrass. A dramatic increase in broad scale bare areas was also observed from 2004 to 2005 at TFlats. These changes from vegetated to bare primarily occurred in the eastern and southern sections of the study site (Figure II.14), where large accumulations of macroalgae were often found. Where non-anthropogenic loss of seagrass vegetation occurred (Figures II.14 and II.15), either hydrodynamic processes (fetch and erosion) in shallow water areas or light limitation in deep water appear responsible. Much less propeller/pipeline scarring and channelization was apparent at the East Flats site (Figures II.12, II.13, and II.15) than TFlats , verifying that this site is subjected to much less physico-mechanical, anthropogenic disturbance. Thus, propeller and pipeline scarring, channelization, deposition of drift macroalgae and wrack due to currents, hydraulic conditions, erosion and scour from winter storms or tropical weather systems, or water column light limitation, are considered the major seagrass disturbance processes at work in these sites.

Conclusions

The Seagrass Strategic Monitoring Plan for Texas coastal waters has recommended landscape-scale monitoring to assess health of and disturbance to Texas seagrass beds (Pulich *et al.* 2003). Standard photointerpretation and manual classification procedures on 1:24,000 scale color photography are not practical for providing the necessary information needed to meet these objectives for a broad-scale monitoring program. Thus remote sensing studies of high resolution aerial photography at 2 intensive study sites were undertaken to evaluate automated, digital image analysis methods for delineating landscape features and documenting changes in seagrass beds indicative of human and/or natural disturbances. The 2 sites, East Flats and Terminal Flats, were chosen based on previous work (Pulich *et al.*

1997; Dunton and Schonberg 2002) which inferred that one was a relatively pristine, undisturbed site (East Flats), while Terminal Flats may be highly impacted (at least anthropogenically).

The semi-automated method employed for this project integrated high resolution aerial color photography, RGB to HIS color space transformation, pixel thresholding models, and GIS technology to evaluate change dynamics of bare areas within seagrass beds over time. Map accuracies (i.e., user's accuracy, producer's accuracy, and overall accuracy) ranging from 73% to 95% were obtained with this procedure. It was possible to accurately follow and monitor fine-scale changes in two Texas seagrass bed sites (180 to 250 ha in size) over several years.

It had been anticipated that landscape monitoring of seagrass bed disturbance would require higher resolution photography than 1:24,000 scale data traditionally used for seagrass mapping (TSMP 2003). Several analyses comparing accuracy and effective coverage between 1:9,600 and 1:24K scale photos were performed at the 2 study sites which confirmed this expectation. The major conclusions from this analysis are that bare and patchy polygons less than 2-3 m^2 in size are significantly underestimated at the 1:24,000 scale compared to 1:9,600. Small bare patches (eg. prop scars, small blowouts, erosion foci, etc.) cannot be detected unless the high resolution imagery is used. These small bare patches are the main characteristic of fragmenting seagrass beds, and represent potential sublethal health indicators. Further, macroalgae deposits, which may also indicate an environmental impact to seagrass beds, were also inaccurately delineated and underestimated by 5-20%. Thus, while distribution mapping (presence and absence) may be comparable between the 2 photoscales, landscape analysis of health indicators would require the higher resolution (1:9,600 scale) imagery for adequate detection and statistical measurement.

Integrated monitoring of these landscape indicator features and patterns combined with plant scale (microscale) measurements from field surveys requires an *a priori* hypothesis for testing (Kirkman 1996; Duarte 1999; Lathrop *et al*. 2001; Fonseca *et al*. 2002). If aerial photography or imagery were used as the basis to hypothesize the location and direction of landscape gradients for disturbance processes, then field sampling could be designed to test

for disturbance processes across this gradient. In effect, the 2 scales of monitoring could be integrated through a conceptual model based on a hypothesis of spatial gradients in disturbance processes. Two obvious landscape gradients for sampling are: 1) the shallow- to deepwater gradient which encompasses and reflects water column light attenuation conditions (Lathrop *et al.* 2001), and 2) fetch and hydraulic gradients (Fonseca *et al.* 2002). Geostatistical analysis of the landscape sampling data could then confirm or reject the existence of or impact from the disturbance gradient. Even subtle trends in these landscape indicators (such as bare patch patterns, macroalgae deposits, seagrass species patterns, or unusual bed morphology), should ultimately prove invaluable in detecting declines or loss of health in seagrass beds, long before their complete loss or disappearance.

The overall study findings show that the integration of high resolution digital color photography, RGB color space transformation, pixel threshold models, and GIS technology was successful for monitoring changes in bare and vegetated areas within these Coastal Bend, Texas seagrass beds. The techniques applied here based on 1:9,600 scale photoimagery have documented potential for accurately assessing areas at least the size of those in this study (*ca* 250 ha). Future studies should extend and refine the image processing techniques, evaluate other geographic areas using the integrated landscape gradient approach, and develop landscape metrics of disturbance based on geostatistical analysis of landscape features and their relationship to disturbance processes at the plant scale. It would also be worthwhile to compare this technique using color transformed aerial photography with results using much more expensive multispectral imagery; this would probably prove its cost-effectiveness and effectiveness as a routine source of aerial remote sensed data.

Acknowledgements

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Ward, D.H., Markon, C.J., and Douglas, D.C., 1997. Distribution and stability of eelgrass beds at Izembek Lagoon, Alaska. *Aquatic Botany,* 58, 229-240.

Zainal, A.J.M., Dalby, D.H., and Robinson, I.S., 1993. Monitoring marine ecological changes on the east coast of Bahrain with LANDSAT TM. *Photogrammetric Engineering and Remote Sensing,* 59, 415-421.

Zieman, J.C., 1982. The Ecology of the Seagrasses of South Florida: A Community Profile, FWS/OBS- 82/25, U.S. Fish and Wildlife Service, 185pp.

Figure II.1-Eight target sites in CBBEP study area selected for 1:9,600 scale photographic monitoring [modified from Pulich *et al.* (2003), Texas Seagrass Monitoring Plan].
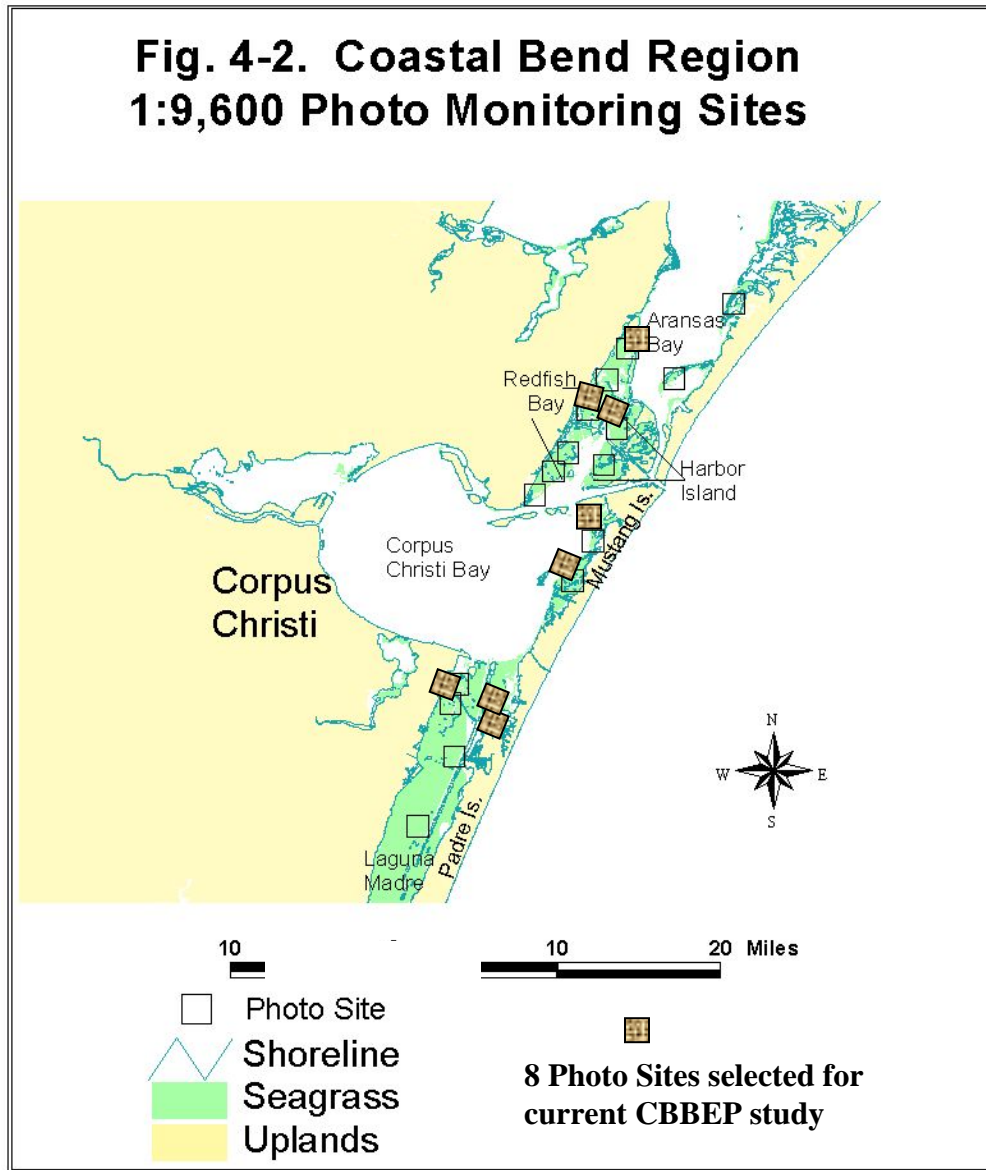
Figure II.2-Site map showing intensive sampling sites for integrated landscape and field seagrass studies in Redfish Bay-North and East Flats. The 1:9,600 scale photographs are shown within a 1:24,000 scale photo footprint.
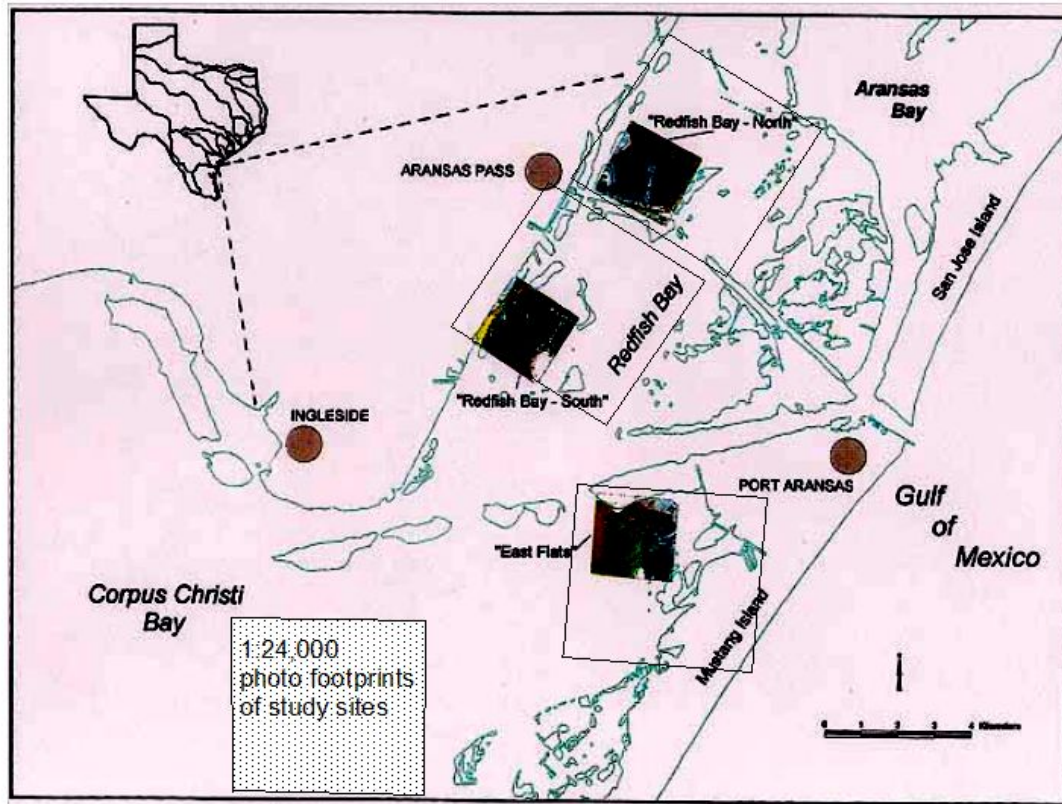
Appendix A

Chapter 2 Figures

II. Monitoring Landscape Indicators of Seagrass Health Using High Resolution Color Aerial Photography

Figure 3. Terminal Flats and South Bay 1:9,600 scale photoimages overlayed on DOQQ of N. Redfish Bay at 1:24,000 scale
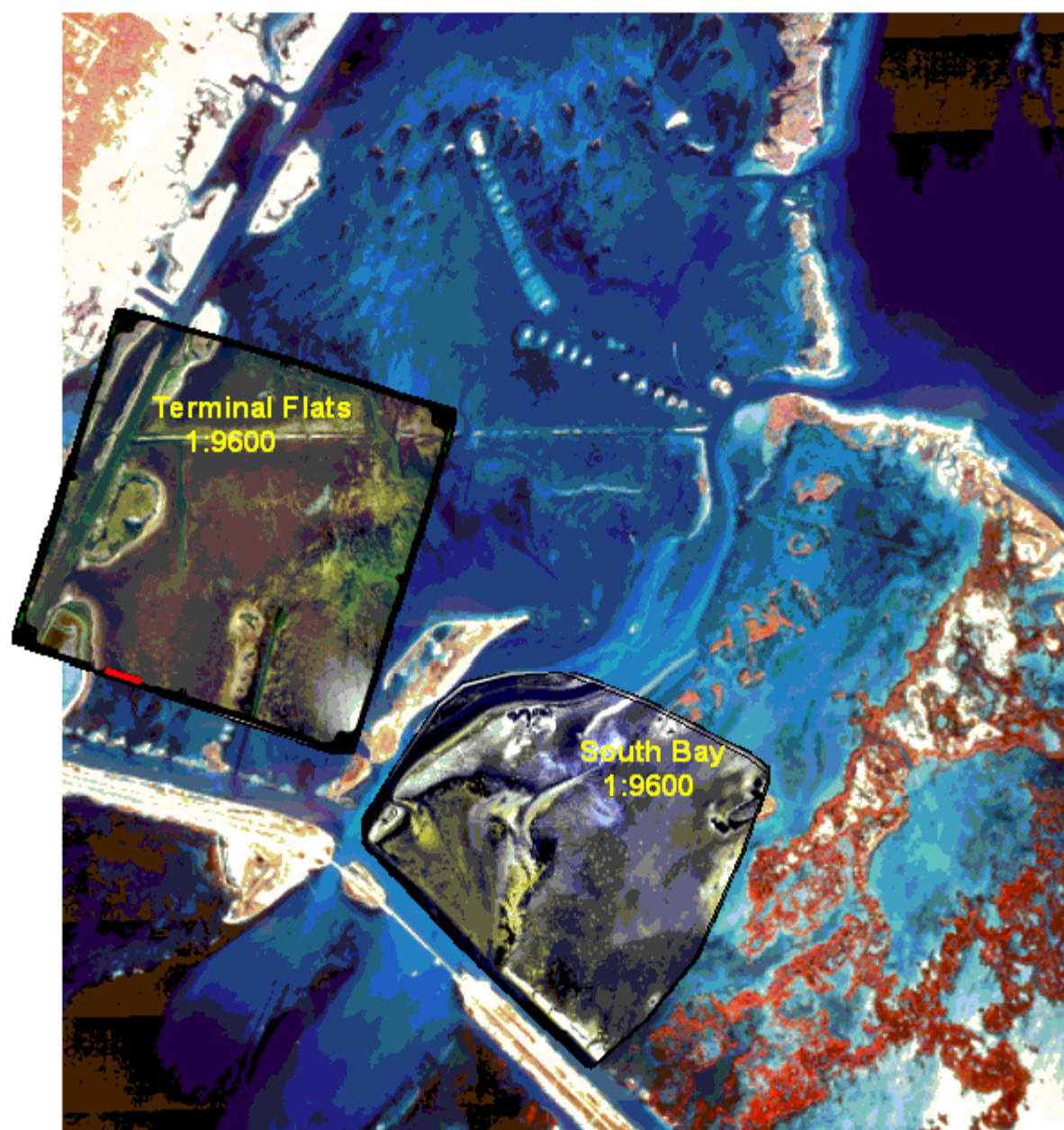
Subset_tflts96_dec04_polygeo2.tif
:Layer_1
:Layer_2
:Layer_3
Subset_sthbaydec04_24k.tif
:Layer_1
:Layer_2
:Layer_3
27097h18.tif
:Layer_1
:Layer_2
:Layer_3
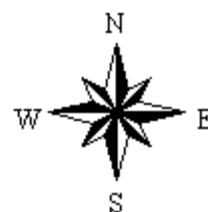
2   0   2   Kilometers

Figure 4. Terminal Flats site, 2004 and 2005.
1:9,600 photographs with ROIs overlaid.
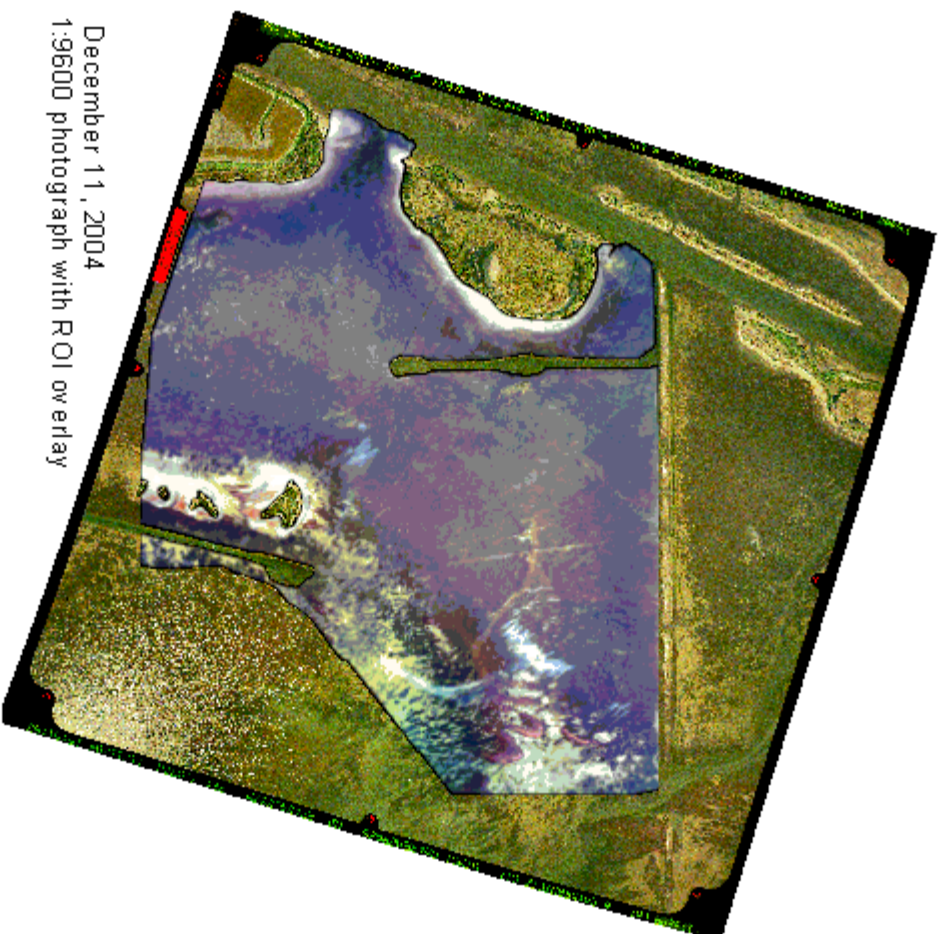
December 11, 2004
1:9600 photograph with ROI overlay

Tfltsdec2004_finalroi.tif
:Layer_1
:Layer_2
:Layer_3

December 11, 2005
1:9600 photograph with ROI overlay

Tflts2005dec_georoi.tif
:Layer_1
:Layer_2
:Layer_3
Georef_01_0005.tif
:Layer_1
:Layer_2
:Layer_3

0.9   0   0.9   Kilometers
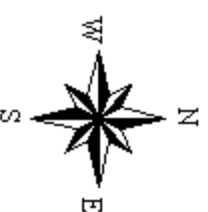
Figure 5. East Flats site, 2004 and 2005.
1:9,600 photograph and ROIs

December 11, 2004
1:9600 photograph with ROI overlay

Estflts9600_2004roi1a.tif
:Layer_1
:Layer_2
:Layer_3

Tfltsdec04_rgbtohsv.jpg
:Layer_1

December 11, 2005
ROI from 1:9600 photograph

Estflts2005roi_geojun20.tif
:Layer_1
:Layer_2
:Layer_3
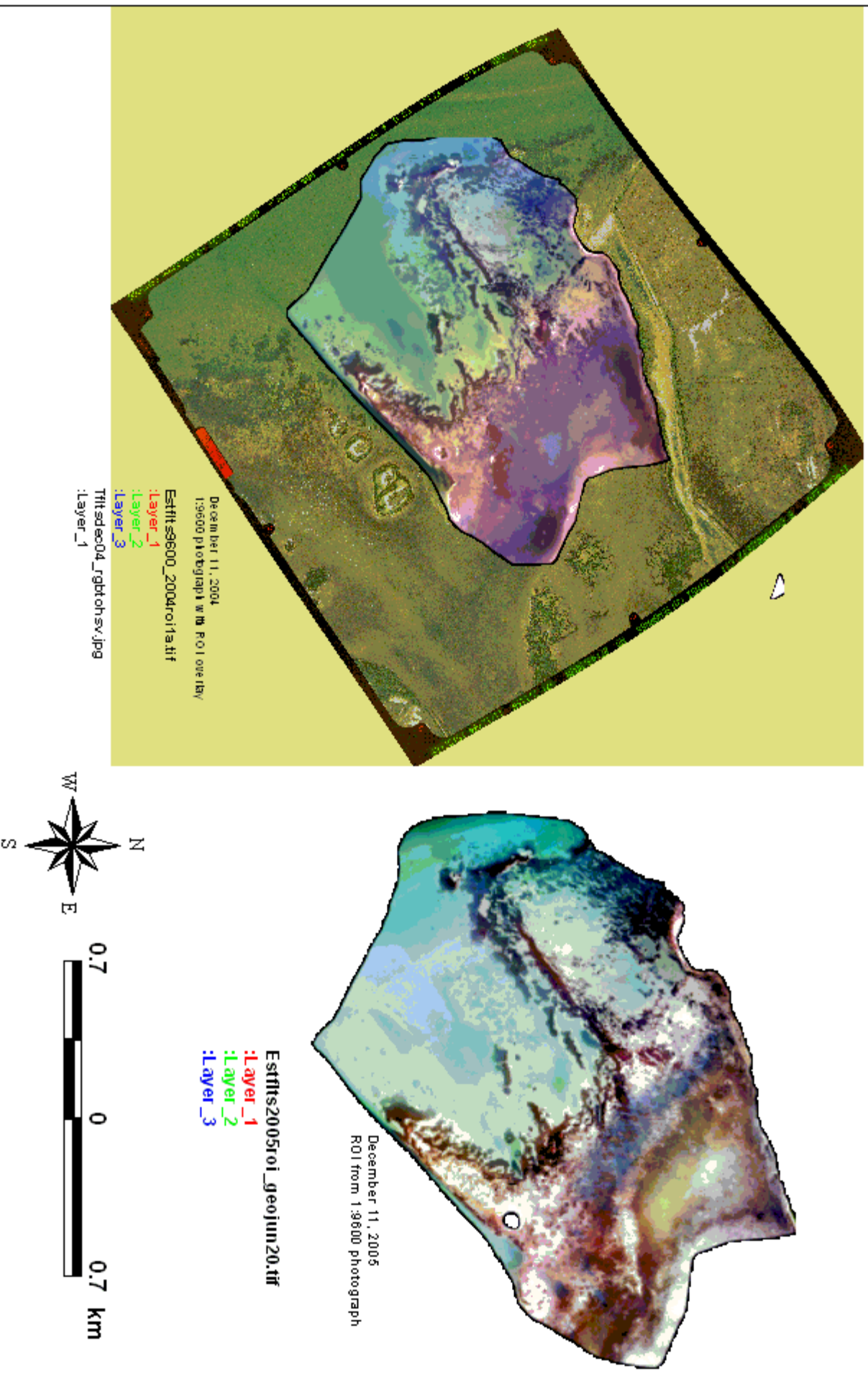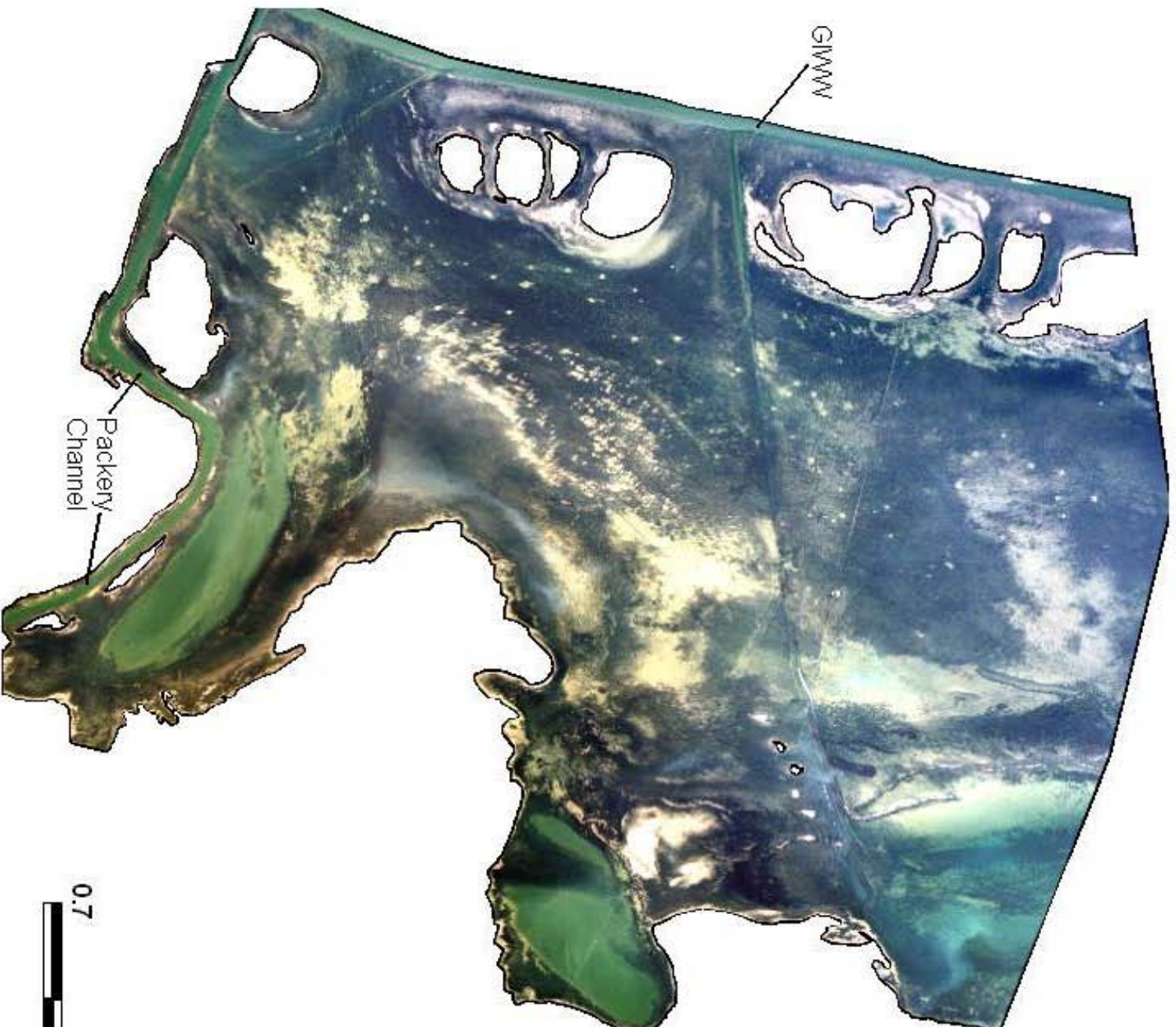
0.7   0   0.7   km

Figure 6.
Packery Channel site,
2004 ROI (1:24,000 scale)

Packery24k_2004finalroi.tif
:Layer_1
:Layer_2
:Layer_3

GIWW

Packery
Channel
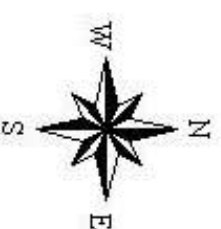
0.7   0   0.7 Kilometers

Figure 7. Terminal Flats site, 2004. Saturation band image of ROI overlaid onto color photo after RGB to HSI transformation.
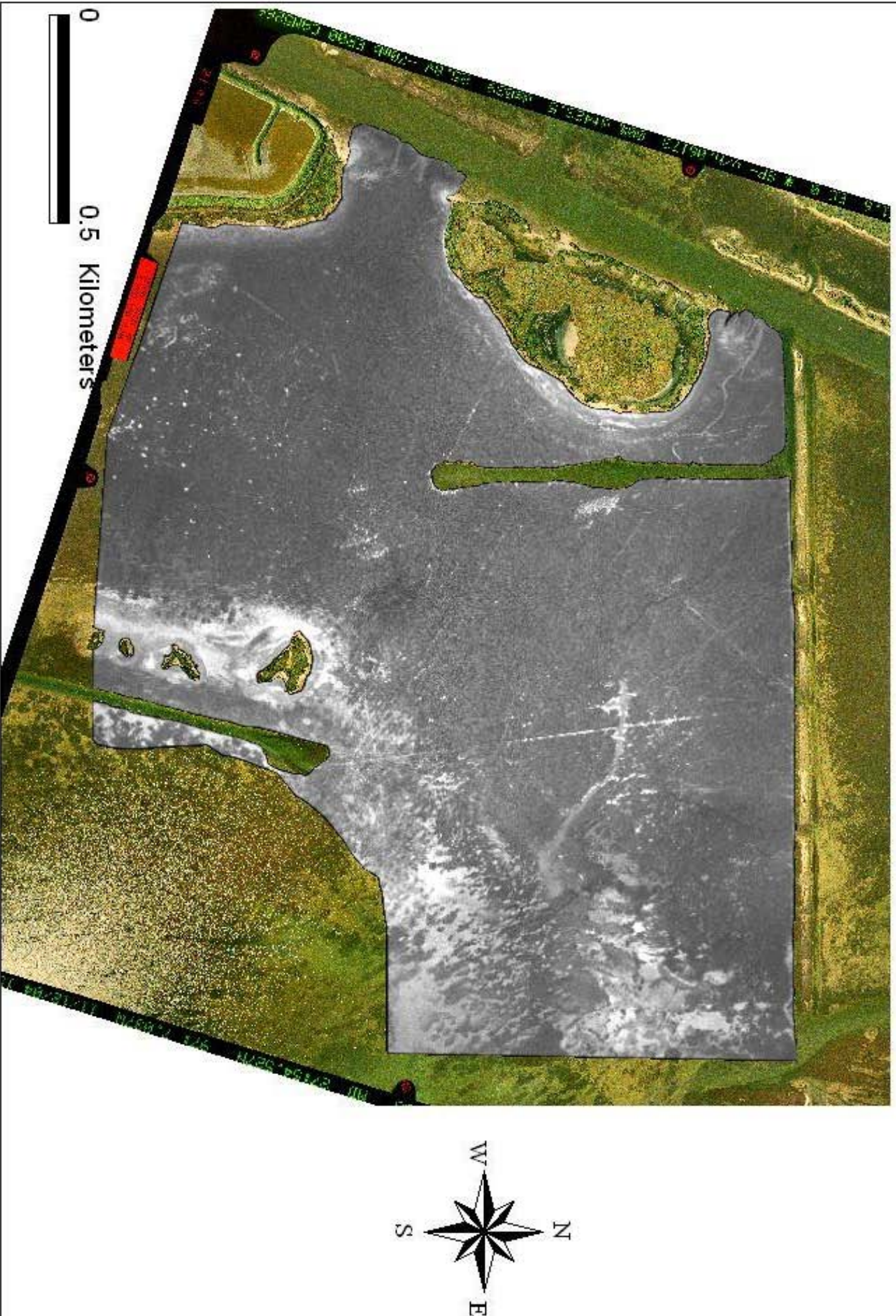
Figure 8. TFlats site 2004. Bare area mask, saturation band image, classified at threshold 38, with enlargement.
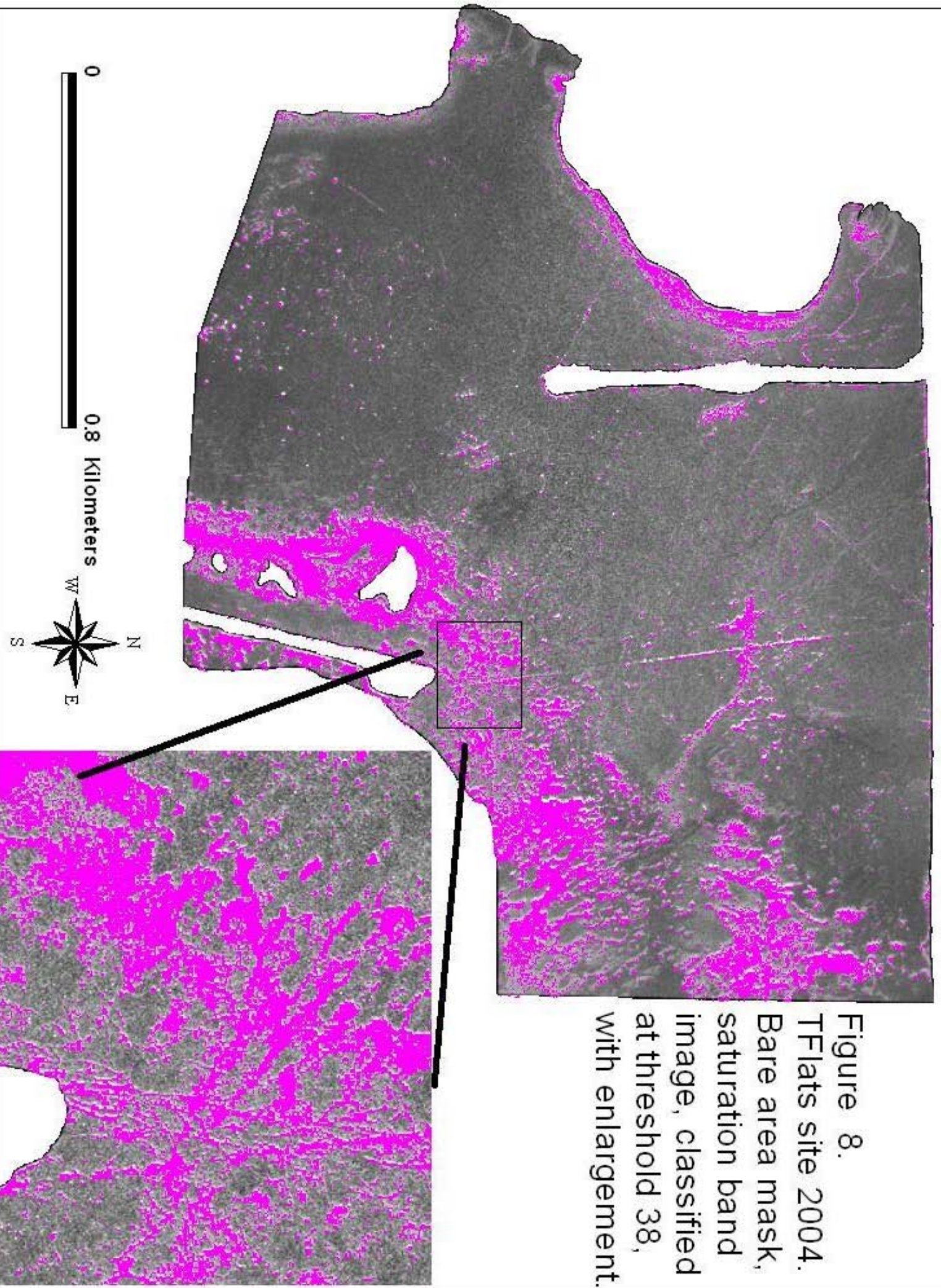
Fig. 9. Terminal Flats, 2005. Enlargement of Bare mask produced by density slicing Value band at dsr 170 threshold. Inset shows whole ROI.

Tflts2005_
valclass_dsr170.tif
☐ Vegetated
🟧 Bare Class

Tflts2005dec
_georoi.tif
:Layer_1
:Layer_2
:Layer_3

N W S E
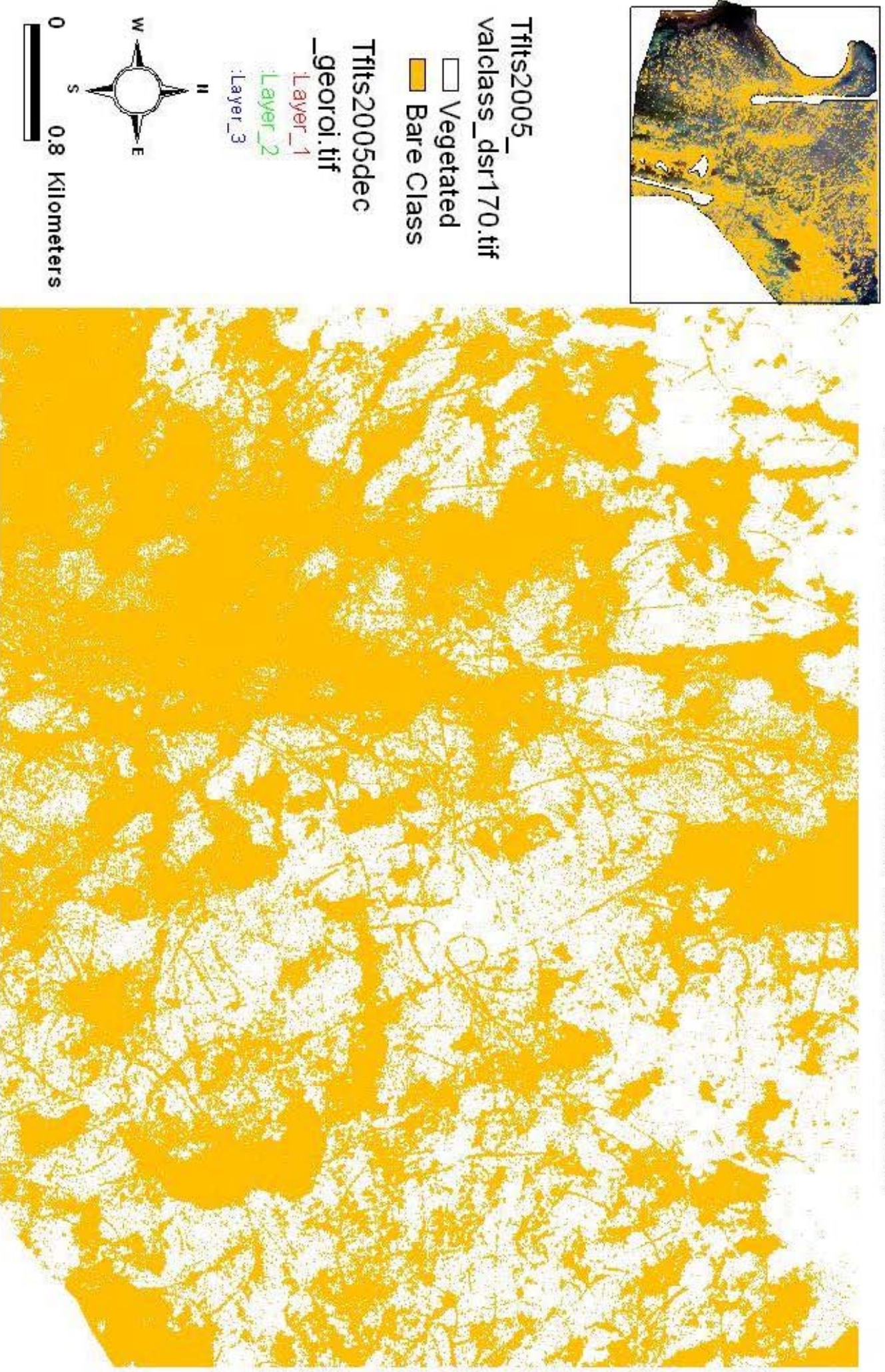
0   0.8   Kilometers

Figure 10. Terminal Flats 2005. Vegetated and bare masks classified from Saturation and Value bands by density slicing.
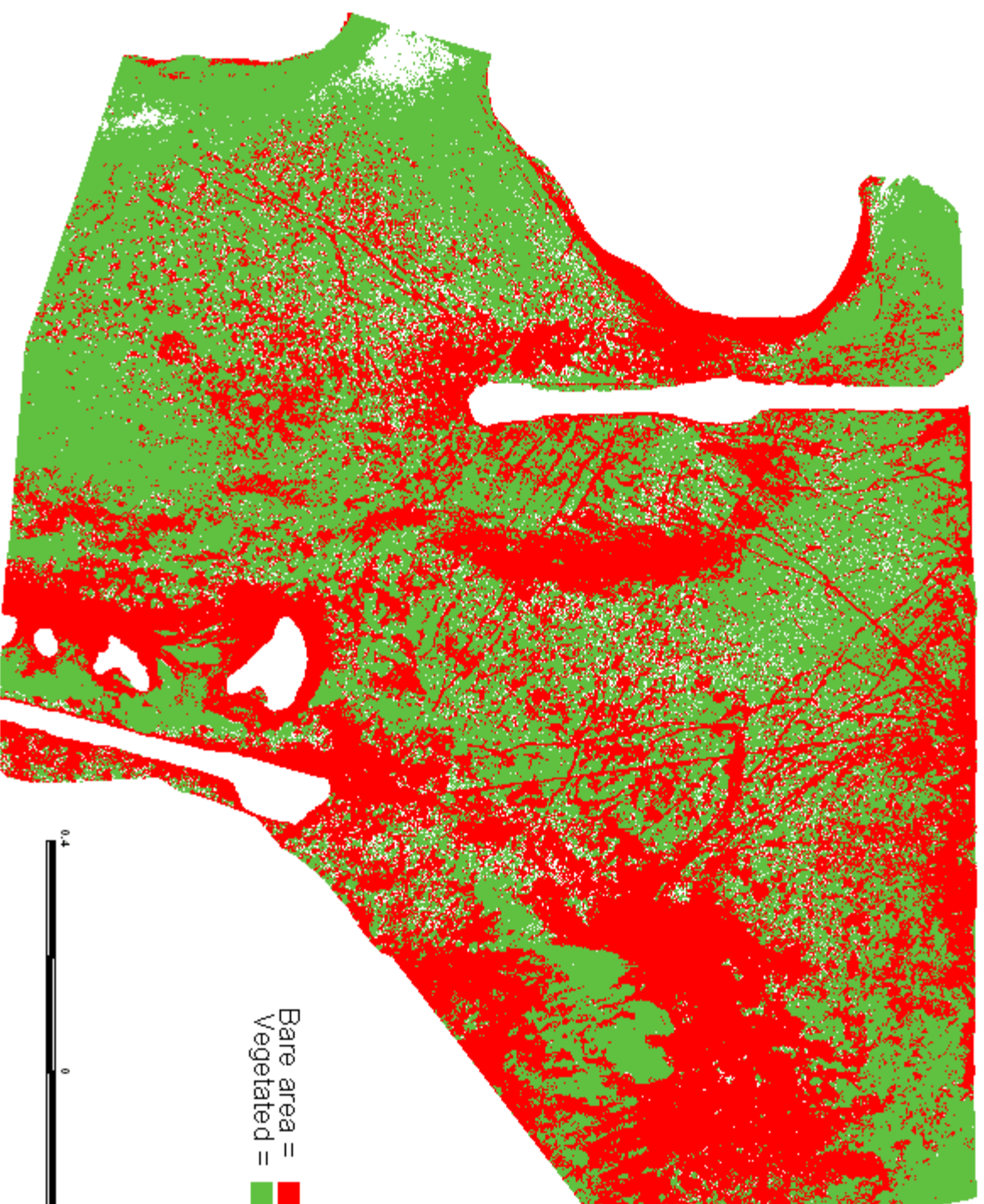
Bare area = Ps_post3.shp
Vegetated = Tflts2005_satvegclass50a.shp

Figure 11. East Flats 2004. Value band image after RGB to HSV color transformation
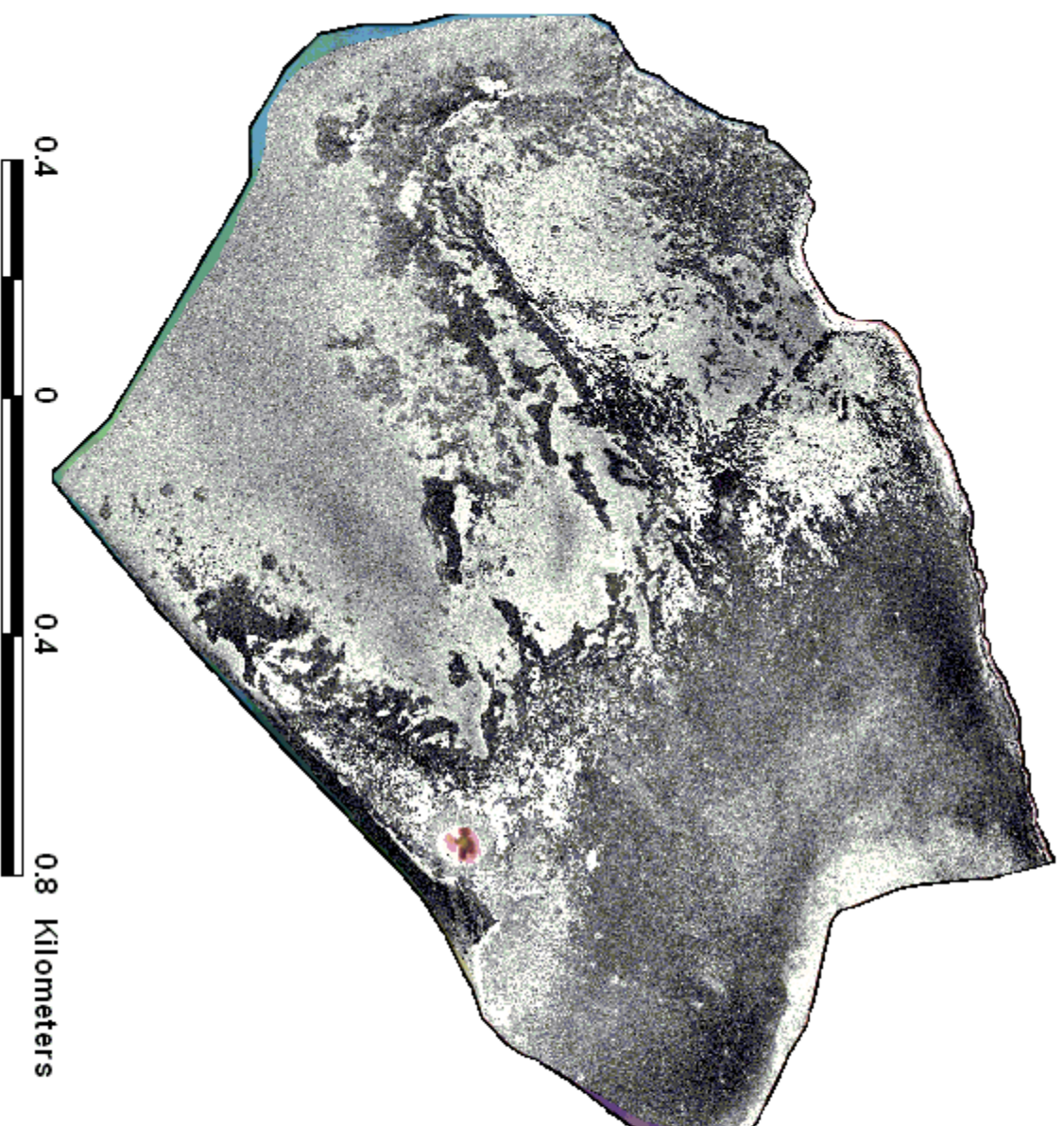
0.4   0   0.4   0.8   Kilometers

Estflts2005_valuebband.tif
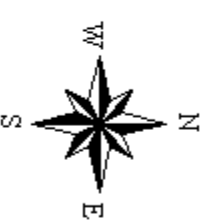:Layer_1
Estflts9600_2004roi1a.tif
:Layer_1
:Layer_2
:Layer_3

Figure 12a. East Flats 2004. Value band image with bare areas after density slicing at dsr threshold of 170
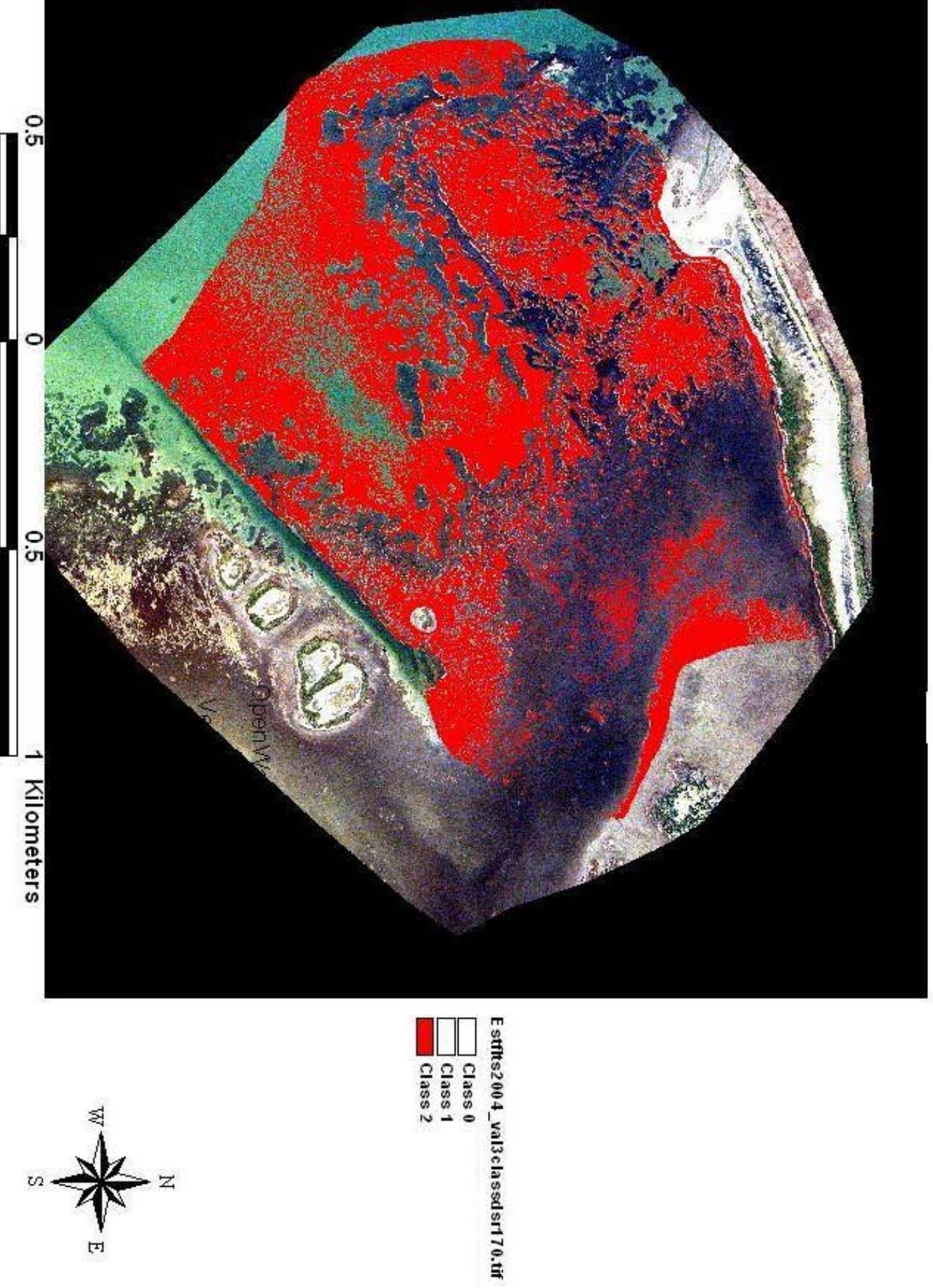
E stfits2004_val3classdisr170.tif

Class 0
Class 1
Class 2

Kilometers
0.5 0 0.5 1

Figure 12. East Flats 2004. Value band .tif image showing bare and vegetated areas after density slicing at dsr 180 threshold.
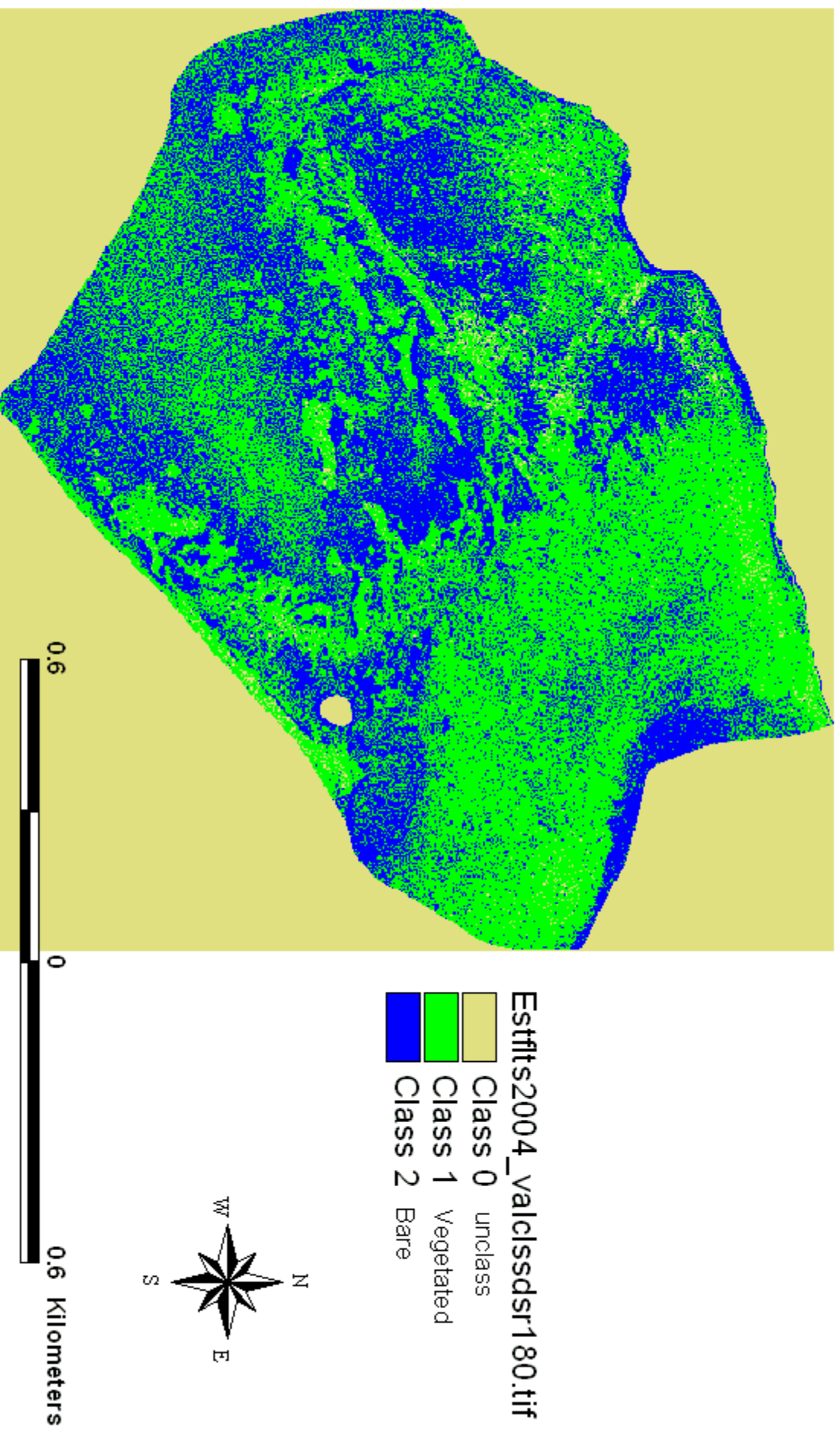
Estflts2004_valclssdsr180.tif

Class 0 unclass
Class 1 Vegetated
Class 2 Bare

0.6   0   0.6   Kilometers

Figure 13. East Flats 2004, classified vegetated and bare areas from density slicing of value (=intensity) band image

Deep Water=
Bare =
Vegetated=

Eflts2004jun20_deepwaterpoly.shp
Eflts2004jun20_medfil3_valdsr185cls6_3.shp
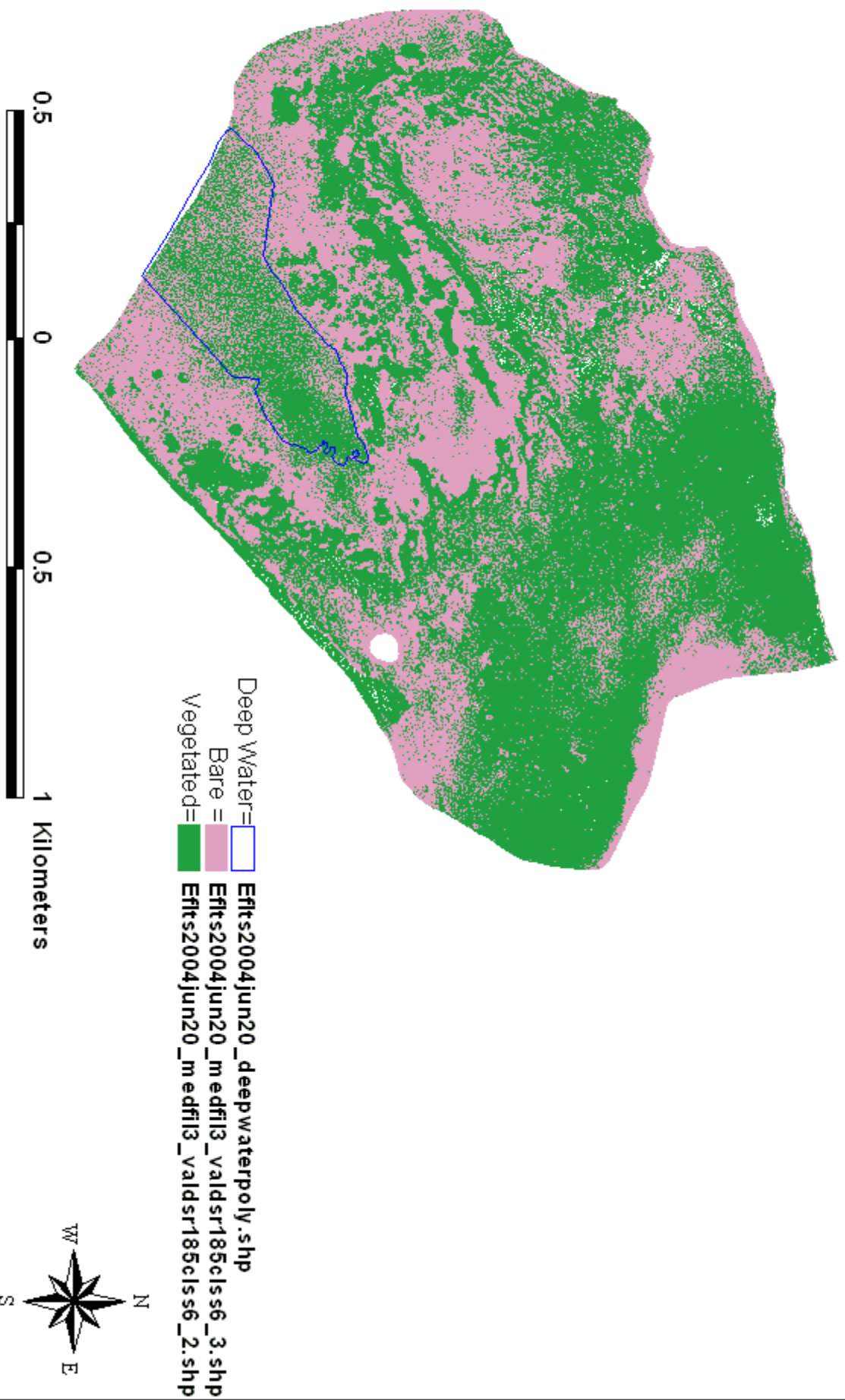Eflts2004jun20_medfil3_valdsr185cls6_2.shp

0.5   0   0.5   1 Kilometers

Figure 14. East Flats site, 2004. Vector file masks of bare and vegetated classes in A) southern, deepwater LUA, and B) northern, shallow LUA.
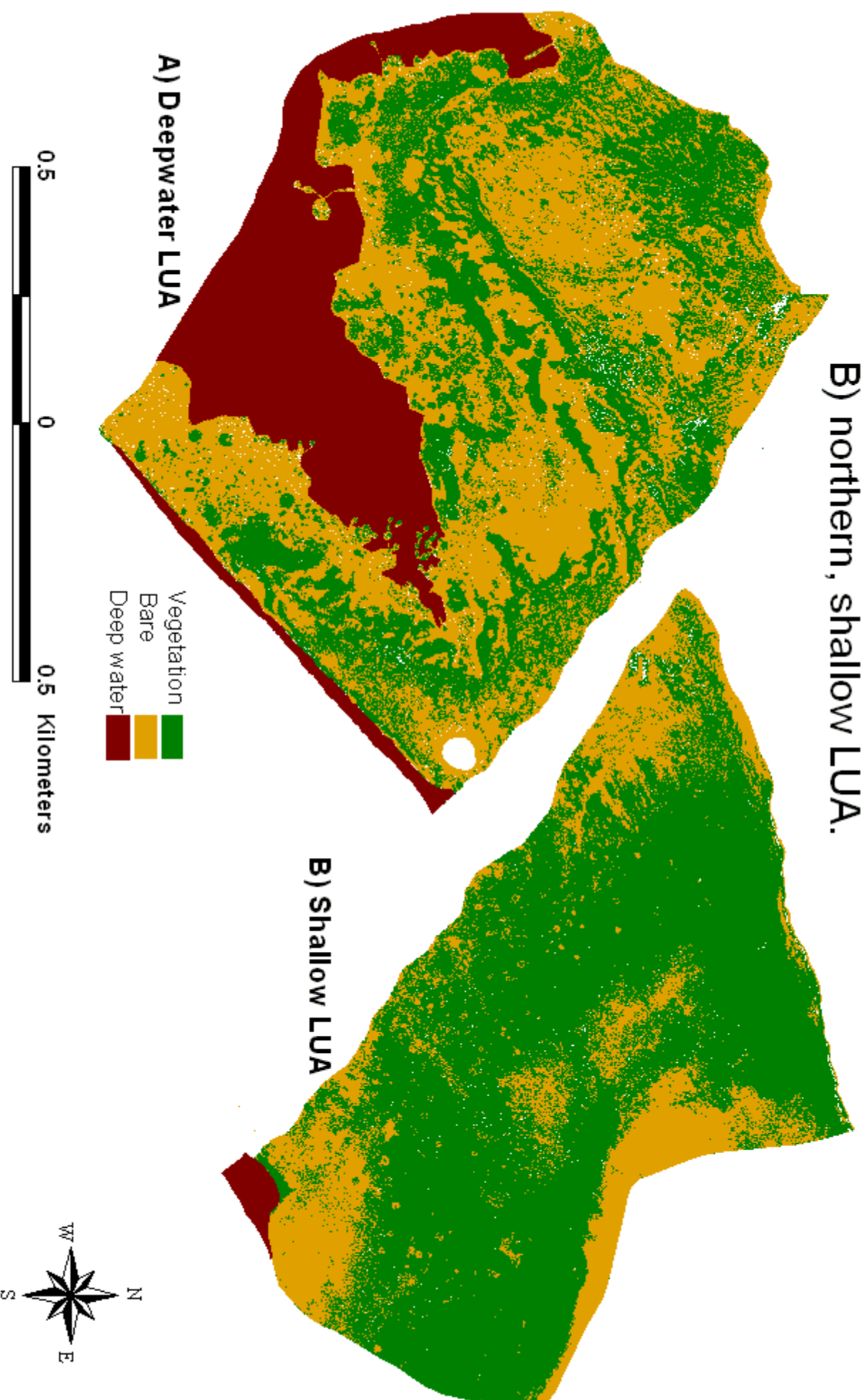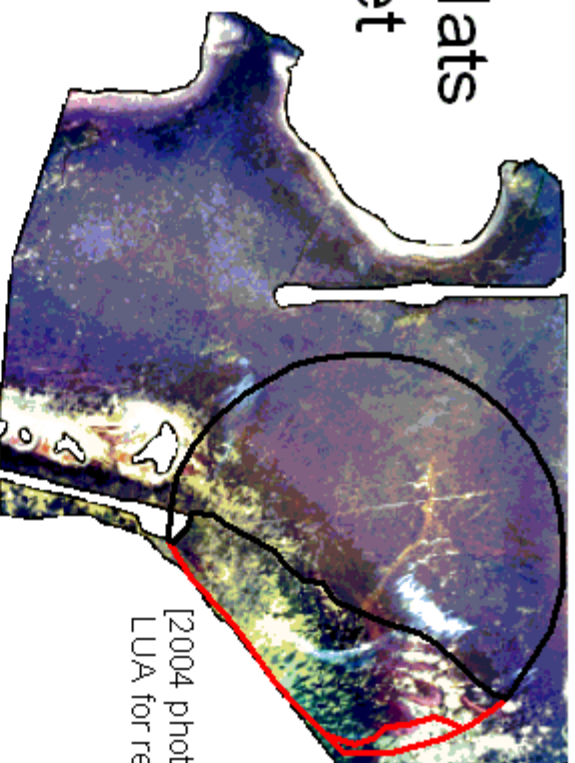
A) Deepwater LUA

B) Shallow LUA

Vegetation
Bare
Deep water

0.5   0   0.5   Kilometers

Figure 15.
Terminal Flats
LUA Subset
Analysis

[2004 photo ROI with LUA for reference]

2003 LUA
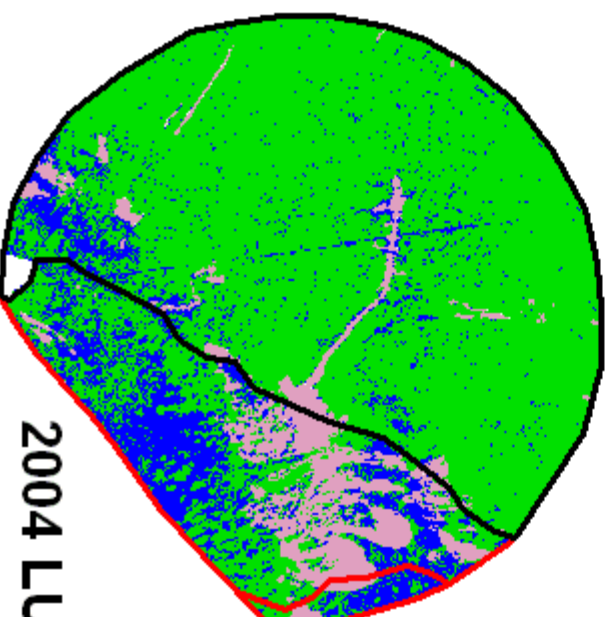

2004 LUA

2005 LUA

Figure 15

Landscape Unit Area (LUA) changes in Bare Patches (blue), Seagrass (green), and Macroalgae (pink) over 3 years at Terminal Flats site, N. Redfish Bay, Texas. LUA is divided into 3 depth zones from left to right (black outline 1-2 ft; black-red outline 2-3 ft; and red outline 3-4 ft). Prevailing fetch is from SSE.

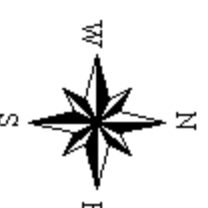| | | | | 0.3 | 0 | 0.3 Km |

Hectares within LUA			
Year	Seagrass	Macroalgae	Bare Area
2003	80.55	6.83	14.12
2004	75.74	9.38	16.22
2005	41.97	7.44	52.53
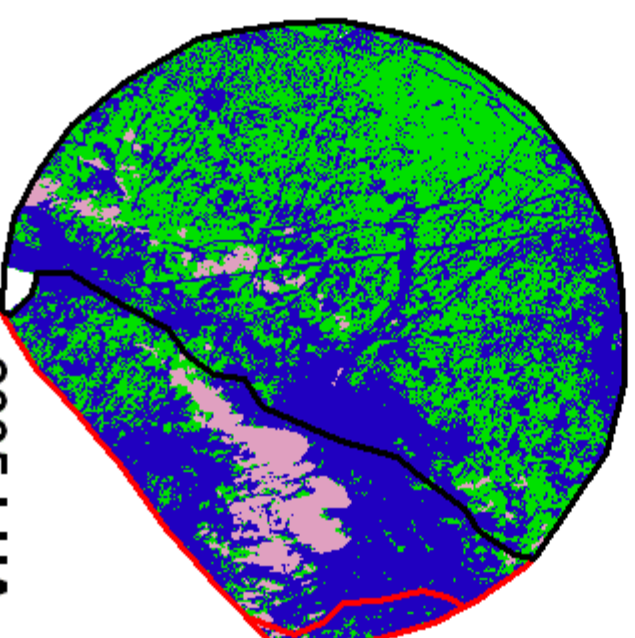
Figure 16. Depth contour data from NOAA-NOS overlaid onto Redfish Bay and East Flats study areas

Terminal Flats

South Bay

East Flats

Depth (m)	Color
0 to +0.3	Dk Blue
0 to -1.0	Lgt Blue
-1 to -2.0	Lgt Green
-2 to -3.0	Yellow
-3 to >-4.0	Red

Tf&eflts_noaadepth.shp

Estflts2004_noaapoly.shp

Tflts9600aoi_ms.shp

Sbayaoipolygon.shp

N
W   E
S

4   0   4   8 Kilometers
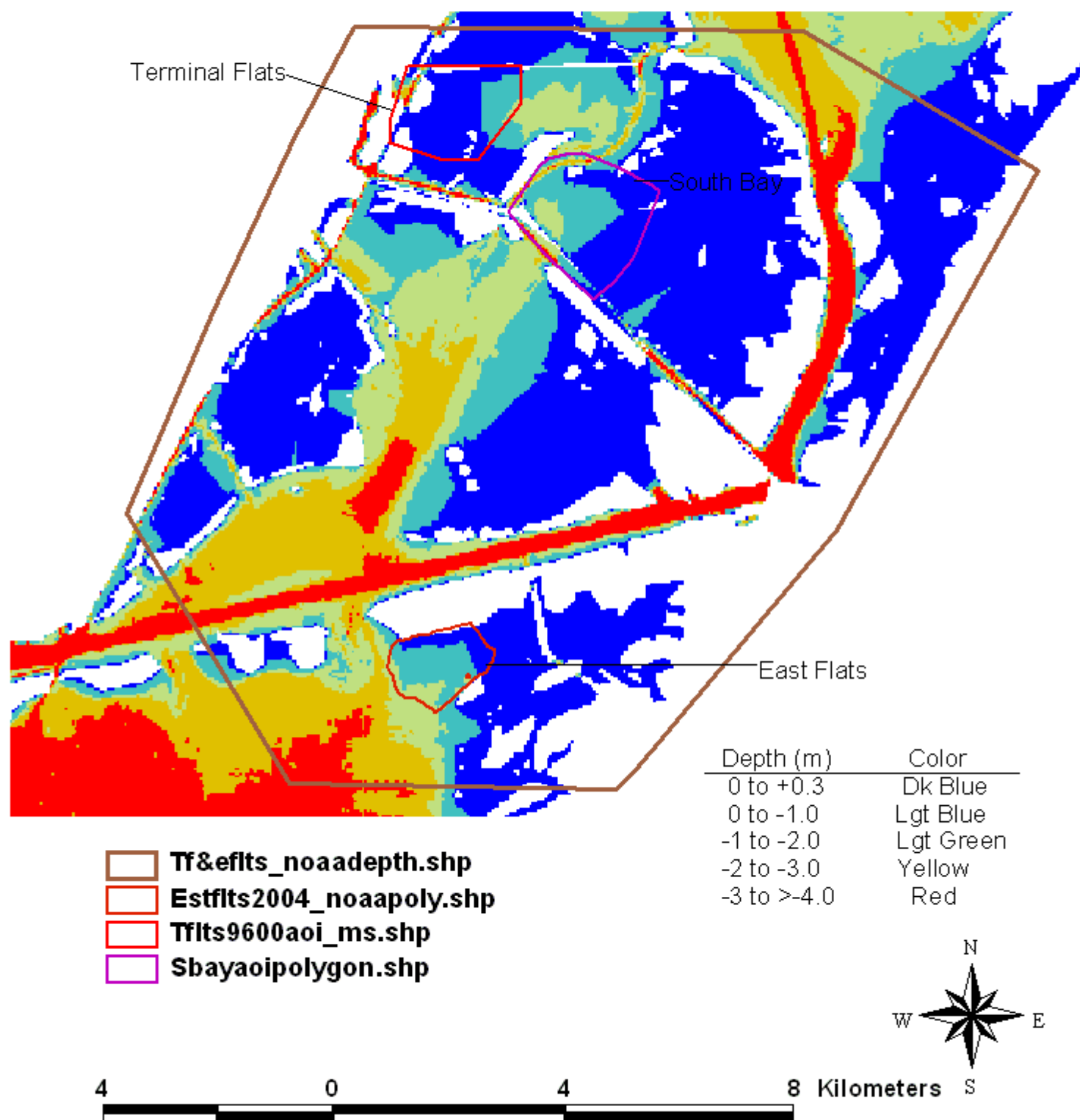
Figure 17. Terminal Flats site. Comparison of macroalgae deposition between 2004 and 2005

2004
Algae = 11.38 ha

2005
Algae = 10.55 ha

Utm tf 9600 algae_rev.shp
Tfltsdec2004_finalroi.tif
:Layer_1
:Layer_2
:Layer_3

2005 tf algae_rev.shp
Tflts2005dec_georoi.tif
:Layer_1
:Layer_2
:Layer_3

0.6   0   0.6   Kilometers

W   N   E   S
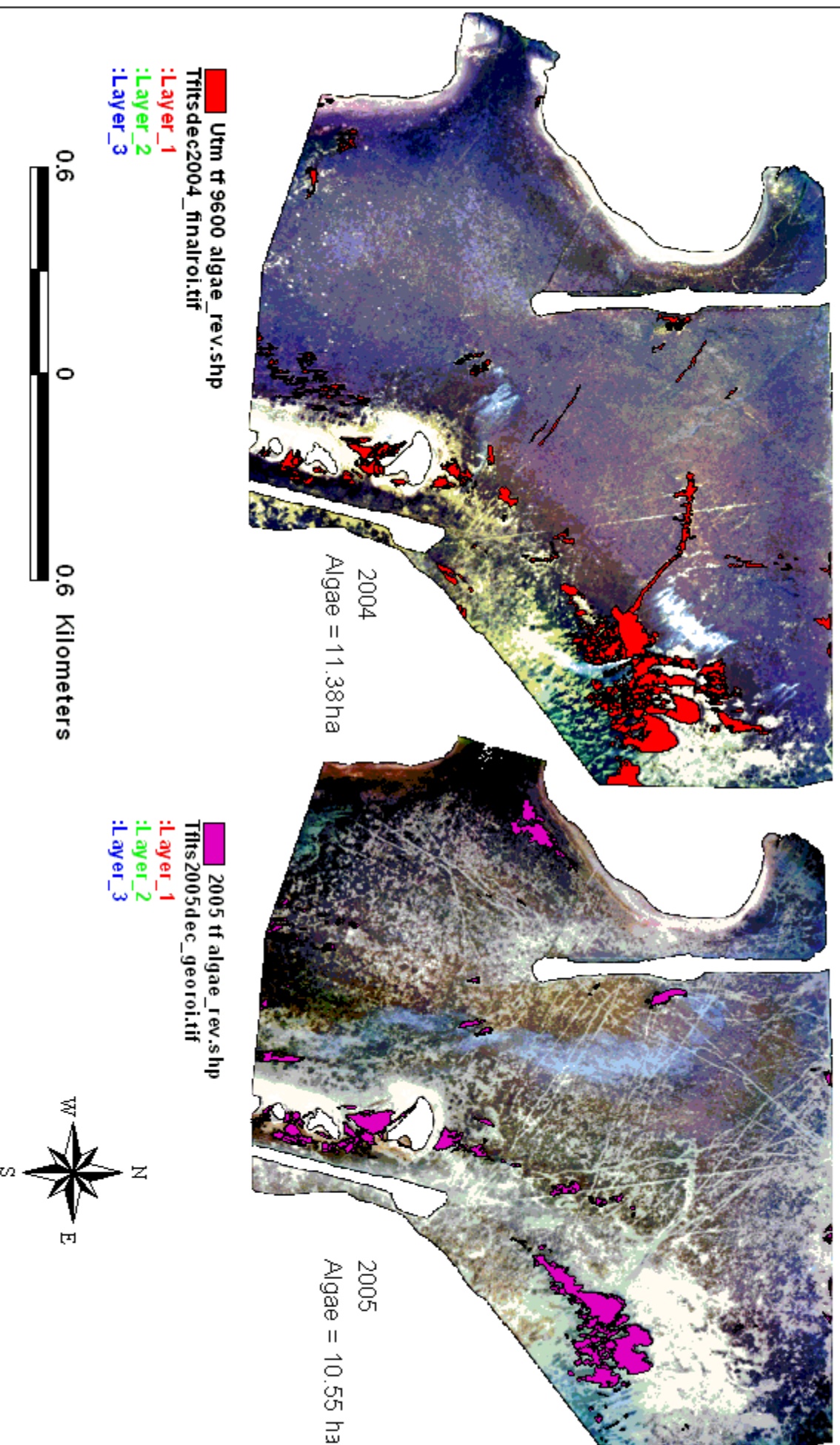
Fig. 18. East Flats site. Comparison of macroalgae deposition between 2004 and 2005.

2004
Algae = 5.422 ha

Estfts04_9600algae.shp
Estfts_2004roijun20.tif
:Layer_1
:Layer_2
:Layer_3

2005
Algae = 6.025 ha

East algae.shp
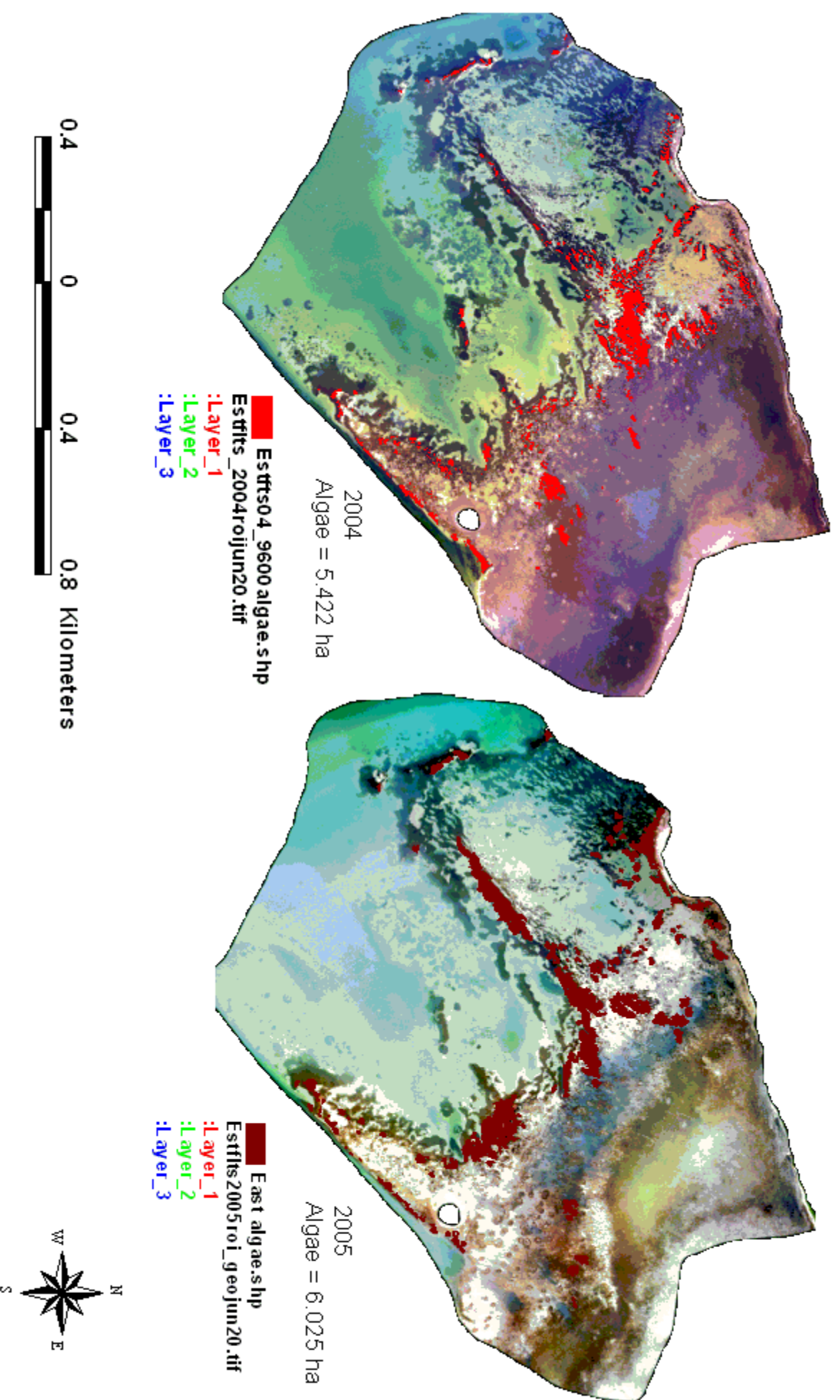Estfts2005roi_geojun20.tif
:Layer_1
:Layer_2
:Layer_3

0.4   0   0.4   0.8 Kilometers

Figure 19. Comparison of Macroalgae delineated
for Terminal Flats site at 1:9,600 or
1:24,000 photoscales.

Algae = 11.38 ha

Algae = 9.47 ha

Utmcopy 9600 tf algae.shp
Tfltsdec2004_finalroi.tif
:Layer_1
:Layer_2
:Layer_3

Utm 24k tf2004algae.shp
Tflts24kdec2004roi_rewarp.tif
:Layer_1
:Layer_2
:Layer_3

0.7   0   0.7   Kilometers

N
W   E
S
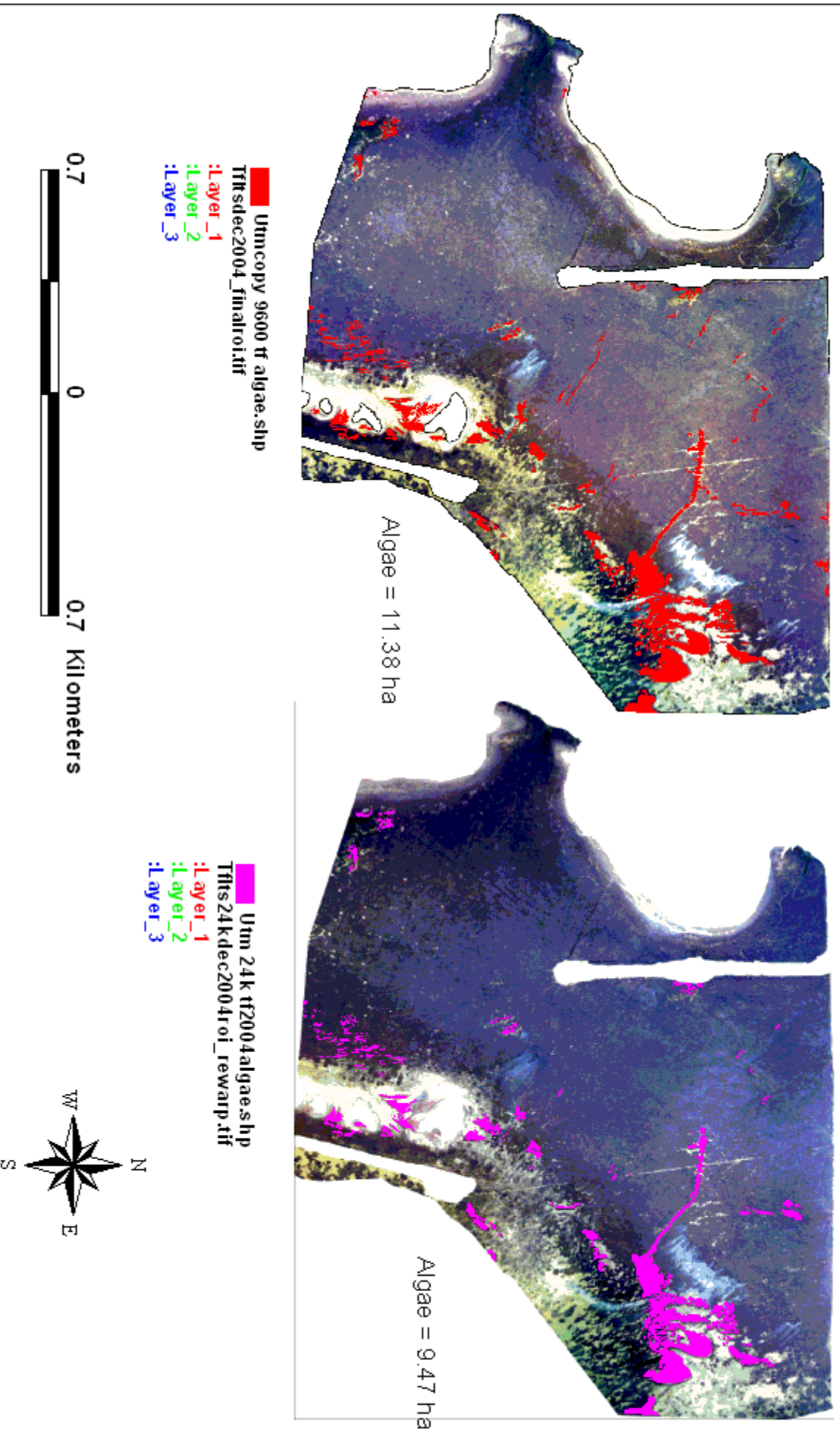
Figure 20. Comparison of Macroalgae delineated for East Flats site at 1:9,600 or 1:24,000 photoscales.

Algae = 5.42 ha

Estfts04_9600algae.shp
Estflts_2004roijun20.tif
:Layer_1
:Layer_2
:Layer_3

0.7   0   0.7 Kilometers

N
W   E
S

Algae = 5.22 ha

Estfts04_24kalgae_merge.shp
Estflt24k_05mngeo0018roi.tif
:Layer_1
:Layer_2
:Layer_3
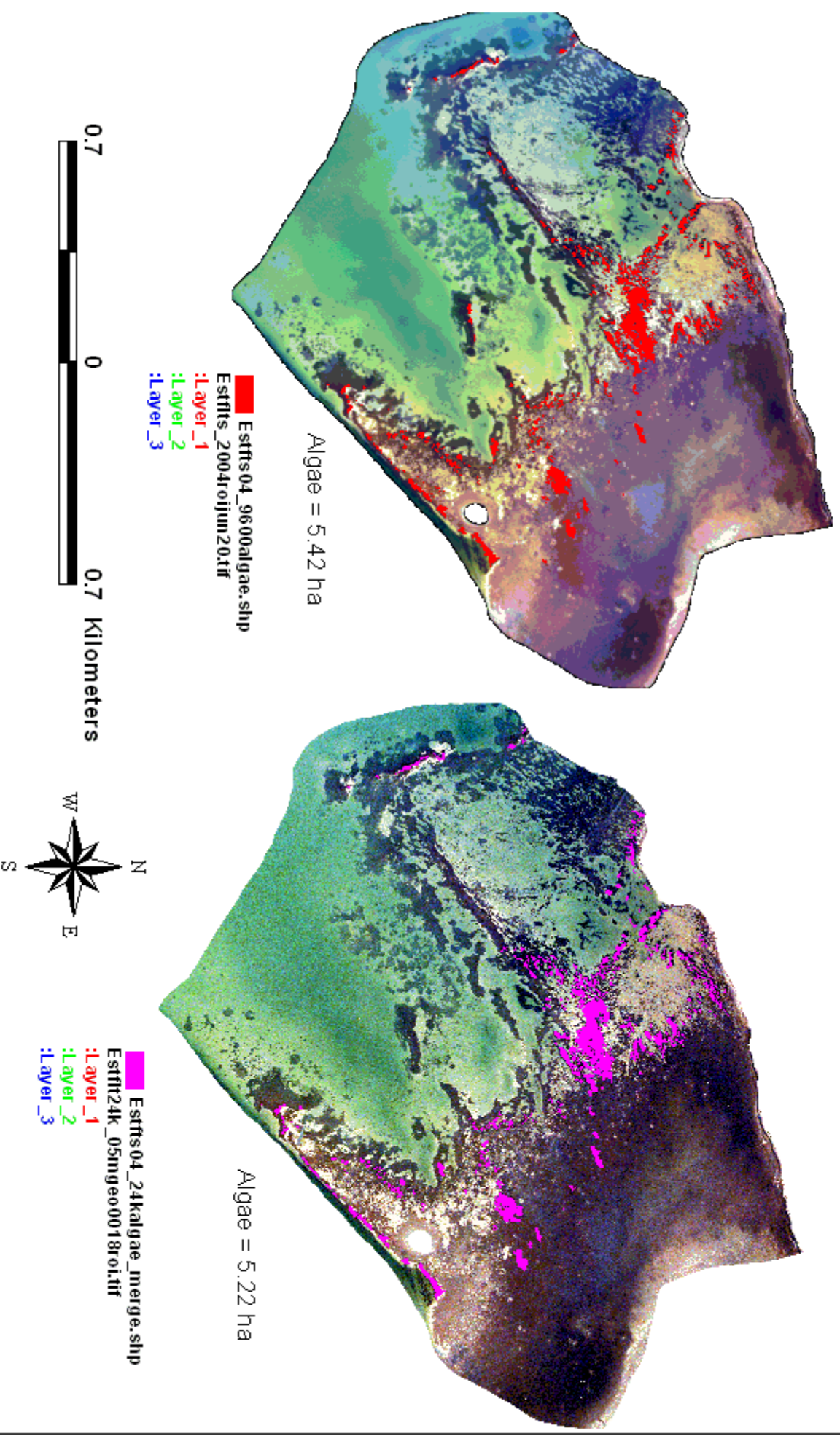
Figure 21. Terminal Flats site, 2004. Comparison of polygons in 13 grids delineated at 1:24,000 or 1:9600 photoscale.

Tf9600dense.shp
Tf9600algae.shp
Tf9600bare_rev.shp
Tfnewpatchy.shp
Tflts_dec2004_finalroi.tif
:Layer_1
:Layer_2
:Layer_3

1:9,600

0.6   0   0.6
0.6   Kilometers

Tf24k_bare_rev.shp
Tfdense_24k.shp
Tfnewpatchy.shp
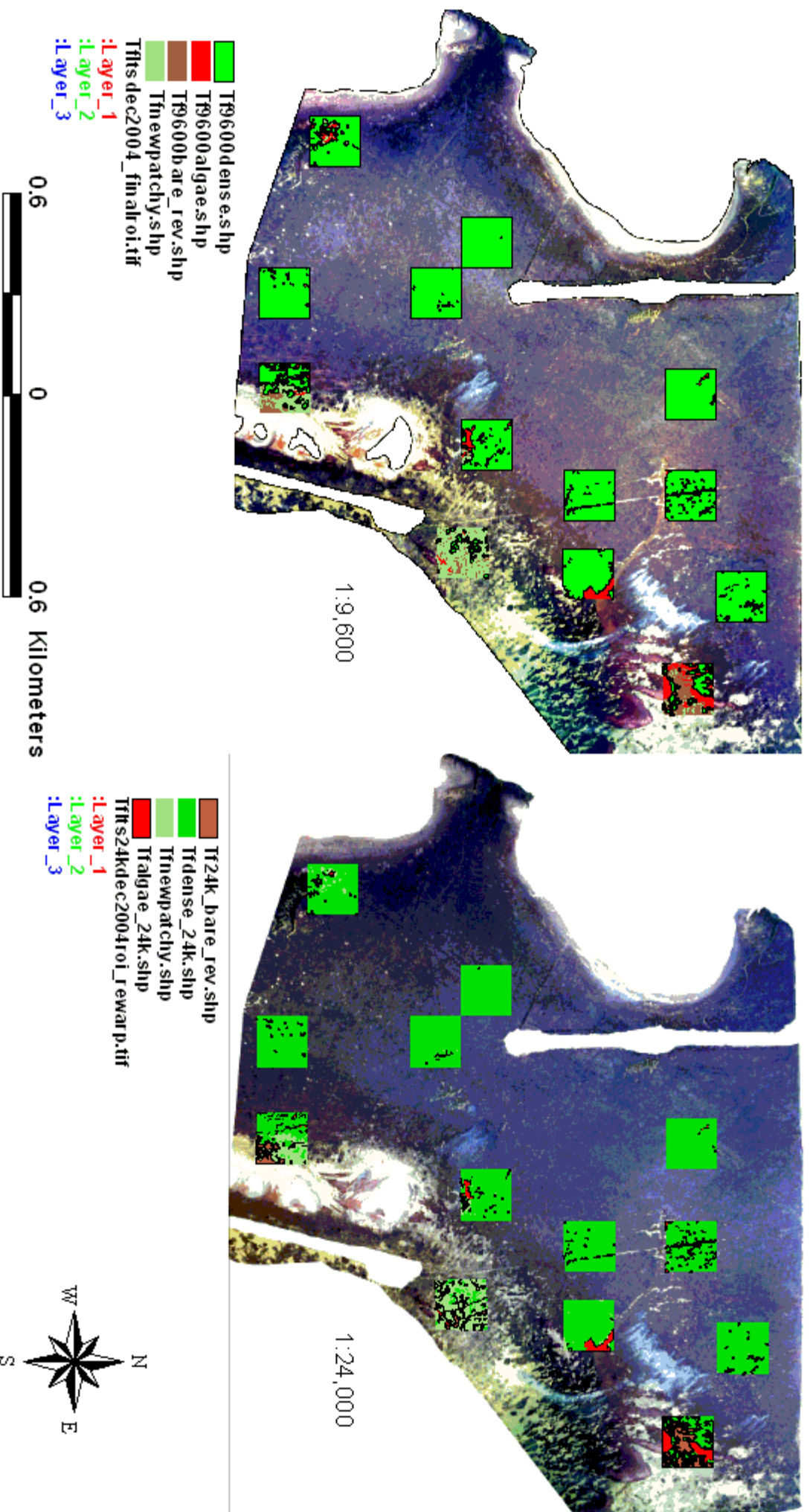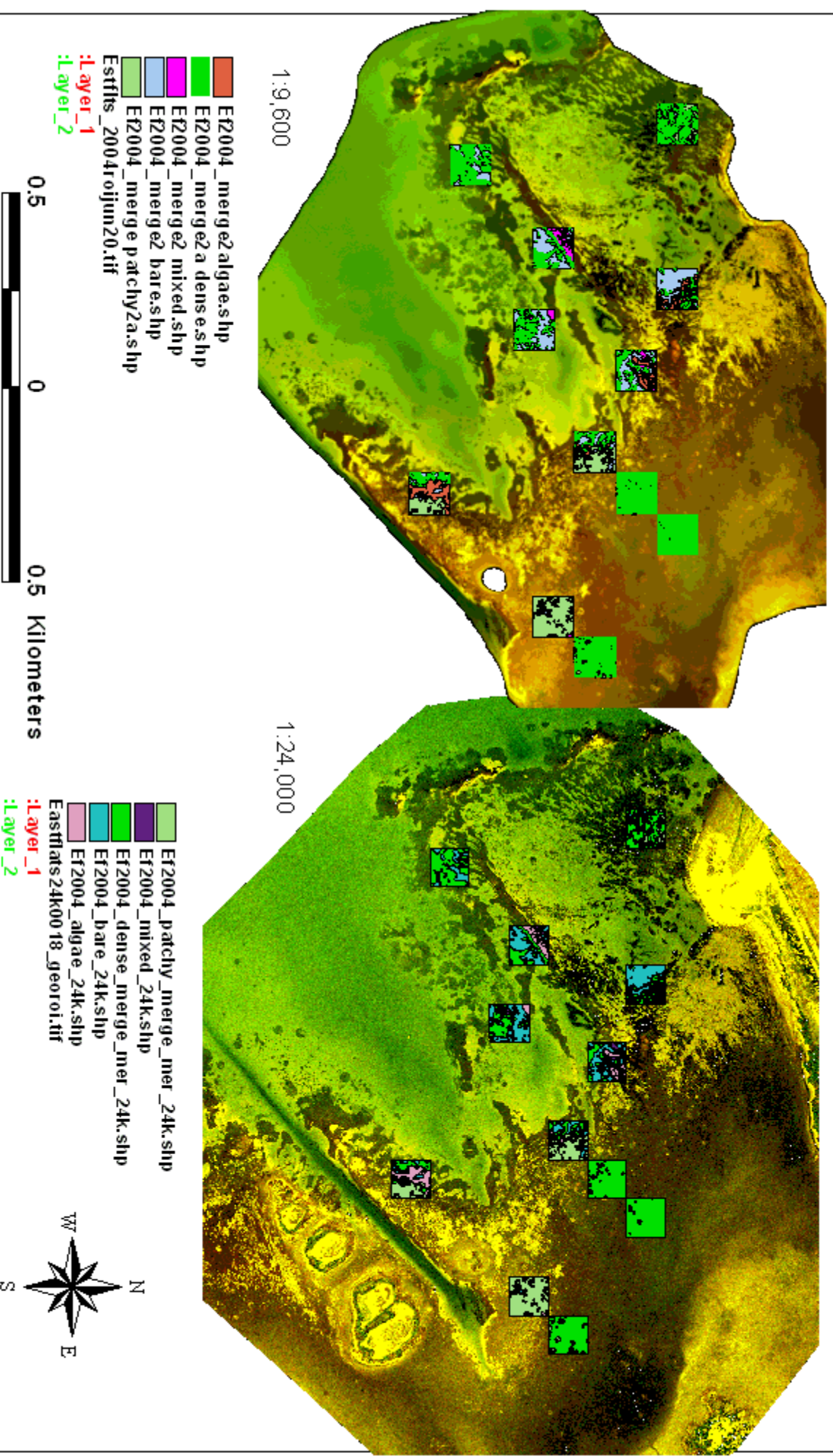Tfalgae_24k.shp
Tflts24kdec2004roi_rewarp.tif
:Layer_1
:Layer_2
:Layer_3

1:24,000

N
W   E
S

Figure 22. East Flats site, 2004, comparison of polygons in 12 grids delineated at 1;9600 or 1:24,000 photoscale.

1:9,600

Estflits_2004rojjun20.tif
Ef2004_merge patchy2a.shp
Ef2004_merge2 bare.shp
Ef2004_merge2 mixed.shp
Ef2004_merge2a dense.shp
Ef2004_merge2algae.shp

:Layer_1
:Layer_2

1:24,000

Eastflats24k0018_georoi.tif
Ef2004_algae_24k.shp
Ef2004_bare_24k.shp
Ef2004_dense_merge_mer_24k.shp
Ef2004_mixed_24k.shp
Ef2004_patchy_merge_mer_24k.shp

:Layer_1
:Layer_2

0.5   0   0.5   Kilometers

N
W   E
S

A SEAGRASS MONITORING PROGRAM FOR TEXAS COASTAL WATERS:

MULTISCALE INTEGRATION OF LANDSCAPE FEATURES WITH PLANT AND WATER QUALITY INDICATORS
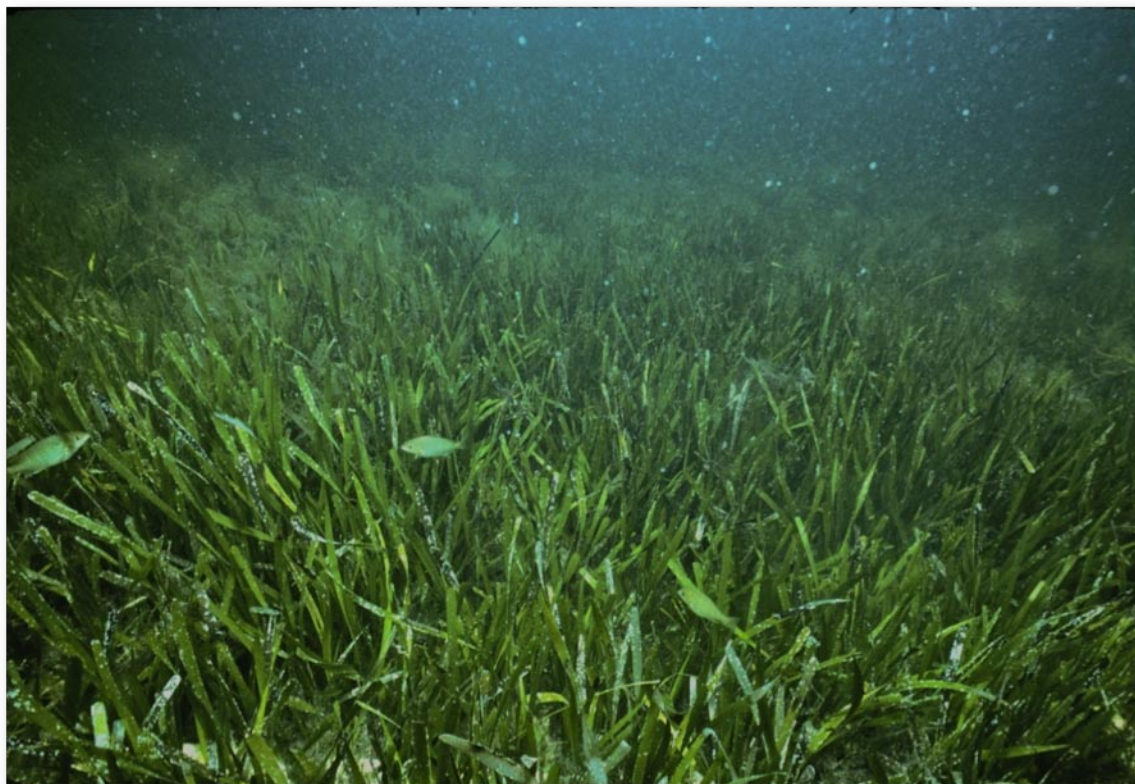
KEN DUNTON[1], WARREN PULICH[2], AND TROY MUTCHLER

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EXECUTIVE SUMMARY

This report outlines an implementation program for monitoring Texas seagrasses following protocols that evaluate seagrass condition based on landscape-scale dynamics. We recommend a **hierarchical strategy for seagrass monitoring** in order to establish the quantitative relationships between physical and biotic parameters that ultimately control seagrass condition, distribution, and persistence. The monitoring protocols are based on conceptual models that link: (1) light and nutrient availability to seagrass condition indicators and landscape level dynamics, including patchiness and depth limit distributions, and (2) physico-mechanical stressors, including hydrodynamic processes and human activities, to landscape feature indicators of seagrass bed degradation. The three-tiered approach follows a broad template adopted by several federal and state agencies across the country, but which is uniquely designed for Texas. This plan accommodates the immense hydrographic diversity in the State's estuarine systems and its associated seagrass habitats, recent advances in seagrass monitoring techniques, and current economic constraints associated with long-term studies. Based on this approach, we describe a multiscale monitoring protocol that, when implemented, integrate plant condition indicators with landscape feature indicators to detect and interpret seagrass bed disturbances. The program includes:

- a remote sensing component at two levels of resolution for status and trends mapping [Tier 1] and high resolution photoimagery analysis for deep edge delineation [Tier 2],

- a regional rapid assessment program using fixed stations sampled annually from a shallow-draft vessel [Tier 2] and,

- an integrated landscape approach that includes permanent stations and transects that are aligned with high resolution photoimagery to examine the presumptive factors associated with changes in seagrass maximum depth limits and patchiness [Tier 3].

Active involvement and support from the Texas Seagrass Monitoring Work Group in all aspects is critical to the implementation of a coast-wide seagrass monitoring program. Tier 1 monitoring has already been implemented by state agencies in cooperation with federal mapping efforts. We envision a program of implementation that encourages cooperation and support among the state and federal agencies responsible for the stewardship of these valuable coastal habitats.
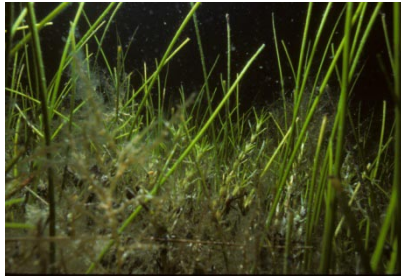
Syringodium filiforme

Aerial view of seagrass beds
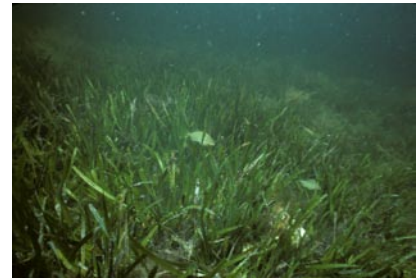
Thalassia testudinum

INTRODUCTION

In 1999, the Texas Parks and Wildlife Department (TPWD), along with the Texas General Land Office (TGLO) and the Texas Commission on Environmental Quality (TCEQ), drafted a Seagrass Conservation Plan that proposed, among other things, a seagrass habitat monitoring program (Pulich and Calnan, 1999). One of the main recommendations of this plan was to develop a coastwide monitoring program. In response, the Texas Seagrass Monitoring Plan (TSGMP) proposed a monitoring effort to detect changes in seagrass ecosystem conditions prior to actual seagrass mortality (Pulich et al., 2003). However, implementation of the plan required additional research to specifically identify the environmental parameters that elicit a seagrass stress response and the physiological or morphological variables that best reflect the impact of these environmental stressors.

Numerous researchers have related seagrass health to environmental stressors; however, these studies have not arrived at a consensus regarding the most effective habitat quality and seagrass condition indicators. Kirkman (1996) recommended biomass, productivity, and density for monitoring seagrass whereas other researchers focused on changes in seagrass distribution as a function of environmental stressors (Dennison et al., 1993, Livingston et al., 1998, Koch 2001, and Fourqurean et al., 2003). The consensus among these studies revealed that salinity, depth, light, nutrient concentrations, sediment characteristics, and temperature were among the most important variables that produced a response in a measured seagrass indicator. The relative influence of these environmental variables is likely a function of the seagrass species in question, the geographic location of the study, hydrography, methodology and other factors specific to local climatology. Because no generalized approach can be extracted from previous research,

3

careful analysis of regional seagrass ecosystems is necessary to develop an effective monitoring program for Texas.

A second approach to determining seagrass condition involves a combination of remote sensing data analysis, coupled with field sampling, to examine plant response at landscape or bed scales (Bell et al., 2006). Field-based sampling of plant condition indicators and environmental variables involves processing a large volume of point samples collected over broadly distributed sampling sites. Concurrent analysis of high-resolution aerial photography or digital imagery can provide an additional layer of resolution to these spatial approaches, but historically this has been labor-intensive, and analytical techniques have needed refinement. However, early detection of impending impairments to seagrass ecosystems may be possible if point measurements of habitat quality and seagrass condition indicators are correlated with prominent landscape features and seagrass bed morphological patterns in high resolution imagery. Such an analysis would help separate hydrodynamic stressors from human impacts that are most often reflected in landscape patterns and apparent in high resolution aerial photography.

Because of the complexity of these systems, it is important to identify the factors that drive seagrass dynamics. At both micro- and bed-scales, stress - response relationships must be examined carefully. Environmental stressors can influence seagrass condition directly, eliciting a positive or negative effect, or they may act indirectly through interaction with other variables. Consequently, identifying causative factors requires deciphering complex interactions at both point- and landscape scales.

Figure III.1 – Texas seagrass monitoring program regions. Regions include Christmas Bay and Galveston Bay in the Trinity-San Jacinto estuary (Region 1), the Matagorda Bay system in the Guadalupe estuary (Region 2), the San Antonio Bay area (Region 3), the Mission-Aransas National Estuarine Research Reserve, including Aransas and Copano Bays (Region 4), south Redfish Bay and southeast Corpus Christi Bay in the Nueces estuary (Region 5), the Upper (Region 6), and Lower Laguna Madre (Region 7).

In a recent Coastal Bend Bays and Estuary Program (CBBEP) study, we used a multi-scale approach to identify the measurements best suited to initiate a seagrass monitoring program for the state of Texas. The overarching goal of this study was to validate a landscape analysis approach to seagrass monitoring and establish protocols to evaluate stress on seagrass systems. Our monitoring protocol builds on data obtained from recent ecosystem studies that included intensive field sampling of environmental variables (Chapter 1) in combination with landscape analyses of true color aerial photoimagery (Chapter 2). Our major objectives addressed (1) the development of a conceptual "working" model that outlines the important linkages among stressors and condition indicators, (2) identification of the relevant environmental and landscape indicators that are responsive to both natural and anthropogenic stressors, and (3) the development of a hierarchical strategy for seagrass monitoring in Texas coastal waters. This plan incorporated the utilization of both new and historical data to establish the natural baselines of condition indicators to enable status and trends assessment of seagrass populations unique to Texas estuarine systems. Our approach was entirely inclusive of the known distribution of seagrasses along the entire Texas coast, from Galveston to the Brazos Santiago Pass near the U.S.-Mexican border (Fig. III.1).

OVERALL PROJECT SCOPE AND OBJECTIVES

The objectives of the recent CBBEP-funded project were to (1) design a monitoring program to detect environmental changes with a focus on the ecological integrity of seagrass habitats, (2) provide insight to the ecological consequences of these changes, and (3) help decision makers (e.g. TPWD, TCEQ, TGLO) determine if the observed change necessitated a revision of regulatory or management policy or practices. We defined ecological integrity as the capacity of the seagrass system to support and maintain a balanced, integrated, and adaptive community of flora and fauna including its historically characteristic seagrass species. Ecological integrity is assessed using a suite of condition indicators (physical, biological, hydrological, and chemical) measured on different spatial and temporal scales.

In this chapter we summarize our preliminary results that provide a framework for discussion and consideration by the Seagrass Monitoring Work Group (SMWG), a State advisory group formed in 2004. This group is composed of knowledgeable scientists and natural resource managers from local universities and a variety of local, state, and federal agencies (e.g. USGS-NWRC, USF&WS, TPWD, TCEQ, TGLO, and USACE). Other sources of information include EPA's R-EMAP (Regional Environmental Monitoring and Assessment Program), which utilized conceptual models as part of the EMAP process, and on-going seagrass monitoring programs in the Florida Keys National Marine Sanctuary (FKNMS, Fourqurean et al,. 2002), Chesapeake Bay (Moore and Reay 2009), Indian River Lagoon in Florida (Mattson 2000), the northeastern United States (Neckles et al., 2010), and Puget Sound, Washington (Dowty et al., 2005). Our products include a conceptual model that can help guide selection of appropriate environmental, water quality and landscape indicators with respect to stressors, the selection of appropriate indicators based on a variety of criteria, and the collection of baseline data associated with the development of a coast-wide monitoring effort to assess seagrass status and trends.

A Conceptual Model (Version 1)

It is important to develop a conceptual model that outlines the linkages among seagrass ecosystem components and the role of indicators as predictive tools to assess seagrass response to stressors at various temporal and spatial scales. Tasks for this objective include the identification of stressors that arise from human-induced disturbances which can result in seagrass loss or compromise seagrass condition (health). For example, stressors that lead to higher water turbidity and light attenuation (e.g. dredging, and shoreline erosion) have been shown to result in lower below-ground seagrass biomass and changes in sediment nutrient concentrations. The linkage between light attenuation and plant response is often evaluated through long-term light measurements, examination of porewater nutrient, sulfide, and dissolved oxygen levels, and the biomass of above- versus below-ground tissues (Fig. III.2).

An exhaustive listing of anticipated stressors, the ecological consequences of stressor action, and how they would be measured are first steps toward indicator identification and selection. Conceptual models can help show the linkages between stressors and their consequences and

7

summarize how a given component functions. These exercises will provide a current understanding of ecosystem processes and cause-and-effect relationships, which are critical to appropriate indicator selection. These models can be built at several different scales to accommodate the complexity of the system, the variety of stressors, and the possible synergisms with natural disturbance events. It is important to integrate scales of time/space with dynamic processes (e.g. nutrient cycling, trophic interactions).
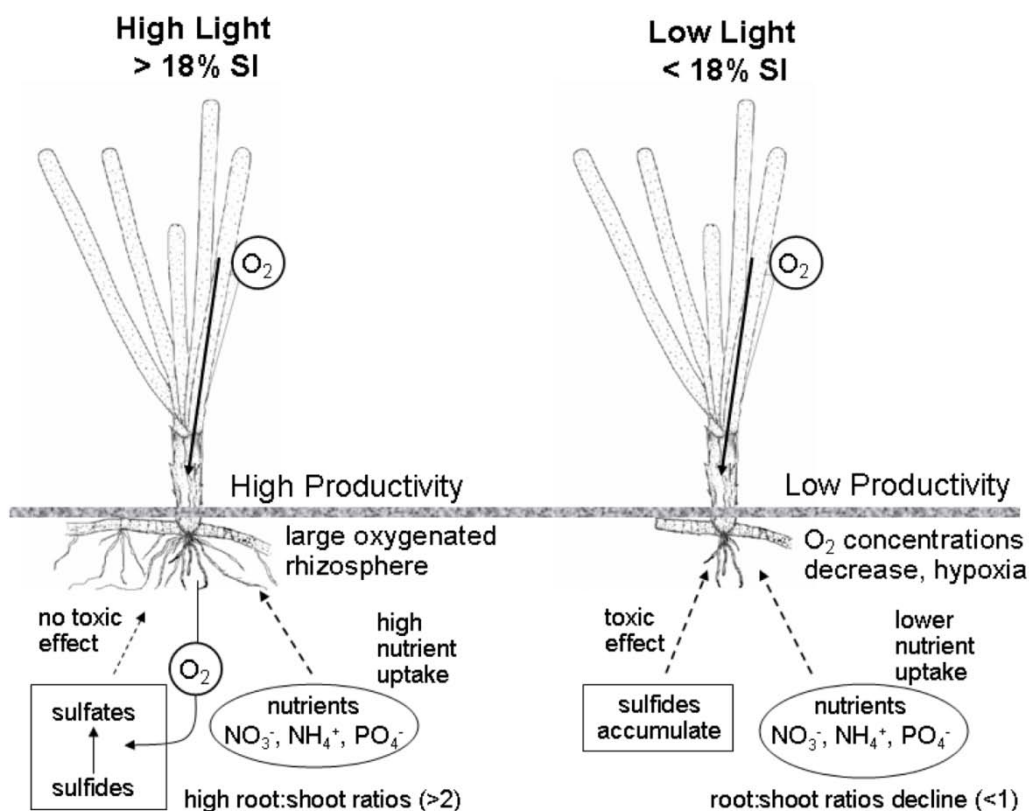

Figure III.2 - Effect of light attenuation on seagrass productivity, sediment chemistry, and root:shoot biomass ratios. Photosynthetic oxygen transported into seagrass roots and rhizomes plays a significant role in the maintenance of aerobic conditions in the rhizosphere. Light attenuation that drops the percent surface irradiance (SI) to less than 18% (for seagrasses in the northwestern Gulf of Mexico) produces less oxygen for below-ground tissue respiration, which can result in build-up of sulfides and ammonium, toxic to seagrasses at high concentrations (from Dunton, unpub. and Mateo et al., 2006).

Environmental and Landscape Indicators

Relevant and measurable environmental, water quality and landscape indicators must be sensitive to human-induced activities and accurately characterize the condition of seagrass communities within the major estuarine systems of Texas. The success of a monitoring program is related to the choice of condition indicators that are (1) reflective of a seagrass ecosystem response, (2) linked to a cause-effect process identified in the conceptual model, and (3) measured at reasonable cost and effort. In our CBBEP project, we provided a list of candidate indicators (Table III.1) based on an evaluation of measurements collected in two estuarine systems between 2003 and 2005 (Dunton et al., 2005). We focused on those indicators with the following properties:

- unambiguously related to conceptual models
- relatively simple to measure and not influenced by observer subjectivity
- consistently responsive to change
- accurately and precisely estimated
- possess measurable changes in magnitude
- natural variability is readily distinguished from background
- societal relevance
- integrative qualities

Table III.1. Some recommended condition indicators for inclusion into an integrated seagrass monitoring program for Texas coastal waters based on Neckles (1994), Dunton et al. (2005), and this study.

Water Quality	Sediment Quality	Seagrass Light Response Indicators	Plant Nutrient Response Indicators
dissolved oxygen	grain size	biomass (above- & below-ground)	C:N:P blade ratios
conductivity, salinity, and temperature	total organic carbon	root:shoot ratio	epiphytic algal species composition and biomass
nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{-3})	porewater NH_4^+	percent cover and related morphometric data (blade width, blade height)	drift macroalgal abundance and composition
chlorophyll *a*		shoot density	$\delta\,^{13}C$ and $\delta\,^{15}N$ of leaf tissues and attached algal epiphytes
total suspended solids (TSS)		chlorophyll fluorescence	
light attenuation (*k*)		species composition	
surface irradiance (%SI)		maximum depth limit	

We plan on utilizing certain candidate indicators in the existing literature for the proposed study. Starting in 2002, core EMAP seagrass indicators were measured (Neckles, 1994) along with additional parameters in Laguna Madre and Redfish Bay from 2002-2004 (Dunton et al., 2005) and in Redfish Bay and East Flats in 2005 (this study). The 2005 project (Chapters 1-2, this report) also addressed landscape indicators for seagrass monitoring to establish protocols for evaluating stress on seagrass systems from landscape-scale dynamics determined from aerial remote sensing data. In addition to the indicators listed in Table III.1, other possible candidates

include leaf scars on individual shoots (to assess growth), assessment of seed reserves, and benthic infaunal diversity.

Table III.2. Indicators and proposed measurement frequency under a Tier 2 (annual) seagrass monitoring program. Note: k = light attenuation, %SI = percent surface irradiance, PDR = Precision Depth Recorder. Asterisks denote minimum criteria for a Tier 2 sampling effort.

Indicator	Field Method
Stressor	
*k, %SI	underwater light sensor
*water transparency	Secchi
*depth	PDR
*temperature, salinity, pH, dissolved oxygen	SONDE
*TSS	water collection
NH_4^+, NO_3-, PO_4^{-2}	water collection
*chl a	in situ fluorescence
drift algal biomass	0.25 m^2 quadrats
sediments (grain size/organics)	benthic cores
algal epiphyte biomass	benthic cores
Seagrass Condition Indicator	
canopy height	benthic cores
shoot density	benthic cores
seagrass biomass	benthic cores
root:shoot ratios	benthic cores
*seagrass species composition	0.25 m^2 quadrats
C:N:P and ^{15}N:^{14}N ratios	benthic cores
*percent cover	0.25 m^2 quadrats

Our recommended list of Tier 2 indicators for annual sampling (Table III.2) is based on data collected in Texas estuarine seagrass systems that is available in over 20 peer-reviewed publications, numerous M.S. and Ph.D. theses, and various unpublished reports. These data represent an extremely valuable source of historical measurements collected over the past two decades in seagrass systems located from Lower Laguna Madre to San Antonio Bay in the Guadalupe Estuary. In addition, information from other seagrass monitoring programs across the U.S. (referenced above) has also proved an invaluable set of resources.

We examined many of these condition indicators at 40 sites in seagrass beds of Redfish Bay and East Flats to determine the strength of their relationship with seagrass biomass, density, cover and community composition (Chapter 1). Strong relationships would have suggested possible stressors as well as identify potential indicators of current and future seagrass condition. We used both univariate and multivariate statistical analyses to assess these relationships and identify candidate variables for inclusion in a monitoring program. All variables except N:P of *Thalassia testudinum* leaves exhibited significant site x sampling date interaction terms, indicating both spatial and temporal variability in Redfish Bay and East Flats. Parametric and nonparametric analyses, however, revealed only modest associations between both abiotic and biotic variables and seagrass measurements.

In many cases, dried seagrass tissues from quantitative samples have been archived and are available for constituent analysis. We are particularly interested in the differences in elemental ratios (carbon, nitrogen, and phosphorus) among estuaries that reflect nutrient availability (see below). At one site in Upper Laguna Madre, seagrass and water quality measurements have been collected continuously since 1989 (Dunton, 1994); the data and seagrass samples from this work are particularly appropriate for inclusion in our evaluation of condition indicators.

An increase in nutrient loading is one water quality change that is most likely to affect seagrass populations as a consequence of human population growth in coastal areas, and has already caused eutrophication of many estuaries. Nutrient concentrations are relatively low in seagrass-dominated environments and therefore, seagrasses are normally nutrient limited by either nitrogen (N) or phosphorus (P) (Fig III.3). Consequently, nutrient addition can shift the competitive balance from seagrasses to faster-growing primary producers, such as

phytoplankton, epiphytes, or benthic macroalgae. Under high nutrient concentrations, estuaries previously dominated by mixtures of turtle grass (*Thalassia*) and manatee grass (*Syringodium*), will revert to more weedy vegetative assemblages characterized by widgeon grass (*Ruppia*) and benthic seaweeds (Fourqurean and Rutten, 2003). Lapointe et al. (2004) found that the $\delta^{15}N$ values of macroalgae accurately identified different sources of nitrogen enrichment, from sewage to fertilizer. Consequently, changes in seagrass tissue stable isotopic composition may reveal the onset of environmental shifts in nutrient availability (Fourqurean et al., 2005) that can ultimately influence seagrass composition.
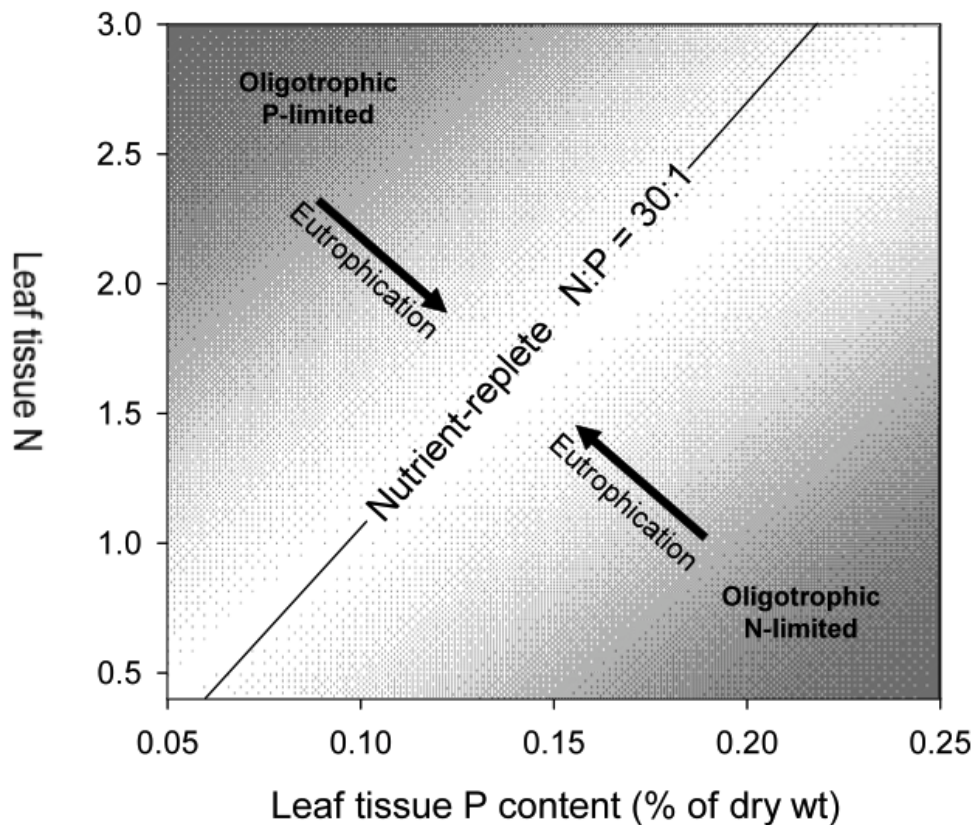
Figure III.3 - A conceptual model of the relationship between seagrass leaf nutrient content and nutrient availability in south Florida (from Fourqurean and Rutten, 2003).

Evidence suggests that these replacements occur over time scales ranging from years to decades. However, indications of a regime shift can be detected early through the monitoring of seagrass (blade) tissue nutrient concentrations, which reflect the relative availability of nutrients in an

13

estuary as integrated over time scales of weeks to months. For example, under nutrient replete conditions, the availability of nitrogen (N) to phosphorus (P) is reflected in a balanced ratio of 30:1 for the seagrass *Thalassia testudinum* in the FKNMS. Since the 8-yr average N:P ratio in *T. testudinum* from Florida Bay is about 38:1, reflective of a P limited environment, a change in this ratio to a value closer to 30:1 is indicative of eutrophication (Fig. III.3). For comparison, N: P ratios of *T. testudinum* collected in the Aransas-Copano Estuary in 2005 are about 32:1 (see Chapter 1, this study). However, Texas estuarine systems appear to possess unique hydrographic characteristics as reflected in the elemental composition of resident seagrasses which have distinctive estuarine specific C:N:P ratios (Dunton, unpub. data).

Similarly, ratios of carbon (C) to nitrogen (C:N) in seagrass tissues are also indicative of nutrient availability in coastal systems, especially in Texas estuaries, since they are seldom P limited. The spatial variability in C:N ratios of *T. testudinum* along the Texas coast reflect the ecological differences of our coastal ecosystems. Texas estuaries possess distinct biogeochemical signatures that are reflected in the chemical composition of the resident biota. For example, the variation in N availability between Lower Laguna Madre and the Aransas-Copano estuaries is reflected in porewater ammonium-N concentrations and plant C:N ratios. The naturally higher N levels in the Aransas system are reflected in both porewater ammonium-N concentrations, which are twice as high in Aransas Bay as Lower Laguna, and lower *Thalassia* C:N ratios in Aransas Bay. Such biogeochemical differences are reflected in morphometric and biomass characteristics (e.g. blade width and length, leaf scars, etc.)*,* which are useful condition indicators. Taken together, the attributes that characterize seagrass populations reflect the natural characteristics of the ecosystem in which they live (Table III.2), and can help identify ecologically distinct regions (Hackney and Durako, 2004).

In addition to the condition indicators noted above, we evaluated a variety of landscape indicators (Table III.3) in an effort to identify those most relevant to long-term seagrass monitoring. We examined various features (e.g. patterns in bed morphology, non-vegetated seabed, drift macroalgae, and hydrodynamic disturbances) from high-resolution true color photography in relation to seagrass plant/habitat parameters (e.g. biomass, species composition, water column and sediment porewater nutrient concentrations). We believe the results of this

work are important to our understanding of seagrass distribution and species composition, seagrass bed fragmentation, and gap (or patch) dynamics. Gaps are often produced through physical and biological disturbances, producing a mosaic of different vegetational assemblages that can be quantified from high resolution aerial imagery. The size (or "grain") of gaps and their extent (coverage) over a study area can be used to characterize spatial dynamics of seagrass beds. This approach will help us distinguish between the effects contributed by physical stressors (e.g. hydrodynamics) versus changes in water quality (e.g. water transparency) with respect to seagrass response indicators (Fonseca et al. 2002, Yamakita and Nakaoka, 2009).

Table III.3. Spatial metrics for landscape feature indicators in a specific seagrass region of interest quantified from 1:9,600 photoimagery at ~ 1m^2 resolution.

Indicator class	Landscape metrics (within region of interest)
Bare Patches	Size frequency, number, shape
Seagrass Assemblage	Size and shape of plant assemblages
Depth Distribution	Seagrass areal coverage (ha) in depth zones, deepest depth (m)
Macroalgae Deposition	Areal coverage (ha)
Seagrass Species' Distribution	Areal coverage (ha) per species

Edge dynamics, which reflect changes in the depth distribution of seagrasses, as revealed from digital aerial imagery, can also be used as an integrative measure of seagrass change, since the maximum depth penetration of seagrasses reflects overall water quality and light conditions. Consideration of landscape indicators must include an analysis of the cost and/or availability of remotely sensed imagery at the resolution required to detect change in critical landscape features (e.g. 1:24,000 vs. 1:9,600 scale) based on the results of this study (see Chapter 2).

Other practical issues pertain to indicator selection and reliability. These include the temporal frame and frequency for sampling (e.g. monthly, seasonal, biannual, annual), replication for statistical validity and hypothesis testing, optimal sample size and shape, measurement units, and cost.

A HIERARCHICAL
STRATEGY FOR SEAGRASS MONITORING

Our third objective focuses on the spatial and temporal variability of baseline indicators from both historical data and new synoptic measurements collected at sites located within seagrass dominated estuaries to establish the critical distributions that define seagrass condition (health) in Texas. Currently, the general distribution of all Texas seagrass habitat is known and encompasses six major Texas estuarine systems located in 10 coastal counties between Galveston and Brownsville (Fig. III.1; SCPT 1999). Our major task for a coast wide monitoring program is the collection of baseline measurements of condition indicators (Table III.2 and III.3), including the acquisition of remotely sensed data made available by other agencies or acquired solely for this monitoring program.

We recommend a sampling protocol for condition indicators identified above following the procedures and standards established by Fourqurean et al. (2001) for the EPA sponsored seagrass status and trends monitoring project in the Florida Keys National Marine Sanctuary (http://www.fiu.edu/~seagrass/), the USGS (for the National Park Service, see Neckles et al., 2010), the National Estuarine Research Reserve System (Moore et al., 2009), and the Puget Sound Submerged Vegetation Monitoring Project (Washington Department of Natural Resources http://www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_nrsh_eelgrass_stress or_response.aspx). Station selection follows the stratified random method of hexagonal tessellation used by TPWD (Fig. III.4); we used this technique to locate permanent monitoring stations within the Lower Laguna and Mission-Aransas study areas under the 2002-2004 R-EMAP program (Dunton et al., 2005) and in this study. The approach ensures that all points within the landscape have an equal probability of being sampled, and that the sampling effort be quasi-evenly distributed across the landscape. Some stratification will be required in order to

sample in seagrass areas and to insure that no particular portion of the sampling area is favored more than another (Volstad et al., 1995). This can be accomplished by using the baseline seagrass maps at 1:24,000 scale that exist for most of these Texas bays (at least back to the early 1990s) and that are available and archived at TPWD. In addition, recent aerial imagery acquired in mid 2000s by NOAA for a coastal benthic mapping program can also be used to confirm the presence and substantial changes in seagrass meadows in several of the CBBEP estuarine bay areas. The analytical protocol for all condition indicators will follow guidelines established by a Quality Assurance Project Plan as approved by the EPA and TCEQ (see Radloff, 2009).

For landscape feature indicators, we recommend the acquisition and analysis of high resolution digital true color aerial photoimagery, at least 1:9,600 scale or larger. In Chapter 2 we addressed several questions related to aerial imagery for seagrass landscapes, including development of semi-automated methods for efficiently analyzing and classifying landscape features, and the critical comparison of scales (1:24,000 vs. 1:9,600) for detection of indicators in the classified scanned imagery. Our results indicated that 1:9,600 scale resolution or better was needed to ensure accurate delineation and quantification of drift macroalgae accumulations, bare patches and gaps of 1-2 m^2, and precise location of the deepwater edge of seagrass beds. These three landscape features are considered most critical for correlating with the plant-scale indicator measurements made by point sampling. With this high resolution imagery, we are able to extend (i.e. extrapolate) our observations from point samples over a larger area. Because each frame of 1:9,600 photography covers a seagrass bed area of approx. 2.2 km by 2.2 km (4.84 km^2), the high resolution imagery has a direct impact on our ability to detect and quantify the extent of landscape indicators chosen for long-term monitoring.
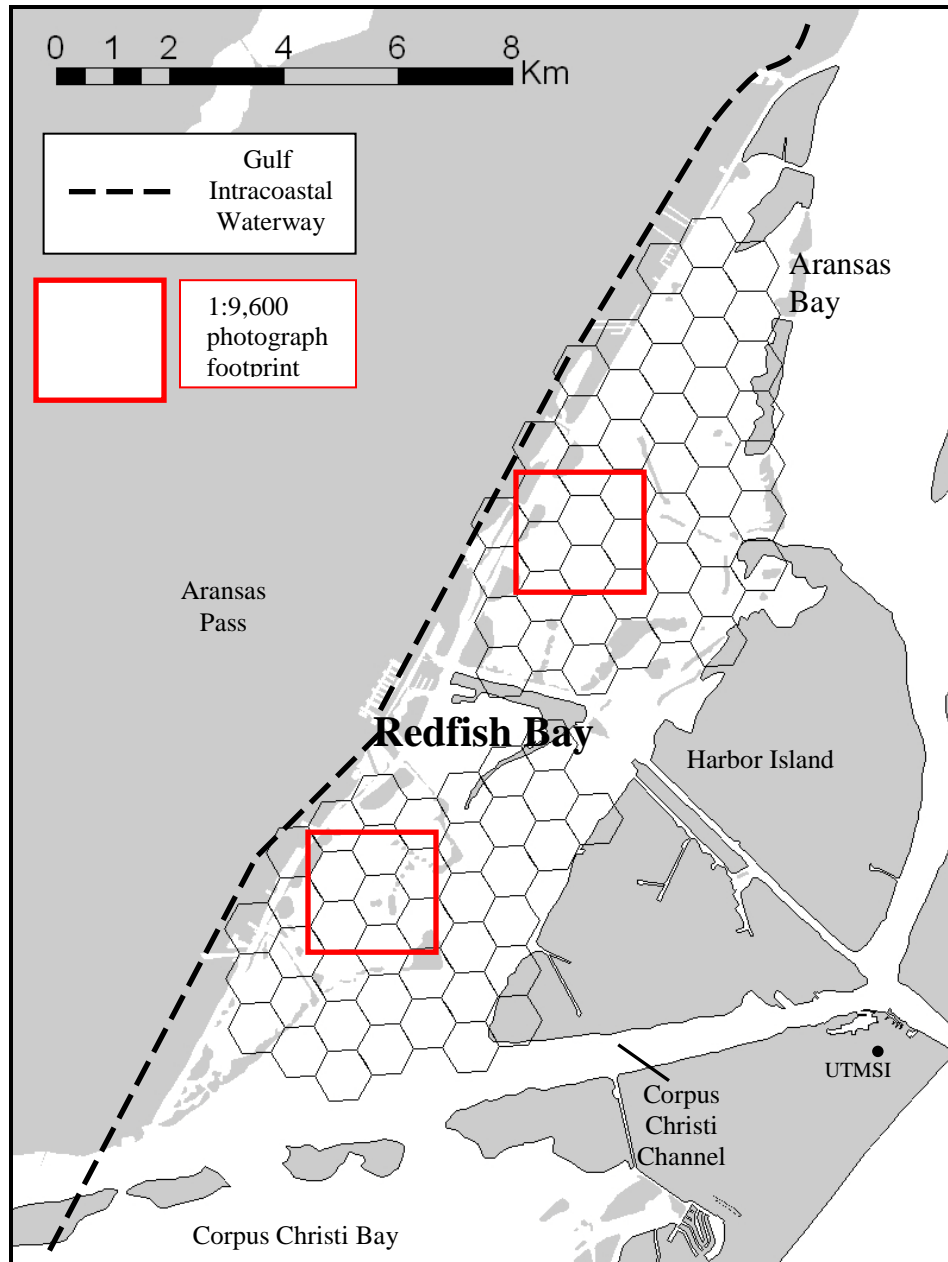
Figure III.4 - A hexagon layer superimposed on Redfish Bay. Hexagons are 500 m wide and contain one random sampling location (see text for details). Footprints of two 1:9,600 scale photographs are overlaid for comparison (adapted from Dunton et al., 2005).

Table III.4. Summary of total seagrass changes for Texas bay systems over four decades. Seagrass values are in hectares with acres in parentheses. Modified from Pulich and Onuf (2007).

Bay System	[1]Late 1950s or mid-1960s	[2]Mid-1970s	[3]1987 or early 1990s	[4]1998
Galveston Bay System				
Galveston/Christmas Bays	590[a] (1,457)	134[a] (331)	113[b] (279)	210[c] (519)
Midcoast Region				
Matagorda Bay			1,099[b] (2,716)	
San Antonio Bay		5,000[d] (12,350)	4,305[d](10,638)	
Coastal Bend Region				
Aransas/Copano			2,871[e] (7,094)	
Redfish Bay and Harbor Island	5,380[e] (13,293)	6,200[e](15,320)	5,710[e] (14,109)	
Corpus Christi Bay			2,568[e] (6,346)	
Laguna Madre System				
Upper Laguna Madre	12,321[f] (30,445)	20,255[g] (50,050)	22,903[h] (56,593)	22,443[i] (55,456)
Lower Laguna Madre	59,153[f] (146,166)	46,558[g] (115,044)	46,624[h] (115,207)	46,174[i] (114,095)
Baffin Bay			2,200[j] (5,436)	

[1] Data for Galveston/Christmas Bays, Redfish Bay, and Harbor Island based on 1956/58 Tobin photography. Data for upper and lower Laguna Madre based on field surveys during mid-1960s.

[2] Data for Galveston/Christmas and Redfish Bay/Harbor Island based on 1975 (National Aeronautics and Space Administration Johnson Space Center (NASA- JSC) photography; San Antonio Bay based on 1974 NASA-JSC photography. Data for upper and lower Laguna Madre based on 1974–75 field surveys.

[3] Data for Christmas, Matagorda, and San Antonio Bay systems from 1987 NASA-Ames Research Center photography. Data for Aransas/Copano, Redfish, and Corpus Christi Bay systems based on 1994 TPWD photography. Data for upper and lower Laguna Madre based on 1988 field surveys. Data for Baffin Bay based on 1992 U.S. Fish and Wildlife Service National Wetlands Inventory photography.

[4] Data for Christmas Bay from 1998 Galveston Bay National Estuary Program photography. Data for upper and lower Laguna Madre from 1998 field surveys.

[a] From Pulich and White (1991).   [b] From Adair and others (1994).   [c] From Pulich (2001).   [d] From Pulich (1991).

[e] From Pulich and others (1997).   [f] Areas computed for this review from McMahan (1965–67). See Laguna Madre vignette.

[g] Areas computed for this review from Merkord (1978).   [h] Areas computed for this review from Quammen and Onuf (1993). See Laguna Madre vignette.   [i] Areas computed for this review. See Laguna Madre vignette.

[j] Areas computed for this review by Texas Parks and Wildlife Department, Coastal Studies Program, Austin, Tex. (unpub. data)

In recognition of the unique differences inherent to Texas estuaries and the availability of reliable historical data (and samples), we propose to establish a database for the distribution of indicator values for each Texas estuarine system (Laguna Madre is additionally divided into Upper and Lower regions). This will ensure that we capture the natural temporal and spatial variability in condition and landscape indicators, especially since not all changes over time are a consequence of human-induced impacts. Changes are intrinsic to natural systems and it is important to document these sources of variation in order to detect and recognize deviations that are extrinsic and related to an anthropogenic disturbance. As described above, recognition of these deviations will be based on the historical distribution of indicators acquired for each particular estuary. The data and archived samples from the 2002-2004 R-EMAP and 2005 CBBEP projects are of particular value, as are data from a variety of published and unpublished sources that potentially relate to the distribution of selected seagrass indicators (see Table III.4).

RECOMMENDED STATEWIDE MONITORING PROGRAM FOR TEXAS

The implementation of a hierarchical strategy for seagrass monitoring reflects the need for comprehensive information on seagrass status, change, and condition. The basis of this approach is to provide an early warning of emerging ecological problems and provide a basis for establishing water quality criteria for seagrass conservation (Bricker and Ruggiero, 1998). In recognition of the financial constraints and resources associated with a seagrass monitoring program, we recommend a landscape level approach for estimating seagrass status and trends, physiological condition, and linkages to environmental processes. This approach is adapted from a very similar program developed by USGS to monitor estuarine seagrass populations in New England for the National Park Service (Neckles et al. 2002; 2010). The Tier 3 approach proposed here has been adopted by the National Estuarine Research Reserve (NERR) as the official monitoring protocol for mapping and monitoring submerged aquatic vegetation in the Reserve System (Moore et al. 2009; NERRS Research and Monitoring Plan 2006-2011). Similar protocols have been established for quantification of seagrass dynamics on a global scale (http://www.SeagrassNet.org; Short et al., 2006). This design incorporates changes in spatial

distributions from 1:24,000 scale remotely sensed data (Tier 1), rapid in situ spatial assessment in conjunction with optional high resolution (at least 1:9600 scale) aerial photo imagery (Tier 2), and fixed transects with permanent sampling stations (Tier 3).

Tier 1: System-Wide Mapping from Remotely Sensed 1:24,000 Scale Imagery

We propose to utilize remote sensing at two levels of resolution in order to compile status and trend maps for the study area. The primary purpose of Tier 1 is to characterize seagrass distribution over large spatial scales by remote sensing using 1:24,000 scale imagery. However, high resolution imagery (1:9,600) should be acquired when intensive monitoring is employed under Tier 2 or 3.

Standard system-wide mapping methods are used to identify seagrass meadow locations in all major Texas bays and coastal lagoons. The approach includes acquisition of remotely sensed images at 1:24,000 scale (digital true color), georectification of imagery, collection of ground truth data, interpretation of the images and delineation of vegetative areas, and importing the data into a GIS format for accuracy assessment, change detection, and reporting. The 1:24,000 scale photography acquisition and mapping should occur at about five year intervals.

Tier 2: Regional Rapid Assessment, Fixed Station Locations

Under Tier 2, broad-scale surveys in a large bay or lagoon are used to characterize the system based on specific biotic and abiotic properties of the water column, seagrasses, and sediments. Such measurements are absolutely critical to the development of a knowledge base that is *estuarine specific*, providing a foundation of data for the development of water quality and transparency criteria based on a large number of replicate samples for a selected site or area. Tier 2 monitoring is often integrated with existing high-resolution (Tier 3) studies at designated stations within a site and high resolution (1:9,600) aerial imagery (Fig. III.4). The approach incorporates random station selection in a stratified design that produces a somewhat even

dispersion of stations across the site or area of interest. Dunton et al. (2005) successfully used a grid of tessellated hexagons for random station selection in Laguna Madre and Redfish Bay with excellent results (Kopecky and Dunton 2006, Fig. III.5).
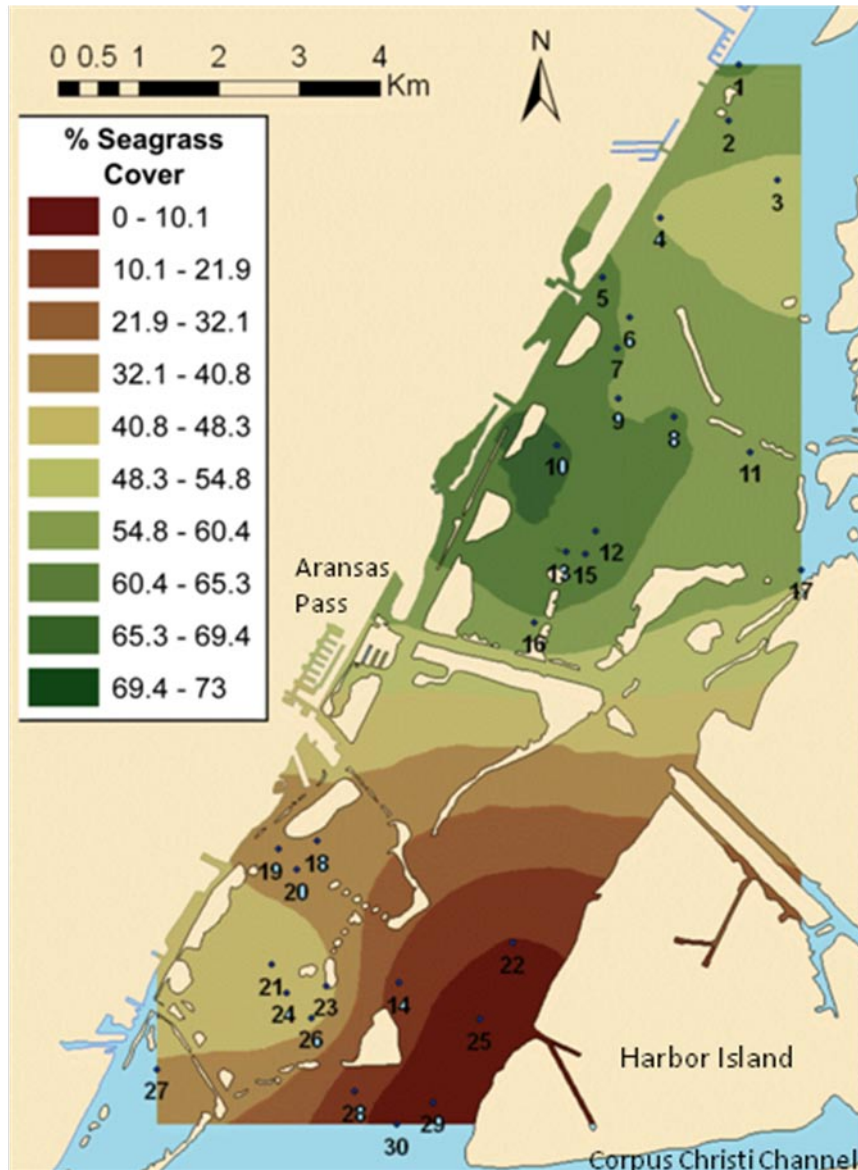

Figure III.5 - Interpolated average percent seagrass cover in Redfish Bay based on data collected at 30 randomly selected stations within each of 30 hexagons (see Fig. III.4; from Dunton et al., 2005).

Spatial design

The Tier 2 design utilizes a grid of tessellated hexagons within each regional bay system following Neckles et al. (2010). This approach forms the basis for high replication of parameters and the selection of probability-based sampling locations. In Redfish Bay, hexagons were 500 m on a side and covered 0.65 km^2, with one random sampling station located within each hexagon (Fig. III.4). The size of the hexagons within each bay system is largely dictated by sampling logistics and feasibility (e.g. 750 m hexagons may be required for Laguna Madre). The selection of stations is limited to a maximum depth of 2 m (MSL) in all regions of the Texas coast unless there is clear evidence of seagrass penetration to deeper depths in a given region (e.g. Lower Laguna Madre). This same approach has been utilized by Neckles et al. (2010) to detect changes in seagrass condition over time in Little Pleasant Bay, MA and Great South Bay, Long Island.

In addition to ground-based measurements, 1:9,600 scale, or larger, high resolution true color aerial photography can be used to assess spatial landscape indicator patterns and produce metrics for patchiness, macroalgae accumulations, and deepwater edges of existing seagrass meadows, especially in fringing habitats (Table III.4). Overlaying footprints (2.2 km x 2.2 km; 4.84 km^2) of high resolution 1:9,600 photographs over the hexagon grid (Tier 2) is employed for assessment of spatial patterns in patchiness, dense macroalgae deposits, and depth distribution of existing seagrass meadows. Because hexagons are 500 m on a side (0.65 km^2 in area), approximately 7.4 contiguous hexagons can be contained within one 1:9,600 scale photograph (Fig. III.4). The positions of randomly selected hexagon sampling points in Tier 2 are used to determine the location for acquisition of 1:9,600 photographs.

Sampling Strategy and Methods (adapted from Neckles et al., 2010)

- Annual sampling is performed during or shortly following peak seagrass standing crop (mid to late summer).

- For statistical rigor, use a repeated measures design with fixed sampling stations to maximize ability to detect change.

- Navigate to pre-selected stations with a GPS accuracy of 4 m or better.

- Stations are defined as the area within a 10-m radius of the GPS location.

- Hydrographic measurements are collected with a data sonde prior to deployment of any benthic sampling equipment.

- Water quality is determined from replicate water samples collected at each station. Water transparency is calculated from simultaneous measurements of photosynthetically active radiation (PAR) at the surface and at a measured depth using spherical quantum sensors and the Beer Lambert equation for calculation of the diffuse attenuation coefficient (k_d).

- Retrieve four replicate samples per station (for indicators listed in Table III.2) from each cardinal direction directly from the vessel. Previous work has shown that the probability of achieving a bias is less than 5% of the overall mean with only four subsamples (Neckles et al., 2011).

- Estimate percent cover within $0.25m^2$ quadrats using an underwater digital camera mounted to quadrat frame, or in shallow water, through direct observation through the water. If water transparency is extremely poor (Secchi < 1 m), make direct in situ measurements of the bottom with a mask and snorkel.

- Obtain morphometric data, biomass, shoot density, sediment characteristics, etc. using a ca. 9 cm coring device (or larger for *Thalassia*) deployed from the vessel.

- For each core sample, record the maximum leaf length of each shoot and the overall canopy height based on 80% of the leaf material and ignoring the tallest 20% of the leaves).

- All measurements and samples are collected by a crew of two from a shallow-draft vessel. Each region likely requires a commitment of one to three 12-hr days, with the exception of the Upper and Lower Laguna (up to 10 days each).

- Other monitoring programs have demonstrated that such an approach, when all sampling stations are considered together within a regional system, results in > 99% probability that the bias in overall estimates will not interfere with detection of change.

Data Analysis

- Use ArcGIS software to manage, analyze, and display spatially referenced point samples, and interpolate surfaces of all measured parameters biomass on integrated temporal and spatial scales using techniques of kriging interpolation (estimates the value of unsampled points as the weighted average of values from a given number of the closest points, giving more weight to closer points).

- Set the shoreline as an impermeable boundary (i.e. value of unsampled points is based only on sampled points within the same section of the region).

- Display the results of percent cover estimates based on Braun-Blanquet classes (Fourqurean et al., 2002).

- Utilize repeated measures ANOVA to determine if significant inter-annual spatial or temporal changes are occurring within a region.

Tier 3: Integrated Landscape, Permanent Stations

Tier 3 studies are conducted at a relatively small number of stations and consist of experimental studies and intensive monitoring for assessment of baseline conditions within a specific region. Tier 3 work is designed to address specific hypotheses in response to measured environmental change. Such studies provide an opportunity to link the presumptive factors responsible for changes in seagrass landscape indicators as detected by high resolution 1:9,600 imagery (patch formation, advances and/or retreats from deep edges, color changes that may reflect abundance of drift macroalgae or algal epiphytes) to changes in water quality and/or seagrass condition

indices that are measured either continuously or frequently at permanent stations. Dunton et al. (2005) conducted high resolution monitoring at several sites, from Laguna Madre to Redfish Bay. Monitoring occurs at least annually in mid-summer, but has been often conducted quarterly.

Design

Sampling methods are generally consistent with either SeagrassNet, a global monitoring program developed to investigate and document the status of seagrass resources worldwide (Short et al., 2006), or NERR protocols (Moore et al., 2009). In either case, quadrats (0.25 m^2) are positioned along three transects placed either parallel (SeagrassNet) or perpendicular (NERR) to the shoreline (Figs. III.6). Under the NERR protocol, the permanently established transect must bisect transitional or marginal seagrass beds that are characterized by any of one of the following features: an obvious deep edge, patchiness, or a distinct depth gradient.

At each Tier 3 station, plots are sampled non-destructively for percent cover by each species or cover category (e.g., bare ground, detritus) within a 0.25 m^2 area (Fig. III.7). In some beds, SAV clonal patchiness may require a much larger sampling area than 0.25 m^2. In addition to cover estimates, shoot or stem density and maximum canopy height should be determined for each species within each plot. If the vegetation is very dense then the plot may be sub-sampled for density, height and leaf or shoot width as needed.

An area reserved for the sampling of other factors such as sediment nutrients, porewater sulfide, sediment deposition, etc. should be located at a 1 m fixed distance from the transect line point oriented 180° from the vegetation sampling plot. Voucher specimens including flowers, fruits, and below-ground material of each species and their various morphological variants should be sampled and appropriately preserved.
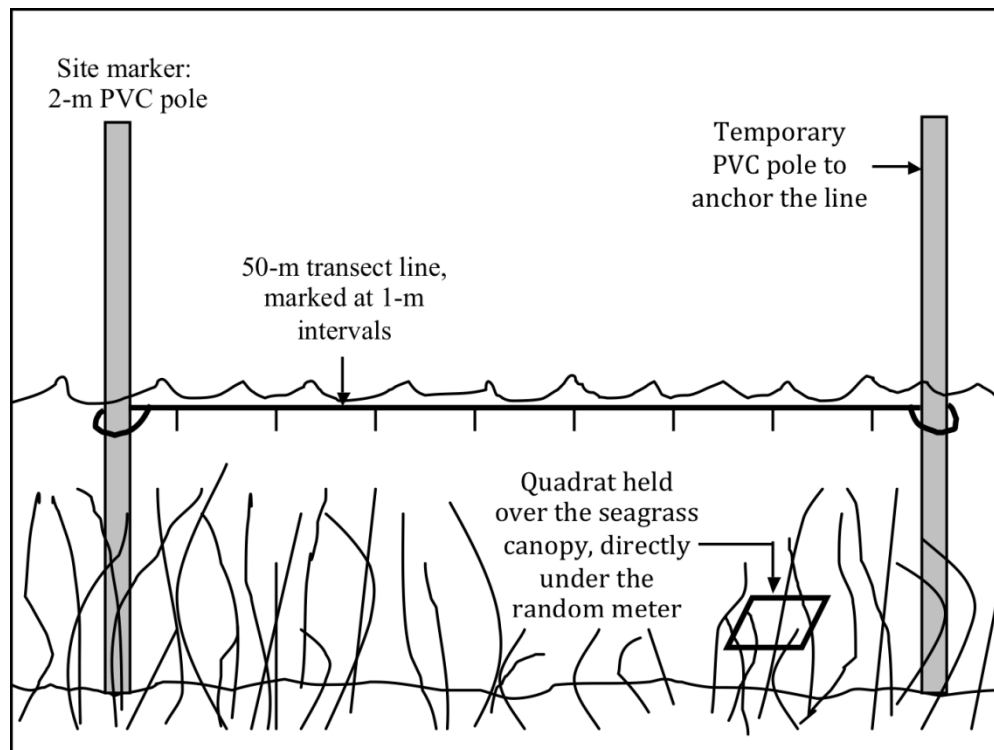
Figure III.6 – Example of permanent transects for NERR (in red; Moore et al., 2009) and SeagrassNet (blue; modified from Short et al. 2002). NERR annual transects are a minimum of 10 m apart, are 100 m long and extend past the edge of the seagrass bed. Seven to ten sampling locations along each annual transect are shown as red circles. White quadrats on blue transect lines parallel to the shoreline reflect the SeagrassNet protocol.

Figure III.7 - Each permanent site includes three 50 to 100-m transects over which samples are collected within permanent quadrats.

Notes on Transect Sampling

- Transect visits are conducted annually during the period of peak biomass, usually mid-summer.

- Ten permanent $0.25m^2$ quadrats are randomly located along each transect following the sampling protocol as outlined in Chapter 1.

- Biomass, epiphyte cover, above- and below-ground tissue samples, seed reserves, and sediment characteristics are determined from an adjacent core sample (0.5 m distant from the quadrat).

- Continuous measurements of light, temperature, and salinity are collected at one representative site in each region through deployment and periodic maintenance of dataloggers and appropriate sensors.

- If high resolution imagery is available, the transects are aligned with the 2.2 km x 2.2 km footprint of 1:9,600 aerial photography. As noted above, because hexagons are 500 m on a side, approximately seven contiguous hexagons can be sampled within one 1:9,600 photograph for assessment of spatial patterns in patchiness, dense macroalgae deposits, and depth distribution of existing seagrass meadows.

Patchiness and Location of the Deep Edge

- Patchiness and deep edges are critical landscape-level parameters. The deep edge estimate integrates long-term water transparency and both parameters are observed in 1:9,600 imagery.

- A quantitative measure of "patchiness" (referred to as "grain" by Pielou 1977) is computed in the simplest form by considering seagrasses as a two-phase mosaic (i.e., a surface composed of two types of polygons—with and without seagrasses). We can define patchiness to be the number of patch/gap transitions along each transect.

- Deep edges of beds are first verified by diving; transects start at the deep edge and traverse the bay in a direction perpendicular to shore toward shallower depths.

- Measurements of in situ PAR reflect minimum light requirements of plants at the deep edge (Dunton has conducted high resolution monitoring for PAR since 1989 at one site in Upper Laguna Madre). This is important, as Duarte (2007) recently found that seagrasses in turbid waters appear to have higher light requirements than plants living in clear waters. This is related to a number of stressors, both in the water column and in the sediments.

Experimental Studies

One of the major objectives of Tier 3 measurements are to address the causal relationships between water quality stressors and seagrass response as assessed by any number of condition indices. An understanding of stress/response relationships is often best achieved through intensive, hypothesis-driven experimental studies that address research needs for Texas seagrasses (Pulich and Calman, 1999). A fundamental understanding of the mechanisms and response indicators is required for Tier 3 studies, since measurements often occur across temporal and spatial scales. Ultimately, response variables are largely determined by an overarching question or hypothesis, incorporating additional parameters that could possibly include:

- seed reserves
- growth
- benthic faunal diversity
- sediment chemistry, including sulfides
- organic chemical contaminants (e.g. herbicides)
- leaf chlorophyll fluorescence
- reproduction and demography
- seagrass deep edges
- genetic diversity
- light fields

As such, the studies conducted under Tier 3 sampling are likely to employ innovative approaches to achieving a better understanding of stress/response relationships, with an expectation of publication of results in peer-reviewed journals.

APPLICATIONS TO COASTWIDE SEAGRASS MONITORING IN TEXAS

Data Analysis and Future Products

- Tier 1 observations identify large scale patterns in seagrass distribution and changes over time.

- Tier 3 observations can help interpret larger scale landscape patterns observed in Tier 1 and 2.

- Data gathered from Tier 3 monitoring can be applied to calibrate a biomass model based on percent cover and canopy height.

- Percent cover and canopy height are measured through the Tier 2 rapid assessment, and thus provides an opportunity to interpolate those measurements into a prediction of biomass on a regional scale.

- Determine the physiological indicators that identify the effects of light stress on seagrass photosynthetic tissues.

- The response and sensitivity of seagrass tissue constituents to anthropogenic nutrient loadings is very important.

- Develop a linear regression model of k_d (PAR) as a function of both TSS and chlorophyll (Gallegos, 2001).

- Determine the response of drift and seagrass epiphytic algal response to nutrient loading with respect to algal species composition and tissue constituents (Collado-Vides et al., 2007).

- Integrate the abiotic and biotic components to provide an overall assessment of seagrass condition (i.e. an Index of Biological Integrity).

Program Management

- Active involvement and support from the Seagrass Monitoring Work Group (SMWG) in all aspects of the program is critical. Workshops that include participants active in other nationally recognized seagrass monitoring programs is equally important. Overall coordination of Tier 2 and Tier 3 activities are probably best served by a SMWG subcommittee in partnership with TPWD.

- The environmental, landscape, and biological data gathered on this project should be compiled into a multifunctional data management system (DMS), as outlined in the TSGMP by Pulich et al. (2003). A DMS template will facilitate data access for analysis and mapping purposes using standard GIS procedures to visualize, integrate, and interpret spatial datasets (Pulich et al., 2000). Web-based data dissemination should be an integral part of the DMP.

- Maintain partnerships with local groups to continue to assess the status of seagrasses along the Texas coast.

- This proposed hierarchical strategy for seagrass monitoring has a broad scope that should be implemented for the entire Texas coast with partner support (e.g. MANERR, National Park Service, USGS-NWRC, other universities).

- Seven seagrass monitoring regions are proposed for Texas as follows. Regions are selected based on local physiography, geomorphological characteristics, hydrography and circulation, and the spatial or contiguous extent of the seagrass beds (Table III.5).

Table III.5. Proposed seagrass monitoring regions for the Texas coast based on current distribution (Fig. III.1) and data compiled by Pulich and Onuf (2007).

	Region	Description
1	Galveston Bay	Christmas Bay, West Galveston Bay
2	Matagorda Bay system	Includes East Matagorda Bay, west Matagorda Bay, secondary bays of Cox, Carancahua, Powderhorn and others
3	San Antonio Bay system	Espiritu Santo Bay, San Antonio Bay, Mesquite Bay
4	Mission-Aransas (MA)-NERR	Includes Aransas/Copano Bays, St. Charles Bay, Aransas National Wildlife Refuge shoreline, San Jose Island, North Redfish Bay, Terminal Flats, and north Harbor Island
5	Corpus Christi Bay system	South Redfish Bay, East Flats, Mustang Island, Shamrock Island, north side of Kennedy Causeway, Nueces Bay
6	Upper Laguna Madre	Nine Mile Hole and parts of Baffin Bay, from the Land Cut north to the Kennedy Causeway, as bordered by Padre Island National Seashore
7	Lower Laguna Madre	Land Cut south to Brazo Santiago Pass and including South Bay

Fall 2010

Region 4: Tier 2 and Tier 3 sampling will begin in Aransas and Copano Bays under a long-term commitment from the MANERR. Ken Dunton will provide expertise, assist with program development, populate the seagrass monitoring database, and initiate the integrated field monitoring program.

Region 6: Tier 2 and Tier 3 sampling will also commence in the Upper Laguna Madre (from Nine-Mile Hole to just north of Bird Island Basin) in the area encompassed by the Padre Island National Seashore park boundary. The effort, funded by the National Park Service (NPS), is coordinated with identical seagrass monitoring in the Gulf Islands National Seashore as directed by Ken Dunton (UTMSI in Texas) and Ken Heck (DISL in Alabama).

Proposed Tasks for 2011 and Beyond

Some specific objectives include (in prioritized order):

1. Establish a DMS (partners include MANERR, NPS, and TPWD). Enter data from EPA R-EMAP study and CBBEP (this report) into the database. Provide web access.

2. Analyze existing collections of seagrass tissue for C:N:P and ^{15}N:^{14}N ratios from Laguna Madre and the CBBEP study area for entry into seagrass database.

3. Revise the conceptual models (SMWG).

4. Initiate the integrated hierarchal sampling program (Tiers 2) in selected regions of the CBBEP study area.

5. Synthesis and expansion of monitoring to include all seven seagrass regions across the entire coast of Texas.

6. Acquire 1:24,000 photography statewide in cooperation with state and federal programs.

7. Summarize physical and chemical habitat requirements for Texas seagrasses based on existing data.

8. Develop programs that monitor submerged habitat at higher spatial and temporal resolution. Gather experimental evidence on cause-effect interactions for conceptual model development. Address functionality, habitat quality, and wildlife usage.

9. Hold an annual workshop to summarize trends and relationships between seagrass condition indicators and water column properties, identify problems, and suggest appropriate responses by State agencies.

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