SEAGRASS

Disclaimer

The following methodologies have been adopted for monitoring seagrass in Texas waters. Before conducting any seagrass monitoring activities, it is imperative to coordinate this work with the Texas Commission on Environmental Quality (TCEQ) Surface Water Quality Monitoring team and the Texas Parks and Wildlife Department (TPWD) Water Quality Program. During any seagrass monitoring activity, it is imperative that live seagrass beds be disturbed as little as possible. Texas law prohibits the uprooting of seagrass; propeller scars left by carelessly driven boats take years to recover <https://tpwd.texas.gov/landwater/water/habitats/seagrass/>.

Objective

This chapter describes the protocols used by the TCEQ, TPWD, and cooperators at the University of Texas Marine Science Institute (UTMSI) for monitoring seagrass condition and the collection of data in support of the Texas Seagrass Monitoring Program. These protocols adhere to a widely-accepted three-tiered hierarchical monitoring strategy adapted for Texas (Dunton et al., 2011). The routine seagrass monitoring methods included in this section consist of Tier 2 protocols, intended to provide annual rapid assessments of hydrography, seagrass areal coverage, species distributions, and plant physiological conditions over a broad scale (individual bay or bay system) based on many fixed stations. Tier 2 methods measure parameters for potential stressors and plant condition indicators (Table 1).

Table 1. Indicators and field methods for specific seagrass stressors and condition indicators.

Indicator		Field Method
Stressors	physicochemical (water temperature, salinity, pH, dissolved oxygen, specific conductance, conductivity)	multiprobe
	total depth	survey rod
	water transparency	Secchi disk
	Photosynthetically active radiation (PAR) - percent surface irradiance (% SI), light attenuation coefficient(*k*)	underwater LI-COR, Inc. spherical quantum sensors
	total suspended solids (TSS)	water sample
	chlorophyll *a*	in situ phycoerythrin fluorescence (multiprobe) and water sample*
Condition Indicators	canopy height	0.25 m^2 quadrats
	seagrass species composition	0.25 m^2 quadrats
	Seagrass percent cover	0.25 m^2 quadrats
	C:N:P and ^{15}N:^{14}N ratios	seagrass leaf blades

* Water samples for chlorophyll *a* will be collected at a subset (e.g. every fifth site) of seagrass sites to corroborate phycoerythrin sensor measurements.

Equipment

Equipment requirements are primarily dictated by the environment in which seagrasses are located. They have high light requirements and are generally restricted to shallow waters (< 1.5 m) in Texas because of relatively high turbidity. Most seagrass sampling stations will be accessed by boat, requiring a shallow draft boat capable of carrying 3-4 people and equipment. To avoid damage to seagrass beds from anchors, shallow water pole-type anchors are preferred.

Required Equipment

- multiprobe capable of measuring dissolved oxygen (DO), water temperature, salinity, pH, conductivity, specific conductivity, and chlorophyll *a* (phycoerythrin total algae sensor)
- survey rod or alternative method to measure total depth of water at station
- Secchi disk for measuring water transparency
- LI-COR, Inc. equipment for measuring photosynthetically active radiation (PAR) parameters, percent surface irradiance (%SI) and diffuse light attenuation coefficient (*k*) underwater
- 1 L cubitainers/plastic bottles for total suspended solids (TSS) and chlorophyll *a* water samples
- 0.25 m^2 quadrat (divided into 100 cells) for estimating percent cover, canopy height, and seagrass species
- ice chest(s) with ice for preserving water samples and seagrass blade C:N:P samples
- measuring board or alternative method for measuring seagrass leaves (to nearest 0.1 cm) for estimation of canopy height
- watch or phone for determining sample times
- Global Position System (GPS) unit for navigating to station coordinates (minimum 10 m accuracy)

Optional Equipment

- snorkels/masks for estimating seagrass percent cover, seagrass canopy height, and seagrass species underwater
- water shoes/boots for protection against oyster shells, stingrays, or other underwater hazards
- wetsuit for protection against prolonged exposure to cool water temperatures, wind, or sun
- protective gear for sun exposure
- weighted belt to aid staying underwater for estimating percent cover, canopy height, and seagrass species

Seagrass Sampling Period

Sampling should be conducted during an index period from August 1 – October 31 which would include, or shortly follow, the peak seagrass standing crop. The data reported to SWQMIS will have an associated Monitoring Type code of BS (Biased Season) because the sampling is scheduled for a certain time of year.

Sample Design and Station Selection

The Tier 2 design utilizes an aerial imagery seagrass distribution GIS layer for regional bay systems, corroborated with in-situ verification, tessellated using hexagons, following Neckles et al. (2012). This approach forms the basis for high replication of parameters and the selection of probability-based sampling locations. Hexagonal grids may be 500 or 750 m per side and cover 0.65 and 1.46 km^2, respectively, dependent on the size of the bay (e.g. smaller hexagons for Redfish Bay system and larger for the Laguna Madre). A minimum percent, or threshold, of seagrass coverage within a hexagon is determined to provide sufficient resolution to assess change over time and allow for a practical monitoring effort.

Within each chosen hexagon, a randomly selected pair of coordinates is assigned for a primary sampling point. If, during the initial visit to a pair of coordinates, the point is found to be in a bare area, but seagrasses exist within 100 m of the point, the station is moved to the new location with seagrasses and the new coordinates recorded. A secondary set of coordinates is also selected for each hexagon in the event there are no seagrasses at or within 100 m of the primary point. This ensures some degree of flexibility but maintains the objective of a stratified but random approach used across the State.

After stations, primary and/or secondary, have been sampled once, they are considered permanent and will be re-visited annually to allow for trend analyses regardless of the presence of seagrass.

Sequence of Sampling Activities

Once the station is located by navigating to the established coordinates in each hexagon, station information will be recorded on field forms. Weather and water conditions, and other pertinent observations, including revised station coordinates, are recorded in the field. Tidal stage and days since last significant rainfall will be recorded by the project manager upon completion of trip.

Water quality sampling activities should be performed in the following order to avoid re-suspending sediments:

- physicochemical measurements, collected with a multiprobe which has been calibrated prior to field use
- Secchi depth
- water column depth at the station
- grab water samples for TSS and chlorophyll *a*
- PAR measurements

Multiprobe calibration, field sampling for Secchi depth, physicochemical measurements, and water collection for TSS will be conducted according to methods described in TCEQ SWQM Procedures, Volume 1 (TCEQ, 2012). Methods for sensor-derived chlorophyll follow UTMSI protocols.

Seagrass species determination, percent seagrass coverage, and leaf length measurements for canopy height estimation, using the quadrat, and blade collection for C:N:P analyses follow the collection of water quality parameters.

Field sheets are reviewed for completeness prior to leaving the station.

Photosynthetically Active Radiation (PAR) Measurements

Water transparency is derived from measuring PAR (ca. 400 to 700 nm wavelength) using two LI-193SA spherical quantum sensors mounted on a lowering frame at a fixed 25 cm depth difference (Figure 1). The sensors are connected to an LI-1500 light sensor logger (LI-COR Inc., Lincoln, NE) which displays real-time data from up to three channels (Figure 2). The LI-1500 may also be programmed to perform math functions, average data, and log all operations (LI-COR, 2016b). Simultaneous measurements of irradiance, one just below the surface of the water and the other at the fixed depth (if depth at station allows) are recorded on the field form for each of five replicates.

Care should be taken to reduce extraneous sources of reflected light (from boats or clothing) and that the lowering frame and sensors are held vertically and level within the water column. PAR data are collected under similar meteorological conditions to ensure consistent readings.

The measured PAR data are used to calculate percent surface irradiance (% SI) and the diffuse light attenuation coefficient (*k*) post-sampling. Percent surface irradiance is calculated from the averaged surface and depth replicates ($\%SI = I_z/I_0 * 100$) where I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively. Diffuse light attenuation is calculated using the transformed Beer Lambert equation: $K_d = -[\ln(I_z/I_0)]/z$ where K_d is the attenuation coefficient (m^{-1}) and I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (m) and at the surface, respectively.

Refer to the LI-1500 Light Sensor Logger: Instruction Manual for use, configuration of settings, and logging and viewing data procedures. LI-COR spherical quantum sensors are calibrated using a National Institute of Standards and Technology (NIST) light source. Each sensor has unique calibration constants required for underwater use. Recalibration of sensors is recommended every two years (LI-COR, 2016a). Refer to the LI-COR Underwater Radiation Sensors: Instruction Manual for use, care, and maintenance procedures.

Figure 1. Example of a lowering frame with paired LI-193SA spherical quantum sensors.

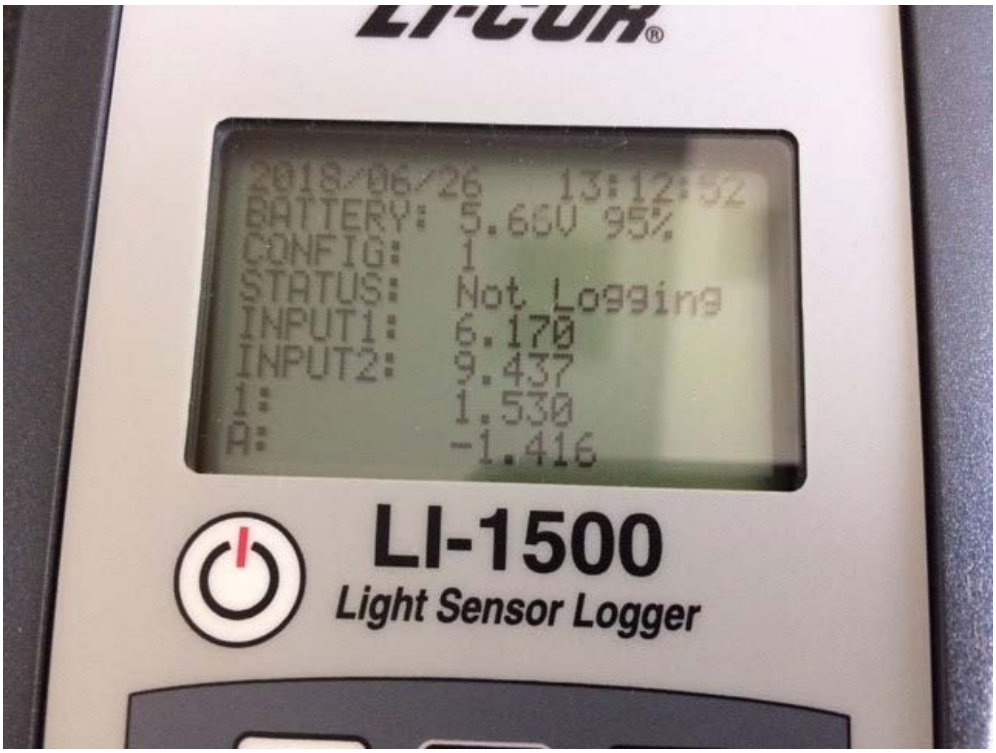


Figure 2. Example of an LI-1500 light sensor logger display. The logger can be programmed to continuously display irradiance measurements at the two depths simultaneously.

Seagrass Percent Cover Estimation

After water samples and additional water quality information have been collected, a 0.25 m^2 quadrat subdivided into 100 gridded squares using monofilament line is used to estimate percent seagrass cover or bare areas (Figure 3). The quadrat is thrown into the water within the 10 m radius sampling area surrounding the boat at the designated locations, (1) forward starboard, (2) aft starboard, (3) aft port, and (4) forward port (Figure 4). The quadrat is tossed without regard to where seagrass might be more, or less, abundant to avoid biasing percent cover measurements.

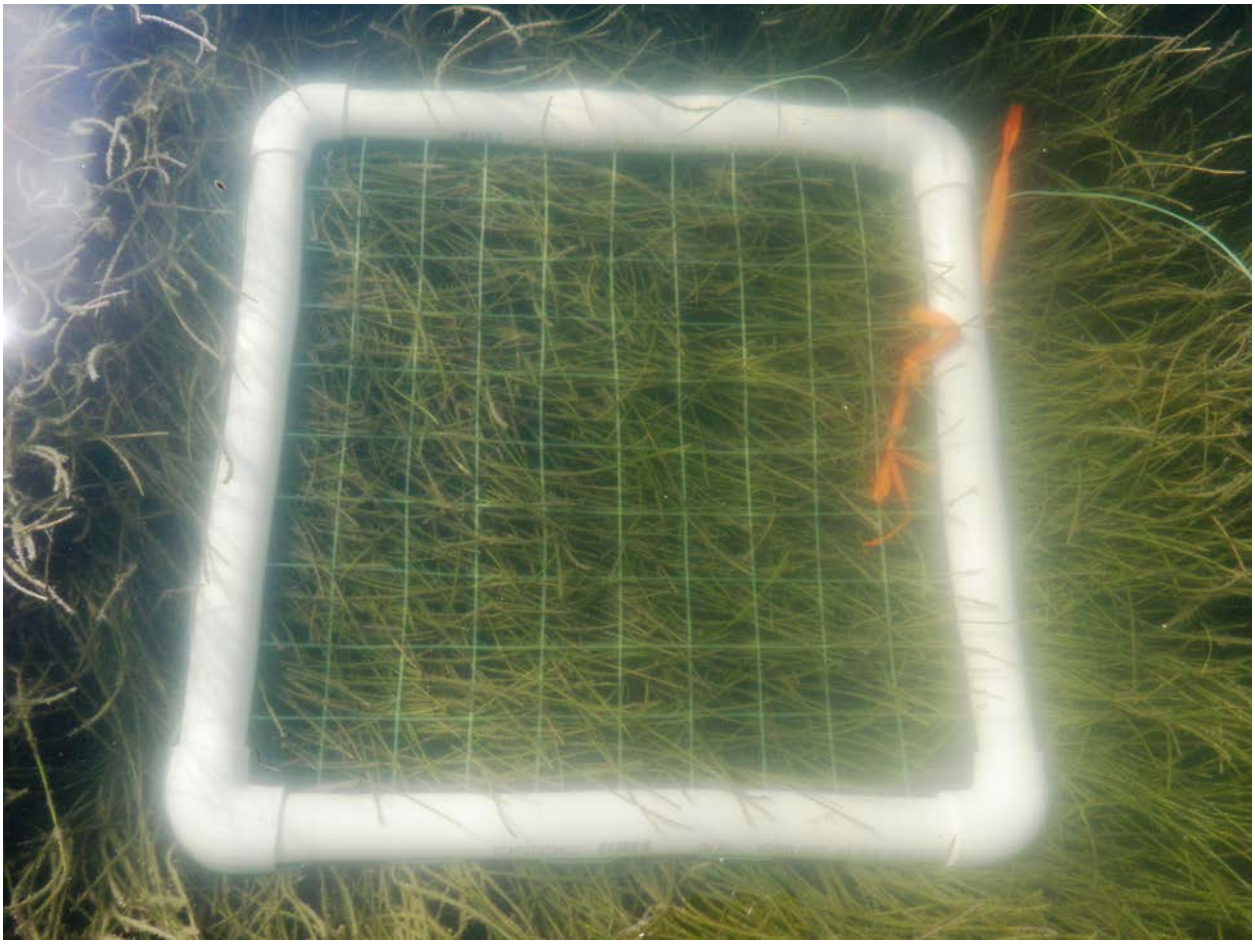


Figure 3. A 0.25 m^2 quadrat, divided into 100 cells, for use in estimating percent seagrass cover and bare areas.

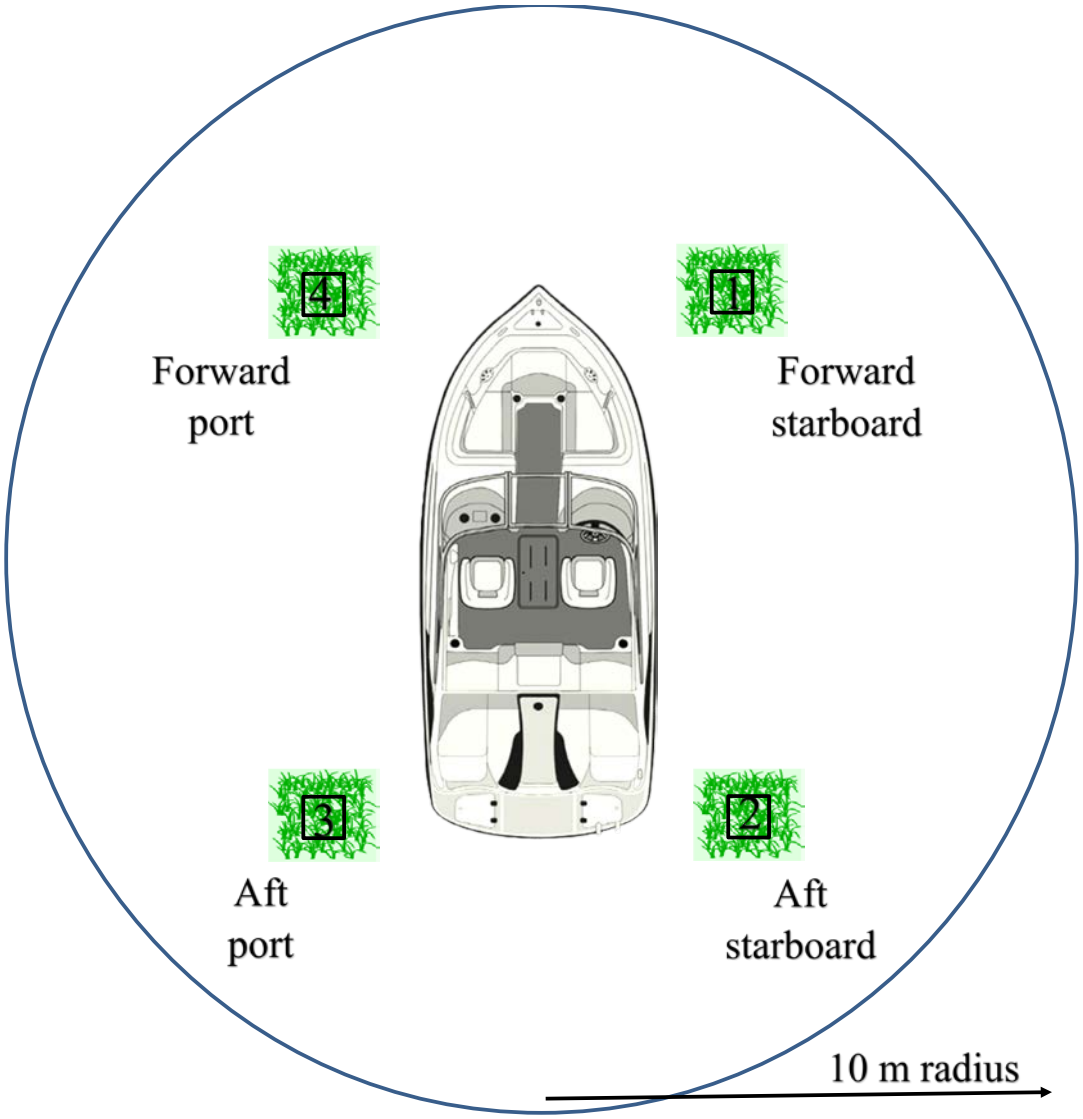
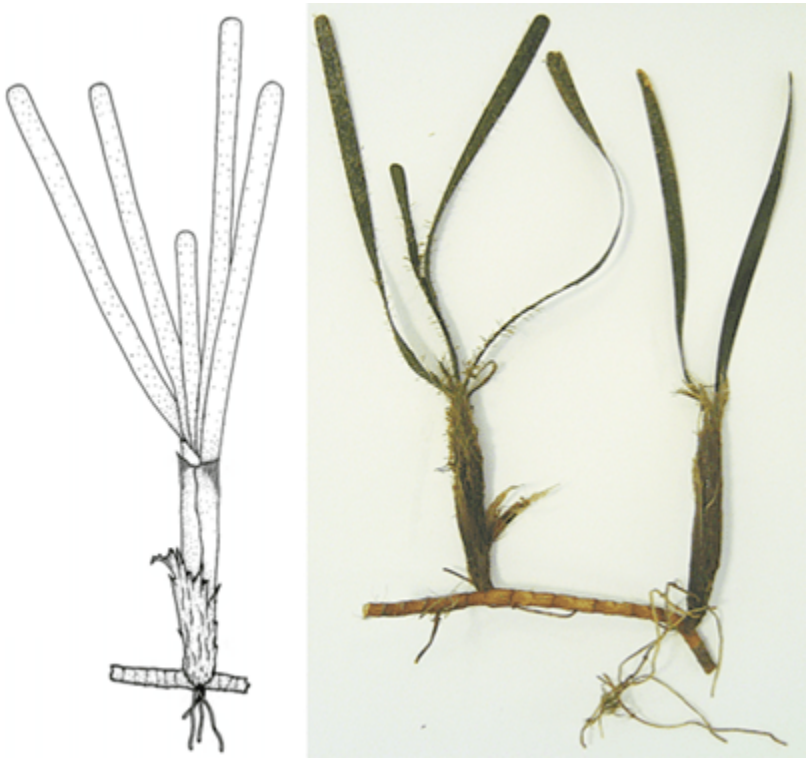


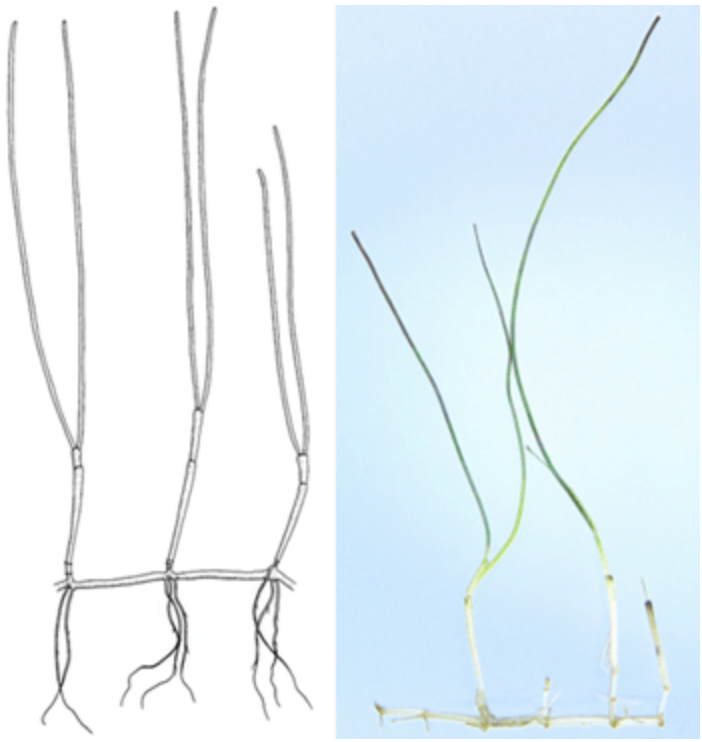
Figure 4. Sampling station area and quadrat placement for estimating seagrass percent cover and canopy height.

The quadrat area will be carefully cleared of wrack (dead seagrass leaves that have been shed by the plants and no longer rooted), macroalgae, or other material which may obscure the view of the seagrass. Care should be taken not to remove shallowly rooted live plants. Depending on water depth and clarity, this may require using a viewing scope and/or snorkeling mask, or estimating cover tactilely within the quadrat. At a given station, the method that allows the best view of the substrate within the quadrat will be used. Percent cover is defined as the percent of the total quadrat area that is obscured by each species of seagrass present or bare area when viewed from directly above. There are five seagrass species found along the Texas coast, *Halodule wrightii*, *Thalassia testudinum*, *Syringodium filiforme*, *Halophila engelmannii*, and *Ruppia maritima* (Figure 5).

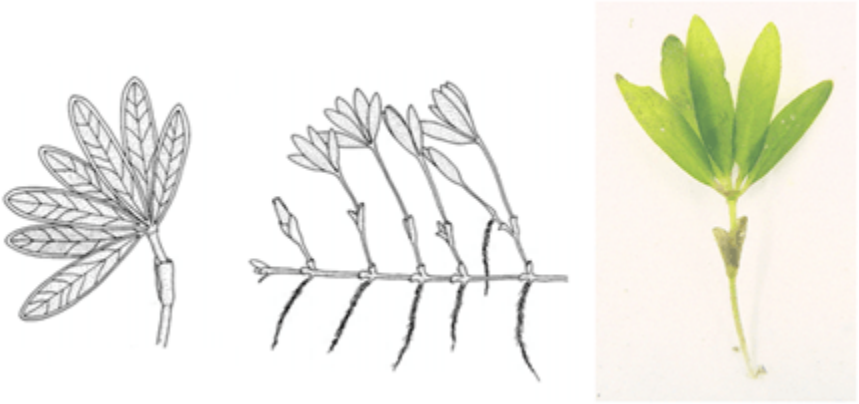
Halodule wrightii (shoal grass) *



Thalassia testudinum (turtle grass) *



Syringodium filiforme (manatee grass) *



Halophila engelmannii (star grass) *

* Drawing modified from Phillips and Menez (1988); photo by Ken Dunton

Ruppia maritima (widgeon grass)
Drawing modified from U.S. Fish and Wildlife Service, Florida Museum of Natural History; photo by Ken Dunton

Figure 5. Five seagrass species found along the Texas coast.

A photo guide is provided for several examples of percent cover (Figure 6). Cover estimates are standardized based on this photographic reference guide and used in observer calibration/training. Percent cover should be recorded to the nearest 5%. Percent cover categories are each of the five seagrass species and Bare.

The total of all species plus percent of the quadrat area that is bare should equal 100%.

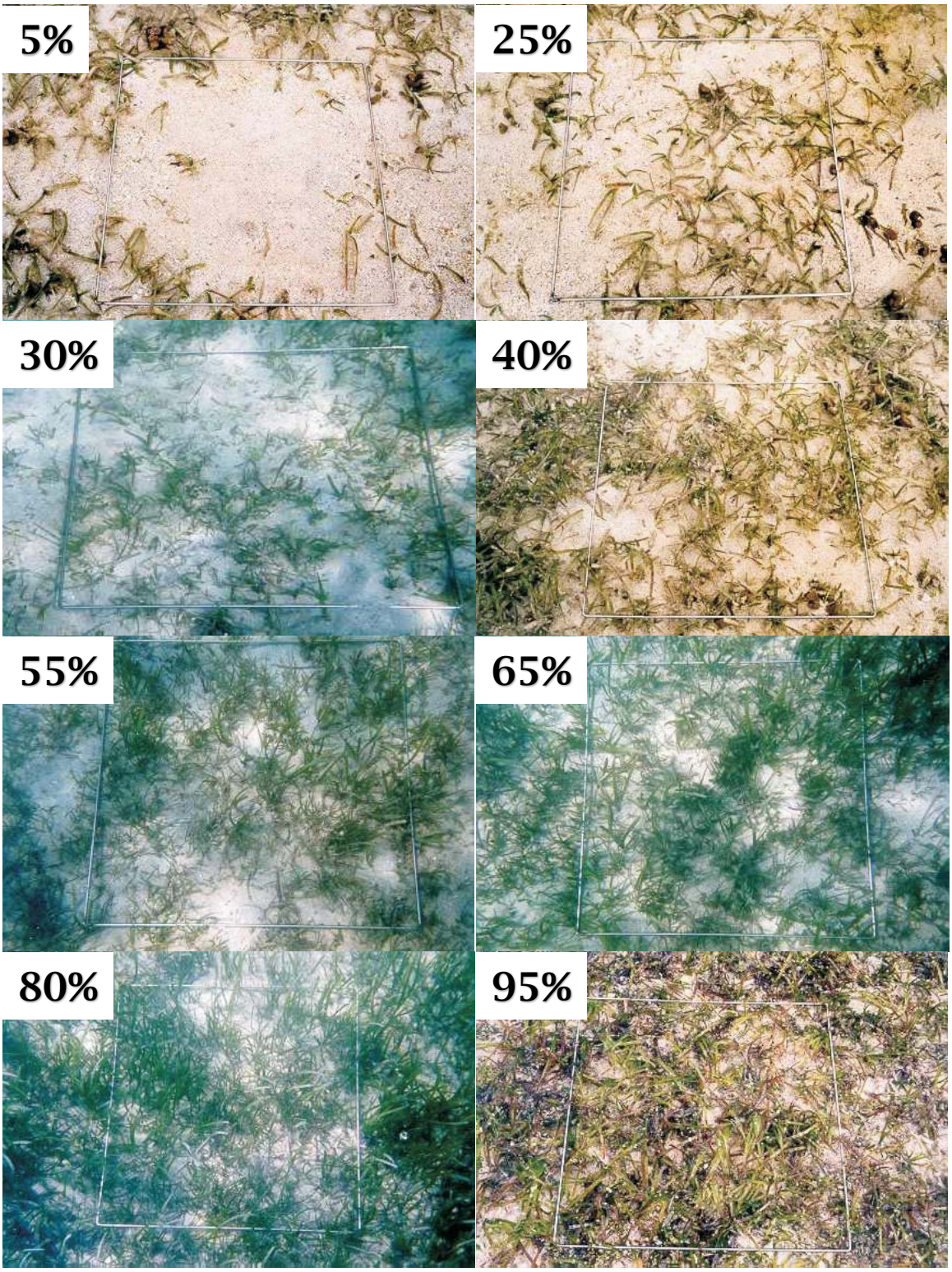


Figure 6. Examples of percent cover standards from the Seagrass Watch organization. Species depicted in this guide may differ from Texas species. (McKenzie, Campbell, and Roder, 2003).

Seagrass Canopy Height Estimation

Seagrass canopy height will also be collected from gently uprooted rhizomes at each of the four designated locations around the boat. Average canopy height will be estimated from randomly selected leaves for each species present. Measurements are taken from the beginning of the shoot where it is photosynthetic to the tip of the shoot (Figure 7). For *Ruppia maritima*, leaf length is not measured on a single shoot. Instead the rhizomatous shoot is measured beginning at the point where it begins to branch as a photosynthetic shoot to the end of the longest branch (Figure 8).

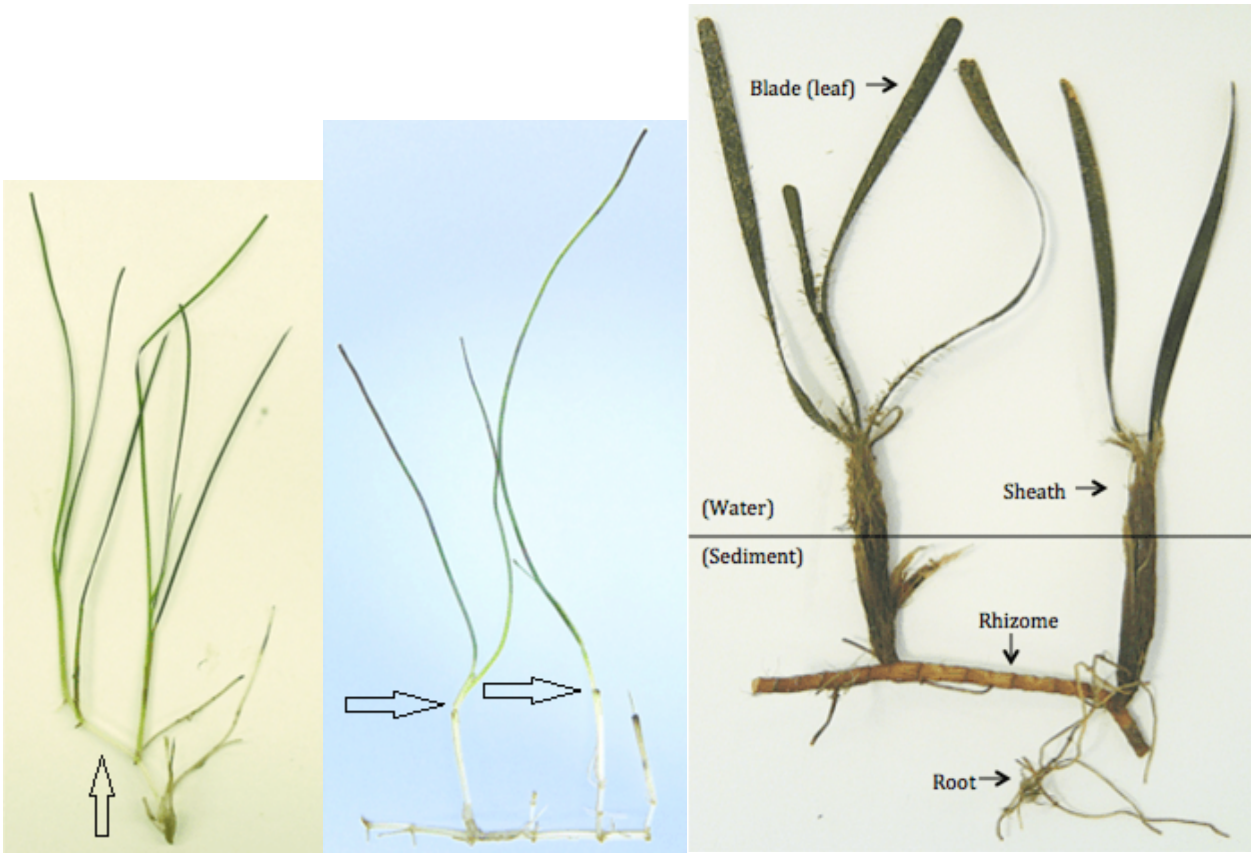


Photo by Ken Dunton

Figure 7. Estimation of canopy height from randomly selected leaves. Only the photosynthetic portion of the leaf is measured.

Photo by Ken Dunton

Figure 8. Example of branching of a *Ruppia maritima* shoot.

Shoot Collection For C:N:P Analyses

After estimation of percent cover and canopy height, if *Thalassia testudinum* or *Halodule wrightii* are present, a minimum of (5) shoots and (20) shoots, respectively, should be collected for submittal to the UTMSI laboratory for seagrass blade C:N:P and stable C and N isotope ratio analyses. Samples will be placed in a Ziploc bag and kept cool on ice and transported to the lab within 3-5 days.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data sheets (see Attachment) or in user specified field notebooks. The following will be recorded for all visits (before or after sampling):

1. Location information - bay name, station coordinates, seagrass hexagon number, TCEQ monitoring station (*all pre-printed*)
2. Date, time (SWQMIS end time), personnel involved
3. Detailed observational data, including:
    - weather conditions, including temperature, estimated wind speed, cloud cover, etc.
    - water conditions, including color, odor, foam, algae, etc. (*optional*)
    - human uses observed, including angling, swimming, boating, etc. (*optional*)
    - tide stage (*completed post-sampling*)
    - days since last significant rainfall (*completed post-sampling*)

4. Sample Event, Field sample Set, Chemical Sample Set, RFA, and Seagrass Sample Set (*all pre-printed*)
5. Multiprobe measurements at 0.3 m (and 1.0 m for stations with > 1 m total depth), Secchi depth, and total water depth
6. Any applicable notes (*optional*)
7. Verification of water sample collection for TSS and Chlorophyll *a* (*optional*)
8. PAR measurements, five replicate pairs of just below surface and at 25 cm depth (*at extremely shallow stations, the depth measurement may not be possible*) and Average of surface and depth measurements (*completed post-sampling*)
9. Calculation of percent surface irradiation and diffuse light attenuation (*completed post-sampling*)
10. Any applicable notes (*optional*)
11. Percent cover for any seagrass species or Bare areas, and total (*must equal 100*) for each of four quadrat positions around the boat
12. Any notes regarding presence of wrack, drift macroalgae, oyster bed, etc. (*optional*)
13. Seagrass canopy height (shoot measurements) for each species present in quadrats (*5 per quadrat, if available*)
14. Average (to 0.1 cm) of shoot lengths for each species present (*completed post-sampling*)
15. Verification of *Thalassia* or *Halodule* samples collected for C:N:P analyses
16. Any applicable notes (optional)

Recording Data

For the purposes of this section and subsequent sections, all field and laboratory personnel follow the basic rules for recording information as documented below:

- Loose-leaf field notes and field forms may be recorded legibly in indelible ink (preferred) or pencil with no erasures, modifications, write-overs or multi-line cross-outs. Bound field notes, forms and in-house field and lab records (multiprobe calibration logs, bench logs, etc.,) must be recorded in indelible ink with no modifications, write-overs or multi-line cross-outs.
- Field forms should be printed on waterproof paper such as Rite in the Rain paper.
- Errors are to be corrected with a single line-through followed by initials and a date.
- Close-out incomplete pages with an initialed and dated diagonal line.

Sample Handling and Custody

At a minimum, water sample containers are labeled with the RFA tag number, Station ID, date, and time of collection. Samples are placed on ice immediately after collection and transported from the field to the laboratory.

Minimum sample volume, container types, preservation requirements, and holding time requirements may vary, depending on laboratory and field QA/QC measures. Typical requirements are published in TCEQ's SWQM Procedures, Volume 1 (TCEQ, 2012).

References

Dunton, K H., W. Pulich, Jr. and T. Mutchler. 2011. A Seagrass Monitoring Program for Texas Coastal Waters: Multiscale Integration of Landscape Features with Plant and Water Quality Indicators. Final report to Coastal Bend Bays & Estuaries Program.

Florida Museum of Natural History, University of Florida. <https://www.floridamuseum.ufl.edu/southflorida/habitats/seagrasses/species>

LI-COR, Inc. 2016a. LI-COR Underwater Radiation Sensors: Instruction Manual. Lincoln, NE. 22 pp.

LI-COR, Inc. 2016b. LI-1500 Light Sensor Logger: Instruction Manual. Lincoln, NE. 32 pp.

McKenzie, L.J., Campbell, S.J. and Roder, C.A. 2003. Seagrass-Watch: Manual for Mapping & Monitoring Seagrass Resources by Community (citizen) volunteers. 2nd Edition. Cairns, Queensland. 100 pp.

Neckles, H. A., et al. 2012. Integrating scales of seagrass monitoring to meet conservation needs. *Estuaries and Coasts* 35:23-46.

Phillips, RC and Menez, EG. 1988. Seagrasses. *Smithsonian Contributions to the Marine Sciences* 34: 1–104.

TCEQ. 2012. Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods.

Texas Statewide Seagrass Monitoring Program. A Seagrass Monitoring Program for Texas Coastal Waters. <http://www.texasseagrass.org/>

Field Sheet (with examples of pre-populated information)

WEST BAY		Site Lat/Long: **29.22343, -95.02795**			Seagrass Hexagon: **31**	TCEQ Monitoring Station: **21861**	
Date		Time		Personnel			
Weather (temp, wind, cloud cover, etc.)							
Water conditions (color, odor, foam, algae, etc.)							
Human use (angling, swimming, boating, etc.)							
Post-sampling	Tide stage (89972):				Days since last significant rainfall (72053):		
Sample Event: **1439960**	Field Sample Set: **14592667**		Chemical Sample Set: **14592668**		RFA: **1604393**	Seagrass Sample Set: **14592669**	

Parameter	**Parameter Code**	**Surface (0.3 m)**	**At 1.0 m depth (for stations >1 m total depth)**
Water temperature (°C)	00010		
Specific conductivity (µS/cm)	00094		
Conductivity (µS/cm)	47004		
Salinity (ppt)	00480		
Dissolved oxygen (%)	00301		
Dissolved oxygen (mg/L)	00300		
pH	00400		
Chlorophyll (µg/L)	NA		
Secchi visibility (m)	00078		
Total water depth (m)	82903		

Notes:

Water Sample Collection	Total Suspended Solids (TSS) 1L container ☐	Chlorophyll *a* (optional) 1L container ☐

PAR measurements (LI-1500 output)	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Surface (I_0) – Input 1					
Depth at 25 cm (I_z) - Input 2					
%SI – Math 1 (Input 2/Input 1)					
(K) – Math A (Attenuation coefficient)					

Percent surface irradiance ($\%SI = I_z / I_0 * 100$) **Recorded post-sampling**		Diffuse light attenuation ($K_d = -[\ln(I_z/I_0)]/z$) **Recorded post-sampling**	

Notes:

Seagrass Percent Cover		Percent cover of each seagrass species within 0.25 m^2 quadrat (total of all species plus bare should equal 100% for each quadrat).						
Position		***Halodule***	***Thalassia***	***Syringodium***	***Halophila***	***Ruppia***	**Bare**	**Total**
1	Forward-starboard							
2	Aft-starboard							
3	Aft-port							
4	Forward-port							
Notes: (on wrack, drift macroalgae, oyster bed, etc.)								

Seagrass Canopy Height	Gently uproot seagrass rhizomes within the quadrat at all four positions around the boat and measure the photosynthetic portion of five random shoots from each species present to the nearest 0.1 cm.

Quadrat position (replicate)	***Halodule***	***Thalassia***	***Syringodium***	***Halophila***	***Ruppia***
Forward-starboard (1)					
Forward-starboard (2)					
Forward-starboard (3)					
Forward-starboard (4)					
Forward-starboard (5)					
Aft-starboard (1)					
Aft-starboard (2)					
Aft-starboard (3)					
Aft-starboard (4)					
Aft-starboard (5)					
Aft-port (1)					
Aft-port (2)					
Aft-port (3)					
Aft-port (4)					
Aft-port (5)					
Forward-port (1)					
Forward-port (2)					
Forward-port (3)					
Forward-port (4)					
Forward-port (5)					
Mean of leaf lengths (0.1 cm) (post-sampling)	**44582**	**44581**	**44585**	**44583**	**44584**
C:N:P samples collected[1] □					

Notes:

[1] For *Thalassia* at least 5 shoots and for *Halodule* at least 20 shoots although more may be collected if necessary. However, if there are not enough shoots available, the UTMSI will attempt to analyze what is available.