SECTION 3: FIELD SAMPLING DESIGN FOR SEAGRASS MONITORING

I. Intensive Field Surveys

The Seagrass Monitoring Workgroup recognized the fundamental need for establishing a rigorous, statistically sound field sampling and survey program. The development of such a program must first address questions concerning field sampling design and the seagrass ecosystem parameters or indicators to be measured. In addition, field sampling must be based on a clearly defined conceptual model that identifies the appropriate seagrass ecosystem parameters for measurement. While constraints on such a program are recognized (namely manpower and costs), monitoring objectives require a reasonable balance between operational constraints and the need for scientifically accurate indicator data, at a sufficient density of coverage.

Seagrass Health and Environmental Indicators. Many indicators of seagrass plant health and environmental quality have been identified in previous monitoring planning studies and workshops. Several published references in particular deal with proposed seagrass indicators and field survey protocols. Neckles (ed) (1994) summarized the proceedings of a monitoring workshop on Gulf of Mexico seagrasses sponsored by EPA –Office of Research and Development (Environmental Monitoring and Assessment Program) and USGS (formerly National Biological Survey) National Wetlands Research Center. This document provides a basic foundation for seagrass monitoring programs, and provides many recommendations for seagrass indicators and ecosystem health parameters, field survey and mapping techniques, as well as related research needs. The Chesapeake Bay Program had earlier identified submerged vegetation monitoring parameters and procedures for temperate eelgrass (*Zostera*) under its renowned estuarine management system (Batiuk et al. 1995). Washington State's Department of Natural Resources (Norris et al. 2000) has also recently designed a seagrass monitoring model for Puget Sound eelgrass which is based on rigorous statistical criteria. While each of these plans contains pertinent, generic information, none is directed specifically towards Texas subtropical seagrass communities. Geographic differences, in particular, can dictate the modifications required to customize the monitoring procedures to Texas seagrasses.

Seagrass parameters can be divided generally into biotic (seagrass abundance, morphology, physiology, and tissue composition) and habitat condition (physicochemical, hydrographic, and habitat) indicators. Most are standard plant ecology and estuarine environmental parameters derived from published seagrass productivity and growth models (Dennison et al. 1993, Dunton et al. 2002). Such models are powerful tools for understanding the complex ecological and biogeochemical relationships between seagrasses and their environment. The exact suite of parameters monitored can depend on the specific conceptual model chosen. Since the efficacy of many parameters has not yet been rigorously substantiated for the Texas monitoring program, this planning document treats parameters as potential indicators until results from the Texas R-EMAP study described below are available.

Seagrass Plant Growth Conceptual Model

This section briefly describes the seagrass plant model that forms the basis for potential monitoring parameters indicative of Texas' seagrass health. A conceptual model to predict trends in biomass when seagrasses are exposed to different environmental conditions was previously developed to assess plant responses to dredging events in Laguna Madre (Dunton et al. 2002). This model was formulated to have both above- and below-ground components and to be applicable to the three dominant seagrass species in the Laguna, turtlegrass (*Thalassia testudinum)*, shoal grass (*Halodule wrightii*) and manatee grass (*Syringodium filiforme)*. The model is driven by incident light and incorporates carbon transport from above- to below-ground tissue. A comprehensive sediment diagenesis model is coupled to the seagrass biomass model allowing the incorporation of important sediment toxicity effects (Fig. 1).

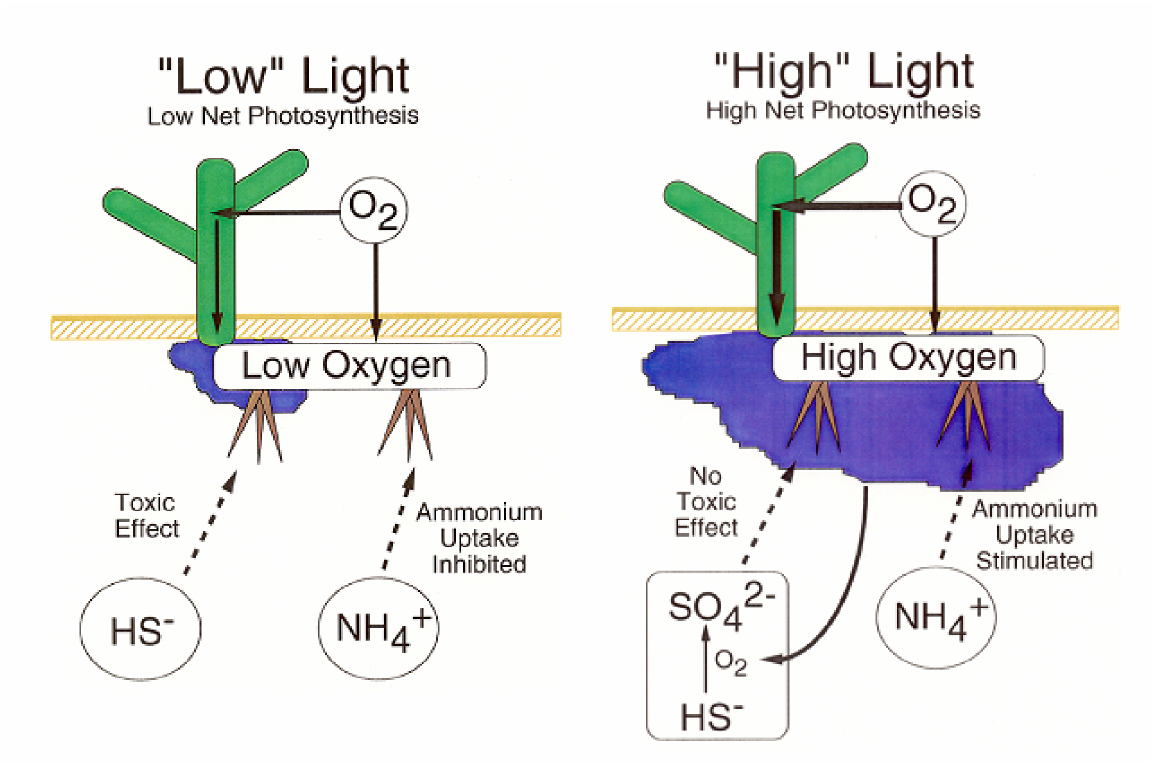

Figure 1. The components of the seagrass model incorporate both the above- and below-ground portions of plant biomass and the changes in sediment geochemistry that occur in relation to underwater light fields.

More sophisticated seagrass models have been developed to examine the flow of carbon and nitrogen in plant tissues. Carbon flow represents energy flow while nitrogen

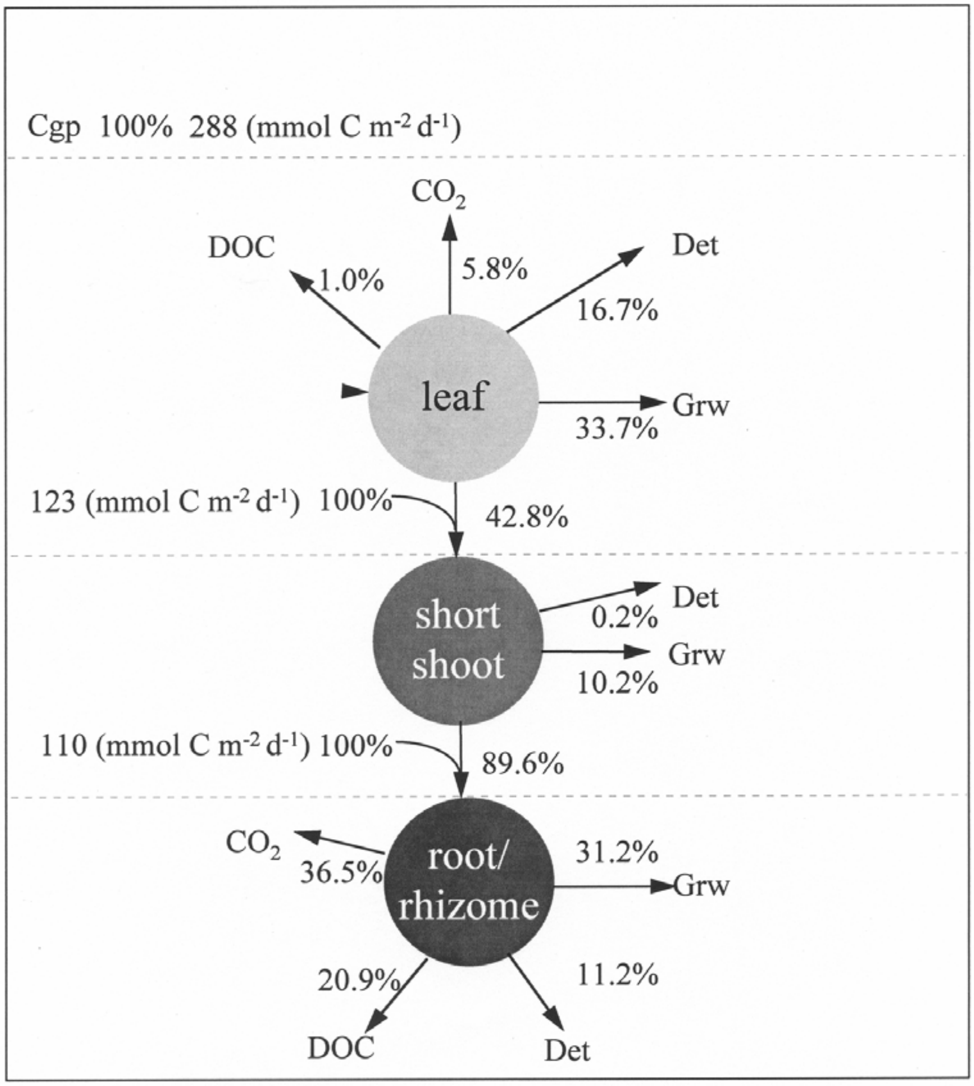

Figure 2. *Thalassia* carbon flow diagram. Arrows indicate direction and numbers (in bold) show the amount of the flow (mmol C m^{-2} d^{-1}) to other seagrass components, such as respiration (CO_2), excretion (DOC), detritus (Det), and growth (Grw) based on a net carbon input from gross primary production (Cgp) of 288 mmol C m^{-2} d^{-1}.

flow is a surrogate for the nutritional state of the plant. Dunton et al. (2002) used field measurements and literature values of production, growth and turnover rates to develop the data and constraint systems. Model results for *Thalassia testudinum* indicate that assimilated carbon was equally partitioned between leaves and below-ground tissues and that the flow was unidirectional during the summer months (Fig. 2). Losses to dissolved organic carbon (DOC) from the root/rhizome module were substantial and may contribute to the high DOC concentrations measured in the sediments. Lee and Dunton (2000) noted that nitrogen assimilation occurred in the below-ground module and model results indicate that internal recycling, particularly from the leaves, was important.

Losses of dissolved organic nitrogen (DON) were minimal, indicating that *Thalassia* uses nitrogen efficiently.

II. Field Sampling Design and R-EMAP Project

Field survey sampling design for Texas areas is currently being evaluated in a R-EMAP pilot project conducted by University of Texas Marine Science Institute, funded by EPA, Region 6, and its Office of Research and Development. Indicators and sampling design are being evaluated by researchers at the University of Texas by applying geostatistical data analysis methods to randomly selected seagrass sampling sites consistent with the recommended EPA-REMAP approach. Results from this project will form the basis for establishing a coastwide field monitoring grid tailored to Texas seagrass beds. Indicator measurements and sampling protocols will be recommended after data from their study have been subjected to rigorous statistical analyses.

The Region 6 REMAP study will identify the indicators that provide the most critical information on water quality criteria and are relevant to successful maintenance and growth of seagrasses. This study will generate data to assess the relative value of various indicators with respect to cost, inherent variability on spatial and temporal scales, and field effort. The study focuses on two estuarine systems, the Mission-Aransas and lower Laguna Madre. The two systems are distinctly different in terms of salinity, nutrient loadings, and freshwater inflows. Yet both support extensive seagrass meadows that contain all five species of seagrasses common to the Texas coast (*Halodule wrightii, Ruppia maritima, Thalassia testudinum, Syringodium filiforme,* and *Halophila engelmanii*). Consequently, a monitoring program that proves successful in accounting for seagrass changes in this pilot investigation will be robust in application to other systems. In both systems, a large amount of baseline data collected in conjunction with previous seagrass studies will be utilized for indicator development through geostatistical analysis.

This study addresses the following questions:

- What key indicators (biotic and abiotic) are most sensitive to causative changes in water quality and best reflect the health of submerged seagrass beds?
- Which seagrass indicators provide the most critical information over spatial and/or temporal time scales? Are some more cost-effective than others?
- Which suite of indicators would be most appropriate, based on their statistical strength, for inclusion into an Index of Biological Integrity (IBI)?
- What monitoring design should be established in Texas seagrass beds for probabilistic sampling that allows for rigorous statistical geospatial analysis? What are some of the options?
- Over what time scales (from every sixty days to annual) is sampling most appropriate on a cost-benefit basis? What temporal scales provide the most sensitivity to environmental change?

- How concentrated should sampling efforts become? What spatial sampling density is sufficient to capture the inherent variability in the system? What is the trade-off between replication, the number of stations, and cost?

This project involves sampling 30 sites within each of two estuarine systems, the Mission-Aransas and the lower Laguna Madre. Within each of the two study areas, core EMAP seagrass indicators are measured along with additional parameters that have been identified based on recent research activities (Table 1). This effort will require the development of a detailed bathymetric base map in digital form using Geographic Information System (GIS) software.

Table 1. Core EMAP coastal indicators considered at each permanent sampling site.

Water Quality	**Sediment Quality**	**Seagrass Light Response Indicators**	**Plant Nutrient Response Indicators**
Dissolved oxygen	grain size	biomass (above- & below-ground)	C:N:P blade ratios
Conductivity, salinity, and temperature	total organic carbon	root:shoot ratio	epiphytic algal species composition and biomass
Nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{-3})	pore water NH_4^+	leaf area index; blade width	drift macroalgal abundance/composition
Chlorophyll *a*	Redox?	shoot density	
total suspended solids (TSS)	Depth to reducing layer?	chlorophyll fluorescence	
light attenuation (*k*)	Sulphide?	species composition	
Surface irradiance (%SI)		maximum depth limit	

All sixty sites will be sampled every six months from July 2002 through February 2004. At each site, a rapid visual assessment technique developed early in the twentieth century by plant sociologist Braun-Blanquet (Braun-Blanquet 1972) is used to assess the abundance of seagrass and macroalgae. This method is used in the EPA sponsored seagrass status and trends monitoring project in the Florida Keys National Marine Sanctuary (FKNMS). It is very quick, requiring only minutes at each sampling site, yet is robust and highly repeatable, thereby minimizing among-observer differences, and has recently been applied to seagrass research. At each site, a 50-m-long transect is established by driving steel rods into the substratum at both ends of the transect. Each time a site is visited, the transect is marked with a 50-m rope from the site marker towards the south. During each sampling period, ten quadrats (0.25 m^2) are placed along each transect at pre-determined random distances from one of the marker rods. A new set of random sampling positions are chosen before each visit to a site. Each quadrat is examined using SCUBA or snorkeling equipment. All seagrass species occurring in the quadrat are listed, and a score based on the cover of the species in that quadrat is assigned (see Table 2). Cover is defined as the fraction of the total quadrat area that is obscured by a particular species when viewed from directly above. From the observations of cover in each quadrat at a site, three statistics will be computed for each species: density, abundance and frequency following the detailed procedures of Fourqurean et al. (2001).

Table 2. Braun-Blanquet abundance scores (S). Each seagrass species will be scored in each quadrat according to this scale (from Fourqurean et al., 2001). (Shoot density applies to *Thalassia* only).

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

Data collected during the 18-month field effort will be incorporated into a geospatial database in various GIS layers for assessment and statistical analysis. These data layers will include seagrass distribution and measurements from a variety of indicators. Since indicators are clearly linked to an underlying ecological process, geostatistical analyses can be used effectively to evaluate the power and reliability of a given indicator.

The expected benefits of these activities include:

- Evaluation of the relative importance of various seagrass indicators for a state-wide seagrass monitoring program.
- Demonstration of a seagrass monitoring program in two distinctly different Texas estuaries characterized by widespread cover and diversity of seagrasses.
- Creation of a website linked to the EMAP website to provide other individuals and programs access to data on seagrass health and distribution.

Beginning in 2003, data will be analyzed and interpolated using Geostatistical Analyst, an ArcInfo 8.1 extension. Geostatistical techniques, which involve kriging or cokriging methods (for multivariate cases), can be used to create prediction surfaces. Several methods are available in the Geostatistical Analyst extension. Understanding the combination of spatial and temporal trends in data requires a combination of techniques from geospatial analysis using GIS and time series analysis. Some of the issues are the following:

- A given indicator at a particular location may vary seasonally (e.g. dissolved oxygen) or have a value that shows little seasonal variation (e.g. total suspended solids).
- There may be consistent differences from one year to the next in the level of an indicator.
- The indicators are linked by physical, chemical and biological relationships.

The techniques proposed for use in this study to clarify these relationships are:

- Geostatistical analysis of point information to generate spatial maps of expected concentrations of variables and their standard error of estimate. The Geostatistical Analyst extension of ArcInfo 8.1 will be used for this purpose. This technique has been successfully employed in a study of the benthic community in the Western Arctic ocean (Jonsdottir, et al., 2000).
- Fourier analysis of indicators showing seasonal variations. This is fairly easy to accomplish using regular regression methods, which can be combined with annual variables to examine combinations of year-to-year trends and seasonal variations within a year.
- Regression or other mathematical or physical models to describe the interrelationships of the indicator variables. This analysis will likely be programmed in Visual Basic to operate on data in ArcInfo 8.1 or a combination of ArcInfo 8.1 and Excel.

One goal of this study is to identify an indicator variable or combined set of variables that reliably identifies the health of seagrass beds. One method of doing this is an Index of Biological Integrity (IBI), which is a weighted set of indicator values that provide a score. Gradations of the score from high to low are then used to characterize regions of health of the seagrass population. Such indicators need to be:

- Statistically sound in the sense that they discriminate between sampled conditions in a statistically significant manner.
- Scientifically sound in the sense that the variables included in the IBI are rational.
- Reasonable in the sense that they may be used by resource staff to determine an IBI value following a standardized protocol within a reasonable amount of time with limited resources. Statistical summarization techniques are helpful in forming such indices but common sense selection of reasonable variables should always take precedence.

Incorporating Study Results into the Seagrass Monitoring Plan

After the Steering Committee Workgroup reviews and analyzes results from the R-EMAP study, decisions will be made to select the most definitive, cost-effective indicators for routine, coastwide monitoring surveys. These surveys will be designed for each bay system to cover all areas where seagrass occurs or potentially could occur; thus primarily shallow subtidal areas will be monitored. While a minimum suite of environmental parameters and bioindicators can be established for routine monitoring based on cost considerations, special studies will warrant more sophisticated measurements in certain cases.

A robust sampling grid will be applied to each bay based on evaluation of sample numbers needed to provide statistically valid results. The Steering Committee tentatively anticipates that some form of an EMAP global spatial grid developed according to the generalized random tessellation stratified (GRTS) design will be employed as the basic random sampling strategy (EPA/ORD/NHEERL 2002). The equal area sampling grid cells from GRTS satisfy probabilistic sampling requirements, but also support other critical options for monitoring seagrass target populations in coastal waters. Statistical issues such as uneven seagrass sample distributions, stratified or nested subsampling, and monitoring over time at special study areas, can be accommodated under this design. EPA-National Coastal Assessment Program staff and Texas research scientists will officially certify the sampling scheme at this final stage.