LONG-TERM SEAGRASS MONITORING PROGRAM FOR TEXAS COASTAL WATERS:

ANNUAL PROJECT PLAN

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Table 1: List of Acronyms

CBBEP	Coastal Bend Bays & Estuaries Program
DM	Data Manager
DMS	Data Management System
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
iRODS	Integrated Rule-Oriented Data System
MA-NERR	Mission-Aransas National Estuarine Research Reserve
NBS	National Bureau of Standards
NIST	National Institute of Standards Technology
NPS	National Park Service
NSF	National Science Foundation
OSHA	Occupational Safety and Health Administration
PAR	Photosynthetically Active Radiation
PDR	Precision Depth Recorder
QA	Quality Assurance
QA/QC	Quality Assurance/Quality Control
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RPD	Relative Percent Difference
RFU	Relative Fluorescence Units
SI	Surface Irradiance
SOP	Standard Operating Procedure
TACC	Texas Advanced Computing Center
TCEQ	Texas Commission on Environmental Quality (ne TNRCC)
TGLO	Texas General Land Office
TPWD	Texas Parks and Wildlife Department
TSGMP	Texas Seagrass Monitoring Plan
TSS	Total Suspended Solids
UTMSI	University of Texas Marine Science Institute

Background

This proposal outlines an implementation program for monitoring Texas seagrasses in the Mission-Aransas, Nueces, and Laguna Madre estuaries following protocols that evaluate seagrass condition based on landscape-scale dynamics. We will adopt the hierarchical strategy for seagrass monitoring outlined by Neckles et al. (2011) to establish the quantitative relationships between physical and biotic parameters that ultimately control seagrass condition, distribution, persistence, and overall health. The three-tiered approach proposed here 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). Based on this approach, we describe a multiscale monitoring protocol that, when implemented, integrates plant condition indicators with landscape feature indicators to detect and interpret seagrass bed disturbances.

The program is focused on Tier-2 monitoring that includes a regional rapid assessment using fixed stations sampled annually from a shallow-draft vessel. This research will monitor long-term health of Texas seagrass from the Mission-Aransas system to the Lower Laguna Madre. It constitutes a Tier-2 state-wide effort to assess seagrass condition and distribution that began summer 2011 (see **http://www.texasseagrass.org/**). The program receives direct funding and/or in-kind logistic support from long-term commitments made by the General Land Office through the CMP grants Program, the Coastal Bend and Bays Estuaries Program, the National Park Service, and the Mission-Aransas National Estuary Research Reserve. The effort is unprecedented in its breadth and scope and will serve as an invaluable database of existing seagrass resources available for various local, state, and national groups.

The objectives of this proposed work are 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 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.

The primary questions that we will address in the 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?

Introduction

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 and overall 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.

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.

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 is a high priority for future research.

Overview and Criteria

To align with other monitoring programs (Neckles et al., 2011), we used a grid of tessellated hexagons (500 or 750 m) as the means for selecting sampling locations (see Figure 2). Within each equal-sized contiguous hexagon, a station was randomly selected as per Neckles et al. (2011). We plan to continue monitoring these Tier-2 stations on an annual basis. There are approximately 565 Tier-2 sites in the current state-wide monitoring program. At each Tier-2 site, as described in Dunton et al. (2011) and Neckles et al. (2011), we will collect rapid assessment data on seagrass species composition and abundance and monitor potential stressors (see Table 2). The project objective is to collect data that complies with TCEQ rules for seagrass monitoring programs. The measurement methods to support the project are specified in Table 3.

Table 2. Seagrass Condition Indicators

Indicators and proposed field methods under a Tier-2 (annual) seagrass monitoring program. Note: *k* = light attenuation, %SI = percent surface irradiance, PDR = Precision Depth Recorder, CNP = Carbon, Nitrogen, Phosphorus. Asterisks denote minimum criteria for a Tier-2 sampling effort (Dunton et al., 2011). A double asterisk denotes optional sampling.

	Indicator	Field Method
Stressor		
	*k, %SI	Underwater PAR scalar sensors
	water transparency	Secchi
	*depth	PDR
	*temperature, salinity, pH, dissolved oxygen	YSI 600XL SONDE
	TSS	water collection
	chl *a*	in situ fluorescence
Seagrass Condition Indicator		
	*canopy height	0.25 m^2 quadrats
	*seagrass species composition	0.25 m^2 quadrats
	*percent cover	0.25 m^2 quadrats
	**CNP and ^{15}N:^{14}N ratios	clipping

Table 3. Performance specifications.

Water quality parameter measurement performance specifications for both Upper Laguna Madre and Corpus Christi Bay. Parameters measured using the YSI 600XL SONDE are denoted with an asterisk (*). RFU = Relative Fluorescence Unit

Analysis	Matrix	Units	Range	Resolution	Accuracy
pH*	water	Standard units	0 – 14 units	0.01 unit	±0.2 unit
Dissolved oxygen (mg/L)*	water	mg/L	0 – 50 mg/L	0.01 mg/L	0 to 20 mg/L: ± 0.2 mg/L or 2% of reading, whichever is greater; 20 to 50 mg/L: ±6% of reading
Dissolved oxygen (% sat)*	water	%	0 – 500%	0.1%	0 to 200%: ±2% of reading or 2% air saturation, whichever is greater; 200 to 500%: ±6% of reading
Specific conductance*	water	µS/cm	0 - 100 µS/cm	0.001 - 0.1 µS/cm	±0.5% of reading + 0.001 µS/cm
Salinity*	water	ppt	0 – 70 ppt	0.01 ppt	±1% of reading or 0.1 ppt, whichever is greater
Chlorophyll*	water	µg/L	~0 to 400 µg/L; 0 to 100 RFU	0.1 µg/L Chl; 0.1% RFU	----
Temperature*	water	°C	-5 to +50°C	0.01°C	±0.15°C
Secchi depth	water	meters	0 – 25 m	0.10 m	----
Total Suspended Solids (TSS)	water	mg/L	0 – 350	0.01 mg/L	±0.1 mg/L
Surface irradiance	water	%	0 - 100	0.1 %	±0.1
Light attenuation	water	m^{-1}	0.2 – 4.0	0.05	±0.1
Photosynthetically -active radiation	water	µmol m^{-2} sec^{-1}	0 - 3000	Typically 7 µA per 1000 µmol s^{-1} m^{-2} in water.	± 5% in air traceable to NBS
Depth*	water	meters	0 to 30 ft, 9.1 m	0.001 ft, 0.001 m	±0.01 ft, 0.003 m
C:N:P; ^{15}N:^{14}N	tissue	ratio	5 – 30; 1 – 15 ‰	0.1 unit; ±0.05‰	0.2 unit; ±0.3‰

Documentation and Records

Microsoft SQL/Server is a standard relational database that provides robust and reliable storage, archiving and querying capabilities for tabular data. Microsoft Access was used by Dunton et al. (2005) for compilation of historical data on benthic biomass in the western arctic, and is designed to be used by single individuals managing their own data. Data from Microsoft Excel files are entered into SQL/Server using a standardized data loader whose structure conforms to the internal schema in which the data will reside on the database. SQL/Server also has a powerful utility called SQL/Server Integration Services, which is a programming environment that allows for extracting data from various ASCII, Excel, Access or other databases, transforming the data into the Observations Data Model schema, and loading it into the database.

We will use a relational database schema called the Observations Data Model (ODM) developed in the NSF-supported cyberinfrastructure project for the hydrologic sciences, where it is used extensively for storing observations of physical, chemical and biological data in water and benthic environments, including coastal bays. The Observations Data Model uses a star schema in which every discreet observation is treated as a separate entity and stored in one large DataValues table. The ODM has a set of tools for examining the data as graphs and tables and, if necessary, editing the data to correct errors. Data may be stored in versions to permit the maintenance of both raw observations and quality-controlled final datasets.

A seagrass project website, **http://www.texasseagrass.org/**, has been developed to share these results with other scientists, with regulators, the public, and with stakeholders. The website is linked to the Integrated Rule-Oriented Data System (iRODS) on the Corral Server of the Texas Advanced Computing Center (TACC), a secure gridbased software system for managing data on the web. The ArcGIS Online community will be used for sharing geographic data and mapping support will be provided via a project geodatabase.

Sampling Design

Tier-2 Sampling

Tier-2 protocols, which are considered Rapid Assessment sampling methods, are adapted from Neckles et al. (2011). Tier-2 sampling will begin in late summer and continue each year. Annual sampling is performed during or shortly following peak seagrass standing crop, which falls mid- to late summer. For statistical rigor, we will use a repeated measures design with fixed sampling stations to maximize our ability to detect change.

Stations are pre-selected and are located while in the field using a GPS device with an accuracy of 4 m or better. We consider stations as the area within a 10 m radius of the GPS location. Once at the station, hydrographic measurements including water depth, conductivity, temperature, salinity, dissolved oxygen, chlorophyll fluorescence and pH are collected with a YSI 600XL data sonde prior to deployment of any benthic sampling equipment. Visibility is measured using a Secchi disk. Concentrations of Total Suspended Solids (TSS) are calculated from analysis of water samples collected from each station. Water transparency is derived from simultaneous measurements of photosynthetically active radiation (PAR) just below the surface and at a pre-measured depth using LI-COR spherical quantum scalar sensors on a specially designed lowering frame. We use the Beer Lambert equation for calculation of the diffuse attenuation coefficient (k_d). Continuous measurements of light, temperature, and salinity are collected using a LI-COR datalogger and spherical quantum sensor deployed at a permanent site (LM-151) within the study area. Measurements of water column chlorophyll and TSS will be used to determine their relative contributions to k_d, since both are the major light absorbing components in the water column.

Seagrass species composition and percent cover are measured using four replicate quadrat samples per station. Replicates will be positioned in each cardinal direction from the vessel for visual measurements of the seagrass bed. Previous work has shown that the probability of achieving a bias is less than 5% of the overall mean with only four subsamples (Neckles, pers. comm.). We will estimate percent cover through direct vertical observation of the seagrass through the water using a 0.25 m^2 quadrat framer that is subdivided into 100 cells.

The project approach permits baseline data collection while preparing for more in depth Tier-3 data collection that will elucidate causal relationships among variables. 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.

Sampling Methods

Field and Lab Procedures

Sonde Measurements

A YSI 600XL data sonde will be used for hydrographic measurements at each sampling station. The sonde will be checked on a daily basis for accuracy in the laboratory prior to field sampling. We will recalibrate as necessary to insure that all parameters are reading within their published performance (Table 3). At each sampling station, the sonde will be lowered into the water from

the side of the boat so that the instrument probes are completely submerged. Parameter measurements are recorded once readings have stabilized for each depth. At stations deeper than 1 m, additional measurements will be taken above the sediment-water interface. Care will be taken to avoid agitating the seagrasses or sediments since this can re-suspend microalgae and compromise the accuracy of the *in situ* chlorophyll probe. During transport between sampling stations the sonde is wrapped in a damp towel to preserve the integrity of the probes. In the field, dissolved oxygen levels are checked for accuracy based on 100% saturation at the water-atmosphere interface and re-calibrated as necessary. Upon return to the laboratory, a post-calibration check will be performed on salinity, pH, dissolved oxygen, and chlorophyll. See Section B7 for calibration procedures. If sonde probes cannot be calibrated or do not maintain their calibration they will be replaced.

Percent Cover Calculations

We will use direct observation to estimate percent cover of both seagrasses and benthic macroalgae. Four quadrats will be used per station from the vessel as previously described. Each quadrat is examined underwater by a field assistant. All seagrass species occurring in the quadrat are listed and counted. Cover is defined as the fraction of the total quadrat area (as reflected by the number of cells) that is obscured by a particular species when viewed from directly above.

Percent Surface Irradiance and Light Attenuation

Measurements of percent surface irradiance (% SI) and the diffuse light attenuation coefficient (*k*) are made from simultaneous measurements of ambient sub-surface and underwater irradiance at a fixed depth. Measurements of photosynthetically active radiation (PAR = ca. 400 to 700 nm wavelength) are collected using spherical quantum sensors that provide input to a LI-COR datalogger (LI-COR Inc., Lincoln, Nebraska, USA using a LI-193SA sensors. Measurements of % SI and *k* are based on five or more replicate determinations of instantaneous PAR collected synoptically by surface and underwater sensors and recorded by the datalogger. Distance between the two sensors is kept constant (30 cm) since they are fixed to the lowering frame. Care is taken to reduce extraneous sources of reflected light (from boats or clothing) while taking measurements.

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.

Total Suspended Solids

Concentrations of Total Suspended Solids (TSS) are calculated from analysis of water samples collected from each station. The methods outlined below are adapted from EPA Method #160.2. This method is applicable to drinking, surface, and saline waters, in addition to domestic and industrial wastes. The practical range of determination is 4 mg/L to 20,000 mg/L. TSS analyses will be completed under the auspices of CBBEP but not TCEQ.

Field Procedures for TSS

Water samples are collected using previously acid-washed, pre-labeled 1 L polyethylene containers, and rinsed in the field three times prior to addition of the collected water samples. If non-representative particulates (e.g., leaves, sticks, and detritus) are observed in the water sample, it will be dumped out and a fresh sample will be taken. Bottles are sealed, placed on ice, and returned to UTMSI for further analysis. Transport to the laboratory will not exceed 6-8 hours. 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.

C:N:P Ratios

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

Solorzano and Sharp (1980) as described by Fourqurean et al. (1992). Molar C:P, C:N, and N:P ratios are then calculated for evaluation of temporal and spatial trends.

Sampling failures could occur if any or all samples are not taken at a particular sampling station. It is the responsibility of the Field Manager to ensure that samples for every parameter specified are taken at each sampling station before the boat leaves for the next station. Recording data on pre-printed waterproof data sheets helps to ensure that all samples are taken, since there are areas blank areas for data entry that correspond to each sampling parameter. If any sampling failure occurs, corrective action will be taken as deemed appropriate by the Principal Investigator.

Isotope Analysis

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

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

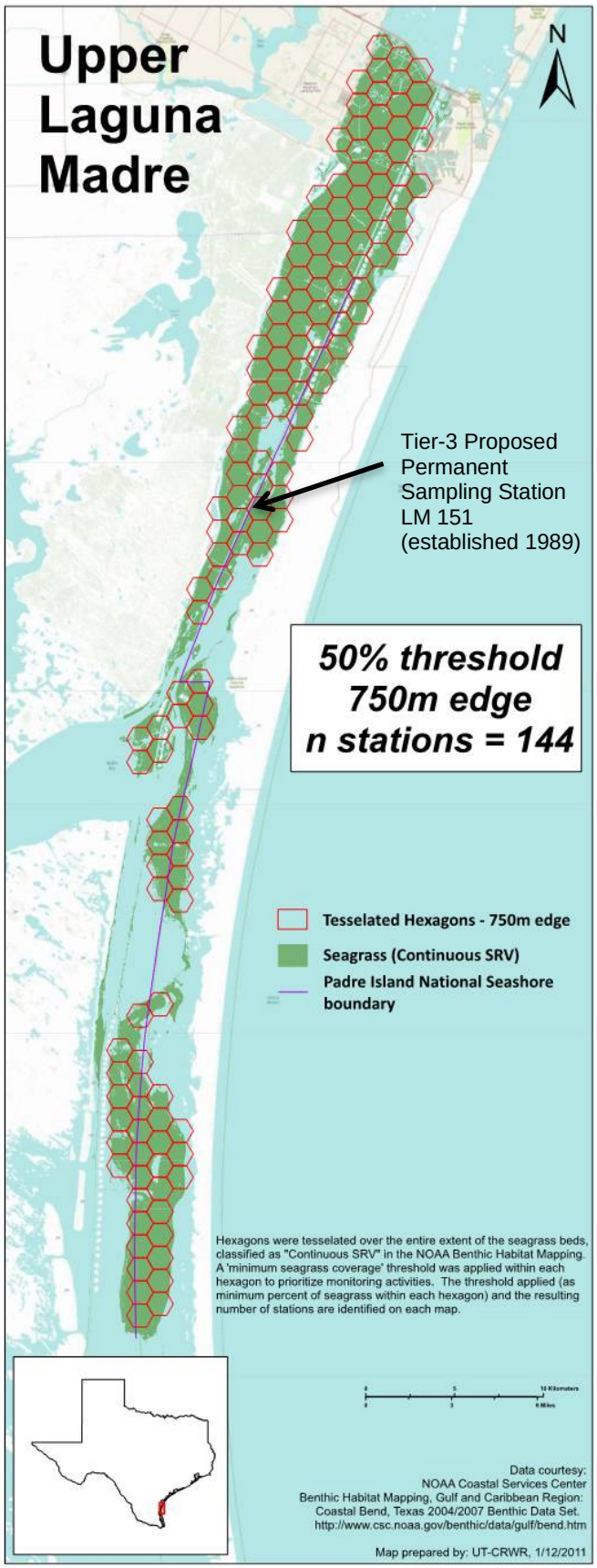

Figure 1. Upper Laguna Madre

A hexagon layer superimposed on the seagrass resources (green areas) of the Upper Laguna Madre. Hexagons (red lines) have an edge distance of 750 m. Hexagons were tessellated over the entire extent of the seagrass beds in south Texas that were classified as "Continuous SRV" in the NOAA Benthic Habitat Mapping. A 'minimum seagrass coverage' threshold was applied within each hexagon to prioritize monitoring activities. The 50% threshold applied (as minimum percent of seagrass within each hexagon) and the resulting number of stations (n = 144 in this example) are clearly identified on the map.

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