Assessment of Seagrass Habitat Quality and Plant Condition in Texas Coastal Waters: 2011 and 2012

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ABSTRACT

This report details the results of a statewide seagrass monitoring program for Texas that was undertaken in the summers of 2011 and 2012. Rapid assessment (Tier-2) sampling was completed at 567 sampling stations in four systems: the Mission-Aransas National Estuarine Research Reserve (MANERR), Corpus Christi Bay (CCB), and the Upper (ULM) and Lower (LLM) Laguna Madre. In the CCB/MANERR site, water clarity decreased and light attenuation increased from 2011 to 2012. Despite this, seagrass percent coverage in this site was nearly identical from 2011 to 2012. In the ULM site, water clarity also decreased from 2011 to 2012, although seagrass percent coverage increased. In the LLM site, declines in chlorophyll, salinity, and total suspended solids all contributed to an increase in water clarity from 2011 to 2012. While this site also experienced increases in seagrass percent cover, it is important to note that some areas in this region remain devoid of seagrasses. This multi-year examination of seagrass distribution and water quality data along the Texas coast can serve as a valuable tool for management officials because of its capability to highlight specific areas of both seagrass growth and decline.

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

Conservation efforts should seek to develop a conceptual model that outlines the linkages among seagrass ecosystem components and the role of indicators as predictive tools to assess the seagrass physiological 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 plant physiological condition. For example, stressors that lead to higher water turbidity and light attenuation (e.g. dredging and shoreline erosion) are known to result in lower below-ground seagrass biomass and alterations to sediment nutrient concentrations. It is therefore necessary to evaluate long-term light measurements, the biomass of above- versus below-ground tissues and the concentrations of nutrients, sulfides and dissolved oxygen in sediment porewaters when examining the linkages between light attenuation and seagrass health.

This study implements a program for monitoring seagrass meadows in Texas coastal waters following protocols that evaluate seagrass condition based on landscape-scale dynamics. These protocols adhere to the hierarchical strategy for seagrass monitoring outlined by Neckles et al. (2011) and serve to establish quantitative relationships between physical and biotic parameters that ultimately control seagrass condition, distribution, persistence, and overall health. Our monitoring approach follows a broad template adopted by several federal and state agencies across the country, but which is uniquely designed for Texas (Dunton et al., 2011) and integrates plant condition indicators with landscape feature indicators to detect and interpret seagrass bed disturbances.

The objectives of this study were to (1) implement long-term monitoring 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. various state and federal agencies) determine if the observed change necessitates a revision of regulatory policy or management 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 was assessed using a suite of condition indicators (physical, biological, hydrological, and chemical) measured on different spatial and temporal scales.

The primary questions addressed in the 2011 and 2012 annual Tier-2 surveys include:

1) What are the spatial and temporal patterns in the distribution of seagrasses over annual and decadal scales?
2) What are the characteristics of these plant communities, including their species composition and percent cover?
3) How are any changes in seagrass percent cover and species composition, related to measured characteristics of water quality?

METHODS

Sampling Summary

Tier-2 protocols, which are considered Rapid Assessment sampling methods, are adapted from Neckles et al. (2011). Tier-2 sampling began in late summer and was completed by September 2011 and 2012. For statistical rigor, a repeated measures design with fixed sampling stations was implemented to maximize our ability to detect future change. Neckles et al. (2011) demonstrated that the Tier-2 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.

Site Selection

The Tier-2 sampling program is intended to compliment ongoing remote sensing efforts. Sites were therefore selected from vegetation maps generated with aerial and satellite imagery during the 2004/2007 NOAA Benthic Habitat Assessment. The vegetation maps were then tessellated using polygons, and sample locations were randomly selected within each polygon (Figure 1). Only polygons containing > 50 % seagrass coverage were included in the 2011 and 2012 sampling efforts. In 2012, fourteen sampling stations were added in Little Bay within the MANERR.

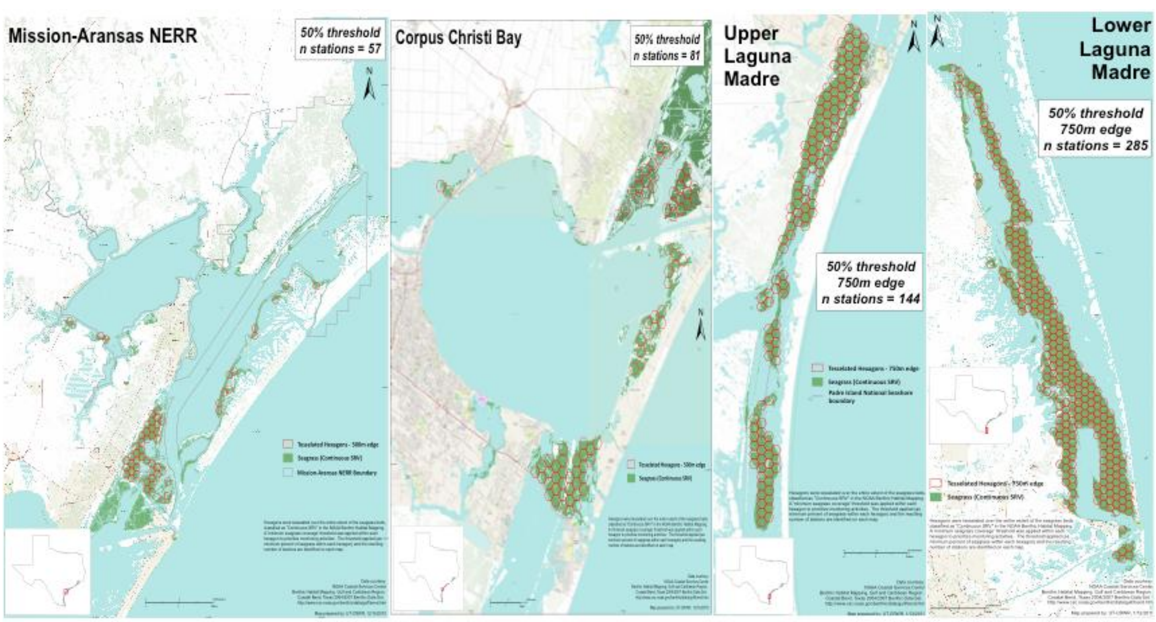


Figure 1. Tessellated boundaries of submerged vegetation delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

Water Quality

All sampling stations were located in the field using a handheld GPS device to within a 10 m radius of the pre-determined station coordinates. Upon arrival to a station, hydrographic measurements including water depth, conductivity, temperature, salinity, dissolved oxygen, chlorophyll fluorescence and pH were collected with a YSI 600XL data sonde. Water samples were obtained at each station for determination of Total Suspended Solid (TSS) concentration (See Appendix A.1). Water transparency was derived from measurements of photosynthetically active radiation (PAR) using two LI-COR spherical quantum scalar sensors attached to a lowering frame (See Appendix A.2). All sonde measurements and water samples were obtained prior to the deployment of benthic sampling equipment.

Seagrass Coverage

Species composition and areal coverage were obtained from four replicate quadrat samples per station at each of the four cardinal locations from the vessel. Percent cover of areal biomass was estimated by direct vertical observation of the seagrass canopy through the water using a 0.25 m^2 quadrat framer subdivided into 100 cells. Previous research has demonstrated that the probability of achieving a bias is less than 5% of the overall mean when using only four subsamples (Neckles, pers. comm.).

Plant Tissue Condition

Seagrass leaf tissue was collected at every station containing a vegetated bottom. Upon removal, all tissue samples were immediately placed on ice in sealed plastic containers and transported to the University of Texas at Austin Marine Science Institute (UTMSI). Leaf tissue samples were then dried to a constant weight in a 60°C oven and homogenized using a mortar and pestle. Subsamples of leaf tissue were sent to the University of California at Davis for determination of leaf tissue carbon content, nitrogen content, $\delta^{13}C$ and $\delta^{15}N$ (See Appendix A.3). Leaf tissue phosphorous content was determined at UTMSI (See Appendix A.3). All plant tissue analysis was limited to *Halodule wrightii*, as this species was the most prevalent and widely distributed among sample sites. A single species was chosen to reduce confounding factors attributed to differences in plant physiology among species and to provide a reliable metric amenable to spatial comparisons.

Spatial Data Analysis and Interpolation

ArcGIS software (Environmental Systems Research Institute) was used to manage, analyze, and display spatially referenced point samples and interpolate surfaces for all measured parameters. An inverse distance weighted method was used to assign a value to areas (cells) between sampling points. A total of 12 sampling stations were identified from a variable search radius to generate the value for a single unknown output cell (100 m^2). All data interpolation was spatially restricted to the geographic limits of the submerged vegetation map created during the 2004/2007 NOAA Benthic Habitat Assessment.

RESULTS

Water Quality

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2011

The CCB/MANERR site exhibited a depth of 51.3 ± 23.2 cm (mean ± standard deviation; Table 1, Figure 2a), and was the most shallow of the three regions surveyed in 2011. This site also exhibited the smallest spatial variation in depth. Salinity varied the least among sampling stations at this site, with a mean of 41.8 ± 3.9 (Table 1, Figure 3a). Very low salinity values (<10) were observed along the southern border of Copano Bay, while several regions of hypersaline water were documented along the northeastern border of Redfish Bay and the easternmost boundary of CCB. This region had the second highest dissolved oxygen concentration, with a mean of 6.39 ± 2.00 mg L^{-1} (Table 1, Figure 4a). The lowest dissolved oxygen concentrations were documented in the northeastern boundary of CCB, within East Flats. One sampling station had a dissolved oxygen concentration indicative of hypoxic conditions (<2 mg L^{-1}), while four additional stations revealed concentrations below 3 mg L^{-1}. Lastly, the CCB/MANERR site had the lowest and most variable pH values (7.85 ± 0.38; Table 1, Figure 5a) and the only acidic pH value (6.79) for any region. The pH was lowest in the Aransas and Copano Bays and gradually increased southwards into CCB.

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2012

The CCB/MANERR site had an increased depth (64.5 ± 27.8 cm; Table 1, Figure 2b) in 2012 as compared to 2011. As in 2011, this site was the most shallow of the three regions. Salinity values decreased from 2011 (38.5 ± 3.9; Table 1, Figure 3b), and showed a general decrease from north to south throughout the site, except one area on the west coast of Mustang Island in Corpus Christi Bay. The CCB/MANERR region had the highest dissolved oxygen concentration of the three sites, with a mean of 7.11 ± 2.32 mg L^{-1} (Table 1, Figure 4b). The lowest dissolved oxygen values were again observed in the northeast of CCB, with the highest dissolved oxygen values (> 12 mg L^{-1}) in Redfish Bay and South Bay. The pH of the region was the second highest in 2012, and increased from 2011 (8.00 ± 0.28; Table 1, Figure 5b). Unlike 2011, none of the sampling stations had an acidic pH value. A different trend was seen with regards to pH in 2012, with pH decreasing from north to south in Redfish Bay.

Upper Laguna Madre 2011

The ULM site had a mean depth of 81.7 ± 42.0 cm (Table 1, Figure 2a) and varied the most spatially among the three sites surveyed. The ULM exhibited the highest salinity of all sites with a mean of 49.3 ± 6.9 (Table 1, Figure 3a). It is interesting to note that the maximum salinity values were observed south of Baffin Bay on the eastern side of the land cut. This area is essentially in the center of the Laguna Madre and lies at the greatest distance from a significant tidal inlet or freshwater source. As a result, these high salinity values are likely attributed to long water residence times. The ULM had the lowest mean dissolved oxygen concentration of any site (5.33 ± 2.06 mg L^{-1}; Table 1, Figure 4a). Hypoxic conditions were observed at five sampling stations, while an additional nine stations recorded dissolved oxygen concentrations less than 3 mg L^{-1}. The highest dissolved oxygen concentrations were observed at the northern mouth of Baffin Bay, and the lowest concentrations were found to the south of Baffin Bay and the interior ULM between Baffin and Corpus Christi Bays. Finally, the ULM recorded a mean pH of (7.96 ± 0.34; Table 1, Figure 5a), with the lowest values generally observed at Mustang Island State Park and to the west of the Intracoastal Waterway.

Upper Laguna Madre 2012

The water depth in the ULM region increased slightly in 2012, with a mean depth of 86.3 ± 43.4 cm (Table 1, Figure 2b), though a spatial depth pattern was nearly identical to that of 2011 was observed. Overall salinity values decreased slightly from 2011, with a mean of 48.3 ± 3.2 (Table 1, Figure 3b), though areas in the south did show substantial increases. Dissolved oxygen concentrations were again the lowest of the three sites in the ULM, although values were higher than those of 2011, with a mean of 6.52 ± 2.17 mg L^{-1} (Table 1, Figure 4b). The spatial pattern of dissolved oxygen concentrations was very different from that of 2011. The highest dissolved oxygen concentrations were near the mouth and to the south of Baffin Bay, and the lowest values were in the northern region of the ULM. The pH of this region was the highest of the three (8.20 ± 0.23; Table 1, Figure 5b), and elevated from 2011. Areas that experienced high dissolved oxygen concentrations also showed the highest pH values. The lowest pH values were observed just north of the mouth of Baffin Bay.

Lower Laguna Madre 2011

The LLM contained the deepest seagrass habitat of the three regions surveyed with a mean depth of 84.1 ± 36.2 cm (Table 1, Figure 2a). This region was the least saline with a mean salinity of 41.6 ± 3.9 (Table 1, Figure 3a). The lowest salinities were observed in areas to the north of the Port Mansfield and Brazos Santiagos Passes. Highest salinities were observed in the El Realito Bay and in the interior section of the LLM, furthest from navigational passes. The LLM region contained the highest mean dissolved oxygen concentration of any region (7.17 ± 1.28 mg L^{-1}; Table 1, Figure 4a). There were no documented instances of hypoxia and only one site recorded a dissolved oxygen concentration < 3 mg L^{-1}. Dissolved oxygen concentrations were highest to the south of the Port Mansfield Pass and lowest to the east of Laguna Atascosa Wildlife Refuge and within Lake Verdolaga. Lastly, the LLM exhibited the highest and least variable pH of all three regions (8.09 ± 0.25; Table 1, Figure 5a), with a slight decrease in pH from north to south.

Lower Laguna Madre 2012

The mean depth (83.5 ± 35.7 cm; Table 1, Figure 2b) of the LLM sample sites was within 1% of the depth reported in 2011. Overall, there was a slight decline in mean salinity (39.6 ± 3.6; Table 1, Figure 3b) in 2012. The largest salinity decline was observed north of the Laguna Atascosa Wildlife refuge on the western boundary of the LLM. There was a moderate increase in salinity in 2012 south of the Brazos Santiagos Pass. Dissolved oxygen declined slightly in 2012 (6.76 ± 1.33 mg L^{-1}; Table 1, Figure 4b), with the most notable decreases occurring in the southeastern LLM. However, there were still no documented cases of hypoxia and none of the sites exhibited a dissolved oxygen concentration < 3 mg L^{-1}. The pH (7.91 ± 0.28; Table 1, Figure 5b) declined throughout the entire LLM region in 2012. The northern limit of the LLM near Padre Island National Seashore and the waters east of the Laguna Atascosa Wildlife Refuge recorded the greatest declines in pH.

Table 1. Summary of water quality parameters by region and year.

		Depth (cm)		Salinity		Dissolved Oxygen (mg L^{-1})		pH	
		2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
CCB/MANERR									
	Mean	51.3	64.5	41.8	38.5	6.39	7.11	7.85	8.00
	Standard Deviation	23.2	27.8	3.9	3.9	2.00	2.32	0.38	0.28
	N	138	150	138	151	137	151	138	151
ULM									
	Mean	81.7	86.3	49.3	48.3	5.33	6.52	7.96	8.20
	Standard Deviation	42.0	43.4	6.9	3.2	2.06	2.17	0.34	0.23
	N	144	144	142	140	142	139	143	140
LLM									
	Mean	84.1	83.5	41.6	39.6	7.17	6.76	8.09	7.91
	Standard Deviation	36.2	35.7	3.9	3.6	1.28	1.33	0.25	0.28
	N	285	284	284	284	282	239	285	282

Water Column Optical Properties

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2011

The CCB/MANERR site was characterized by the highest water clarity of all sites with a mean downward attenuation coefficient (K_d) of 0.70 ± 0.53 m^{-1} (Table 2, Figure 6a). The highest attenuation values were generally recorded at the northeastern boundary of Aransas Bay and in the westernmost CCB near Portland. Light attenuation is likely attributed to chlorophyll in the water column, as this site had the highest average chlorophyll (4.62 ± 2.86 μg L^{-1}; Table 2, Figure 7a) and lowest average TSS (13.7 ± 8.7 mg L^{-1}; Table 2, Figure 8a) concentrations of any site. Based on the mean K_d value observed in 2011, seagrasses in this region become limited by light availability at a depth of 2.44 m (assuming a surface irradiance of 2000 μmol photons m^{-2} s^{-1} and a minimum light requirement of 18% of surface irradiance).

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2012

The CCB/MANERR site had higher values of light attenuation in 2012 than 2011 (0.85 ± 0.77; Table 2, Figure 6b), but had the best water clarity of the three regions. Very high attenuation was recorded in Nueces Bay near Portland, and also in the south of Copano Bay and the southwest of Redfish Bay. There was a decrease of water column chlorophyll from 2011 (3.90 ± 4.17 μg L^{-1}; Table 2, Figure 7b). Areas with high chlorophyll seemed to be aligned with high attenuation, with the exception of southwest Redfish Bay. The lowest average TSS was again observed in the CCB/MANERR region, with an average of 16.3 ± 13.6 mg L^{-1} (Table 2, Figure 8b). The average amount of TSS in the water column increased from 2011, possibly explaining the increase in light attenuation. Highest TSS was again found in areas with the highest attenuation and chlorophyll (Nueces Bay near Portland and the south of Copano Bay).

Upper Laguna Madre 2011

The ULM exhibited a mean K_d of 0.73 ± 0.33 m^{-1} (Table 2, Figure 6a), which was the least variable among sites. Similarly, water column chlorophyll (3.92 ± 2.07 μg L^{-1}; Table 2, Figure 7a) and TSS (13.8 ± 7.1 mg L^{-1}; Table 2, Figure 8a) also exhibited the least amount of spatial variation. Interestingly, the highest K_d values are located to the southeast of Laguna Larga and correspond to an area of high chlorophyll concentrations. Based on the mean K_d value observed in 2011, seagrasses in this region become limited by light availability at a depth of 2.34 m (assuming a surface irradiance of 2000 μm photons m^{-2} s^{-1} and a minimum light requirement of 18% of surface irradiance).

Upper Laguna Madre 2012

The mean K_d of the ULM rose substantially in 2012 (1.11 ± 0.61 m^{-1}; Table 2, Figure 6b), and was the greatest of the three regions. Extremely low light attenuation was observed to the south of Baffin Bay, while several patches to the north of Baffin Bay had very high K_d values (> 2.0 m^{-1}). Chlorophyll in the water column also increased greatly, with an average of 5.56 ± 4.80 μg L^{-1} (Table 2, Figure 7b). Several areas north of Baffin Bay showed extremely high chlorophyll values > 15 μg L^{-1}. Water column chlorophyll in the ULM was more than double than in the LLM, and nearly double as found in the CCB/MANERR. In the ULM, TSS also increased markedly from 2011 (19.6 ± 13.1 mg L^{-1}; Table 2, Figure 8b), especially to the south of Baffin Bay. The TSS values for the ULM were the highest of any of the three regions. It is likely that both the extremely high TSS and chlorophyll in the water column contributed to the large amount of attenuation in the ULM site.

Lower Laguna Madre 2011

The LLM had the lowest and most variable water clarity of any region (K_d = 1.39 ± 0.97 m^{-1}; Table 2, Figure 6a). This region also recorded the lowest mean chlorophyll (2.75 ± 3.05 μg L^{-1}; Table 2, Figure 7a) and highest mean TSS (26.1 ± 33.4 mg L^{-1}; Table 2, Figure 8a) concentrations of any region. The highest attenuation coefficients were observed at the southern entrance to the land cut, southwest of Port Mansfield and to the northeast of the Laguna Atascosa Wildlife Refuge. Each of these locations also exhibited very high concentrations of water column chlorophyll and TSS, which is undoubtedly responsible for the observed spatial patterns in light attenuation. Based on the mean K_d value observed in 2011, seagrasses in this region become limited by light availability at a depth of 1.24 m (assuming a surface irradiance of 2000 μm photons m^{-2} sec^{-1} and a minimum light requirement of 18% of surface irradiance).

Lower Laguna Madre 2012

Water clarity improved markedly in 2012 (K_d = 1.07 ± 0.62 m^{-1}; Table 2, Figure 6b) and was less variable throughout the region. The higher water clarity is attributed to declines in both mean chlorophyll (2.02 ± 1.97 μg L^{-1}; Table 2, Figure 7b) and mean TSS (18.1 ± 13.7 mg L^{-1}; Table 2, Figure 8b). The highest attenuation coefficients were still observed at the southern entrance of the land cut, southwest of Port Mansfield, and to the northeast of the Laguna Atascosa Wildlife Refuge. However, these areas of high light attenuation declined in both extent and severity from 2011 to 2012. The largest declines in both chlorophyll and TSS occurred in the waters surrounding the Laguna Atascosa Wildlife Refuge. Based on the mean K_d value observed in 2012, seagrasses in this region become limited by light availability at a depth of 1.62 m (assuming a surface irradiance of 2000 μm photons m^{-2} sec^{-1} and a minimum light requirement of 18% of surface irradiance).

Table 2. Summary of water transparency property indicators by region and year.

		K_d (m^{-1})		Chlorophyll *a* ($\mu g\ L^{-1}$)		Total Suspended Solids ($mg\ L^{-1}$)	
		2011	**2012**	**2011**	**2012**	**2011**	**2012**
CCB/MANERR							
	Mean	0.70	0.85	4.62	3.90	13.7	16.3
	Standard Deviation	0.53	0.77	2.86	4.17	8.7	13.6
	N	35	68	134	151	138	151
ULM							
	Mean	0.73	1.11	3.92	5.56	13.8	19.6
	Standard Deviation	0.33	0.61	2.07	4.80	7.1	13.1
	N	100	95	142	140	143	144
LLM							
	Mean	1.39	1.07	2.75	2.02	26.1	18.1
	Standard Deviation	0.97	0.62	3.05	1.97	33.4	13.7
	N	176	174	277	283	283	282

Seagrass Coverage and Species Distributions

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2011

Seagrasses covered a mean area of 68.6 ± 29.8 % (Table 3, Figure 9a) of the benthos in the CCB/MANERR site. The seagrass assemblage was dominated by *Halodule wrightii* (39.8 ± 35.2 %; Table 3, Figure 10a), followed by *Thalassia testudinum* (23.7 ± 35.3 %; Table 3, Figure 11a), *Syringodium filiforme* (4.0 ± 14.1 %; Table 3, Figure 12a), *Ruppia maritima* (1.0 ± 5.4 %; Table 3, Figure 13a) and *Halophila engelmanii* (0.1 ± 0.7 %; Table 3, Figure 14a). Four sampling stations did not contain any vegetation and 5.8% of the stations in this site contained less than 10% seagrass coverage. Seagrass coverage was lowest along St. Joseph Island, near the Ingleside Naval Base and in the Nueces Bay near Portland. *Halodule wrightii* was distributed throughout the CCB/MANERR region, with the exception of Redfish Bay, where *Thalassia testudinum* dominated. Established *Thalassia testudinum* populations are likely excluding *Halodule wrightii* from expanding into this area. The CCB/MANERR generally contained a tall seagrass canopy (23.0 ± 9.0 cm; Table 4, Figure 15a), which was highest along the western portions of Redfish Bay. The average canopy height of this site was the tallest of the three, likely due to the large amount of *Thalassia testudinum*.

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve 2012

Seagrasses covered a mean area of 74.2 ± 30.2 % (Table 3, Figure 9b) of the CCB/MANERR benthos, a substantial increase from 2011. *Halodule wrightii* continued to dominate the seagrass assemblage (42.2 ± 39.6 %; Table 3, Figure 10b), followed by *Thalassia testudinum* (23.2 ± 35.4 %; Table 3, Figure 11b), *Syringodium filiforme* (7.1 ± 19.9 %; Table 3, Figure 12b), *Ruppia maritima* (2.3 ± 11.8 %; Table 3, Figure 13b), and *Halophila engelmanii* (0.5 ± 3.9 %; Table 3, Figure 14b). The largest assemblages of *Thalassia testudinum*, *Ruppia maritima* and *Halophila engelmanii* were observed in this region. Thirteen of the fourteen stations within the newly sampled Little Bay did not contain any vegetation. In addition to this, three other sampling stations throughout the CCB/MANERR did not have any vegetation, and 2.9 % of the stations in this site contained less than 10 % seagrass coverage. Seagrass coverage was again lowest in Nueces Bay near Portland, as well as some areas in the west of Redfish Bay, the west of Copano Bay, and north of Cedar Bayou. *Halodule wrightii* seemed to be distributed in the eastern portion of this region, with *Thalassia testudinum* distributed in the western portion. The CCB/MANERR had a slight increase in average seagrass canopy height (25.3 ± 11.2 cm; Table 4, Figure 15b), with the tallest canopy again observed in the west of Redfish Bay. The CCB/MANERR site once again had the tallest seagrass canopy of the three sites.

Upper Laguna Madre 2011

Seagrasses covered a mean area of 75.2 ± 30.9 % (Table 3, Figure 9a) of the benthos in the ULM site. The seagrass assemblage was again dominated by *Halodule wrightii* (60.9 ± 36.9 %; Table 3, Figure 10a), followed by *Syringodium filiforme* (13.6 ± 26.1%; Table 3, Figure 12a), *Ruppia maritima* (0.3 ± 1.9%; Table 3, Figure 13a), *Halophila engelmanii* (0.3 ± 1.5%; Table 3, Figure 14a), and *Thalassia testudinum* (0.1 ± 0.6%; Table 3, Figure 11a). Seven sampling stations in this site did not contain any seagrass coverage, and 6.9% of the stations contained less than 10% seagrass coverage. Seagrass coverage was lowest to the southeast of the Nueces-Kleberg County line and south of Baffin Bay to the east of the land cut. *Halodule wrightii* was found throughout the ULM, but was lower in abundance to the east of Chapman Ranch. However, this area contained extensive cover of *Syringodium filiforme*. It does not initially appear that *Syringodium filiforme* is excluding *Halodule wrightii* because these two species were observed interspersed throughout the ULM. Lastly, the ULM site contained the greatest coverage of *Halophila engelmanii*, with a distinguished population located to the east of Laguna Larga. The ULM site had an average seagrass canopy height of 20.1 ± 8.8 cm (Figure 15a), with a general decrease in canopy height from north to south throughout the site.

Upper Laguna Madre 2012

Seagrass cover increased in the ULM to 87.7 ± 25.5 % (Table 3, Figure 9b). The seagrass assemblage was again dominated by *Halodule wrightii* (73.1 ± 36.0 %; Table 3, Figure 10b), followed by *Syringodium filiforme* (14.4 ± 29.1 %; Table 3, Figure 12b), *Halophila engelmanii* (0.1 ± 1.1 %; Table 3, Figure 14b), and *Ruppia maritima* (0.1 ± 0.9 %; Table 3, Figure 13b). *Thalassia testudinum* was not observed at any of the sampling stations (Figure 11b). While no seagrass was present at four of the sampling stations, all remaining stations contained at least 10 % seagrass coverage. The increase in percent cover seems to be mostly attributed to the increase observed in *Halodule wrightii* coverage. Areas in the northwest part of the ULM region and the southernmost edge of ULM near the land cut had minimal to low seagrass coverage. The ULM site had an increase in average seagrass canopy height (22.4 ± 10.3 cm; Table 4, Figure 15b), with canopy height again decreasing from north to south throughout the site.

Lower Laguna Madre 2011

Seagrasses covered a mean area of 45.9 ± 40.0 % (Table 3, Figure 9a) of the benthos in the LLM region. The seagrass assemblage was dominated by *Halodule* *wrightii* (25.5 ± 35.7 %; Table 3, Figure 10a), followed by *Thalassia testudinum* (18.4 ± 33.4 %; Table 3, Figure 11a), *Syringodium filiforme* (1.4 ± 7.6 %; Table 3, Figure 12a) and *Ruppia maritima* (0.6 ± 3.5 %; Table 3, Figure 13a). *Halophila engelmanii* was not observed at any of the stations (Table 3, Figure 14a). Fifty of the sample sites were completely absent of vegetation and 14.4 % of sample sites contained < 10 % seagrass coverage. Seagrass coverage was most notably absent around the Port Mansfield pass and in the areas surrounding the Laguna Atascosa Wildlife Refuge. Seagrass species appeared to show spatial segregation within this region as *Halodule wrightii* dominated areas to the north of the Laguna Atascosa Wildlife Refuge and *Thalassia testudinum* dominated areas to the south. *Syringodium filiforme* was also documented within this region, but was restricted to areas surrounding the Brazos Santiagos Pass. The LLM site had the lowest average canopy height of all the sites (15.7 ± 9.8 cm; Table 4, Figure 15a). The tallest canopies were observed in the south, which was dominated by *Thalassia testudinum*.

Lower Laguna Madre 2012

Seagrasses increased to cover a mean area of 50.4 ± 38.4 % (Table 3, Figure 9b) of the benthos in 2012. The seagrass assemblage was still dominated by *Halodule wrightii* (30.3 ± 37.3 %; Table 3, Figure 10b), followed by *Thalassia testudinum* (17.8 ± 31.8 %; Table 3, Figure 11b), *Syringodium filiforme* (2.2 ± 11.1 %; Table 3, Figure 12b) and *Ruppia maritima* (0.4 ± 4.7 %; Table 3, Figure 13b). No *Halophila engelmanii* was observed in this region (Table 3, Figure 14b). *Halodule wrightii* and *Syringodium filiforme* increased in cover, while *Thalassia testudinum* and *Ruppia maritima* decreased slightly in cover compared to 2011 measurements. The overall increase in seagrass was most evident to the northeast of Port Mansfield and on the eastern boundary of the LLM across from the Laguna Atascosa Wildlife Refuge. *Halodule wrightii* accounted for the increase in cover northeast of Port Mansfield, while *Thalassia testudinum* accounted for the increase in cover in the southeast LLM. Lastly, seagrass canopy height increased throughout in the LLM in 2012, with the largest increases observed on the western boundary of the LLM to the north and south of the Laguna Atascosa Wildlife Refuge. The LLM site again had the lowest average seagrass canopy height, but did experience an increase from 2011 (18.0 ± 8.6 cm; Table 4, Figure 15b). Tallest canopies were again observed in the southern areas dominated by *Thalassia testudinum*.

Table 3. Summary of plant areal coverage by species all study regions in 2011 and 2012.

		All species (% cover)		*H. wrightii* (% cover)		*T. testudinum* (% cover)		*S. filiforme* (% cover)		*R. maritima* (% cover)		*H. engelmanii* (% cover)	
		2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
CCB/MANERR													
	Mean	68.6	74.2	39.8	42.2	23.7	23.2	4.0	7.1	1.0	2.3	0.1	0.5
	Std. Dev.	29.8	30.2	35.2	39.6	35.3	35.4	14.1	19.9	5.4	11.8	0.7	3.9
	N	138	137	138	137	138	137	138	137	138	137	138	137
ULM													
	Mean	75.2	87.7	60.9	73.1	0.1	0.0	13.6	14.4	0.3	0.1	0.3	0.1
	Std. Dev.	30.9	25.5	36.9	36.0	0.6	0.0	26.1	29.1	1.9	0.9	1.5	1.1
	N	144	144	144	144	144	144	144	144	144	144	144	144
LLM													
	Mean	45.9	50.4	25.5	30.3	18.4	17.8	1.4	2.2	0.6	0.4	0.0	0.0
	Std. Dev.	40.0	38.4	35.7	37.3	33.4	31.8	7.6	11.1	3.5	4.7	0.0	0.0
	N	285	283	285	283	285	283	285	283	285	283	285	283

Table 4. Summary of average seagrass canopy height for each region and year.

		Average seagrass canopy height (cm)	
		2011	**2012**
CCB/MANERR			
	Mean	23.0	25.3
	Standard Deviation	9.0	11.2
	N	134	135
ULM			
	Mean	20.1	22.4
	Standard Deviation	8.8	10.3
	N	137	136
LLM			
	Mean	15.7	18.0
	Standard Deviation	9.8	8.6
	N	235	241

CONCLUSIONS

Corpus Christi Bay/Mission-Aransas National Estuarine Research Reserve

Overall, the CCB/MANERR site showed a decline in water clarity and greater light attenuation from 2011 to 2012, which should make the area less amenable to seagrasses. Areas of high light attenuation correlated to areas of high TSS and water column chlorophyll, such as Nueces Bay near Portland and the south of Copano Bay. These same areas had very low seagrass percent cover. Despite the decline in water clarity, both dissolved oxygen and pH increased, and seagrass percent coverage throughout the whole site remained nearly identical from 2011 to 2012. *Halodule wrightii* tends to dominate in the east of this site, and *Thalassia testudinum* dominates in the west. Areas in the east of Aransas Bay showed an increase in seagrass percent coverage, as did an area east of Ingleside. Decreases in seagrass percent coverage were seen east of Aransas Pass, in Copano Bay, and in Cedar Bayou.

Upper Laguna Madre

Overall, water quality in the ULM site became much less amenable to seagrasses. Light attenuation rose drastically, likely due to elevated chlorophyll and TSS in the water column. Despite the decrease in water clarity, seagrass percent cover increased throughout the region. A substantial increase was seen in *Halodule wrightii* and a slight increase in *Thalassia testudinum*. The southernmost part of the site and northwestern areas near the Nueces-Kleberg County line and Corpus Christi Naval Air Station did not have any seagrasses present in 2011 or 2012. Attenuation was extremely high near the Nueces-Kleberg County line in 2012, and moderate in 2011, although chlorophyll and TSS levels were low. It is unclear why no seagrasses are growing near the Corpus Christi Naval Air Station, or in the southernmost tip of the site.

Lower Laguna Madre

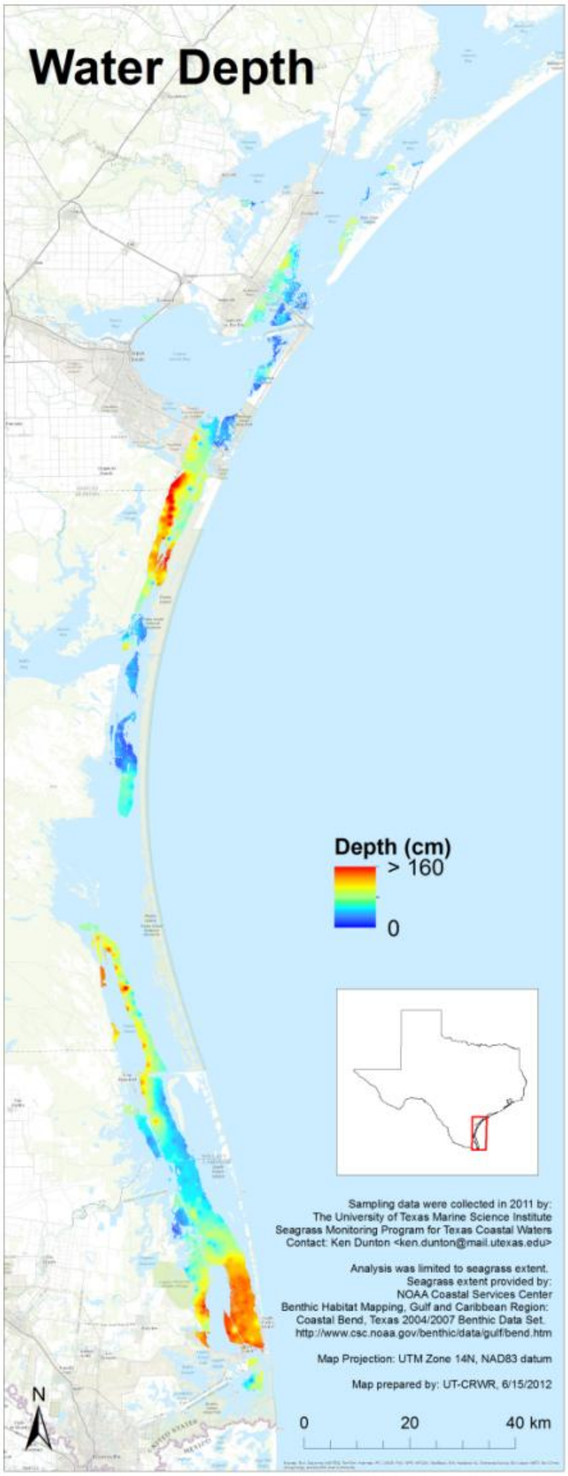
Overall, the water quality in the LLM site became more amenable to seagrasses in 2012 compared to 2011. Mean salinity, chlorophyll and TSS all declined in 2012, which coincided with an increase in water clarity. The improved water clarity is likely responsible for the increases in both seagrass cover and canopy height observed in 2012. The largest changes in both water quality and seagrass distributions were documented in the areas surrounding the Laguna Atascosa Wildlife Refuge and Port Mansfield. It is possible that both of these areas are influenced by fluctuating hydrologic contributions of the Arroyo Colorado, but additional research is required to fully ascertain this relationship. Finally, although conditions improved in 2012, it is important to note that the waters northeast of the Laguna Atascosa Wildlife Refuge were still largely devoid of seagrass. This region was described as vegetated habitat in the NOAA 2004/2007 Benthic Habitat Assessment but continues to exhibit high TSS concentrations and very low water clarity.

ACKNOWLEDGEMENTS

This project was made possible through visionary funding from the National Park Service (NPS), the Mission-Aransas National Estuarine Research Reserve (MANERR), the Texas Coastal Bend Bays and Estuaries Program (CBBEP), and the Texas General Land Office (TGLO) through the Texas Coastal Management Program. We are especially grateful to our program managers Martha Segura and Joe Meiman (NPS), Sally Palmer and Ed Buskey (MANERR), Ray Allen (CBBEP), and Melissa Porter (TGLO). Field work was overseen by Kim Jackson, and largely carried out by tireless efforts of graduate students from the University of Texas Marine Science Institute including Karen Bishop, Philip Bucolo, Kelly Darnell, and Nathan McTigue. All interpolations and maps were created by the University of Texas at Austin Center for Research in Water Resources.

FIGURES

2a.



2b.

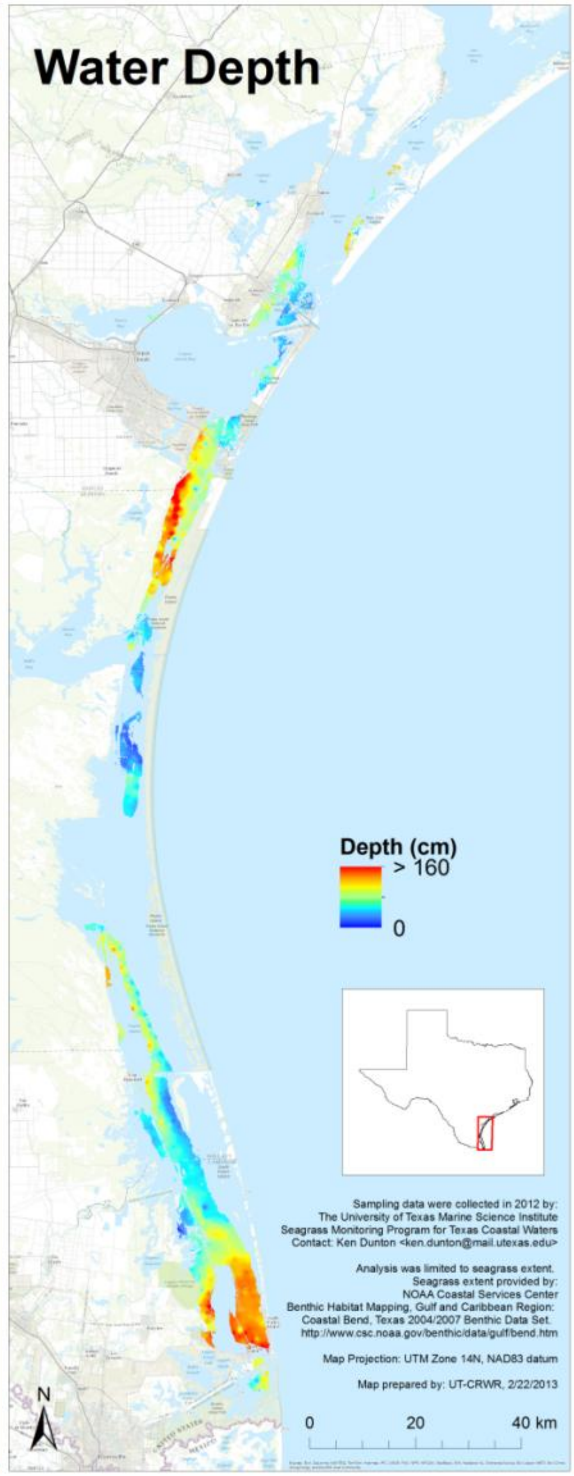
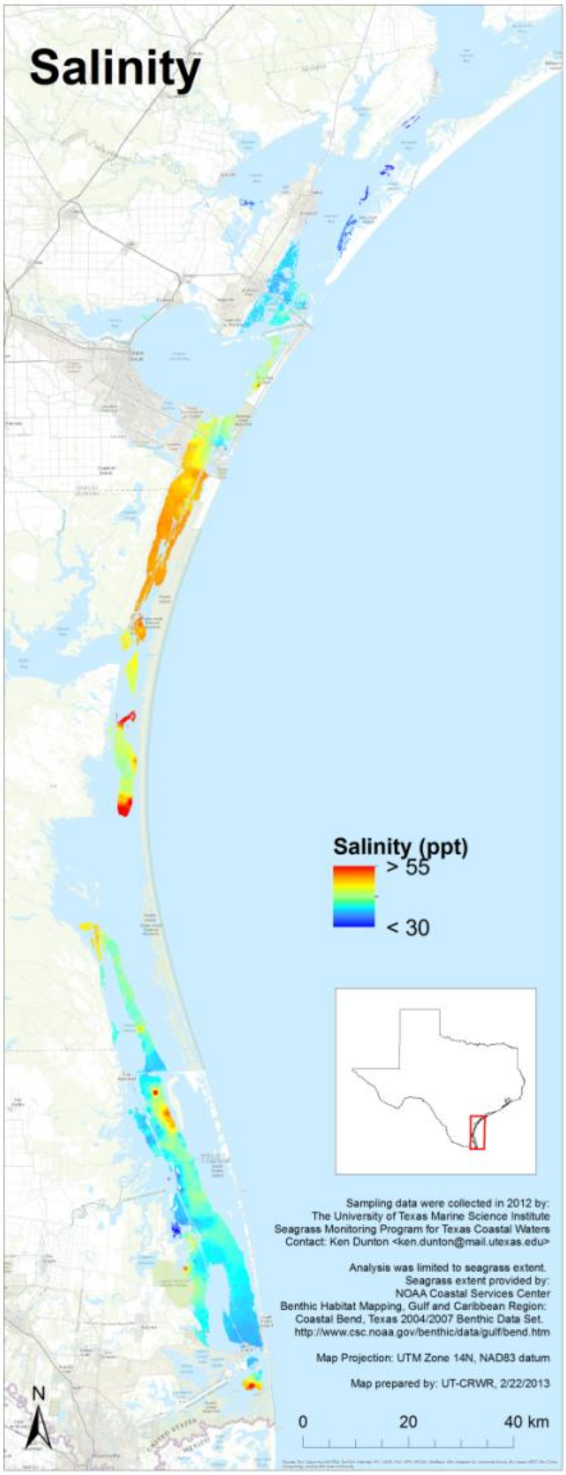


Figure 2. Spatial representations of water depth for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

3a.



3b.

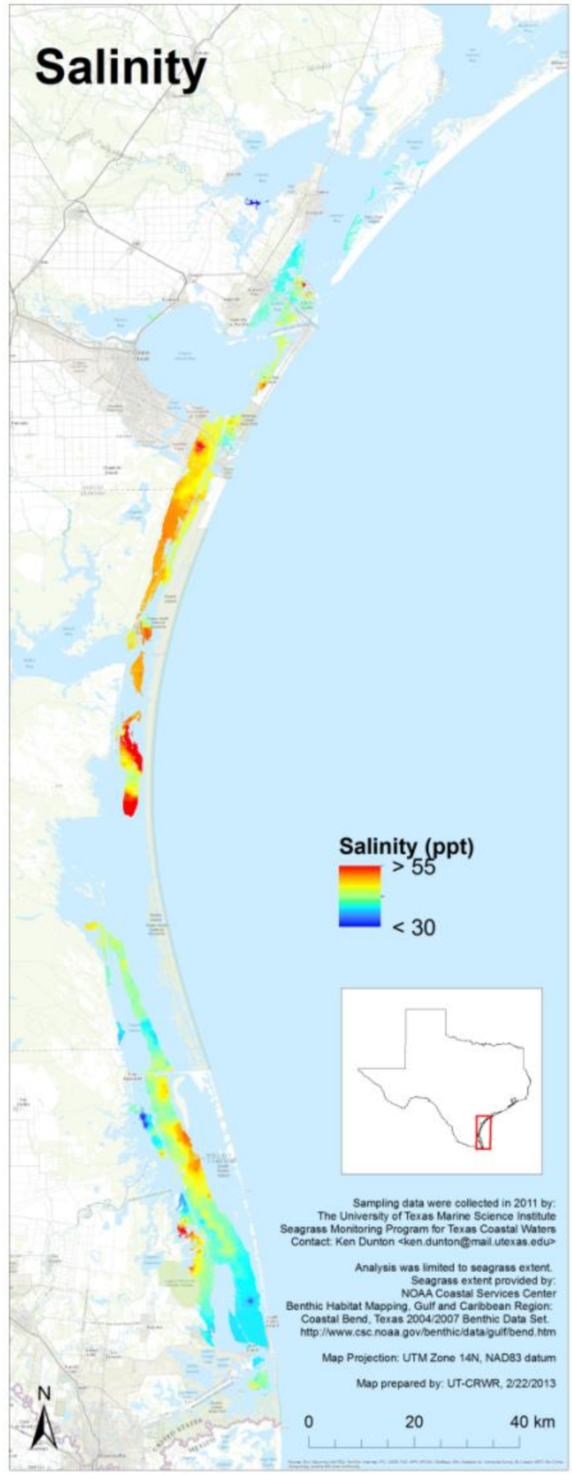


Figure 3. Spatial representations of salinity for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

4a. 4b.

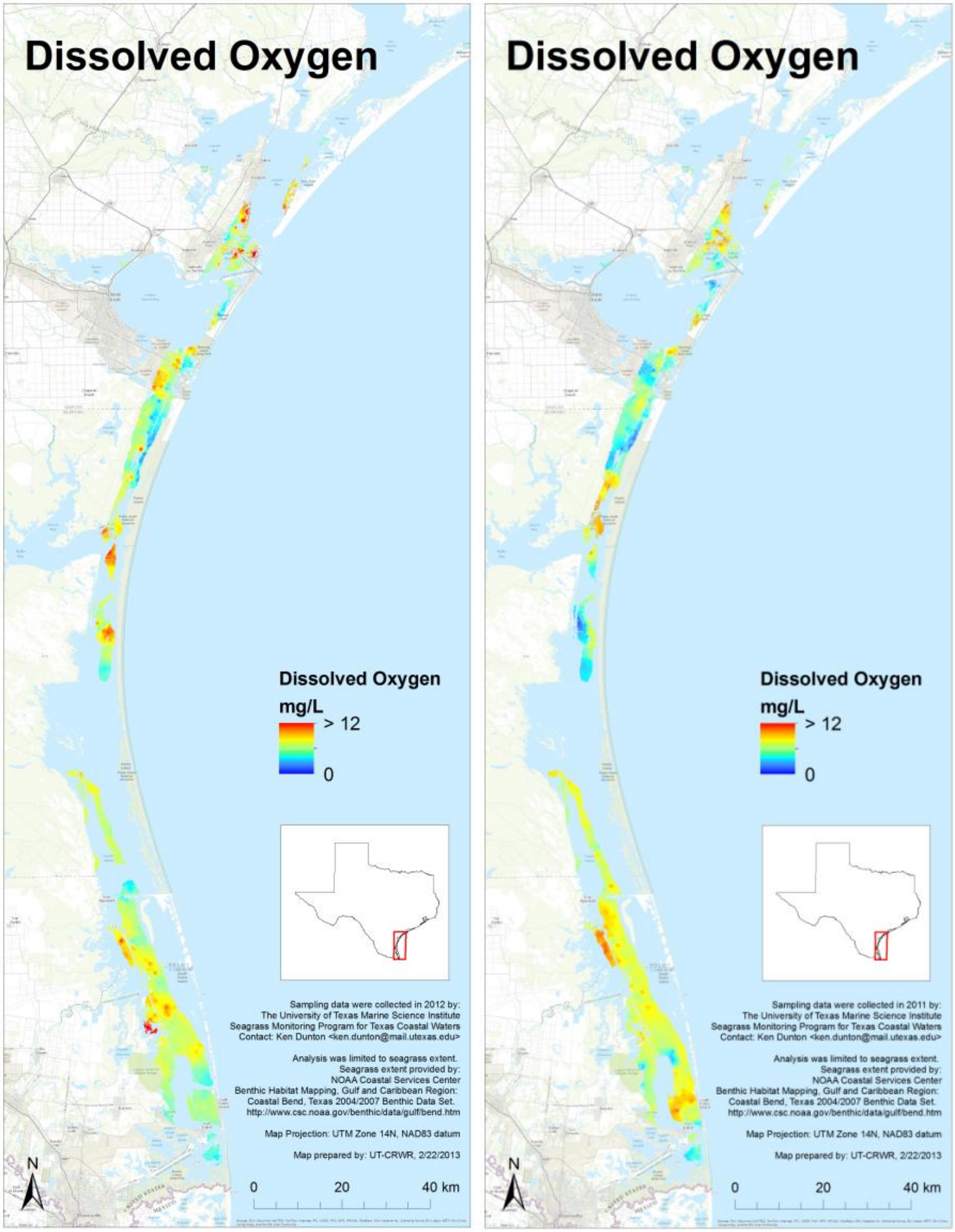
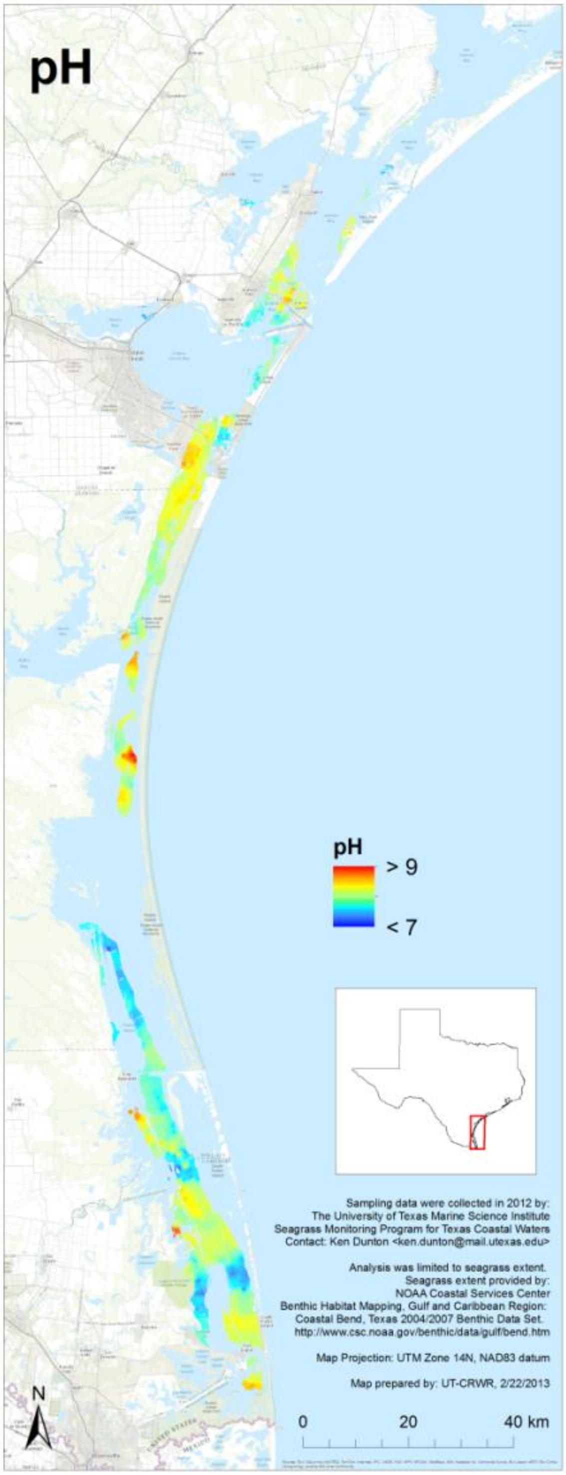


Figure 4. Spatial representations of dissolved oxygen for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

5a.



5b.

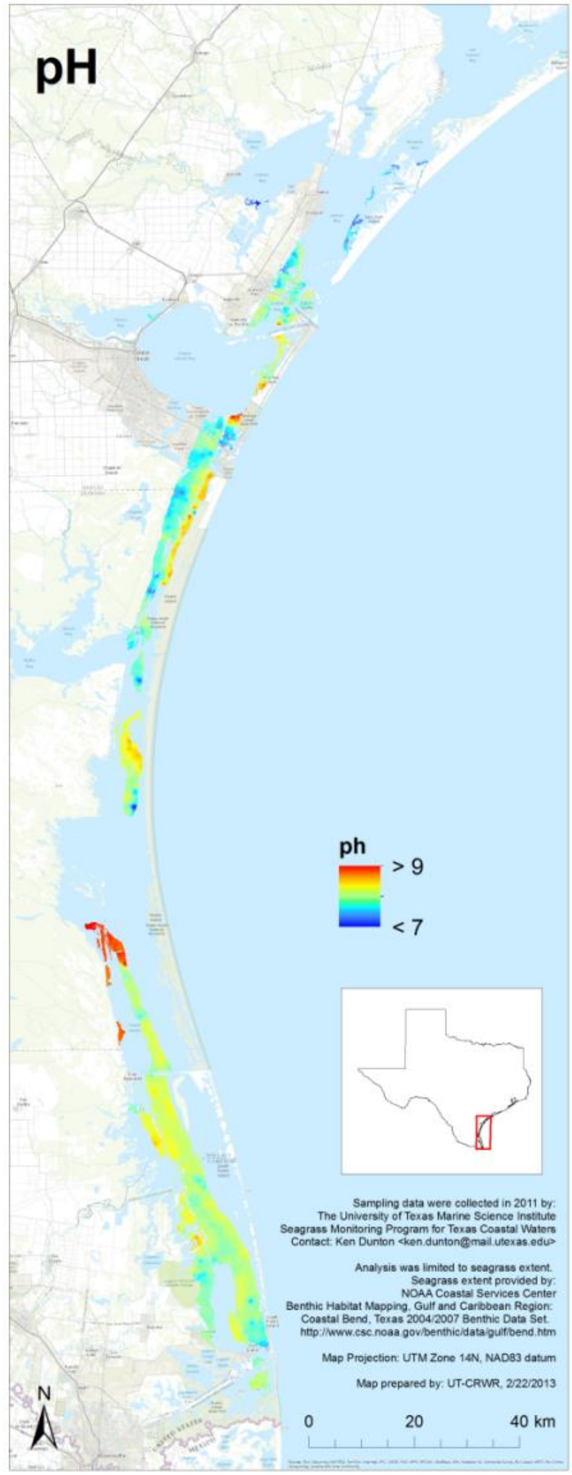
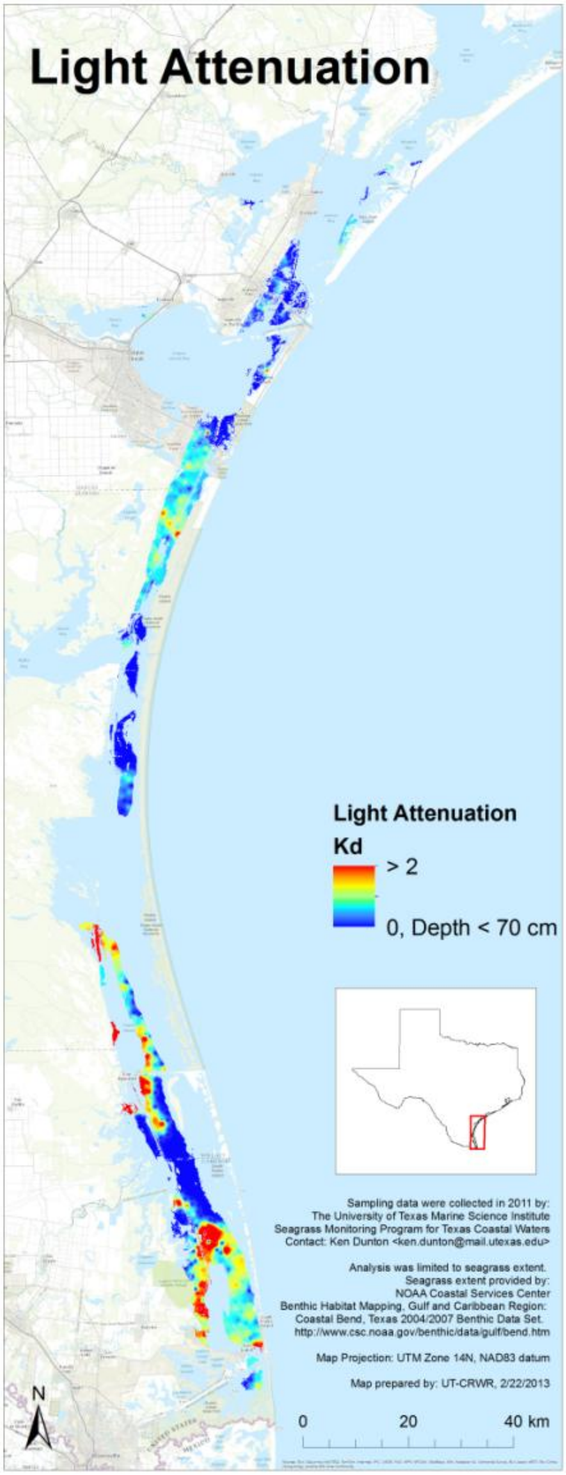


Figure 5. Spatial representations of pH a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

6a.



6b.

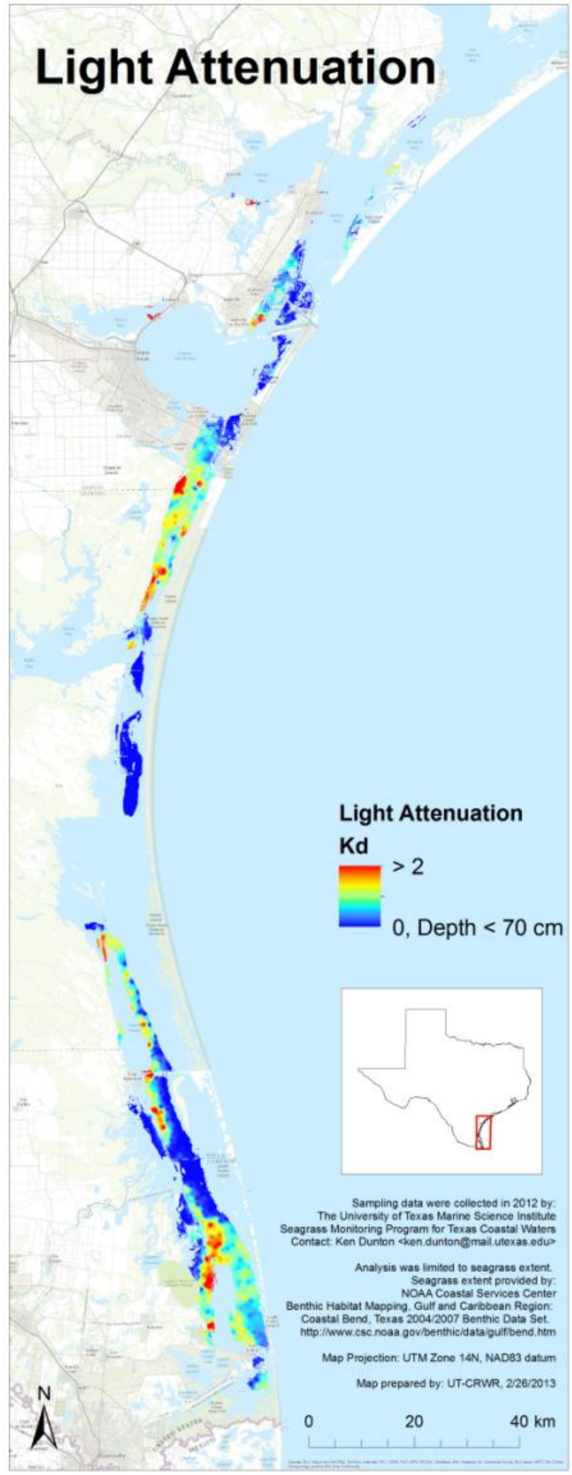
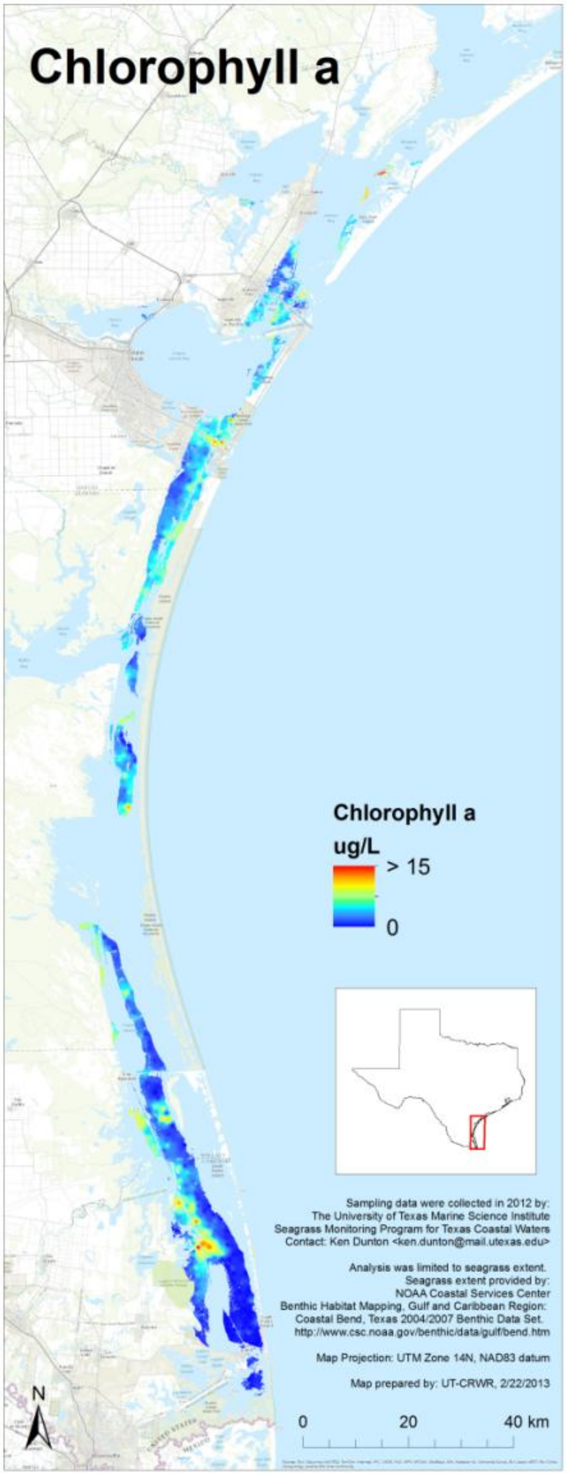


Figure 6. Spatial representations of light attenuation for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

7a.



7b.

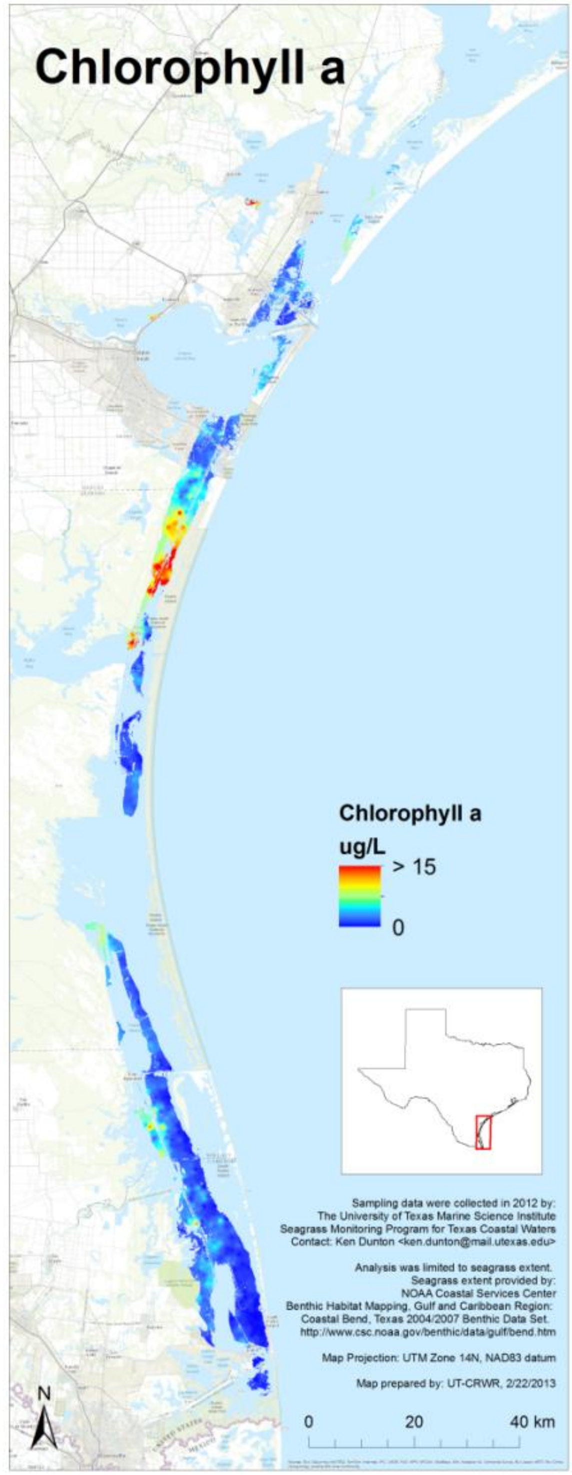


Figure 7. Spatial representations of chlorophyll a for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

8a. 8b.

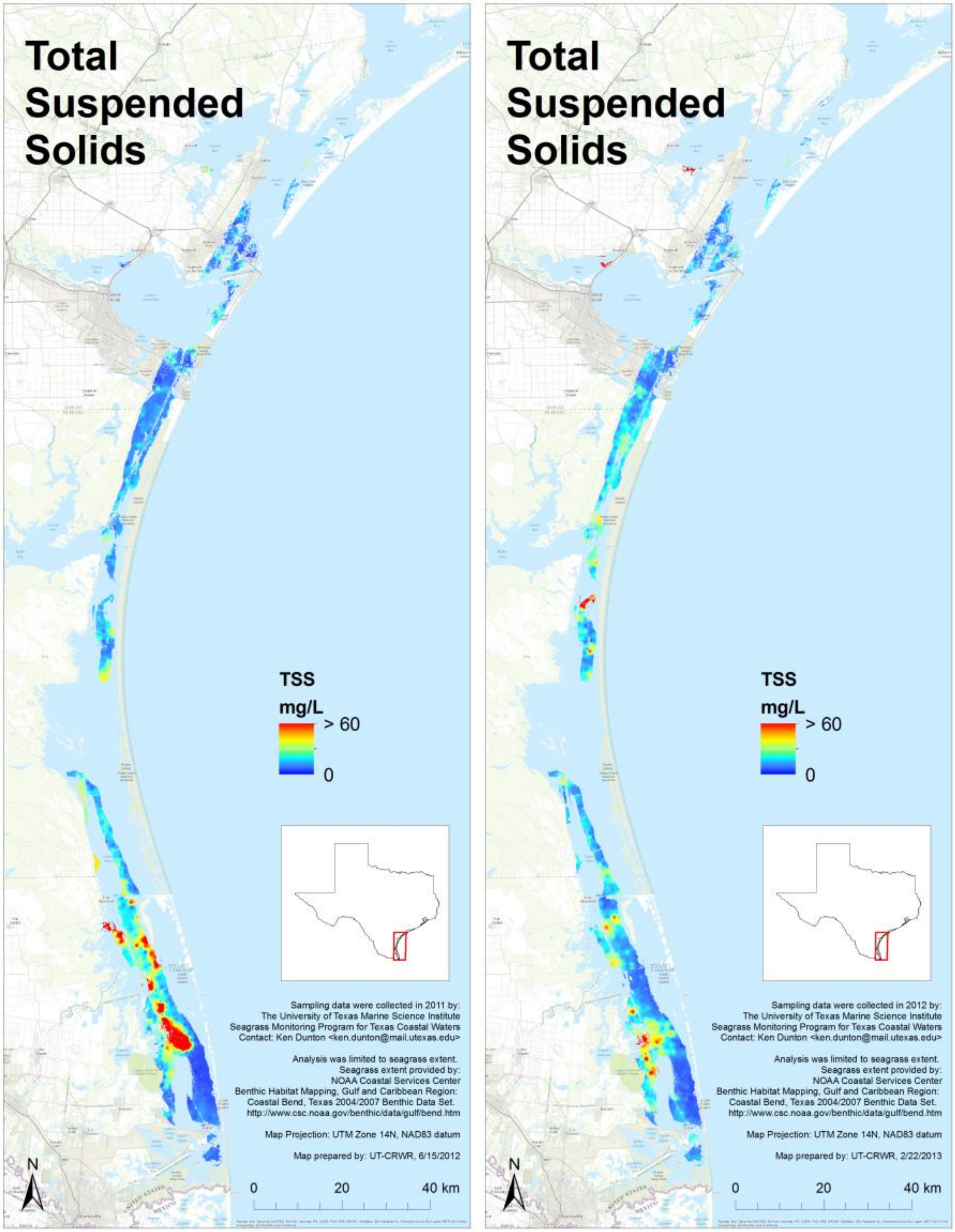


Figure 8. Spatial representations of total suspended solids for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

9a. 9b.

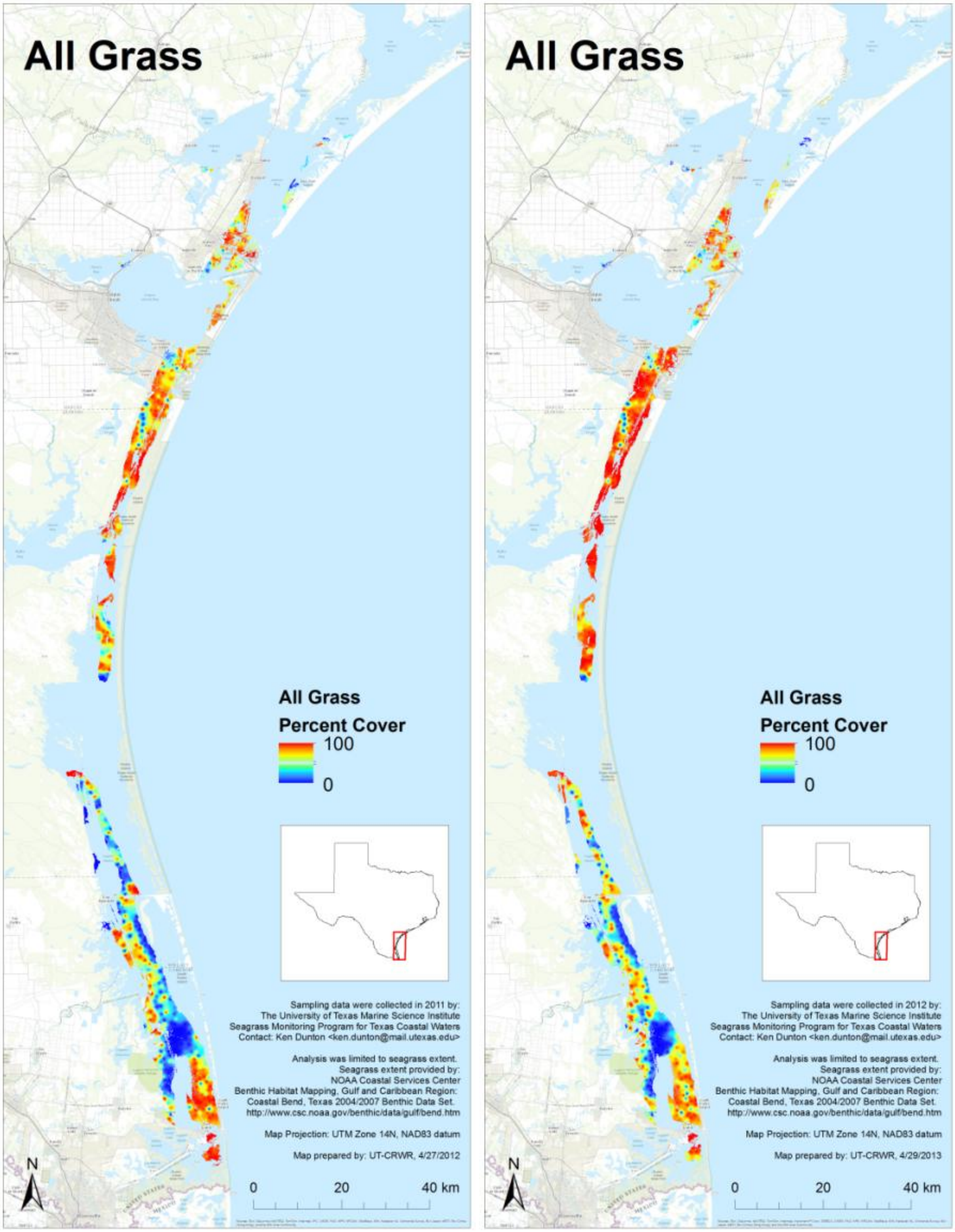
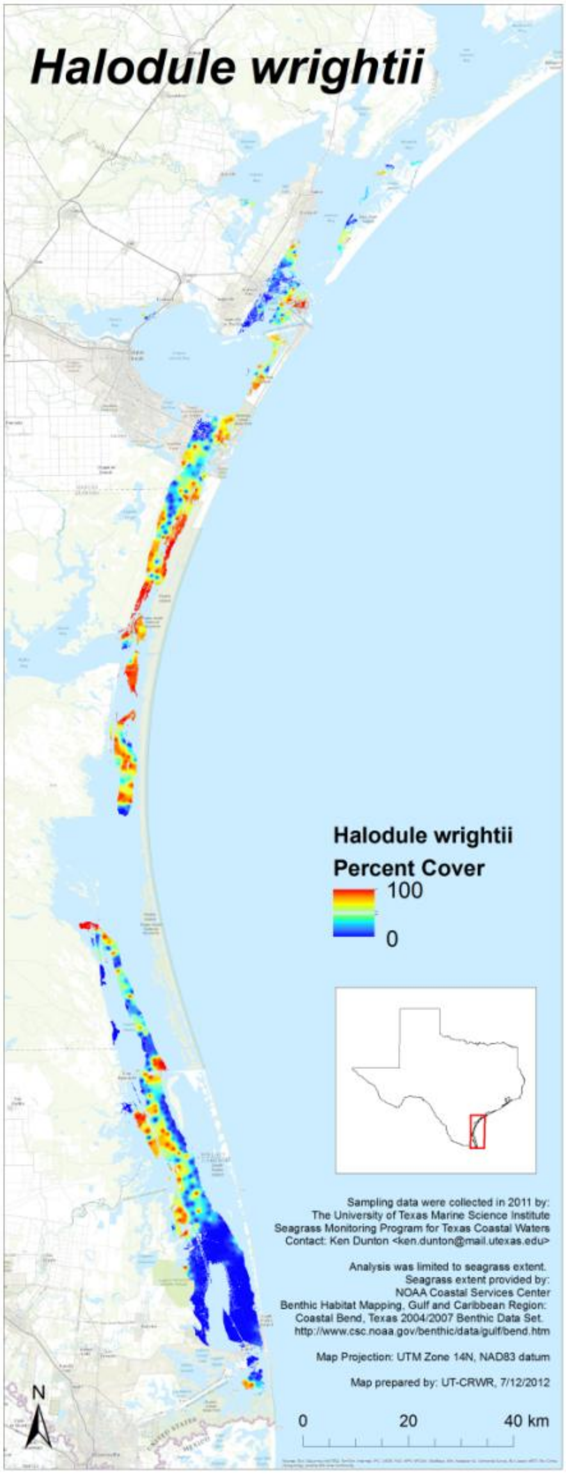


Figure 9. Spatial representations of percent cover for all seagrass for a) 2011, b) 2012). The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

10a.



10b.

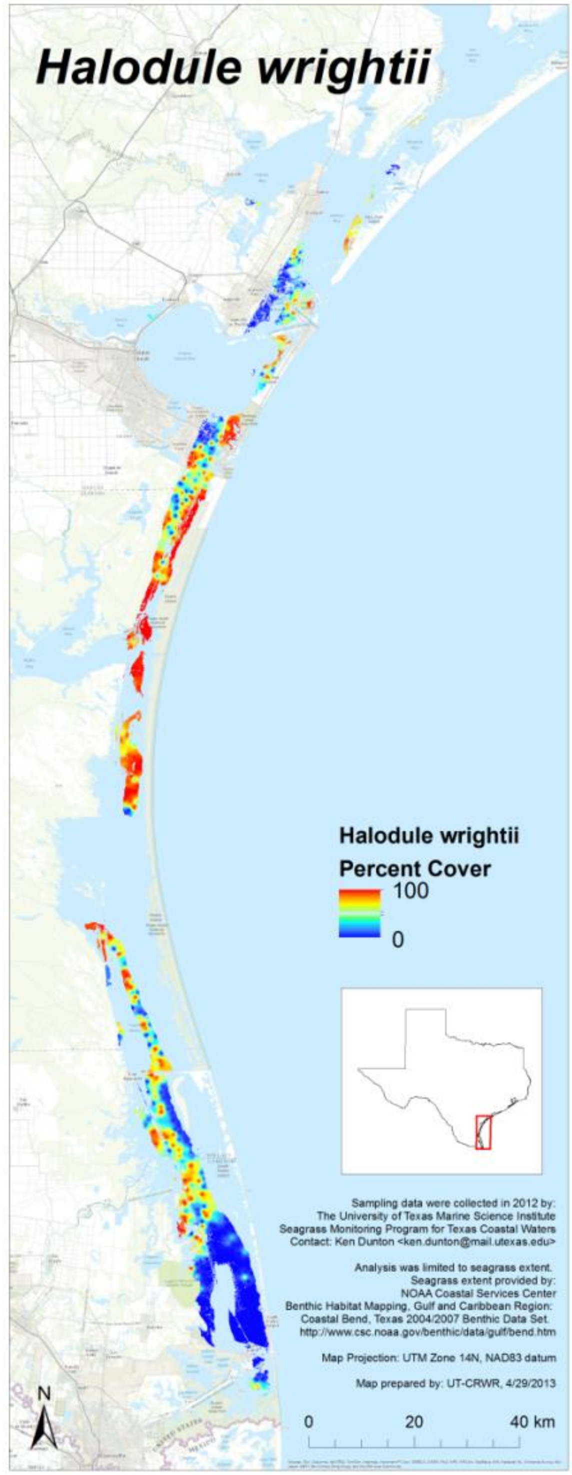
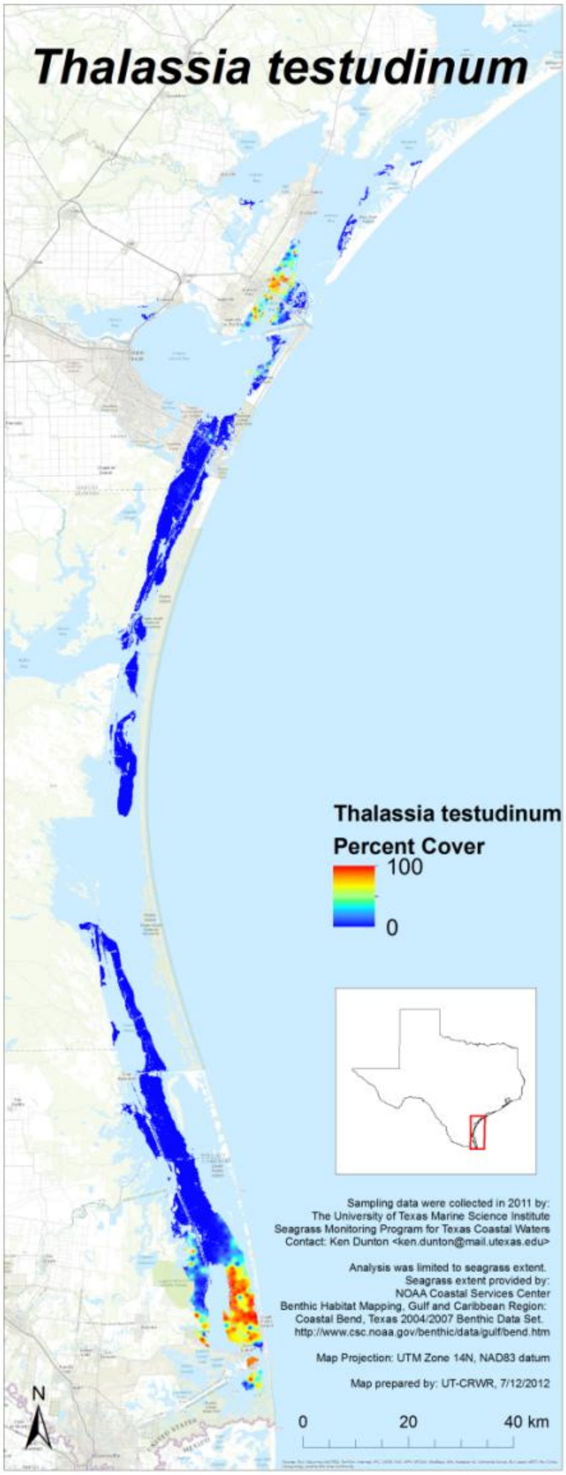


Figure 10. Spatial representations of percent cover for *Halodule wrightii* for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

11a.



11b.

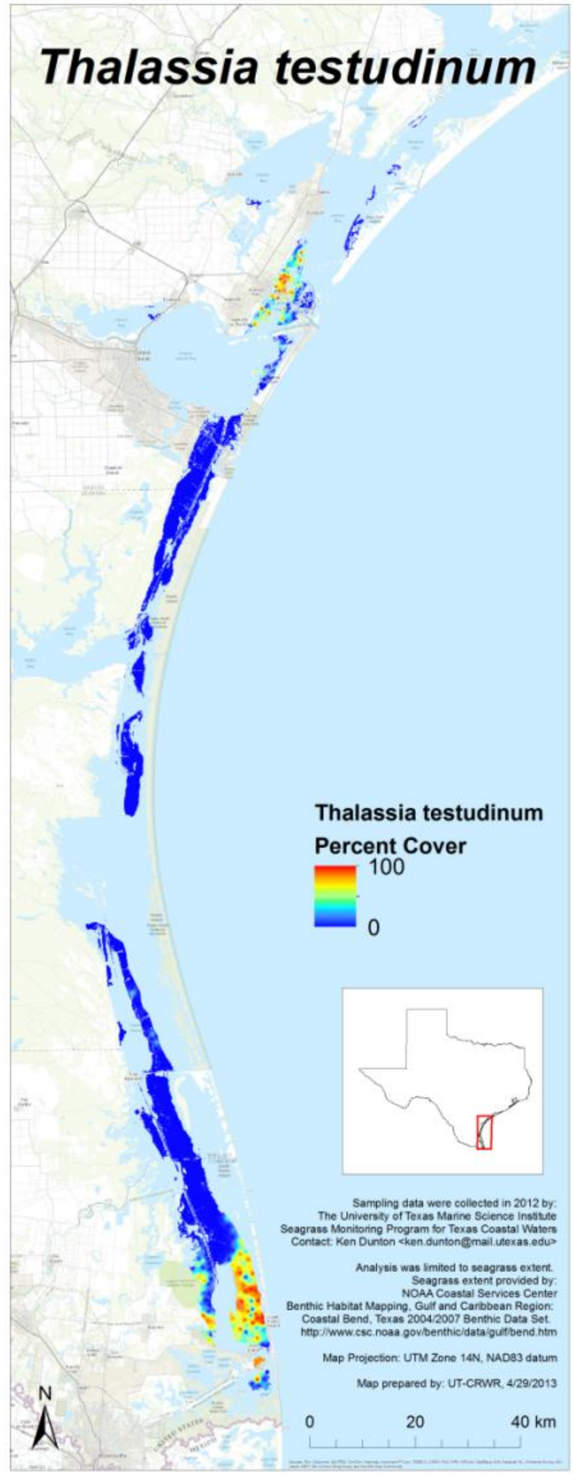
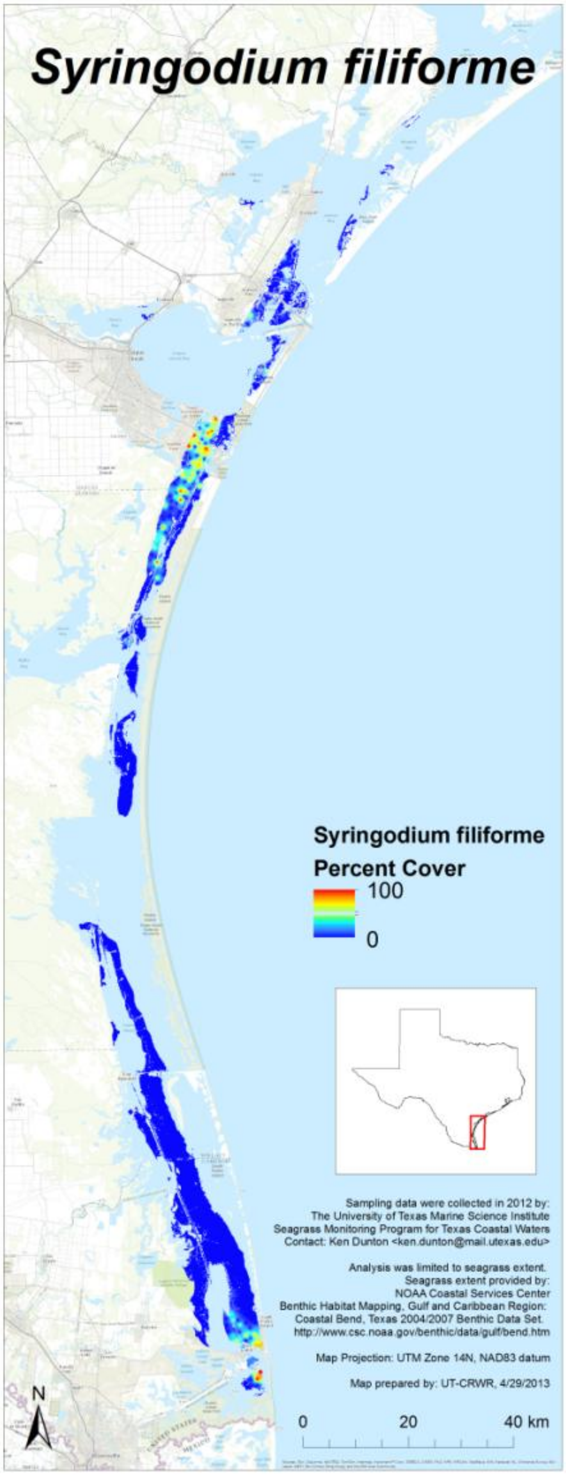


Figure 11. Spatial representations of percent cover for *Thalassia testudinum* for a) 2011, b) 2012, and *Thalassia testudinum* for c) 2011, 2) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

12a.



12b.

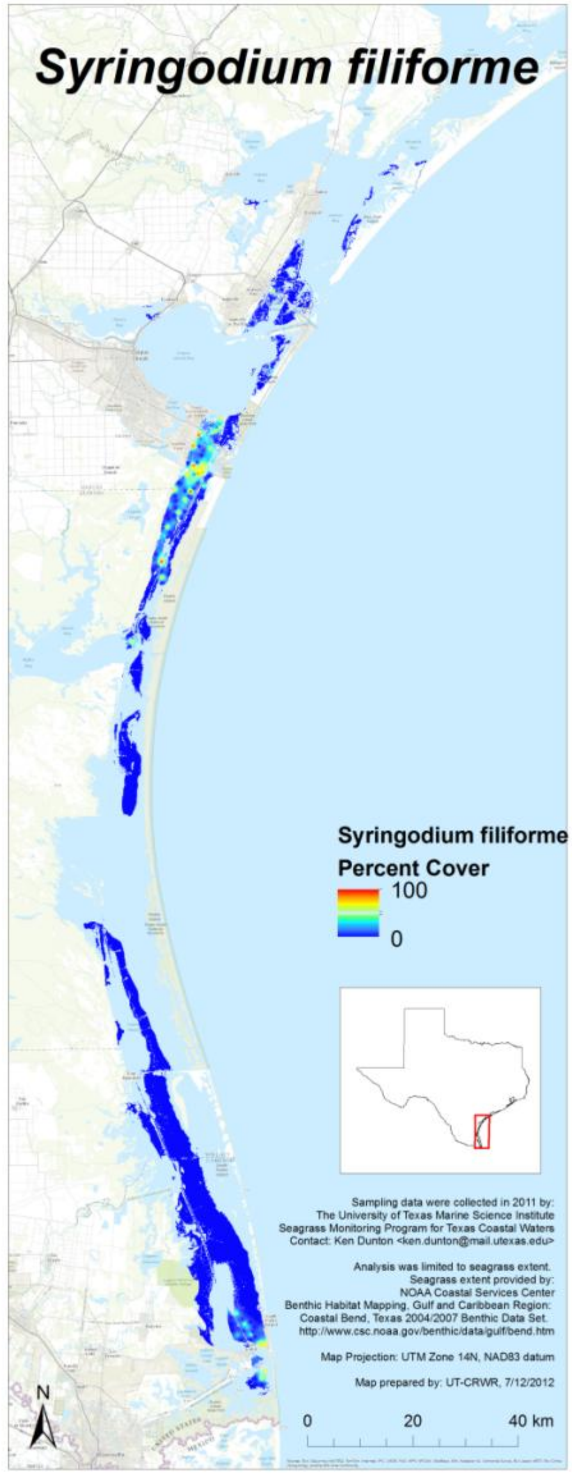
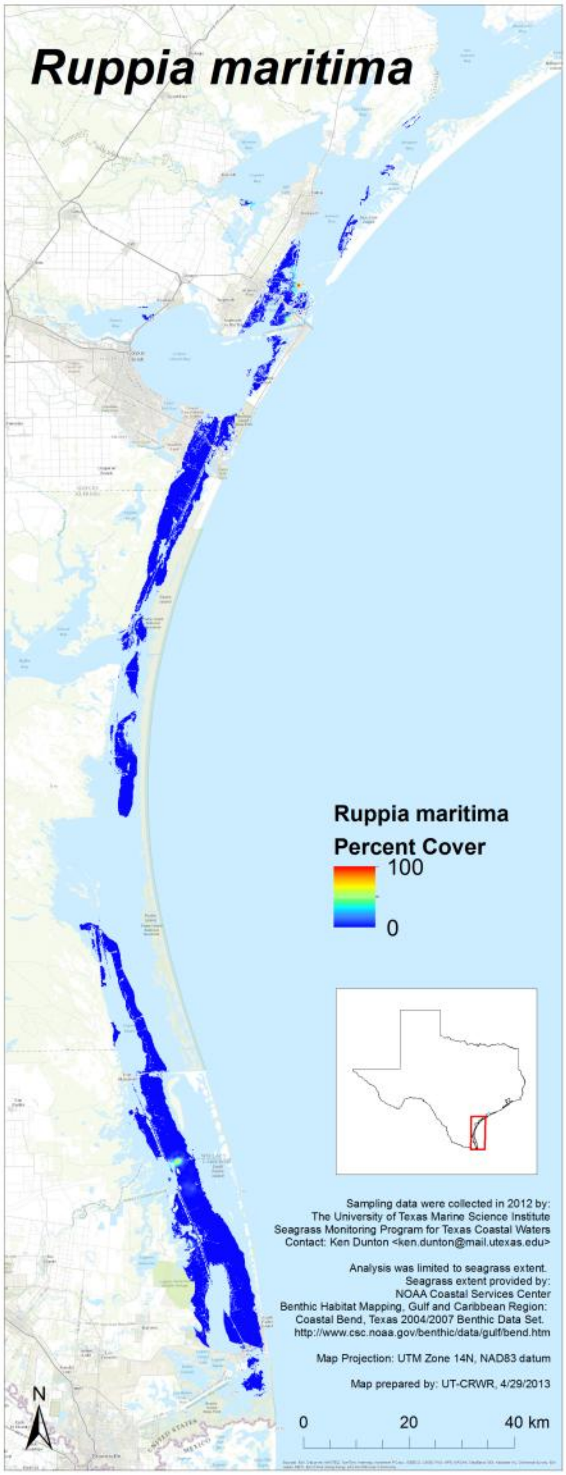


Figure 12. Spatial representations of percent cover for *Syringodium filiforme* for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

13a.



13b.

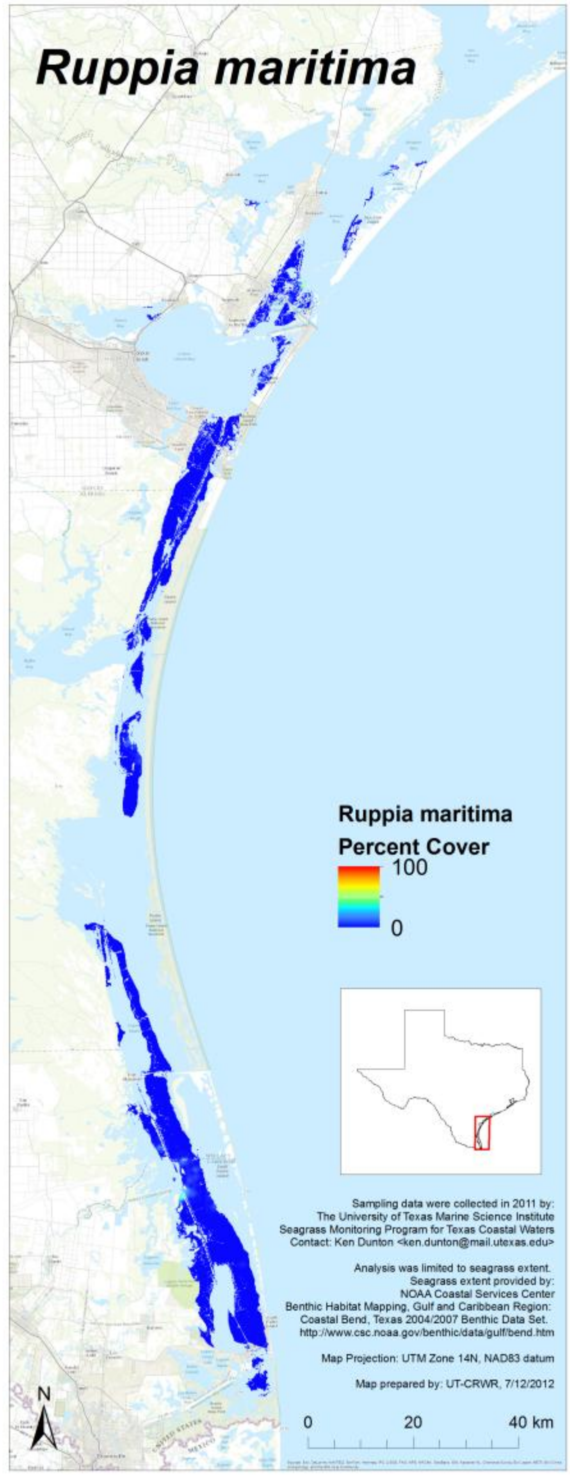


Figure 13. Spatial representations of percent cover for *Ruppia maritima* for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

14a. 14b.

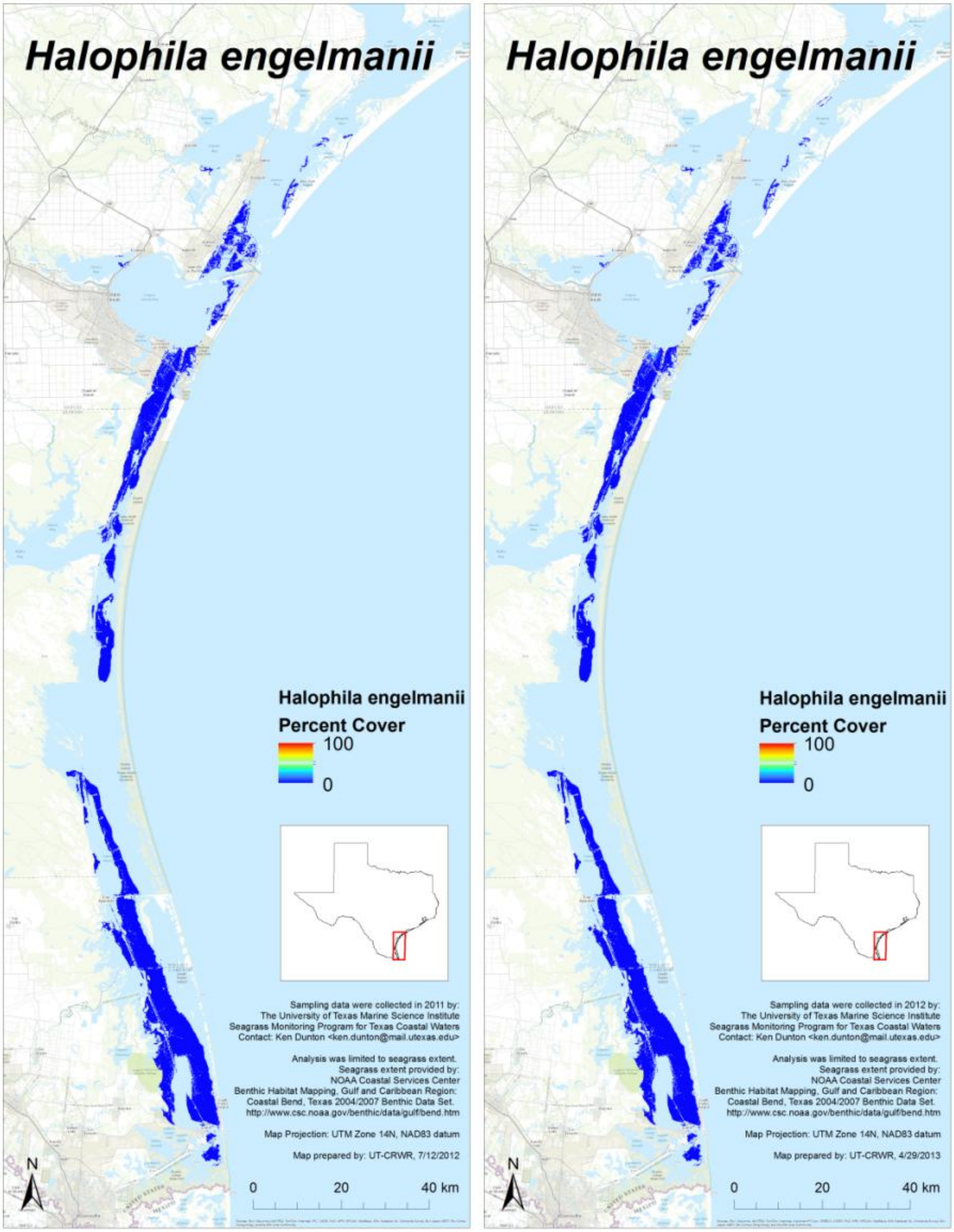
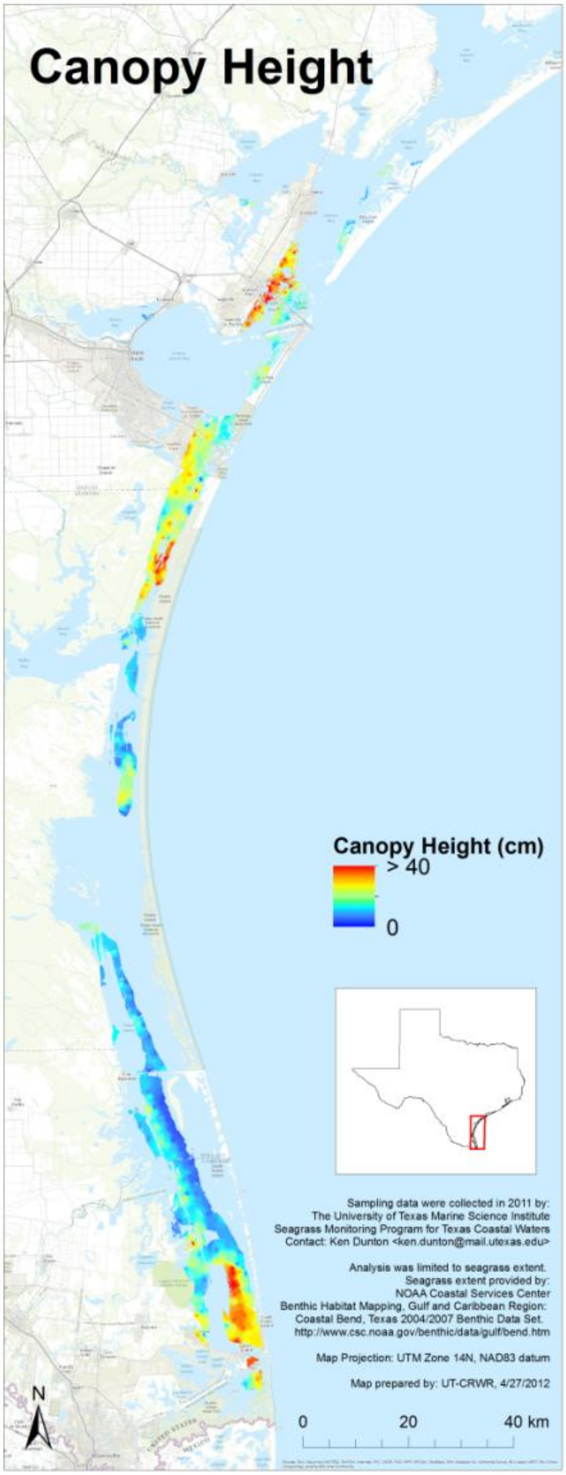


Figure 14. Spatial representations of percent cover for *Halophila engelmanii* for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

15a.



15b.

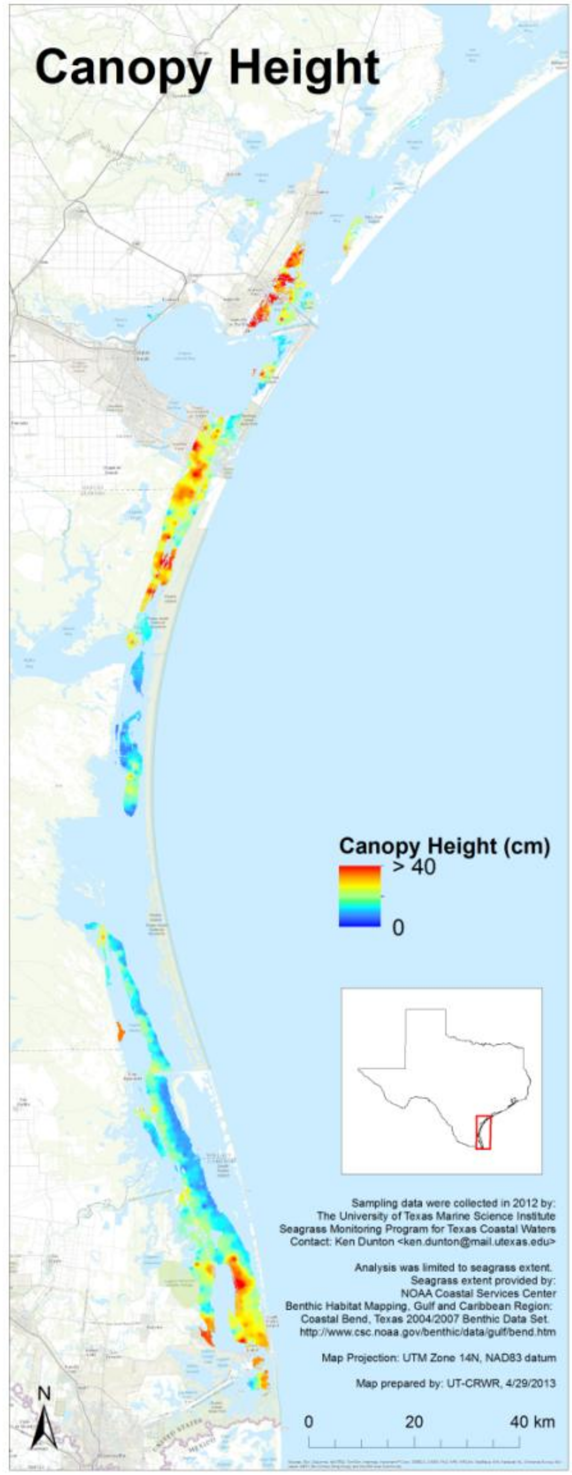


Figure 15. Spatial representations of canopy height for a) 2011, b) 2012. The spatial data interpolation is limited to the boundaries of seagrass habitat delineated during the 2004/2007 NOAA Benthic Habitat Assessment.

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APPENDIX: METHODS

A.1 Total Suspended Solids

Developed by: Kenneth Dunton and Kimberly Jackson
Adapted from: EPA METHOD #: 160.2
Approved by: TPWD (2010)

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

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

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

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

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

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

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

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

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

5) Assemble the filtering apparatus and begin suction.

6) Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected to the filter using a graduated cylinder. Pour into funnel.

7) Remove all traces of water by continuing to apply vacuum after sample has passed through.

8) With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

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

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

6.0 Calculations

Calculate non-filterable residue as follows, where: A = weight of filter (or filter and crucible) + residue in mg B = weight of filter (or filter and crucible) in mg C = mL of sample filtered

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

A.2 Percent Surface Irradiance and Light Attenuation

Developed by: Kenneth Dunton and Kimberly Jackson
Last Revised: December 2009
Approved by: EPA (2002) and TPWD (2010)

Field Measurements

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

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

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

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

Percent surface irradiance available at the seagrass canopy will be calculated as follows:

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

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

A.3 Seagrass Tissue Nutrient and Isotopic Analysis

Developed by: Kenneth Dunton, Kimberly Jackson, Christopher Wilson, Karen Bishop and Sang Rul Park
Last updated: December 2009
Approved by: EPA (2002) and TPWD (2010)

Tissue C:N Content, δ C^{13} and δ N^{15}

Newly formed leaves (the youngest leaf in a shoot bundle) are gently scraped and rinsed in tap water to remove algal and faunal epiphytes. The rinsed tissue samples are then dried to a constant weight at 60 °C and homogenized by grinding to a fine powder using a mortar and pestle. Tissue samples are analyzed for carbon and nitrogen concentrations and isotopic values using either a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (UC-Davis; precision 0.2 ‰ for ^{13}C and 0.3 ‰ for ^{15}N) or a Carlo Erba 2500 elemental analyzer coupled to a Finnigan MAT DELTAplus isotope ratio mass spectrometer 23 (UTMSI; precision 0.3 ‰).

Tissue Phosphorous Content

Tissue Preparation

Newly formed leaves (the youngest leaf in a shoot bundle) are gently scraped and rinsed in tap water to remove algal and faunal epiphytes. The rinsed tissue samples are then dried to a constant weight at 60 °C and homogenized by grinding to a fine powder using a mortar and pestle.

Required Reagents

A. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid

1. Dissolve 22.5 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}$. $4H_2O$] in 400 mL DW (a).
2. Dissolve 1.25 g ammonium metavanadate (NH_4VO_3) in 300 mL hot DW (b).
3. Add (b) to (a) in a 1 L volumetric flask, and let the mixture cool to room temperature.
4. Slowly add 250 mL concentrated nitric acid (HNO_3) to the mixture, cool the solution to room temperature, and bring to 1 L volume with DW.

B. Acid Mixture for Tissue digestion

1. Dilute165.6 mL concentrated hydrochloric acid (HCl, 37%, sp.gr.1.19) in DW, mix well, let it cool, and bring to 1 L volume with DW (2N HCl)

C. Standard Stock Solution

1. Dry about 2.5 g potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 1 hour cool in desiccator, and store in a tightly stoppered bottle.
2. Dissolve 0.1361 g dried potassium dihydrogen phosphate in DW, and bring to 1 L volume with DW. This solution contains 1,000 µM (Stock Solution).
3. Dilute 10 ml Stock Solution to 100 ml final volume by adding DW. This solution contains 100 µM (Stock solution).

Table 1. Table containing volume ratios for standard curve.

<table>
<tr><th>Concentration (µM)</th><th>DW (ml)</th><th>KH_2PO_4 (ml)</th><th>Stock solution</th></tr>
<tr><td>0.0 (Blank)</td><td>1.500</td><td>0.000</td><td rowspan="5">100 µM</td></tr>
<tr><td>25</td><td>1.125</td><td>0.375</td></tr>
<tr><td>50</td><td>0.750</td><td>0.750</td></tr>
<tr><td>75</td><td>0.375</td><td>1.125</td></tr>
<tr><td>100</td><td>0.000</td><td>1.500</td></tr>
<tr><td>250</td><td>1.125</td><td>0.375</td><td rowspan="2">1,000 µM</td></tr>
<tr><td>500</td><td>0.750</td><td>0.750</td></tr>
</table>

Prior to conducting a standard curve analysis:

4. Add 1.5 ml AVM.
5. After 30 minutes, read the absorbance of samples at 410 nm wavelength **(use a blank with ammonium-vanadomolybdate reagent – 1.5 ml DW + 1.5 ml AVM)**.

Procedure

This procedure is based on Chapman and Pratt (1961).

1. Weigh 0.010 - 0.015 g portions of ground plant material in a 30 - 50 mL porcelain crucibles or Pyrex glass beakers.
2. Place porcelain crucibles into a cool muffle furnace, and increase temperature gradually to 550°C. Continue ashing for 5 hours after attaining 550°C.
3. Shut off the muffle furnace and open the door cautiously for rapid cooling. When cool, take out the porcelain crucibles carefully.

4. Dissolve the cooled ash in 1 mL portions 2 N hydrochloric acid (HCl) and mix with a plastic rod.
5. After 15 - 20 minutes, add 4 mL DW.
6. Mix thoroughly, allow to stand for about 30 minutes, and use the supernatant or filter through Whatman No. 42 filter paper, discarding the first portions of the filtrates (Optional)

Measurement

1. Pipette 1.5 mL of the digest filtrate or aliquot of the dissolved ash (depending on the procedure used) into 15 ml glass tube, and then add 1.5 ml ammonium-vanadomolybdate reagent.
2. After 30 minutes, read the absorbance of samples at 410 nm wavelength **(use a blank with ammonium-vanadomolybdate reagent – 1.5 ml DW + 1.5 ml AVM)**.
3. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations
4. Read P concentration in the unknown samples from the calibration curve.

Calculation

Percentage total Phosphorus in plant

$$\% P = C\ (\mu mole/L) \times (1\ L/1{,}000\ ml) \times 5\ ml \times (30.9738\ \mu g/\mu mole) \times (1\ g/1{,}000{,}000\ \mu g) \times (1/g\ sample)$$

5 ml = Total volume of the digest/aliquot **(In this method, total volume is 5 ml (**1 mL of 2 N HCl + 4 ml of DW)

g sample = weight of dry plant used (g)

Citation

Chapman, H.D., Pratt, P.F., 1961. Methods of Analysis for Soils, Plants and Water. Univ. California, Berkeley, CA, USA