Quality Assurance Project Plan

Seagrass Response to Wastewater Inputs: Implementation of a Seagrass Monitoring Program in Two Texas Estuaries

Revision 2
January 13, 2010

Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744-3291

Effective Period: This Quality Assurance Project Plan (QAPP) is effective from January 1, 2010 through March 31, 2011

Questions concerning this quality assurance project plan should be directed to:

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A1. Approval Page

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Patricia L. Radloff, Ph.D. Date
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Charles Wood Date
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Ashley Summers 1-20-10
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GIS Analyst

Warren Pulich, Jr.
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Kimberley Jackson

Kimberly Jackson Date

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A1. Approval Page

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Kirk Cammarata Date

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Project Manager

Hollis Pantalion

Hollis Pantalion 1/15/10

Hollis Pantalion Date
Environmental Laboratory Services
Quality Assurance Officer

The TPWD Water Quality Program will secure written documentation from each sub-tier project participant (*e.g.*, subcontractors, other units of government, laboratories) stating the organization's awareness of and commitment to requirements contained in this quality assurance project plan and any amendments or revisions of this plan. The Water Quality Program Quality Assurance Project Officer will maintain this documentation as part of the project's quality assurance records, and will be available for review.

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List of Acronyms

AWRL	Ambient Water Reporting Limit
BP	Band Pass
CBBEP	Coastal Bend Bays and Estuary Program
CFR	Code of Federal Regulations
CL	Confidence Limit
COC	Chain-of-Custody
DO	Dissolved Oxygen
EPA	U.S. Environmental Protection Agency
GCP	Ground Control Point
GLO	General Land Office
GPS	Global Positioning System
IHS	Intensity-Hue-Saturation
LCRA	Lower Colorado River Authority
LCS	Laboratory Control Standard
µM	micro-Moles
MDL	Method Detection Limit
NAIP	National Agricultural Imagery Program
OP	ortho-Phosphate
PAR	Photosynthetically Active Radiation
PDOP	Position Dilution of Precision
PMT	Photomultiplier Tube
QA	Quality Assurance
QAM	Quality Assurance Manual
QAO	Quality Assurance Officer
QAPO	Quality Assurance Project Officer
QAPP	Quality Assurance Project Plan
QC	Quality Control
RL	Reporting Level
RPD	Relative Percent Difference
SE	Standard Error
SI	Surface Irradiance
SOP	Standard Operating Procedure
SWQM	Surface Water Quality Monitoring
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TSS	Total Suspended Solids
TAMU-CC	Texas A & M University – Corpus Christi
TIF	File format used to display images
TCEQ	Texas Commission on Environmental Quality
TNRIS	Texas Natural Resource Information Service
TPWD	Texas Parks and Wildlife Department
TSU	Texas State University
UTMSI	University of Texas Marine Science Institute

A3. Distribution List

Texas Parks and Wildlife Department
4200 Smith School Road
Austin, TX 78744

Patricia L. Radloff, Ph.D., Project Manager
Cindy Contreras, Quality Assurance Project Officer
Charles Wood, TPWD Quality Assurance Officer
Ashley Summers, GIS Analyst
Adam Whisenant, Regional Biologist
Jennifer Bronson, Regional Biologist

Warren Pulich, Jr., Ph.D.
2300 Westway Circle
Austin, TX 78704

Warren Pulich Jr., Ph.D., Seagrass Consultant

Krawietz Aerial Photography
P.O. Box 191
Bulverde, TX 78163-0191

Richard Krawietz

Marine Science Institute
University of Texas at Austin
750 Channel View Drive
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Kenneth H. Dunton, Ph.D., Project Director
Kim Jackson, Lab and Field Manager
Chris Wilson

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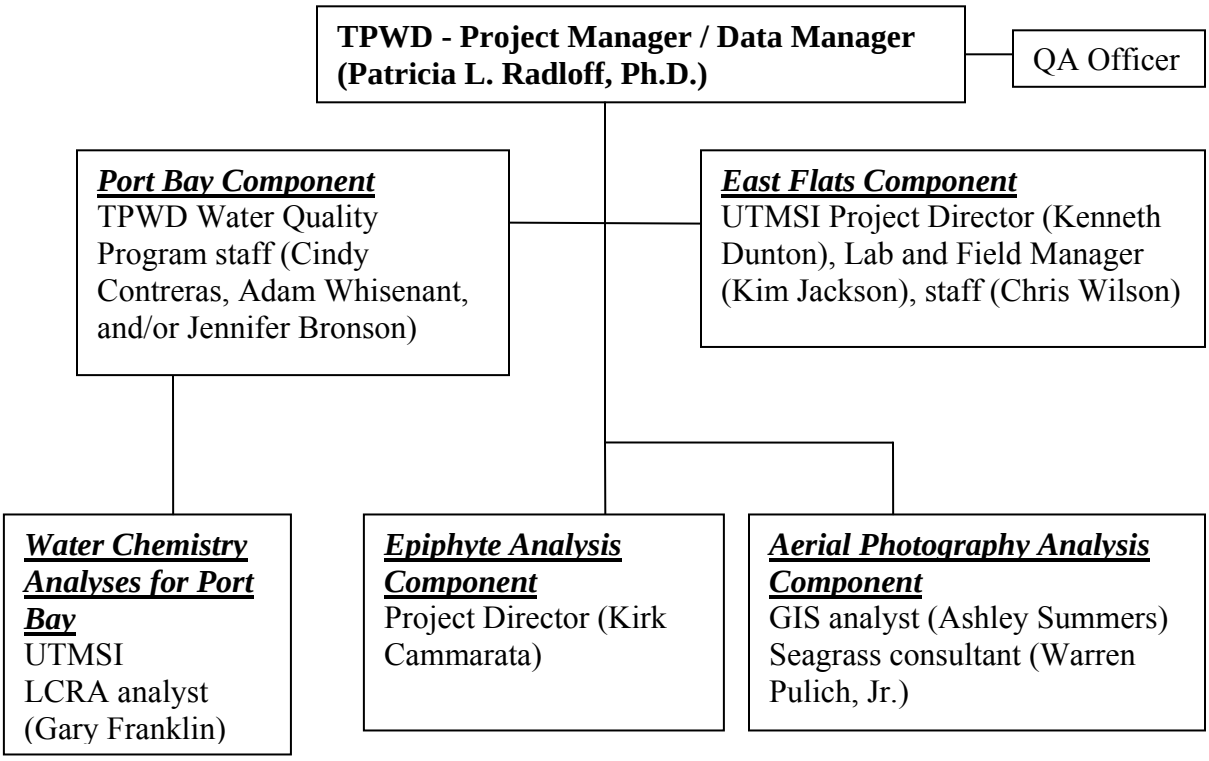
Kirk Cammarata, Ph.D.

Lower Colorado River Authority
Environmental Laboratory Services
P.O. Box 220, Austin, TX 78767
Gary Franklin
Hollis Pantalion

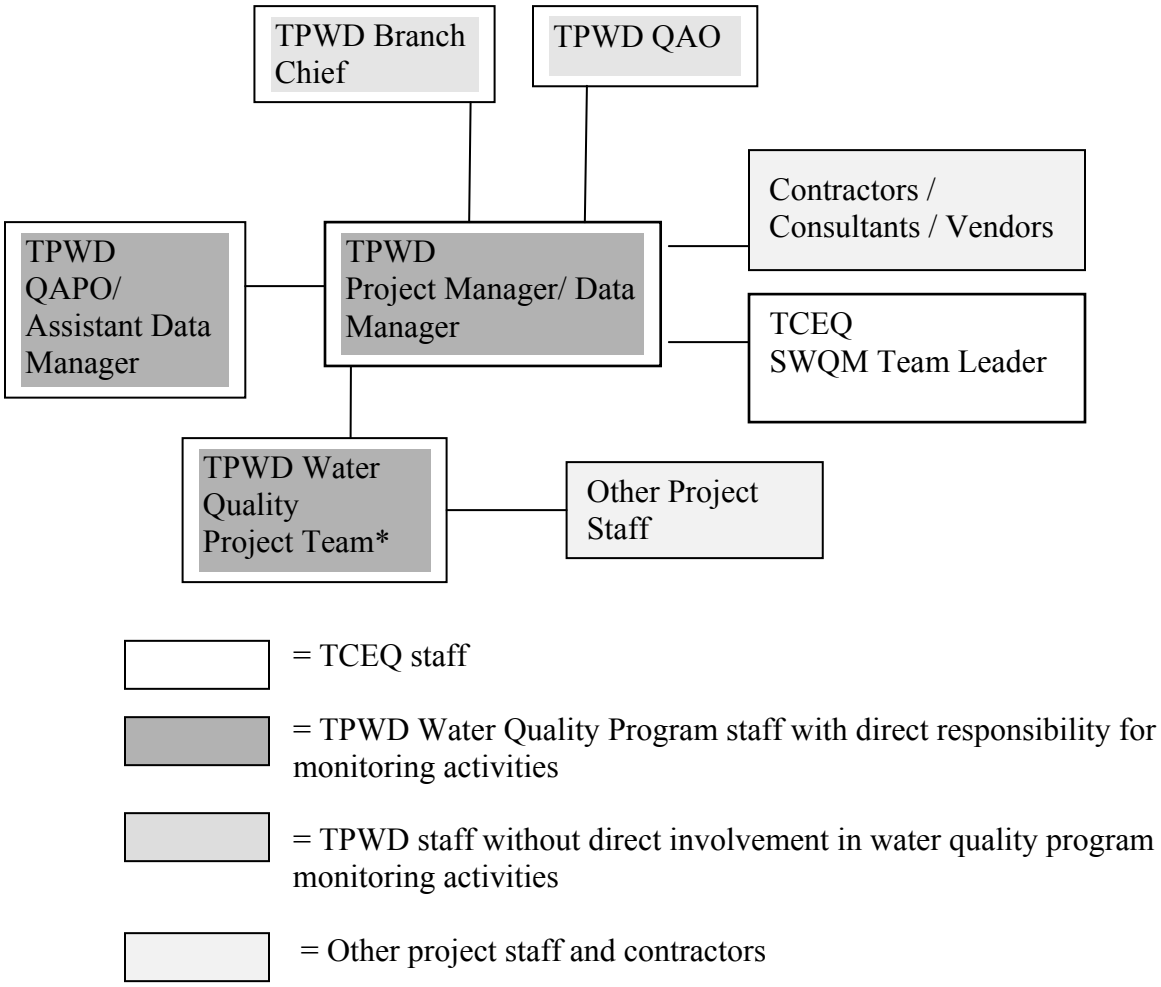
A4. Project/Task Organization

Figure 1. A4 - Key organizations and personnel.



This seagrass monitoring study is funded by a General Land Office (GLO) Coastal Management Program grant to Texas Parks and Wildlife Department (TPWD), which is the overall project manager. The project will use aerial photography, monitoring of seagrass condition and water quality indicators, and epiphyte analysis to study seagrass in two project areas. Dr. Kenneth Dunton, Marine Science Institute, University of Texas at Austin (UTMSI), will direct the component on East Flats. TPWD Water Quality Program staff will conduct the component on Port Bay. Water and sediment chemistry analyses will be conducted by UTMSI for East Flats and by UTMSI and the Lower Colorado River Authority (LCRA) for Port Bay. Seagrass, epiphyte and macroalgae biomass laboratory work will be conducted by UTMSI for East Flats and TPWD for Port Bay. Elemental analyses and isotope ratios will be conducted by UTMSI for both project locations. Dr. Kirk Cammarata of Texas A&M University – Corpus Christi (TAMU-CC) will conduct fluorescence analyses of seagrass epiphyte accumulation for both project locations. Aerial imagery will be acquired for both project locations by Krawietz Aerial Photography and analyzed by Ashley Summers of TPWD in consultation with Dr. Warren Pulich, Jr.

Figure 2. A4 - Schematic project organization chart and lines of communication.



* TPWD's Project Team includes Water Quality Program staff and those under their direct supervision

Texas Parks and Wildlife Department

Patricia L. Radloff, Ph.D.
TPWD Water Quality Program Leader - Project Manager
The Project Manager is responsible for ensuring that all project activities are performed, including planning, documenting project goals, preparation and approval of a Quality Assurance Project Plan (QAPP), and adherence to a QAPP, to ensure that data of known quality are collected. The project manager submits an annual project report to the TPWD QA Officer at an annual meeting of Project Managers, QAPOs, and relevant program managers and supervisory staff. The project manager coordinates assessments and confirms the implementation and effectiveness of corrective actions and documents such actions. The project manager and/ or QAPO submit data to the Texas Commission on Environmental Quality (TCEQ) in an approved format.

Cindy Contreras
TPWD Water Quality Program Coordinator - Quality Assurance Project Officer (QAPO)
The primary duty of the QAPO is to assist the Project Manager in the development and implementation of the QAPP and in the performance and review of project quality assurance assessments. The QAPO is also responsible for recommending to the appropriate staff any modifications that may be needed to improve the effectiveness and efficiency of the TPWD quality system as a whole and for his or her assigned projects. The QAPO is required to attend the annual meeting of Project Managers and QAPOs. The QAPO also may conduct field monitoring and collect data for TPWD Water Quality Program activities, following procedures outlined in QAPP and any referenced Standard Operating Procedures (SOPs) and performing necessary field calibrations and other quality assurance measures as specified.

Adam Whisenant
TPWD Water Quality Program Regional Biologist
Water Quality Project Team
Conducts field monitoring and collects data for TPWD Water Quality Program activities. Follows procedures outlined in QAPP and any referenced SOPs. Performs necessary field calibrations and other quality assurance measures as specified.

Jennifer Bronson
TPWD Water Quality Program Regional Biologist
Water Quality Project Team
Conducts field monitoring and collects data for TPWD Water Quality Program activities. Follows procedures outlined in QAPP and any referenced SOPs. Performs necessary field calibrations and other quality assurance measures as specified.

Charles Wood
TPWD Quality Assurance Officer, FY 2010
The Quality Assurance Officer is responsible for review and approval of QAPPs. Relies on each Project Manager to be responsible for project quality assurance efforts. The QA Officer has authority to reject any environmental data that does not meet quality assurance standards, and reports directly to the Deputy Executive Director for Operations, or his or her designee, on quality assurance matters. Convenes an annual quality planning and assessment meeting involving project managers, QAPOs and other staff as appropriate.

Ashley Summers
TPWD GIS Analyst
Analyzes orthorectified digital imagery to develop landscape analysis for study areas.

University of Texas Marine Science Institute

Kenneth H. Dunton, Ph.D.
Professor, Department of Marine Science
Research Professor, Marine Science Institute
East Flats Project Director

Advises and guides design of project. Assists TPWD in developing protocols for project and training staff. Collects and provides data from East Flats component of study. Analyzes elemental composition and stable isotope ratio of samples from East Flats and Port Bay components of study. Reports results to TPWD on a regular basis.

Kim Jackson
UTMSI Lab and Field Manager
Assists in training TPWD staff to conduct seagrass sampling. Collects and provides data from East Flats component of study. Analyzes elemental composition and stable isotope ratio of samples from East Flats and Port Bay components of study.

Chris Wilson
UTMSI Project Officer
Conducts field monitoring and collects data for UTMSI activities. Follows procedures outlined in QAPP and any referenced SOPs. Performs necessary field calibrations and other quality assurance measures as specified.

Texas A&M University - Corpus Christi

Kirk Cammarata, Ph.D.
Associate Professor of Biology
Advises TPWD on sampling protocols for seagrass epiphyte fluorescence measurements. Conducts fluorescence assessment of seagrass leaf samples to characterize and quantify epiphyte growth. Reports results of analysis on a regular basis to TPWD. Assists in interpreting results of epiphyte fluorescence assessment.

Lower Colorado River Authority

Alicia Gill
LCRA Environmental Laboratory Services Manager
Responsible for overall performance, administration, and reporting of analyses performed by LCRA's Environmental Laboratory Services.

Hollis Pantalion
LCRA Environmental Laboratory Services QA Officer
Responsible for the overall quality control and quality assurance of analyses performed by LCRA's Environmental Laboratory Services. Monitors the implementation of the QAM/QAPP within the laboratory to ensure complete compliance with QA data quality objectives.

Gary Franklin
LCRA Environmental Laboratory Services Project Manager
Responsible for quality assurance of analyses performed by LCRA's Environmental Laboratory Services. Responsible for laboratory and field staff corrective action communication with the LCRA QAO. Oversees analysis of water and sediment samples. Reports results to TPWD on a regular basis.

Krawietz Aerial Photography

Richard Krawietz
Acquires and delivers orthorectified digital imagery consistent with attached work plan.

Warren Pulich, Jr. Ph.D.

Warren Pulich, Jr., Ph.D.
Seagrass Consultant
Trains TPWD staff to analyze orthorectified digital imagery to perform landscape analysis for study areas. Advises and guides acquisition of aerial imagery and groundtruthing activities. Reviews intermediate and final analysis products.

A5. Problem Definition /Background

Seagrass beds serve as critical nursery habitat for estuarine fisheries and wildlife. Seagrass (submerged aquatic vegetation) has been identified as a critical area by the Coastal Coordination Act. Seagrasses provide food for fish, waterfowl and sea turtles, contribute organic material to estuarine and marine food webs, cycle nutrients, and stabilize sediments. They are economically important based on their function in maintaining Gulf fisheries. Increasing coastal development threatens seagrasses.

Three state agencies with primary responsibility for conserving coastal natural resources, Texas General Land Office, the Texas Commission on Environmental Quality, and the Texas Parks and Wildlife Department, signed the Seagrass Conservation Plan for Texas in 1999 (SCPT 1999). Currently, TPWD facilitates quarterly meetings of a Seagrass Monitoring Work Group comprised of experts from academics and government.

This project will leverage the expertise and recommendations of the Seagrass Monitoring Work Group to evaluate seagrass condition in two areas of the central Texas coast, one area more or less "pristine" (East Flats in Corpus Christi Bay) and one area where a wastewater treatment plant is scheduled to begin discharging (Port Bay). Data collected from Port Bay will be compared with data collected from East Flats to help determine whether the effluent limitations that TCEQ has proposed are effective in protecting seagrasses in the vicinity of the discharge.

The study protocol will follow a recent proposal by Dunton and Pulich to the Seagrass Monitoring Work Group (Dunton *et al.* 2007). Identical procedures will be used in East Flats and Port Bay. The study will include three components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) epiphyte analysis. Information from the three components will be integrated to assess the condition of the seagrasses.

Landscape monitoring will be obtained from analysis of 1:9,600 scale color aerial photography. The seagrass stress indicators that will obtained include seagrass coverage, depth limit of seagrass coverage, bare patches (coverage, number, size), and macroalgae deposition (areal

coverage). Dr. Warren Pulich, Jr. of Texas State University - San Marcos, will assist TPWD staff with analysis of landscape data.

For seagrass condition and water quality indicators, this project will use recommendations of Dunton *et al.* (2007), which identified indicators that correlate with effects of stress on seagrasses. This project will be the first test of the protocol. Water quality data that will be collected include nutrients, chlorophyll-*a*, suspended solids, light attenuation, salinity, dissolved oxygen, and temperature. Sediment pore water ammonia samples will also be collected. Seagrass condition indicators that will be studied include total biomass, root-to-shoot biomass ratio, leaf length and width, percent cover and density, carbon and nitrogen isotope ratios (to measure human influence), and ratios of carbon-to-nitrogen in seagrass tissue. TPWD staff will work with Dr. Kenneth Dunton of UTMSI to collect seagrass condition and water quality indicators.

The final component of this study will be the use of a novel technique developed by Dr. Kirk Cammarata of TAMU - Corpus Christi to quantify and analyze epiphytic algae growth on seagrass leaves. In nutrient-enriched waters, epiphytic algae growth may increase; at some point interfering with photosynthesis and causing seagrass loss. Measurements of epiphytic algal density will be a sensitive way to measure impacts of increased nutrient loadings. This study will compare traditional measurements of epiphytic algal biomass (obtained from leaf scrapings) with fluorescence measurements made in Dr. Cammarata's lab (Cammarata *et al.* 2009).

Aerial photography taken shortly following peak seagrass growth season will be obtained at least once during the study. Seagrass condition, water quality indicators and epiphytic algae analysis will be conducted at Port Bay and East Flats in three seasons (spring, summer and fall).

At this time, the wastewater facility on Port Bay has not begun discharging. It is not possible to predict with certainty when the plant will begin operation. If, as expected, the plant is operating during this grant cycle, this project will proceed as described above, with East Flats serving as a reference site for Port Bay. If the plant is not yet operational, then sampling will be conducted as described above at both Port Bay and East Flats. Data from both locations will be used to test the Dunton *et al.* seagrass monitoring protocol. In addition, the Port Bay data will be used as baseline data which will later be compared with data obtained after the plant is operational. A second phase of sampling at Port Bay is not included in this project.

A6. Project/Task Description

Aerial Imagery and Landscape Analysis

High resolution (1:9,600 scale) aerial imagery will be taken of the study sites in East Flats and Port Bay at least once during this project near the time of seagrass "full leaf-on" in November or December.

The aerial photography component of the project consists of:

1. Acquiring true color aerial photography at 1:9,600 scale for East Flats and Port Bay project sites.
2. Groundtruthing vegetation and landscape features.

3. Analysis of 1:9,600 scale photography
4. Determining seagrass coverage, depth limit of seagrass coverage, bare patches (coverage, number, size), and macroalgae deposition (areal coverage).

Originally this project planned to analyze orthorectified digital imagery to determine seagrass coverage, depth limit of seagrass coverage, bare patches (number, shape), and macroalgae deposition (areal coverage). Since making this proposal, TPWD staff have learned that no standard GIS analysis techniques exist for determining the shape of bare patches in seagrass beds and that to attempt such an analysis would be time-consuming. Instead, TPWD staff will analyze for shape by determining the number and size of bare patches. This analysis will give comparable information about seagrass condition. When seagrass is in decline, reticulated, spaghetti-like networks of bare areas appear first. These later coalesce into larger, discrete bare islands (*i.e.* patches). Measures to identify such seagrass fragmentation and decline include counting the seagrass-to-bare transitions along transects (a large number of transitions can indicate decline) and determining the patch size distribution along transects (increasing number or change in size distribution can indicate decline).

Water and Sediment Quality Indicators

Water and sediment quality indicators will be sampled three times to capture seasonal variations at East Flats and Port Bay. Water quality indicators include those measured on an instantaneous basis during sampling of the seagrass beds (dissolved oxygen, salinity, temperature, pH, Secchi depth, percent surface irradiance, light attenuation), and those measured on a longer-term basis (salinity, temperature, depth). Water chemistry measurements include nutrients (ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), ortho-phosphate (OP, PO_4^{-3})), total suspended solids (TSS), and chlorophyll-*a*. Sediment quality indicators (grain size, total organic carbon (TOC)) will be measured once during the study, and sediment pore water ammonium will be analyzed at each sampling event. Long-term measurements of photosynthetically-active radiation (PAR) will be made at East Flats.

Seagrass Condition Indicators

A suite of biological indicators will be assessed at each site. Replicate core samples of seagrasses will provide measures of above and below ground biomass, root-to-shoot ratio, leaf area index, blade width, and shoot density. Seagrass leaf samples from each site will be assessed to determine plant nutrient response indicators, which include C:N blade ratios, and changes in the carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) content of leaf tissues. Algal epiphytes will be harvested from blades using traditional scraping techniques for determination of epiphyte composition and biomass; these results will be compared to the results of Cammarata (see below). Changes in the carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of macroalgae will also be measured. Sites will be digitally videographed.

Seagrass Epiphyte Fluorescence Measurements

Dr. Kirk Cammarata of Texas A&M University – Corpus Christi has developed a novel technique to quantify epiphyte load on seagrass blades. Seagrass blades from collected shoots will be separated from rhizomes and scanned for fluorescence to quantify epiphytes. Images will be analyzed for measures of leaf biomass and epiphyte load and accumulation.

Amendments to the QAPP

Revisions to the QAPP may be necessary to reflect changes in project organization, tasks, schedules, objectives, and methods; to improve operational efficiency; and to accommodate unique or unanticipated circumstances. Amendments are effective immediately upon approval by the TPWD Project Manager, the TPWD QAPO and the TPWD QAO. They will be incorporated into the QAPP by way of attachment and distributed to personnel on the distribution list.

A7. Quality Objectives and Criteria

Aerial Imagery and Landscape Analysis

Determination of seagrass condition from aerial imagery will be performed with high-resolution true color georeferenced digital photography. Aerial imagery will be acquired by Krawietz Aerial Photography per the attached work plan (Appendix A). The mapping protocol relies on photoimage analysis (analogous to photointerpretation) of color photographs, acquired according to strict weather and water conditions that maximize seagrass delineation. Photography will be taken with calm, clear water, under clear skies with full sunlight, usually moderate to low tides, with 1 m or better positional accuracy and 0.3 m resolution. Airborne GPS will be used for georeferencing the imagery. Accuracy may be enhanced by use of control points within the boundaries of each of the areas to be photographed. GPS will be used in groundtruthing to precisely locate landscape features and vegetation to 1 m or better positional accuracy.

Desktop image analysis software, such as ERDAS™, ENVI™ or ArcGIS™, will be used to analyze the orthorectified digital imagery. Table 1 lists the five classes of seagrass landscape indicators as well as proposed spatial metrics. This project will use three classes of indicators (seagrass, macroalgae and bare area) to obtain five metrics: seagrass coverage, bare patches (number, size), depth limit of seagrass coverage, and macroalgae deposition (areal coverage). The minimum mapping unit will be 2 m^2. Classified imagery will have 80 percent or better overall accuracy, assessed using the error matrix technique of Congalton (1991).

Table 1. A7 - Seagrass landscape indicator classes and metrics (Pulich *et al.* 2003).

Landscape Indicator Class	Description	Metrics
Seagrass bed morphology and patterns	Edge shapes and patch sizes of plant beds are often a function of hydraulics (*e.g.* water currents), depth, and localized environmental disturbances. Patchy beds can reflect two types of disturbance responses: a) expanding or colonizing patches of plants, or b) localized fragmentation from physical disturbance, (*e.g.* wave energy or dynamics).	Shape, size, density, & edge symmetry of beds/patches per hectare
Non-seagrass natural features	Random bare patches within large grassbeds could result from storms, tidal currents or fetch, human activities or macroalgae and wrack deposition.	Acreage of macroalgae, bare patches, reefs, tidal channels, sand bars & shoals per hectare.
Human impact features	Landscape features such as propeller scars, pipeline scars, dredged channels and spoil deposits, and industrial activities (*e.g.* aquaculture sites) are examples of human impacts to grassbeds	Linear distance of propeller scars, pipelines, "industrial activities," dredged channels per area of interest.
Spatial distribution of seagrass species	Species composition can reflect successional processes that in turn can result from stressor impacts. Species delineation, however, requires extensive groundtruthing to achieve satisfactory accuracy with the aerial image. Multispectral imagery (digital video or airborne scanner) also enables more accurate delineation.	Percent changes in coverage over landscape area of interest; depth limit; species coverage.
Water column characteristics	Metrics such as currents (flow patterns), turbidity, chlorophyll levels, and chemical components are indicators of water quality or hydraulic stress. However, identification of these parameters usually cannot be done solely from interpretation of the imagery, but requires ancillary field data.	Zones (polygon areas) of turbidity, chlorophyll, other water chemistry.

Table 2. A7 – Water quality parameter measurement performance specifications for East Flats.

Analysis	Matrix	Units	STORET code	Analytical method	Sensitivity	Precision	Expected range
pH	water	standard units	00400	EPA 150.1 and TCEQ SOP	0.01	±0.01	7-9
Dissolved oxygen	water	mg/L	00300	EPA 360.1 and TCEQ SOP	0	±0.01	0-10
Specific conductance	water	µS/cm	00094	EPA 120.1 and TCEQ SOP	0	±1	0-100,000
Salinity	water	ppt	00480	SM 2520 and TCEQ SOP	0.1	±0.01	10-55
Temperature	water	ºC	00010	EPA 170.1 and TCEQ SOP	-5.0	±0.01	10-33
Turbidity	water	NTU	82078	EPA 180.1 and TCEQ SOP	0.1	5%	0 - 1000
Secchi depth	water	meters	00078	TCEQ SOP	NA	NA	NA
Surface irradiance	water	%	NA	Calculation	0.1	±7	5-60
Light attenuation	water	$meters^{-1}$	NA	Calculation	---	±0.1	0.2-4.0
Photosynthetically-active radiation	water	$\mu mol\ sec^{-1}\ meter^{-2}$	NA	UTMSI Protocol	***	5%	***
Depth	water	meters	13850	TCEQ SOP	NA	NA	NA
Days since last significant rainfall	NA	days	72053	TCEQ SOP	NA	NA	NA
Tide stage	water	See TCEQ SOP	89972	TCEQ SOP	NA	NA	NA
24-hr average temperature	water	ºC	00209	TCEQ SOP/Calculation	NA	NA	NA
Maximum daily temperature	water	ºC	00210	TCEQ SOP/Calculation	NA	NA	NA
Minimum daily temperature	water	ºC	00211	TCEQ SOP/Calculation	NA	NA	NA
24-hr water temperature # of measurements	water	#	00221	TCEQ SOP/Calculation	NA	NA	NA
24-hr average salinity	water	ppt	00218	TCEQ SOP/Calculation	NA	NA	NA

Analysis	Matrix	Units	STORET code	Analytical method	Sensitivity	Precision	Expected range
Maximum 24-hr salinity	water	ppt	00217	TCEQ SOP/Calculation	NA	NA	NA
Minimum 24-hr salinity	water	ppt	00219	TCEQ SOP/Calculation	NA	NA	NA
24-hr salinity # of measurements	water	#	00220	TCEQ SOP/Calculation	NA	NA	NA
24-hr average specific conductance	water	µS/cm	00212	TCEQ SOP/Calculation	NA	NA	NA
Maximum 24-hr specific conductance	water	µS/cm	00213	TCEQ SOP/Calculation	NA	NA	NA
Minimum 24-hr specific conductance	water	µS/cm	00214	TCEQ SOP/Calculation	NA	NA	NA
24-hr specific conductance # of measurements	water	#	00222	TCEQ SOP/Calculation	NA	NA	NA
Chlorophyll-*a*	water	µg/L	32211 - spec	EPA 446.0	a	±5%	1-50
Ammonium - nitrogen (NH_4^+ as N) Pore water ammonium – nitrogen (NH_4^+ as N	water sediment pore water	µmol/L µmol/L	NA NA	SM4500-F / UTMSI SOP SM4500-F / UTMSI SOP	0.1 0.1	±0.05 ±0.05	0-20 25-600
Nitrate + nitrite – nitrogen ((NO_3^- + NO_2^-) as N)	water	µmol/L	NA	EPA 353.4 / UTMSI SOP	0.05	±0.5	0-20
ortho-Phosphate- phosphorus (PO_4^{-3} as P)	water	µmol/L	NA	EPA 365.1 / UTMSI SOP	0.05	±0.3	0-5
Total suspended solids Residue, non-filterable	water	mg/L	00530	EPA 160.2 / UTMSI SOP	0.01	±0.5	0-50
Volatile suspended solids called “total organic carbon” by UTMSI	sediment	% dry wt	NA	EPA 160.4 / UTMSI protocol per QAPP	---	---	0-5
Grain size (clay, <0.0039 mm)	sediment	% dry wt	82009	UTMSI protocol per QAPP	1	±5	0 - 100

Analysis	Matrix	Units	STORET code	Analytical method	Sensitivity	Precision	Expected range
Grain size (silt, 0.0039 to 0.0625 mm)	sediment	% dry wt	82008	UTMSI protocol per QAPP	1	±5	0 - 100
Grain size (sand, 0.0625 to 2.0 mm)	sediment	% dry wt	89991	UTMSI protocol per QAPP	1	±5	0 - 100
Grain size (gravel, > 2.0 mm)	sediment	% dry wt	80256	UTMSI protocol per QAPP	1	±5	0 - 100

a - Depends on volume of water filtered.

Table 3. A7 – Water quality parameter measurement performance specifications for Port Bay.

Analysis	Matrix	Units	STORET code	Analytical method	Precision of LCS/LCSD (%RPD)	Bias of LCS (% Rec)	AWRL	LCRA Lab RL	Accuracy of RL (% Rec)	LCRA Lab MDL[2]
pH	water	standard units	00400	EPA 150.1 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Dissolved oxygen	water	mg/L	00300	EPA 360.1 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Specific conductance	water	µS/cm	00094	EPA 120.1 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Salinity	water	ppt	00480	SM 2520 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Temperature	water	ºC	00010	EPA 170.1 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Turbidity	water	NTU	82078	EPA 180.1 and TCEQ SOP	NA	NA	NA	NA	NA	NA
Secchi depth	water	meters	00078	TCEQ SOP	NA	NA	NA	NA	NA	NA
Surface irradiance	water	%	NA	Calculation	NA	NA	NA	NA	NA	NA
Light attenuation	water	$meters^{-1}$	NA	Calculation	NA	NA	NA	NA	NA	NA
Photosynthetically-active radiation	water	$\mu mol\ sec^{-1}\ meter^{-2}$	NA	UTMSI Protocol	5%	NA	NA	NA	NA	NA
Depth	water	meters	13850	TCEQ SOP	NA	NA	NA	NA	NA	NA
Days since last significant rainfall	NA	days	72053	TCEQ SOP	NA	NA	NA	NA	NA	NA
Tide stage	water	See TCEQ SOP	89972	TCEQ SOP	NA	NA	NA	NA	NA	NA
24-hr average temperature	water	ºC	00209	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Maximum daily temperature	water	ºC	00210	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Minimum daily temperature	water	ºC	00211	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
24-hr water temperature # of measurements	water	#	00221	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
24-hr average salinity	water	ppt	00218	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA

Analysis	Matrix	Units	STORET code	Analytical method	Precision of LCS/LCSD (%RPD)	Bias of LCS (% Rec)	AWRL	LCRA Lab RL	Accuracy of RL (% Rec)	LCRA Lab MDL[2]
Maximum 24-hr salinity	water	ppt	00217	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Minimum 24-hr salinity	water	ppt	00219	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
24-Hr salinity # of measurements	water	#	00220	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
24-hr average specific conductance	water	µS/cm	00212	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Maximum 24-hr specific conductance	water	µS/cm	00213	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Minimum 24-hr specific conductance	water	µS/cm	00214	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
24-hr specific conductance # of measurements	water	#	00222	TCEQ SOP/Calculation	NA	NA	NA	NA	NA	NA
Chlorophyll-*a*	water	µg/L	70953 - fluor	EPA 445.0	20	NA	3	2[4]	75-125	0.04
Pheophytin-*a*	water	µg/L	32213 - fluor	EPA 445.0	20	NA	NA	2[4]	75-125	0.04
Ammonia-nitrogen Pore water ammonia - nitrogen	water sediment pore water	mg/L mg/L	00610	EPA 350.1	20	70-130	0.02	0.02[4]	75-125	0.0048
Ammonium (NH_4^+) Pore water ammonium (NH_4^+)	water sediment pore water	µmol/L µmol/L	NA NA	SM 4500-F / UTMSI SOP[3] SM 4500-F / UTMSI SOP[3]	NA NA	NA NA	NA NA	NA NA	NA NA	NA NA
Nitrate + nitrite - nitrogen	water water	mg/L µmol/L	00630 NA	EPA 300.0 EPA 353.4 / UTMSI SOP[3]	20 NA	80-120 NA	0.04 NA	0.04[4] NA	75-125 NA	NA NA
Nitrate-nitrogen	water	mg/L	00620	EPA 300.0	20	80-120	0.02	0.02[4]	75-125	0.003
Nitrite-nitrogen	water	mg/L	00615	EPA 300.0	20	80-120	0.02	0.02[4]	75-125	0.003

Analysis	Matrix	Units	STORET code	Analytical method	Precision of LCS/LCSD (%RPD)	Bias of LCS (% Rec)	AWRL	LCRA Lab RL	Accuracy of RL (% Rec)	LCRA Lab MDL[2]
ortho-Phosphate - P	water	mg/L	00671 (fld filt <15 min)	EPA 365.1 EPA 300.0	20	80-120	0.04	0.04[4]	75-125	0.00249
	water	mg/L	70507 (lab filt >15 min)	EPA 365.1 EPA 300.0	20	80-120	0.04	0.04[4]	75-125	0.00249
	water	µmol/L	NA (fld filt <15 min)	EPA 365.1 / UTMSI SOP[3]	NA	NA	NA	NA	NA	NA
Total suspended solids	water	mg/L	00530	SM 2540 D	20	NA	4.0[1]	1.0	NA	0.5
Volatile suspended solids	water	mg/L	00535	EPA 160.4	20	NA	4.0[1]	1.0	NA	0.5
Grain size (clay, <0.002 mm)	sediment	% dry wt	49900	EPA 600/2-78-054	NA	NA	NA	NA	NA	NA
Grain size (silt, 0.002 to 0.05 mm)	sediment	% dry wt	49906	EPA 600/2-78-054	NA	NA	NA	NA	NA	NA
Grain size (sand, 0.05 to 2.0 mm)	sediment	% dry wt	49925	EPA 600/2-78-054	NA	NA	NA	NA	NA	NA
Grain size (gravel, > 2.0 mm)	sediment	% dry wt	80256	EPA 600/2-78-054	NA	NA	NA	NA	NA	NA
Total organic carbon	sediment	mg/kg	81951	SM 5310 B	30	65-135	NA	1500	65-135	NA

REFERENCES AND NOTES:
EPA = U.S. EPA. Methods for Chemical Analysis of Water and Waste, revised March 1983, Manual #EPA-600/4-79-020. Washington, DC.; U.S. EPA.
TCEQ SOP – Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue, 2008.
SM = American Public Health Association, *et al.* 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Washington, DC

1 - The AWRL is automatically determined by application of the rules for calculation and reporting of the TSS and VSS test results.
2 - The MDL is a measure of method sensitivity and it is defined at 40 CFR Part 136 Appendix B as "the minimum concentration of a substance that can be reported with 99% confidence that the analyte concentration is greater than zero." MDLs can be operator, method, laboratory, and matrix specific. Due to normal day-to-day and run-to-run analytical variability, MDLs may not be reproducible within a laboratory or between laboratories. The regulatory significance of the MDL is that EPA uses the MDL to determine when a contaminant is deemed to be detected and it can be used to calculate a PQL for that contaminant. Where an MDL is specified, LCRA will report values observed at or above the MDL and accompanying quality assurance information.
3 – Samples analyzed by UTMSI. See Table 2 for precision, sensitivity and expected range.
4 – Lab Reporting Level is based upon an aqueous sample where no dilution is required due to matrix or sample quantity.

Methods listed are the preferred method of analysis. Other methods may be employed and the data will be accepted as long as the methods used: (1) meet the sensitivity requirements of the AWRLs, and (2) are contained in 40 CFR 36, the most current version of Standard Methods, or are another reliable procedure as described in this QAPP.

Water and Sediment Quality Indicators

Sample collection and field measurements will be conducted as described in the TCEQ Surface Water Quality Monitoring Procedures Manual (Volumes 1 and 2) (TCEQ 2008, 2007).

Water and sediment quality samples collected at East Flats will be analyzed by UTMSI. Samples collected at Port Bay will be analyzed by UTMSI or LCRA. The measurement performance specifications in Table 2 will be met for parameters collected or measured in East Flats and analyzed by UTMSI and parameters collected in Port Bay and analyzed by UTMSI. The measurement performance specifications in Table 3 will be met for parameters collected or measured in Port Bay and analyzed by LCRA.

East Flats Water and Sediment Quality Objectives

Analyses conducted by UTMSI will use methods and meet specifications as described below and in Table 2.

Sensitivity

Sensitivity is defined as "the capability of a method or instrument to discriminate between measurement responses representing different levels of the variable of interest" and is presented for each measurement in Table 2.

Precision

The precision of data is a measure of the reproducibility of a measurement when a collection or an analysis is repeated. It is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions. If the relative percent difference (RPD) exceeds 20 percent, the data will be flagged or replaced, as described in Section B5.

Bias

Bias, defined as "systematic or persistent distortion of a measurement process that causes errors in one direction" may originate from calibration errors, sample contamination, or unaccounted for interference. Regular instrument calibration, acid-washing of sampling and laboratory containers and wearing of gloves when handling and processing samples will minimize bias. Bias for nutrient analyses is tested regularly by running standard curves of known values. Standard curves are prepared prior to processing each dataset and when using new chemicals. Only standard curves with greater than 98% accuracy are used. Precision, defined as the degree of mutual agreement among individual measurements, represents an estimate of random error. Collectively, bias and precision provide an estimate of total error or uncertainty associated with an individual measured value. Bias and precision goals may not be definable for all parameters due to the nature of the measurement type because "true" or expected values do not exist for some measurement parameters (Table 2).

Accuracy

Accuracy is a measure of the overall agreement of a measurement to a known value; accuracy includes a combination of random error (precision error) and systematic error (bias). A measurement is considered accurate when the value reported does not differ from the true value. Accuracy is verified through the analysis of calibration control standards. Field parameters and

water measured under this QAPP are collected utilizing multiparameter probe instruments, following SWQM SOPs. These instruments are calibrated prior to sampling utilizing standards of known values for pH, conductivity, and dissolved oxygen. The instruments are also checked against these standards following sampling, allowing for an assessment of accuracy. Accuracy of the temperature probe component of the instruments is checked periodically against a NIST traceable thermometer.

Representativeness

Representativeness is defined as "the degree to which the data accurately and precisely represent a characteristic of a population parameter, variation of a property, a process characteristic, or an operational condition." Representativeness applies to the location of sampling or monitoring sites, the collection of samples or field measurements, the analysis of those samples, and the types of samples being used to evaluate various aspects of data quality. Site selection, the appropriate sampling regime, the sampling of all pertinent media, and use of only approved analytical methods will assure that the measurement data represents the conditions at the site.

Comparability

Comparability is defined as "the confidence with which one data set can be compared to another." Comparability of reporting units and calculations, database management processes, and interpretative procedures must be assured if the overall goals of this project are to be realized. Confidence in the comparability of data sets for this project is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format.

Completeness

Completeness is defined as "a measure of the amount of data collected from a measurement process compared to the amount that was expected to be obtained under the conditions of measurement." An aspect of completeness that can be expressed for all data types is the amount of valid data (*i.e.*, not associated with some criteria of potential unacceptability) collected. A criteria ranging from 75 to 90 percent valid data from a given measurement process is suggested as being reasonable for this project.

Port Bay Water and Sediment Quality Objectives

Analyses conducted by UTMSI will use methods and meet specifications as described above and in Table 2. Analyses conducted by LCRA will use methods and meet specifications as described below and in Table 3.

Reporting Limits (RLs)

Ambient water reporting limits, or AWRLs, are not used in this project and are included only for reference. Ongoing ability to recover an analyte at the laboratory Reporting Limit (RL) is demonstrated through analysis of a calibration or check standard at the RL. The RLs for target analytes and performance limits at RLs for this project are set forth in Table 3. However, the reporting limit may be elevated due to dilution of sample to reduce sample matrix interferences,

or if a reduced quantity of sample is received requiring a dilution to meet method quantity requirements. For this project, low levels of certain parameters may reasonably be anticipated. For these parameters, Table 3 lists laboratory Method Detection Limits (MDLs). As per definition, the MDL is a measure of method sensitivity and it is defined at 40 CFR Part 136 Appendix B as "the minimum concentration of a substance that can be reported with 99% confidence that the analyte concentration is greater than zero." MDLs can be operator, method, laboratory, and matrix specific. Due to normal day-to-day and run-to-run analytical variability, MDLs may not be reproducible within a laboratory or between laboratories. Where an MDL is specified, the laboratory will report values observed at or above the MDL and accompanying quality assurance information. TPWD will use best professional judgment in qualifying values below the RL for use in data analysis and reporting

Precision

The precision of data is a measure of the reproducibility of a measurement when a collection or an analysis is repeated. It is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions. Performance limits for laboratory duplicates are defined in Table 3. Performance limits for field duplicates are defined in Section B5.

Bias

Bias is a statistical measurement of correctness and includes multiple components of systematic error. A measurement is considered unbiased when the value reported does not differ from the true value. Bias is verified through the analysis of laboratory control standards prepared with certified reference materials and by calculating percent recovery. Results are plotted on quality control charts, which are calculated based on historical data and used during evaluation of analytical performance. Program-defined measurement performance specifications for laboratory control standards are specified in Table 3.

Accuracy

Accuracy is a measure of the overall agreement of a measurement to a known value; accuracy includes a combination of random error (precision error) and systematic error (bias). A measurement is considered accurate when the value reported does not differ from the true value. Accuracy is verified through the analysis of laboratory spikes and calibration control standards. Performance limits for laboratory spikes and calibration control standards for RLs are specified in Table 3. Field parameters and water measured under this QAPP are collected utilizing multiparameter probe instruments, following SWQM SOPs. These instruments are calibrated prior to sampling utilizing standards of known values for pH, conductivity, and dissolved oxygen. The instruments are also checked against these standards following sampling, allowing for an assessment of accuracy. Accuracy of the temperature probe component of the instruments is checked periodically against a NIST traceable thermometer.

Representativeness

Site selection, the appropriate sampling regime, the sampling of all pertinent media according to TCEQ SOPs, and use of only approved analytical methods will assure that the measurement data represents the conditions at the site. See section B1 for a discussion of site selection criteria.

TPWD understands that it may contribute data to TCEQ for use in the assessment process, without providing all the samples required for assessment.

Comparability

Confidence in the comparability of data sets for this project is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP and in TCEQ SOPs. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format.

Completeness

The completeness of the data is basically a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project that 90% data completion is achieved.

Seagrass Condition Indicators

Seagrass samples will be collected in East Flats and Port Bay as described in Sections B1 and B2. Samples will be analyzed according to the specifications given in Table 4.

Table 4. A7 – Seagrass condition parameter measurement performance specifications.

Analysis	Matrix	Units	STORET Code	Analytical Method	Sensitivity	Precision	Expected Range
Biomass (above and below ground)	plant	g dry / meter^2	NA	UTMSI protocol / QAPP	0.001	±0.001	20-200
Root-to-shoot ratio	plant	---	NA	UTMSI protocol / QAPP	---	±0.01	0.5-8
Leaf area index	plant	meter^2 / meter^2	NA	UTMSI protocol / QAPP	0.0001	±0.0001	0-10
Blade width	plant	mm	NA	UTMSI protocol / QAPP	1.0	0.5	4-8
Shoot density	plant	shoots / meter^2	NA	UTMSI protocol / QAPP	40	±2%	5000-9000
Maximum depth limit	plant	meter	NA	UTMSI protocol / QAPP	4.0	0.1	0.5-2.0
C:N blade ratios	plant	---	NA	UTMSI protocol / QAPP	---	±2%	600:25
Seagrass isotopic abundance $\delta^{13}C$ and $\delta^{15}N$	plant	ppt	NA	UTMSI SOP / QAPP	0.1	±0.01	-8-to -20
Epiphytic algal biomass [a]	plant	% dry weight seagrass	NA	UTMSI protocol / QAPP	0.0001	±0.0001	0.1-100

Analysis	Matrix	Units	STORET Code	Analytical Method	Sensitivity	Precision	Expected Range
Macroalgae isotopic abundance $\delta^{13}C$ and $\delta^{15}N$	plant	ppt	NA	UTMSI SOP / QAPP	0.1	±0.01	-10 to -30
Drift macroalgal biomass	plant	g dry / meter^2	NA	UTMSI protocol / QAPP	0.0001	±0.0001	0.1-100

a – Measured by conventional techniques.
b – May also be measured as g dry / cm^2 for *Thalassia*.

Seagrass Epiphyte Fluorescence Measurements

Seagrass samples will be collected in East Flats and Port Bay as described in Sections B1 and B2 and analyzed by TAMU-CC. The objective is not more than 15% total error in fluorescence quantification of a given sample (Table 5).

Table 5. A7 – Seagrass epiphyte fluorescence measurement performance specifications.

Analysis	Matrix	Units	STORET Code	Analytical Method	Sensitivity	Precision	Expected Range
Red-excited fluorescence	plant	Arbitrary Fluorescence Units (F.U.)	NA	Red-excited fluorescence signal Cammarata protocol/QAPP	5 F.U.	±15%	NA
Scanned leaf area	plant	Number of pixels @ 200 µm	NA	Number of pixels of red-excited fluorescence Cammarata protocol/QAPP	NA	±15%	NA
Epiphyte load	plant	F. U.	NA	Green-excited fluorescence Cammarata protocol/QAPP	5 F.U.	±15%	NA
Normalized epiphyte load	plant	F.U./pixel and F.U./g dry biomass	NA	Calculated Cammarata protocol/QAPP	NA	±15%	1-300
Epiphyte recruitment and growth	plant	F.U. per unit leaf length	NA	Profile of epiphyte accumulation from leaf base Cammarata protocol/QAPP	NA	±15%	NA
Relative contribution green vs. red algal epiphyte load	plant	NA	NA	Calculated ratio of red-excited fluorescence signal to green-excited signal in removed epiphytes Cammarata protocol/QAPP	NA	NA	1-100

A8. Special Training/Certification

New field personnel will receive training in proper sampling and field analysis. Both field and UTMSI laboratory methods are described in standard operating procedures (SOPs) that are made available to all field personnel and analytical laboratories (see Appendix B). Before actual sampling or field analysis occurs, new field personnel will demonstrate to the Project Manager (or designee) their ability to properly calibrate field equipment and perform field sampling and analysis procedures. New laboratory personnel will undergo on-the-job training, which requires them to thoroughly review all methodologies and safety procedures prior to performing any lab work. During the training, personnel will be shown each laboratory procedure by an experienced

staff member. New personnel will practice laboratory procedures under supervision of experienced staff prior to analysis of actual project samples.

Dr. Pulich will provide training in groundtruthing, seagrass classification, and landscape indicator analysis for project personnel by conducting special training sessions.

A9. Documents and Records

In the field, data collected on site will be entered onto preprinted waterproof forms. Notes will be taken regarding date, time, personnel involved, weather conditions, samples collected and unique sample identification numbers. Deviations from standard sampling procedures or unusual occurrences will be noted.

Pertinent project documents and records will be retained as described in Table 6.

Table 6. A9 - Project documents and records.

Document/Record	Location	Retention (yrs)	Format
QAPPs, amendments and appendices	TPWD Project Manager office/ Austin	5 years	Paper, electronic
QAPP distribution documentation	TPWD Project Manager/ QAPO office/ Austin	5 years	Paper, electronic
Field SOPs	TPWD Project Manager / Austin All field offices / UTMSI and TPWD water quality program offices (Austin, Tyler, Waco)	5 years	Paper, electronic if available
Field equipment calibration/maintenance logs	TPWD Project Manager/ Austin All field offices / UTMSI and TPWD water quality program offices (Austin, Tyler, Waco)	5 years	Paper
Field instrument printouts	TPWD Project Manager/ Austin All field offices / UTMSI and TPWD water quality program offices (Austin, Tyler, Waco)	5 years	Paper, electronic
Field notebooks or data sheets	TPWD Project Manager/ Austin All field offices / UTMSI and TPWD water quality program offices (Austin, Tyler, Waco)	5 years	Paper
Field notebooks or data sheets	TPWD Project Manager/ Austin All field offices / UTMSI and TPWD water quality program offices (Austin, Tyler, Waco)	5 years	Paper

Document/Record	Location	Retention (yrs)	Format
Annual Project Report	TPWD Project Manager office/ Austin	5 years	Paper, electronic
Laboratory QA Manuals	Laboratory	5 years	Paper, electronic
Laboratory SOPs	Laboratory	5 years	Paper, electronic
Laboratory calibration records	Laboratory	5 years	Paper, electronic
Laboratory instrument printouts	Laboratory	5 years	Paper, electronic
Laboratory data reports/results	Laboratory	5 years	Paper, electronic
Laboratory equipment maintenance logs	Laboratory	5 years	Paper, electronic
Corrective Action Documentation	Laboratory	5 years	Paper, electronic
Diapositives	TPWD Project Manager office/ Austin	5 years	Film
Orthorectified digital imagery and analysis	TPWD Project Manager office/ Austin	5 years	Paper Electronic

Laboratory Data Reports

Data reports from laboratories will report the test results clearly and accurately. The test report will include the information necessary for the interpretation and validation of data and will include the following:

- name and address of the laboratory
- name and address of the client
- a clear identification of the sample(s) analyzed
- identification of samples that did not meet QA requirements and why (*e.g.*, holding times exceeded)
- date of sample receipt
- sample results
- field split results (as applicable)
- clearly identified subcontract laboratory results (as applicable)
- a name and title of person accepting responsibility for the report

- project-specific quality control results to include LCS sample results (% recovery), LCS duplicate results (%RPD), equipment, trip, and field blank results (as applicable), and RL confirmation (% recovery)
- narrative information on QC failures or deviations from requirements that may affect the quality of results

Electronic Data
Data will be submitted electronically to TPWD as Microsoft Excel files in the format required by TPWD for acceptance into the project database.

B1. Sampling Process Design

Site Selection

Primary consideration is given to accessibility and safety for all project sampling sites. Sites were chosen in coordination with UTMSI staff (Dr. Kenneth Dunton, Kim Jackson, and Chris Wilson) and Dr. Warren Pulich, Jr. based on seagrass presence, condition and adjacent land use (wastewater discharge or reference condition).

The project will use aerial photography, monitoring of seagrass condition and water quality indicators, and epiphyte analysis to study seagrass in East Flats and Port Bay (Figure 3). Site selection was based on two project goals: assessing wastewater permit limits at Port Bay and testing a proposed coastwide seagrass monitoring protocol. East Flats will serve as a reference site for Port Bay. Port Bay seagrasses have not been sampled previously. In contrast, a vast amount of data is available for East Flats, which will facilitate interpretation of data collected in this study.

TPWD and UTMSI staff will each participate in field and laboratory work for this project (Table 7). Water and sediment chemistry analyses will be conducted by UTMSI for East Flats and by UTMSI and LCRA for Port Bay. Seagrass, epiphyte and macroalgae biomass laboratory work will be conducted by UTMSI for East Flats and TPWD for Port Bay. Elemental analyses and isotope ratios will be conducted by UTMSI for both project locations. TAMU-CC will conduct analyses of seagrass epiphyte accumulation for both project locations. Aerial imagery will be acquired and analyzed for both project locations by TPWD in consultation with Dr. Warren Pulich, Jr.

Sample Frequency

Aerial imagery will be obtained at least once during the project. Seagrass condition and water quality indicators will be obtained in three sampling events, scheduled to allow for seasonal variations to be observed.

Transect Location

For seagrass condition and water quality indicators, the study design in both East Flats and Port Bay includes three transects that run roughly perpendicular to the shore and extend to the deep edge of the seagrass beds (Figure 4 and Figure 5). In East Flats, the transects were selected to encompass the deep edge of a healthy seagrass bed and to ensure the presence of multiple

seagrass species (mixed *Halodule* and *Thalassia* meadows). For Port Bay, one transect is located north of the proposed wastewater discharge, one south of the wastewater discharge and one located some distance away. The distant transect is in an area not expected to be impacted by the discharge and will serve as an internal reference. All Port Bay transects encompass the deep edge of a *Halodule* seagrass bed.

The 50-m transects are established by extending a meter tape. Ten quadrats (0.25 m^2) are placed along each transect at pre-determined random distances. Measurements, described below, are associated with a site, a transect and/or a quadrat.

Field Sampling Design

Seagrass condition, water and sediment quality, and seagrass epiphyte fluorescence samples will be taken along or near the selected transects. Water chemistry, instantaneous physicochemical measurements, long-term physicochemical measurements, Secchi depth, and light measurements will be sampled near the deep end of the transect (Figure 6). Seagrass cores, used to obtain seagrass condition measures, will be collected near the transect in an undisturbed area. Seagrass shoots, used for elemental and isotopic analysis and to determine epiphyte density by both conventional (biomass) and fluorescence techniques, will be taken near the transect in an undisturbed area. Sediment samples to determine grain size and total organic carbon will also be taken near the transect. Measurements to determine macroalgae biomass, elemental analysis and isotopic composition, sediment pore water ammonium, and seagrass cover will occur at or near ten randomly placed quadrats along the transects (Figure 7).

Aerial Imagery and Landscape Analysis

Photography Acquisition

Procedures for aerial imagery acquisition are described in the attached work plan for Krawietz Aerial Photography. Aerial imagery will be acquired at least once during the project at sites indicated in East Flats and Port Bay (yellow squares in Figure 3).

Groundtruthing

Ephemeral features such as drift macroalgae make it imperative to perform groundtruthing close to the time the aerial images are acquired. Groundtruthing of vegetation and landscape features in Port Bay and East Flats will be conducted shortly before or after the acquisition of the aerial imagery.

Figure 3. B1 - East Flats and Port Bay study sites showing aerial imagery acquisition areas.

Table 7. B1 – Sample design for East Flats.

Parameter	Indicator	Collected by	Analyzed by	Data Flow	Transects	Replicate per species	Number of Species	Total Replicates	Trips	Samples
Aerial imagery										
Aerial imagery and landscape analysis	Seagrass coverage, depth limit of seagrass coverage, bare patches (coverage, number, size), and macroalgae deposition (areal coverage).	Krawietz Aerial Photo-graphy	TPWD with Pulich	Krawietz provides georeferenced aerial imagery to TPWD	N/A	NA	NA	1	1	1
Water and sediment quality indicators										
Instantaneous physicochemical monitoring	Dissolved oxygen, salinity, temperature, pH, conductance	UTMSI	UTMSI	UTMSI sends TPWD copies of original field sheets and instrument pre- and post-calibration records	3	NA	NA	1	3	9
Light attenuation coefficient (*k*) and percent surface irradiance (% SI)	Photosynthetically active radiation (PAR) at surface & top of seagrass bed (or bay bottom), Secchi depth (3 replicates = 1 sample)	UTMSI	UTMSI	UTMSI sends TPWD copies of original field sheets and instrument pre- and post-calibration records	3	NA	NA	1	3	9
Long-term measurement of PAR	Long-term measurement of PAR (for minimum light requirements for seagrass)	UTMSI	UTMSI	UTMSI sends TPWD copies of original field sheets, instrument pre- and post-calibration records, and an electronic copy of data.	1	NA	NA	1	12	12
Long-term physicochemical monitoring	Salinity, temperature, depth, turbidity	UTMSI	UTMSI	UTMSI sends TPWD copies of original field sheets, instrument pre- and post-calibration records, and electronic copies of raw data	1	NA	NA	1	1	1
Water chemistry	Nitrate plus nitrite, ammonium, ortho-phosphate, chlorophyll-*a,* total suspended solids (TSS)	UTMSI	UTMSI	UTMSI sends copies of original field sheets, electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	2	3	18
Sediment chemistry	Sediment pore water ammonium	UTMSI	UTMSI	UTMSI sends copies of original field sheets, electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	10	3	90

Parameter	Indicator	Collected by	Analyzed by	Data Flow	Transects	Replicate per species	Number of Species	Total Replicates	Trips	Samples
Sediment chemistry	Grain size and TOC - lab analysis of sediment sample	UTMSI	UTMSI	UTMSI sends copies of original field sheets, electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	1	1	3
Seagrass condition indicators										
Seagrass coverage and species	Coverage estimated and seagrass species identified within a 0.25m^2 quadrat	UTMSI	UTMSI	UTMSI sends TPWD copies of original field sheets	3	NA	NA	10	3	90
Seagrass morphology	Core sample yielding above-ground biomass, below-ground biomass, root:shoot, leaf area index, blade width, and shoot density	UTMSI	UTMSI	UTMSI sends TPWD copies of original field and bench sheets	3	3	2	6	3	54
Seagrass condition	Isotopic (^{13}C and ^{15}N) and C:N obtained through elemental analysis	UTMSI	UTMSI	UTMSI sends copies of original field sheets, electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	3	2	6	3	54
Epiphyte biomass	Weight of epiphytes scraped off seagrass leaves	UTMSI	UTMSI	UTMSI sends TPWD copies of original field and bench sheets	3	10	2	20	3	180
Algal nutrient status	Isotopic (^{13}C and ^{15}N) and C:N obtained through elemental analysis	UTMSI	UTMSI	UTMSI sends copies of original field sheets, electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	6	3	54
Macroalgae biomass	Weight of macroalgae collected from 0.0625 m^2 quadrats along transect	UTMSI	UTMSI	UTMSI sends TPWD copies of original field and bench sheets	3	NA	NA	10	3	90
Videography	Videograph of transect	UTMSI	UTMSI	UTMSI sends electronic copy of digital videograph to TPWD.	3	NA	NA	1	3	9
Seagrass epiphyte fluorescence measurements										
Epiphyte biomass and accumulation	Seagrass epiphyte fluorescence measurements	UTMSI	TAMU-CC	UTMSI sends copies of original field sheets to TPWD. TAMU-CC sends copies of scans, electronic lab results and QA information (electronic or hard copy OK) to TPWD	3	9	2	18	3	162

Table 8. B1 – Sample design for Port Bay.

Parameter	Indicator	Collected by	Analyzed by	Data Flow	Transects	Replicate per species	Number of Species	Total Replicates	Trips	Samples
Aerial imagery										
Aerial imagery and landscape analysis	Seagrass coverage, depth limit of seagrass coverage, bare patches (coverage, number, size), and macroalgae deposition (areal coverage).	Krawietz Aerial Photo-graphy	TPWD with Pulich	Krawietz provides georeferenced aerial imagery to TPWD	NA	NA	NA	1	1	1
Water and sediment quality indicators										
Instantaneous physicochemical monitoring	Dissolved oxygen, salinity, temperature, pH, conductance	TPWD	TPWD	TPWD retains original field sheets and instrument pre- and post-calibration records	3	NA	NA	1	3	9
Light attenuation coefficient (*k*) and percent surface irradiance (% SI)	Photosynthetically active radiation (PAR) at surface & top of seagrass bed (or bay bottom), Secchi depth (3 replicates = 1 sample)	TPWD	TPWD	TPWD retains original field sheets and instrument pre- and post-calibration records	3	NA	NA	1	3	9
Long-term physicochemical monitoring	Salinity, temperature, depth, turbidity	TPWD	TPWD	TPWD retains original field sheets, instrument pre- and post-calibration records and electronic copies of raw data	3	NA	NA	1	1	3
Water chemistry	Chlorophyll-*a*, TSS	TPWD	LCRA	TPWD retains original field sheets. LCRA sends electronic and paper results and QA/QC information to TPWD.	3	NA	NA	2	3	18
Water chemistry	Nitrate plus nitrite, ammonium, ortho-phosphate	TPWD	UTMSI	TPWD retains original field sheets. UTMSI sends electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	2	3	18
Sediment chemistry	Sediment pore water ammonium	TPWD	UTMSI	TPWD retains original field sheets. UTMSI sends electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	10	3	90

Parameter	Indicator	Collected by	Analyzed by	Data Flow	Transects	Replicate per species	Number of Species	Total Replicates	Trips	Samples
Sediment chemistry	Grain size and TOC	TPWD	LCRA	TPWD retains original field sheets. LCRA sends electronic and paper results and QA/QC information to TPWD.	3	NA	NA	1	1	3
Seagrass condition indicators										
Seagrass coverage and species	Coverage estimated and species identified within a 0.25m^2 quadrat	TPWD	TPWD	TPWD retains original field sheets	3	NA	NA	10	3	90
Seagrass morphology	Core sample yielding above-ground biomass, below-ground biomass, root:shoot, leaf area index, blade width, and shoot density	TPWD	TPWD	TPWD retains original field and bench sheets	3	3	1	3	3	27
Seagrass condition	Isotopic (^{13}C and ^{15}N) and C:N obtained through elemental analysis	TPWD	UTMSI	TPWD retains original field sheets. UTMSI sends electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	3	1	3	3	27
Epiphyte biomass	Weight of epiphytes scraped off seagrass leaves	TPWD	TPWD	TPWD retains original field and bench sheets	3	10	1	10	3	90
Algal nutrient status	Isotopic (^{13}C and ^{15}N) and C:N obtained through elemental analysis	TPWD	UTMSI	TPWD retains original field sheets. UTMSI sends electronic lab results and QA/QC information (electronic or hard copy) to TPWD	3	NA	NA	6	3	54
Macroalgae biomass	Weight of macroalgae collected from 0.0625 m^2 quadrats along transect	TPWD	TPWD	TPWD retains original field and bench sheets	3	NA	NA	10	3	90
Videography	Videograph of transect	TPWD	TPWD	TPWD retains electronic copy of videograph.	3	NA	NA	1	3	9
Seagrass epiphyte fluorescence measurements										
Epiphyte biomass and accumulation	Seagrass epiphyte fluorescence measurements	TPWD	TAMU-CC	TPWD retains original field sheets. TAMU-CC sends copies of scans, electronic lab results and QA information (electronic or hard copy OK) to TPWD	3	9	1	9	3	81

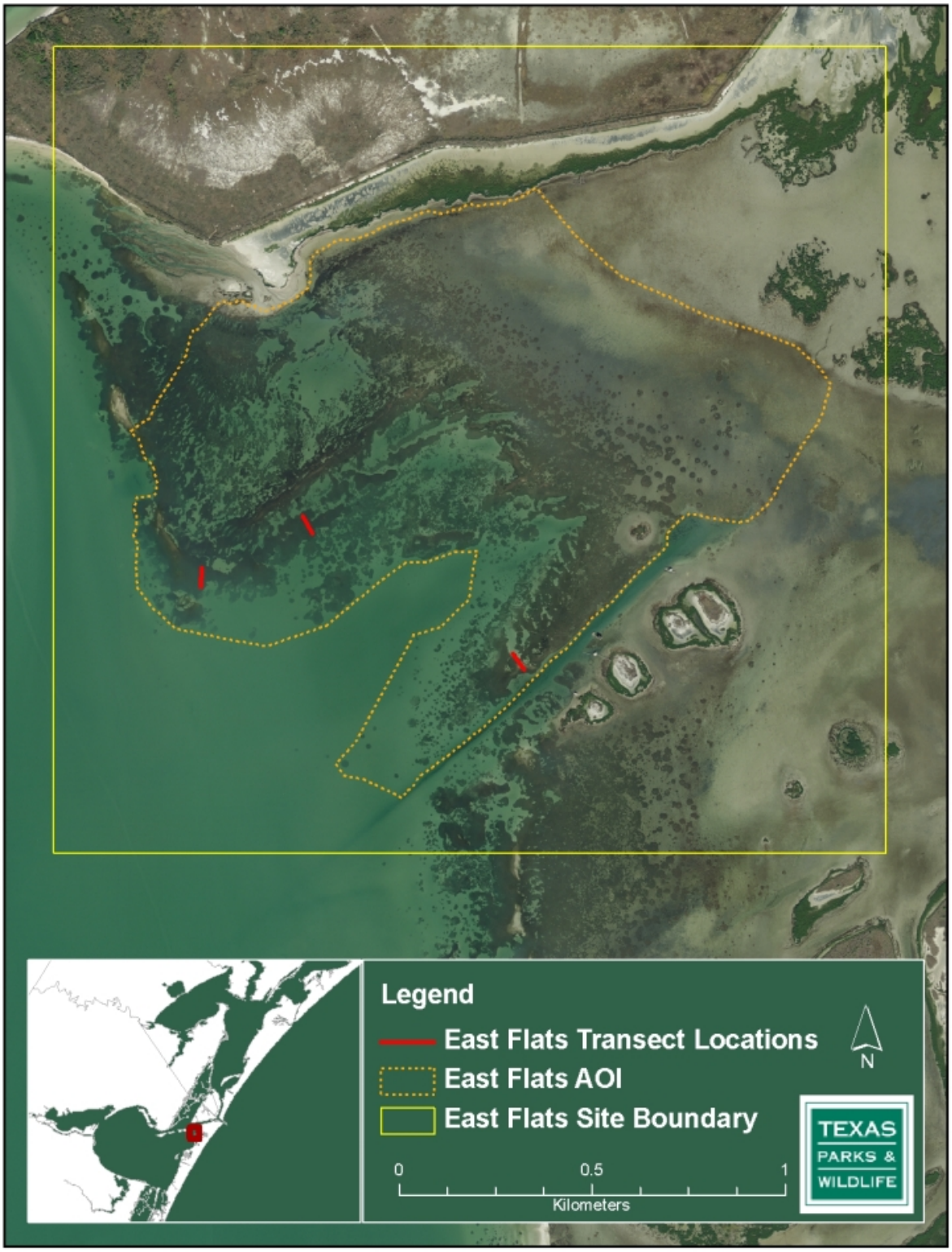


Figure 4. B1 - East Flats aerial image acquisition area, area of interest (AOI) and transects.

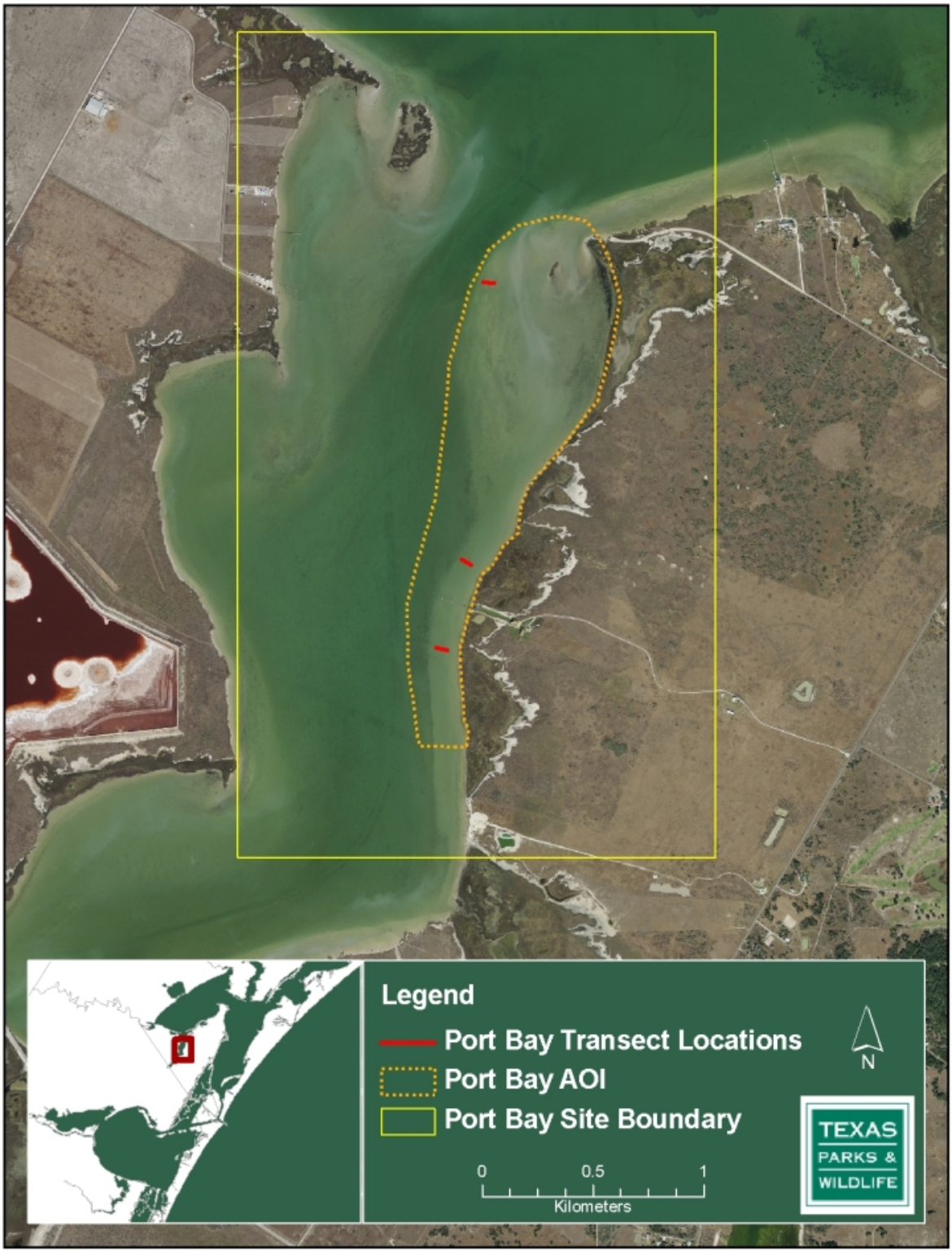


Figure 5. B1 – Port Bay aerial image acquisition area, area of interest (AOI) and transects.

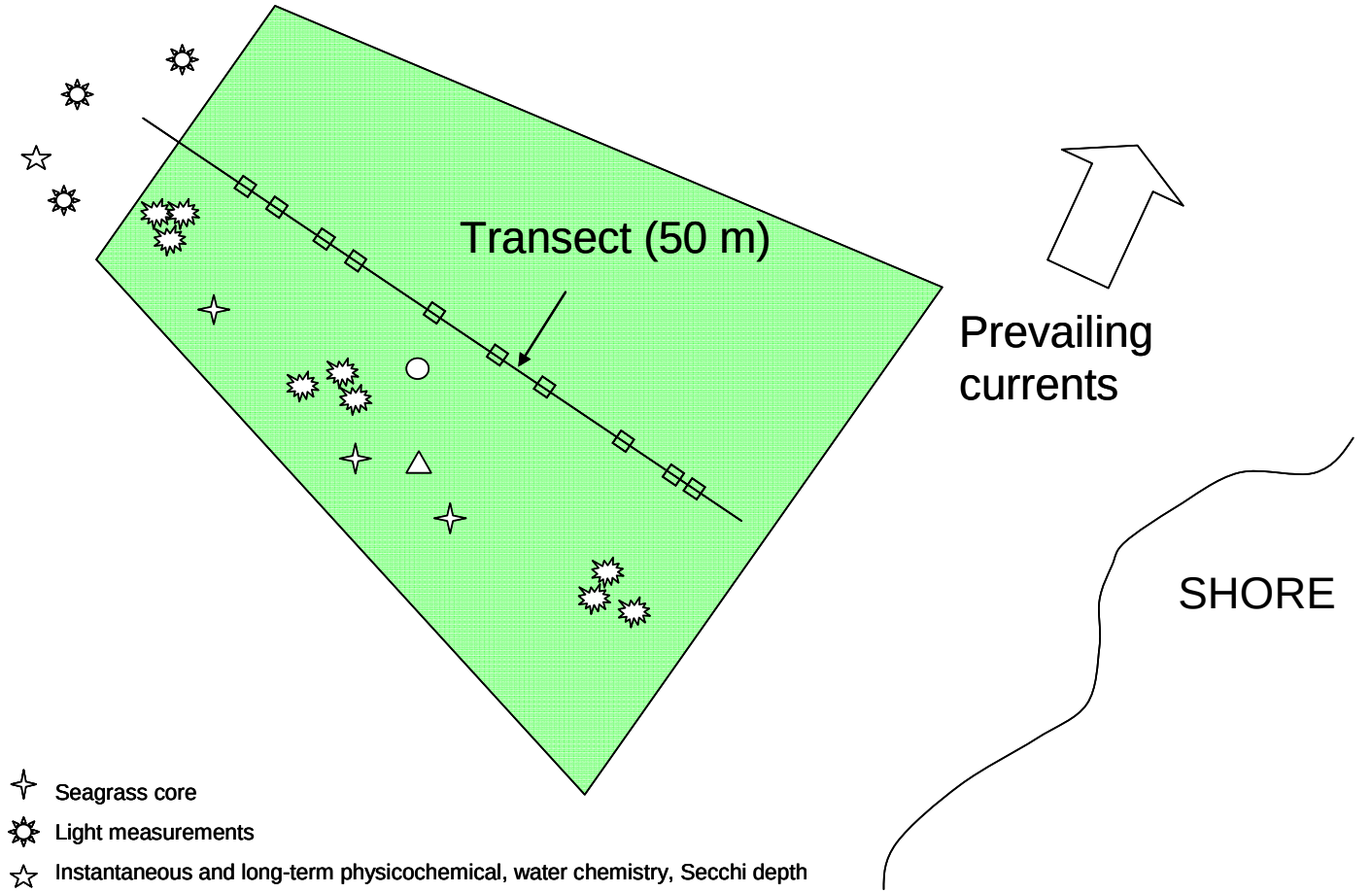


Figure 6. B1 - Schematic of field sampling design.

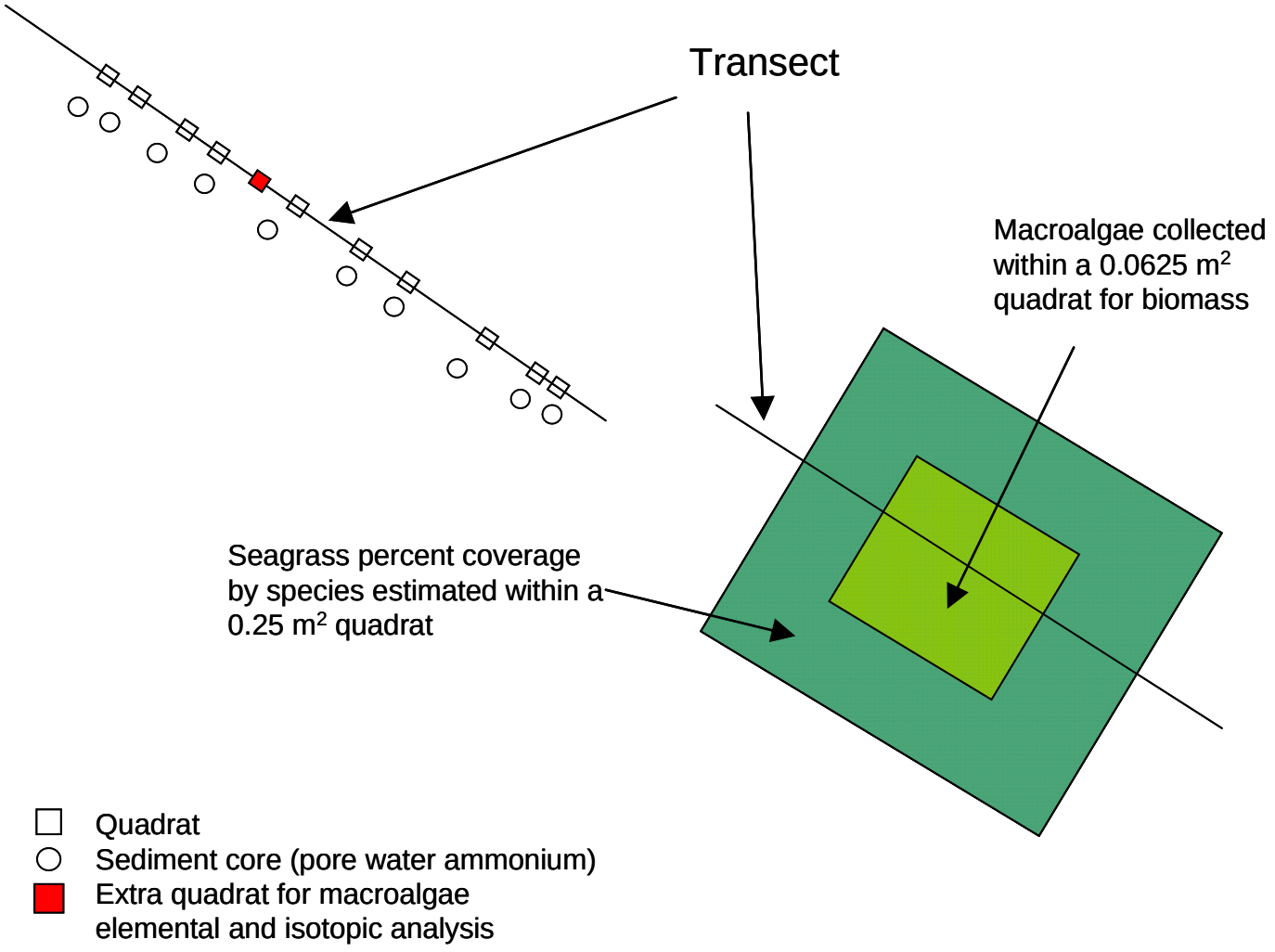


Figure 7. B1 - Close-up of field sampling design showing quadrat.

B2. Sampling Methods

Aerial Imagery and Landscape Analysis

Photography Acquisition

Aerial imagery will be acquired in accordance with the attached work plan. Positional accuracy may be enhanced by use of ground control points, such as white reflective, plastic targets placed within the boundaries of each of the areas to be photographed. GPS will be used to precisely locate control points to 1 m or better positional accuracy. Differential correction of GPS coordinates will be performed by comparing field unit values to reference station values.

Groundtruthing

Groundtruthing sampling will be conducted from shallow-draft boats to identify indicator classes (seagrass species, macroalgae and bare area). For each site (East Flats and Port Bay), 75 points will be obtained. Each point will be representative of an area at least $2m^2$ (and preferably $5m^2$) centered on the point. Seagrass cover will be discriminated visually as either patchy (1-50% cover per m^2) or continuous (51-100% cover per m^2) within the representative area. GPS will be used to locate points to 1 m or better positional accuracy. Differential correction of GPS coordinates will be performed by comparing field unit values to reference station values.

Water and Sediment Quality Indicators

Field Sampling Procedures

Field sampling procedures for water chemistry, Secchi depth, and physicochemical measurements are described in the *TCEQ Surface Water Quality Monitoring Procedures Manual* (Volume 1) (TCEQ 2008).

Two replicate samples will be taken for each of the following measurements: inorganic nitrogen (ammonium (NH_4^+), nitrate (NO_3^-) plus nitrite (NO_2^-)), ortho-phosphate (PO_4^{-3}), total suspended solids (TSS) and chlorophyll-*a*. Samples will be analyzed by UTMSI staff. For Port Bay, pending availability of funds, field splits may also be analyzed by LCRA. UTMSI will collect two replicate samples from East Flats for TSS and chlorophyll-*a* analysis in their laboratory. TPWD will collect single samples from Port Bay for TSS and chlorophyll-*a* analysis by LCRA.

Pore water ammonium will be determined from sediment cores taken near each of the 0.25 m^2 quadrats. Samples will be collected separately in sterile Whirlpak bags. For Port Bay, pending availability of funds, field splits may also be analyzed by LCRA.

TPWD and UTMSI will collect sediment grain size and total organic carbon approximately 16 oz. samples in Whirlpak bags. UTMSI will analyze East Flats samples. LCRA will analyze Port Bay samples. The UTMSI sediment organic carbon analysis seems to correspond most closely to the LCRA volatile suspended solids method. To ensure comparability of data, East Flats field

splits will be analyzed by LCRA and Port Bay samples will also be analyzed by LCRA for volatile suspended solids, pending availability of funds.

Dissolved oxygen, conductivity, salinity, pH and temperature will be measured in the field using pre- and post-calibrated datasondes. Secchi depth and water depth will be measured on each visit at each transect. The maximum depth penetration of seagrasses at each site will be determined from depth measurements made at the deep edge of continuous seagrass meadows closest to the site.

Measurements of percent surface irradiance (% SI) and the diffuse light attenuation coefficient (*k*) will be made from measurements of surface and underwater irradiance as described in Appendix B.

For each type of measurement, field staff will ensure that the site is not disturbed prior to sample collection. Typically, measurements at the deep water end of a transect will be made first (water chemistry samples, instantaneous physicochemical measurements, light measurements and Secchi depth measurements), as these can be made from a boat before any other field work. Next, the transect will be laid and measurements made at the ten quadrats (videography, seagrass coverage, macroalgae biomass, elemental analysis and isotopic analysis samples, and pore water ammonium samples). When transect sampling is completed, seagrass cores and shoots will be collected in the vicinity of the transect. This collection will be done on the "up-current" side of the transect to prevent sample contamination. Finally, a datasonde will be deployed within the mixed surface layer at the deep water end of the transect to obtain long term physicochemical measurements.

High density polyethylene containers will be used to collect water for analysis. When acidification is required the containers will be acidified with concentrated sulfuric acid in the field or a pre-acidified container will be used to reduce the pH to less than 2. The acidified container will be marked with an "X" on the cap, designating that it is chemically preserved. Each container will be labeled per TCEQ SWQM labeling guidelines and chain of custody. All samples will be placed in coolers immediately being at least half full of ice for preservation at 4°C ±2° and transportation to the analytical laboratories within holding times.

Minimum sample volume, container types, preservation requirements, and holding time requirements may vary depending on the laboratory and field QA/QC measures. Typical requirements are given below.

Table 9. B2 – Typical water chemistry sample storage, preservation and handling requirements.

Parameter	Matrix	Container	Preservation	Minimum Sample Volume	Holding Time
TSS/VSS	water	high density polyethylene	<6º C	1000 mL	7 days
ortho-Phosphate	water	high density polyethylene	<6º C	100 mL	48 hrs
Nitrate-nitrogen	water	high density polyethylene	<6º C	100mL	48 hrs
Nitrite- nitrogen	water	high density polyethylene	<6º C	100 mL	48 hrs
Ammonia-nitrogen	water	high density polyethylene	<6º C, pH<2 with H_2SO_4	100 mL	28 days
Chlorophyll-*a*	water	Amber high density polyethylene	<6º C, dark	500 mL	filter < 48 hrs; filter may be stored 21 days
TOC	sediment	glass or Whirlpak	<6º C	100 g	28 days
Grain size (texture)	sediment	glass or Whirlpak	<6º C	100 g	28 days
UTMSI sediment pore water ammonium	sediment	Whirlpak	<6º C	60 mL	30 days frozen

Processes to Prevent Contamination

Procedures outlined in the TCEQ *Surface Water Quality Procedures Manual (Volume 1)* outline the necessary steps to prevent contamination of samples. This includes direct collection into sample containers. Field QC samples (identified in Section B5) are collected to verify that contamination has not occurred.

Seagrass Condition Indicators

Replicate cores will be used for estimates of seagrass condition indicators (above and below ground biomass, root-to-shoot ratio, leaf area index, blade width and length, shoot density) as described in Appendix B. Typically three cores will be taken of each species present at each site. Species present (*i.e.* seagrass species composition) will be determined by visual in situ analysis of plants observed within a 25 m radius of each site. Elemental analysis (C:N), isotopic analysis (^{13}C and ^{15}N) and estimates of algal epiphyte biomass will be made from separate leaf samples of entire shoots taken directly adjacent to the seagrass cores as described in Appendix B.

Macroalgae biomass will be determined from the collection of all algal material within each of ten 0.0625 m^2 quadrats as described in Appendix B.

Seagrass Epiphyte Fluorescence Measurements

Samples for epiphyte fluorescence measurements will be collected near quadrats representing the shallow end, middle and deep end of each transect as described in Appendix C. Three replicate

samples will be obtained for each significant seagrass species at each quadrat (nine samples per seagrass species for each transect). Seagrass samples will be obtained from locations displaced "up-current" from the transects in order to avoid excessive disturbance caused by other sampling activities. Sampling and fluorescence epiphyte measurements will be performed separately for each seagrass species whose abundance is estimated to exceed 20% of the total seagrass coverage at a quadrat site. Thus, if two species are present at >20% of the total seagrass coverage, *separate* samples will be obtained for each seagrass species. Single-species seagrass shoot samples (up to 50) will be obtained by gently pinching or cutting off shoots near their base, handling only at the base to avoid disturbing attached epiphytes and transferring to widemouth sample bottles.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data sheets. The following will be recorded for all visits:

1. Station ID or latitude and longitude information
2. Location
3. Sampling time
4. Sampling date
5. Sampling depth
6. Sample collector's name/signature
7. Values for all measured field parameters
8. Detailed observational data, including:
    a) water appearance
    b) weather
    c) days since last significant rainfall
    d) flow severity
9. Other observational data (*as applicable*), including:
    a) biological activity
    b) pertinent observations related to water quality or stream uses (*e.g.*, exceptionally poor water quality conditions/standards not met; uses such as swimming, boating, fishing, etc.)
    c) unusual odors
    d) specific sample information (number of sediments grabs, type/number of fish in a tissue sample, etc.)
    e) missing parameters (*i.e.*, when a scheduled parameter or group of parameters is not collected)
    f) algal blooms, fish kills, or pollution complaints

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:

1. Legible writing in indelible ink with no modifications, write-overs or cross-outs;
2. Correction of errors with a single line followed by an initial and date;
3. Close-out on incomplete pages with an initialed and dated diagonal line.

Deviations from Sampling Method Requirements or Sample Design, and Corrective Action

Examples of deviations from sampling method requirements or sample design include but are not limited to such things as inadequate sample volume due to spillage or container leaks, failure to preserve samples appropriately, contamination of a sample bottle during collection, storage temperature and holding time exceedance, sampling at the wrong site, etc. Any deviations will invalidate resulting data. Corrective action may include samples being discarded and if possible, re-collected.

B3. Sample Handling and Custody

Aerial Imagery and Landscape Analysis

The Project Manager will make arrangements with the aerial photography vendor to fly the photo mission. The diapositive, orthorectified digital imagery, and supporting documents will be delivered directly by the vendor to the Project Manager.

Water and Sediment Quality Indicators, Seagrass Condition Indicators, and Seagrass Epiphyte Fluorescence Measurements

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. The chain-of-custody (COC) form is used to document sample handling during transfer from the field to the laboratory and among subcontract laboratories. The following information concerning the sample is recorded on the COC form.

1. Date and time of collection
2. Site identification
3. Sample matrix
4. Number of containers
5. Preservative used or if the sample was filtered
6. Analyses required
7. Name of collector
8. Custody transfer signatures and dates and time of transfer
9. Bill of lading (*if applicable*)

Sample Labeling

Samples are labeled on the container with an indelible marker. Label information includes:

1. Site identification
2. Date and time of sampling
3. Replicate number (if applicable)
4. Preservative added, if applicable
5. Designation of field-filtered, if applicable
6. Sample type

Sample Handling
The samples will be transported or shipped to the designated laboratory within the required holding times for the various parameters being analyzed. The sample custody will be transferred to the laboratory custodian and the samples left there for analyses.

Failures in Chain-of-Custody and Corrective Action
Failures associated with chain-of-custody procedures are immediately reported to the TPWD Project Manager. These may include delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TPWD Project Manager, in consultation with the QAPO, will determine if the procedural violation may have compromised the validity of the resulting data and how the issue will be resolved based on best professional judgment. Possible courses of action include, document and proceed; redo the entire sampling event; or selectively analyze the samples. Corrective action documentation is maintained by the TPWD.

B4. Analytical Methods

Aerial Imagery and Landscape Analysis

Landscape features will be delineated from the orthorectified digital imagery using standard image analysis software, such as ERDAS™, ENVI™, or ArcGIS™, to classify the digital imagery into three classes (seagrass, macroalgae and bare area) and to quantify seagrass coverage, depth limit of seagrass coverage, bare patches (number, size), and macroalgae deposition (areal coverage). Classification of Intensity-Hue-Saturation (IHS) transformed data will be used to separate the bare areas in the image from vegetated features (seagrass and macroalgae), producing a two-class image (Figure 8 and Figure 9). The two classes (bare and vegetated) may be stored in separate files for further processing.

The vegetated class will be further processed by separating seagrass and macroalgae. If possible, an automated classification technique will be performed. If the vegetation classes are not sufficiently spectrally distinct to automate the process, manual delineation of macroalgae will be performed. Bare patch metrics (number, size) will be determined by counting the bare-to-seagrass transitions along virtual transects. A "patchiness ratio" will be determined for each transect by dividing the number of transitions by the length of the transect. Additionally, areas of patchy and continuous seagrass cover may be quantified.

The depth limit of seagrass coverage will be obtained using imagery analysis combined with field measurements of depth. Imagery will be analyzed to obtain an approximate contour of the deep edge of seagrass beds. Coordinates of ten points along this contour will be specified. These coordinates will be used to guide field confirmation of seagrass at the deep edge. Measurements of depth at the observed deep edge, along with GPS coordinates, will be obtained. Field measurements will be made either during the groundtruthing sampling or following analysis of imagery. If field measurements are made during groundtruthing, initial estimates of the deep edge will be made using 2009 National Agricultural Imagery Program (NAIP) data (TNRIS 2009). Otherwise, imagery obtained during this project will be used for initial estimates. GPS will be used to precisely locate deep edge points to 1m or better positional

accuracy. Differential correction of GPS coordinates will be performed by comparing field unit values to reference station values.

Specific steps for processing orthorectified digital imagery

a. Apply image-processing techniques to orthorectified digital imagery using ERDAS™, ENVI™, or ArcGIS™ software to separate bare areas from vegetated areas.
b. Conduct further classification analysis on vegetated masks to identify and separate seagrass and macroalgae.
c. Conduct further classification analysis of bare areas to identify number and size of distinct bare patches.
d. Obtain seagrass depth limit estimate by combination of analysis and field measurements. Analyze imagery to identify deep edge of seagrass. Conduct field measurements of depth at specified locations. Estimate average depth of seagrass deep edge.
e. Perform accuracy assessment.

Specific steps for processing of GPS data used for control points and groundtruthing

a. Field notes from each GPS survey point are checked.
b. Download GPS data from the units onto the GIS processing computer and perform postprocessing using Pathfinder Office™ or equivalent software. Differential correction of coordinates is accomplished by comparing field unit values to reference station values.
c. Produce final GPS datasets by converting the coordinate data files into ArcView shapefiles (.shp).

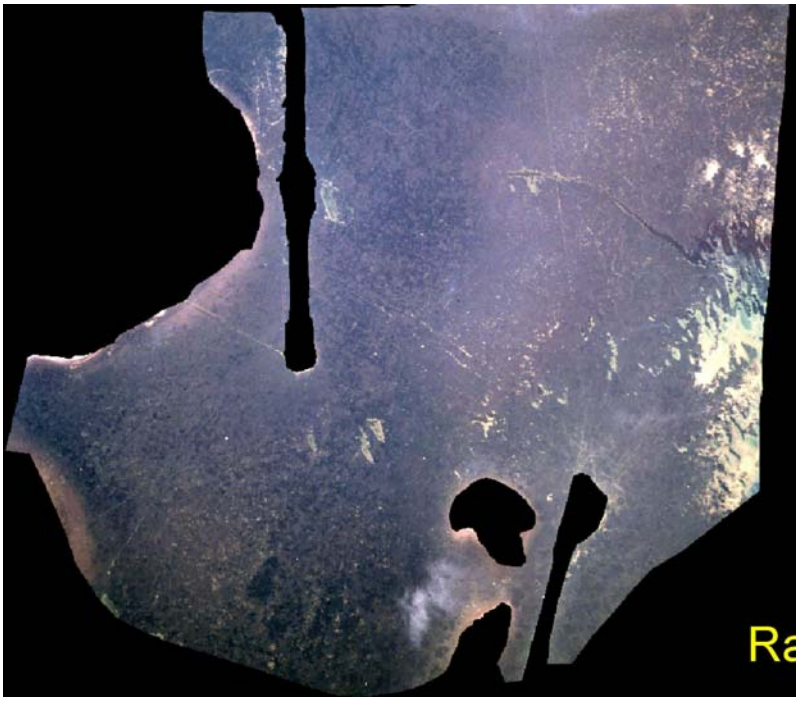


Figure 8. B4 - Sample digital photograph at 1:9,600 **scale of North Redfish Bay (2003).**

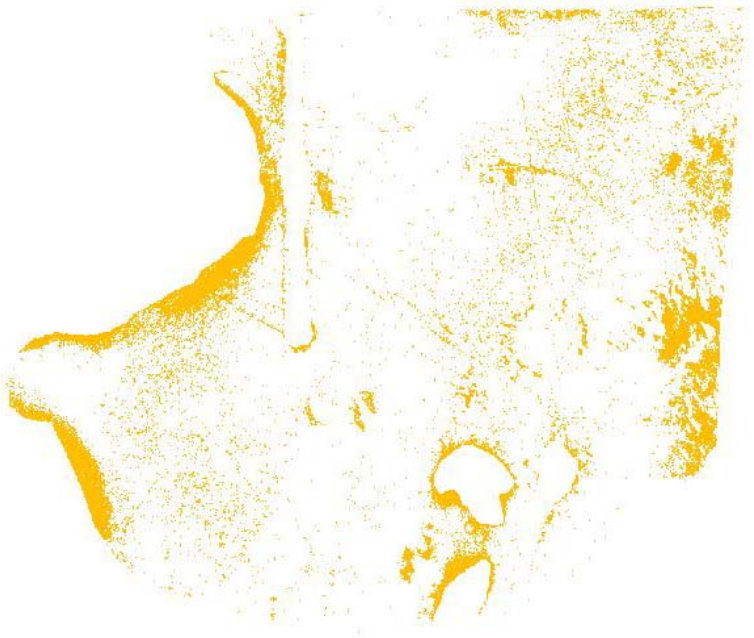


Figure 9. B4 - Image mask, extracted from Figure 8, showing bare landscape features.

Water and Sediment Quality Indicators

Laboratory analysis techniques will be in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the *TCEQ Surface Water Quality Monitoring Procedures Manual*, 40 CFR 136, or other reliable procedures as described in this QAPP.

East Flats

Water and sediment chemistry samples from East Flats will be analyzed by UTMSI using procedures described in Appendix B.

Port Bay

Water and sediment chemistry samples from Port Bay will be analyzed by UTMSI using procedures described in Appendix B or LCRA using methods described in Table 3.

Seagrass Condition Indicators

Seagrass condition indicators will be analyzed using procedures described in Appendix B.

Seagrass Epiphyte Fluorescence Measurements

A novel fluorescence technique will be used to measure the abundance and accumulation profiles of epiphytes on seagrasses according to a method described in Appendix C.

The abundance of epiphytes is believed to be an integrated measure of nutrient conditions in a seagrass bed. This method measures fluorescence of photosynthetic accessory pigments as a

proxy for epiphyte abundance. It enables plotting of incremental epiphyte abundance along the age gradient of the seagrass leaf, providing a record of epiphyte recruitment and growth relative to the growth of the seagrass leaf. The leaf area for a seagrass sample can also be estimated.

This method digitally images epiphytes which absorb light in the green range of the visible spectrum (532 nm) and emit fluorescence at wavelengths between 550 nm and 610 nm. These organisms include cyanobacteria, red algae, diatoms, cryptomonads, brown algae and dinoflagellates. The method is based on the preferential excitation and fluorescence emission of epiphytes relative to the underlying seagrass leaf.

Scanning seagrass leaves by this method does not quantify green algal components of seagrass epiphytes, because the red light needed to excite the chlorophylls of the green algae also excites the seagrass leaf pigments. If epiphytes are removed from the seagrass blade by scraping, then removed epiphytes can be fluoresced and quantified using *both* red and green excitation wavelengths. This provides a measure that includes all of the different types of epiphytic algae, including green. Changes in the relative contributions of green and red algae to total epiphyte abundance can be captured by comparing the ratio of red-excited fluorescence to green-excited fluorescence.

Standards Traceability
All standards used in the field and laboratory are traceable to certified reference materials. Standards preparation is fully documented and maintained in a standards log book. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number; date prepared, expiration date and preparer's initials/signature. The reagent bottle is labeled in a way that will trace the reagent back to preparation.

Analytical Method Modification
Only data generated using approved analytical methodologies as specified in this QAPP will be submitted to the TCEQ.

Failures or Deviations in Analytical Method Requirements and Corrective Actions
Failures in field and laboratory measurement systems involve, but are not limited to, instrument malfunctions, failures in calibration, blank contamination, QC sample problems (*i.e.*, poor spike recoveries), etc. In many cases, the field technician or lab analyst will be able to correct the problem (*i.e.*, via re-calibration or re-analysis). If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the respective supervisor, who will make the determination. If the analytical system failure compromises the sample results, the data will not be reported to the TCEQ as part of this study. The nature and disposition of the problem is documented on the data report which is sent to the TPWD Project Manager. Corrective action documentation is maintained by the TPWD.

B5. Quality Control

Aerial Imagery and Landscape Analysis

The vendor is required to acquire aerial photography according to the attached work plan and meet prescribed specifications of resolution, scale, weather and clear water conditions for accurate seagrass delineation. The project Manager will accept or reject photography based on these specifications.

Thematic accuracy of the classified digital photos must have at least 80% overall accuracy as determined by analysis of groundtruth points. Additional groundtruth points will be obtained if the specified accuracy is not met.

Water and Sediment Quality Indicators

Field QC - The minimum Field QC Requirements are outlined in the *TCEQ Surface Water Quality Monitoring Procedures Manual* (Volume 1). Field instruments (for example, multi-parameter datasondes) will be calibrated against known standards, following the specified procedures, within 24-hours prior to sampling. Standards will not be used if they have expired (exceeded shelf life clearly labeled on standards container). If a field instrument does not pass pre-sampling calibration, it will not be used to collect data. Within 24-hours following sampling, field instruments will be checked against calibration standards to ensure that measurements are within required limits as specified in the TCEQ SOPs. Data collected by instruments which do not meet the post-calibration check requirements will be flagged. Pre- and post- sampling calibrations will be recorded.

Irradiance values will be compared with other sensors calibrated against a National Bureau of Standards (NBS) standard.

Field Split - A field split is a single sample subdivided by field staff immediately following collection and submitted to the laboratory as two separately identified samples according to procedures specified in the SWQM Procedures Manual. Split samples are preserved, handled, shipped, and analyzed identically and are used to assess variability in all of these processes. Field splits apply to conventional samples only and are collected on a 10% basis or one per batch, whichever is greater. The precision of field split results is calculated by relative percent difference (RPD) using the following equation:

$$RPD = (X1-X2)/((X1+X2)/2))*100$$

A 30% RPD criteria will be used to screen field split results as a possible indicator of excessive variability in the collection and analytical system. If it is determined that meaningful quantities of constituent were measured and analytical variability can be eliminated as a factor, than variability in field split results will primarily be used as a trigger for discussion with field staff to ensure samples are being handled in the field correctly. Some sample results or batches of samples may be invalidated based on the examination of all extenuating information.

Professional judgment during data validation will be relied upon to interpret the results and take appropriate action.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria
Detailed laboratory QC requirements and corrective action procedures are contained within the individual laboratory procedures. The minimum requirements that all participants abide by are stated below. Lab QC sample results are submitted with the laboratory data report.

LCRA Analyses
Reporting Limit Verification - To demonstrate the ongoing ability to recover at the reporting limit, the laboratory will analyze a calibration standard (if applicable) at or below the reporting limit on each day samples are analyzed. Two acceptance criteria will be met or corrective action will be implemented. First, calibrations including the standard at the reporting limit will meet the calibration requirements of the analytical method. Second, the instrument response (*e.g.*, absorbance, peak area, etc.) for the standard at the reporting limit will be treated as a response for a sample by use of the calibration equation (*e.g.*, regression curve, etc.) in calculating an apparent concentration of the standard. The calculated and reference concentrations for the standard will then be used to calculate percent recovery (%R) at the reporting limit using the equation:

$$\%R = CR/SA * 100$$

where CR is the calculated result and SA is reference concentration for the standard. Recoveries must be within 75-125% of the reference concentration.

When daily calibration is not required (*e.g.*, EPA Method 624), or a method does not use a calibration curve to calculate results, the laboratory will analyze a check standard at the reporting limit each day samples are analyzed. The check standard does not have to be taken through sample preparation, but must be recovered within 75-125% of the reference concentration for the standard. The percent recovery of the check standard is calculated using the following equation in which %R is percent recovery, SR is the sample result, and SA is the reference concentration for the check standard:

$$\%R = SR/SA * 100$$

If the calibration (when applicable) or the recovery of the calibration or control standard is not acceptable, corrective actions (*e.g.*, re-calibration) will be taken to meet the specifications before proceeding with analyses of samples.

The laboratory will report results of quantitation checks with the data.

Laboratory Control Standard (LCS) - A LCS consists of analyte-free water spiked with the analyte of interest prepared from standardized reference material. The LCS is spiked into laboratory-pure water at a level less than or equal to the mid-point of the calibration curve for each analyte. The LCS is carried through the complete preparation and analytical process. The LCS is used to document the bias of the analytical process. LCSs are run at a rate of one per

batch. Results of LCSs are calculated by percent recovery (%R), which is defined as 100 times the measured concentration, divided by the true concentration of the spiked sample.

The following formula is used to calculate percent recovery, where %R is percent recovery; SR is the measured result; SA is the true result

$$\%R = SR/SA * 100$$

Performance limits and control charts are used to determine the acceptability of LCS analyses. Project control limits are specified in Table 3.

Laboratory Duplicates - A laboratory duplicate is prepared in the laboratory by splitting aliquots of an LCS. Both samples are carried through the entire preparation and analytical process. LCS duplicates are used to assess precision and are performed at a rate of one per batch.

For most parameters, precision is calculated by the relative percent difference (RPD) of LCS duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

$$RPD = (X_1 - X_2)/\{(X_1+X_2)/2\} * 100$$

Laboratory equipment blank - Laboratory equipment blanks are prepared at the laboratory where collection materials for metals sampling equipment are cleaned between uses. These blanks document that the materials provided by the laboratory are free of contamination. The QC check is performed before the metals sampling equipment is sent to the field. The analysis of laboratory equipment blanks should yield values less than the reporting limit. Otherwise, the equipment should not be used.

Matrix spike (MS) - A matrix spike is an aliquot of sample spiked with a known concentration of the analyte of interest. Percent recovery of the known concentration of added analyte is used to assess accuracy of the analytical process. The spiking occurs prior to sample preparation and analysis. Spiked samples are routinely prepared and analyzed at a rate of 10% of samples processed, or one per batch whichever is greater. The MS is spiked at a level less than or equal to the midpoint of the calibration or analysis range for each analyte. Percent recovery (%R) is defined as 100 times the observed concentration, minus the sample concentration, divided by the true concentration of the spike.

The percent recovery of the matrix spike is calculated using the following equation in which %R is percent recovery, SSR is the observed spiked sample concentration, SR is the sample result, and SA is the reference concentration of the spike added:

$$\%R = (SSR - SR)/SA * 100$$

MS recoveries are plotted on control charts and used to control analytical performance. Measurement performance specifications for matrix spikes are not specified in this document.

Method blank - A method blank is an analyte-free matrix to which all reagents are added in the same volumes or proportions as used in the sample processing and analyzed with each batch. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the reporting limit. For very high-level analyses, the blank value should be less then 5% of the lowest value of the batch, or corrective action will be implemented.

Additional method-specific QC requirements - Additional QC samples are run (*e.g.*, sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples) as specified in the methods. The requirements for these samples, their acceptance criteria, and corrective actions are method-specific.

UTMSI Analyses

Data quality will be ensured during the project by the use of standard curves and blanks (NH_4^+, $NO_3^- + NO_2^-$, PO_4^{-3}, chlorophyll-*a*). Standard curves will be established for NH_4^+, $NO_3^- + NO_2^-$, PO_4^{-3} analyses utilizing a blank (nutrient deficient seawater) and at least three analytical standards of increasing concentration, covering the range of expected sample concentrations. Linearity of the standard curve ($R^2 \geq 0.98$) will be established prior to the analysis of samples. Blanks will be used during every analysis to auto-zero the spectrophotometer, and complete standard curves will be performed when new chemicals are used and prior to processing each data set. Select standards will be used during each analysis to check the assumption that the original standard curve continues to be valid. Analysis of standards will occur at the beginning of a sample set and after the last analytical sample. If blanks or initial standards deviate from known concentrations, sample processing will be suspended and procedures will be undertaken to determine the source of error (*i.e.* troubleshoot to determine if the error is due to contamination, bad chemicals, poor technique, a technical error or instrument failure). If the standards run following the last analytical sample deviates from the original values obtained, the last sample analyzed before the check sample (standard) that failed the control limit criteria will be reanalyzed. If the relative percent difference (RPD) between the results of this reanalysis and the original analysis exceeds 20 percent, the instrument is assumed to have been out of calibration during the original analysis and the earlier data should be flagged or replaced. If possible, reanalysis of samples should progress in reverse order until it is determined that there is <20 RPD between initial and reanalysis results. If it is not possible or feasible to perform reanalysis of samples, all earlier data (*i.e.*, since the last successful standard control check) will be flagged.

Failures in Field and Laboratory Quality Control and Corrective Action

Sampling QC excursions are evaluated by the TPWD Project Manager, in consultation with the QAPO. In that differences in field duplicate sample results are used to assess the entire sampling process, including environmental variability, the automatic rejection of results based on control chart limits is not practical. Therefore, some professional judgment will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Blank data are scrutinized very closely. Blank values exceeding the acceptability criteria may automatically invalidate the sample, especially in cases where high blank values may be indicative of contamination which may be causal in putting a value above the standard.

Laboratory measurement quality control failures are evaluated by the laboratory staff. The disposition of such failures is discussed in Section B4 under "Failures or Deviations in Analytical Methods and Corrective Actions." Corrective action documentation is maintained by the TPWD.

Seagrass Condition Indicators

Mean values of seagrass condition (biomass, root-to-shoot ratio, shoot density, blade width, leaf area index), epiphyte biomass and macroalgae biomass measurements will be provided from replicate sample measurements.

C:N ratios will be compared against a known standard (chitin) for every ten samples processed. Mean values of C:N elemental analyses and isotopic analyses will be provided from replicate sample measurements.

Seagrass Epiphyte Fluorescence Measurements

QC procedures for seagrass epiphyte fluorescence measurements are described in Appendix C.

B6. Instrument/Equipment Testing, Inspection and Maintenance

Aerial Imagery and Landscape Analysis

All GPS units will be checked prior to each field survey for proper functioning; and such precautions as proper battery performance will be taken to ensure efficient field survey operations.

Field Equipment

All sampling equipment testing and maintenance requirements are detailed in the *TCEQ Surface Water Quality Monitoring Procedures Manual* (Volume 1). Sampling equipment is inspected and tested upon receipt and is assured appropriate for use. Equipment is maintained in working condition.

LCRA Laboratory

All laboratory tools, gauges, instruments, and equipment testing and maintenance requirements are contained within laboratory QAM(s). Testing and maintenance records are maintained and are available for inspection. Instruments requiring daily or in-use testing include, but are not limited to, water baths, ovens, autoclaves, incubators, refrigerators, and laboratory pure water. Critical spare parts for essential equipment are maintained to prevent downtime.

TAMU-CC Laboratory

Laboratory instruments are maintained in working order. Testing and maintenance records are available for inspection. Refrigerator performance is checked daily.

TPWD Laboratory

The TPWD San Marcos Laboratory is equipped to provide analytical support for chemical and biological analyses, has the suitable facilities to store and prepare samples, and the appropriate

instrumentation and staff to provide data of the required quality within the time period dictated by the project. Operations are conducted using good laboratory practices, including:

- A program of scheduled maintenance of analytical balances, microscopes, laboratory equipment and instrumentation.
- Recording all analytical data in a laboratory notebook or on bench sheets.
- Monitoring and documenting the temperatures of cold storage areas and freezer units.
- Labeling all containers used in the laboratory with date prepared, contents, and initials of the individual who prepared the contents.

UTMSI Laboratory

The UT Marine Science Laboratory is equipped to provide analytical support for chemical and biological analyses, has the suitable facilities to store and prepare samples, and the appropriate instrumentation and staff to provide data of the required quality within the time period dictated by the project. Operations are conducted using good laboratory practices, including:

- A program of scheduled maintenance of analytical balances, microscopes, laboratory equipment and instrumentation.
- Recording all analytical data in bound logbooks in ink.
- Monitoring and documenting the temperatures of cold storage areas and freezer units.
- Verifying the efficiency of fume hoods.
- Labeling all containers used in the laboratory with date prepared, contents, and initials of the individual who prepared the contents.
- Dating and storing all chemicals safely upon receipt. Chemicals are disposed of properly when the expiration date is reached.
- Using a laboratory information management system to track the location and status of any sample received for analysis.

All laboratories at UTMSI are routinely inspected by Environmental Health and Safety personnel from UT-Austin to check adherence to strict University policies on chemical safety and handling. It is the responsibility of the laboratory manager to ensure that safety training is mandatory for all laboratory personnel. The UTMSI laboratory maintains a current safety manual in compliance with the Occupational Safety and Health Administration (OSHA) and the equivalent state or local regulations. The safety manual is available to all laboratory personnel. Proper procedures for safe storage, handling and disposal of chemicals are followed at all times. Chemicals are treated as potential health hazards and good laboratory practices are implemented accordingly.

B7. Instrument Calibration and Frequency

Aerial Imagery and Landscape Analysis

The vendor is required to have a calibrated mapping camera and to supply camera calibration documentation. GPS units will be used to record positional data in the field only when sufficient satellites are available. During field operations, a PDOP reading of 5 or less from the unit ensures this functionality.

Field Equipment
Field equipment calibration requirements are contained in the *TCEQ Surface Water Quality Monitoring Procedures Manual* (Volume 1). Instruments not meeting post-calibration error limit requirements invalidate associated data.

LCRA Laboratory
Detailed laboratory calibrations are contained within the QAM(s). The laboratory QAM identifies all tools, gauges, instruments, and other sampling, measuring, and test equipment used for data collection activities affecting quality that must be controlled and, at specified periods, calibrated to maintain bias within specified limits. Calibration records are maintained, are traceable to the instrument, and are available for inspection. Equipment requiring periodic calibrations include, but are not limited to, thermometers, pH meters, balances, incubators, turbidity meters, and analytical instruments. Calibration records are available for review.

TAMU-CC Laboratory
Excitation laser power and photomultiplier tube (PMT) sensitivity may change over time, so periodic characterization will enable normalization of quantitative fluorescence data. This characterization will be obtained by recording scans of reference fluorophores following procedures described in Appendix C.

TPWD Laboratory
Analytical balances are calibrated annually using a standard weight. LI-COR light sensors are re-calibrated annually to within ± 5% of NBS standards by LI-COR, Inc.

UTMSI Laboratory
Analytical balances are calibrated before each use using a standard weight. LI-COR light sensors are re-calibrated annually to within ± 5% of NBS standards by LI-COR, Inc. These checks are performed routinely and the results recorded in a log kept for each instrument. Routine calibration of pipettes occurs off-site by the pipette suppliers on a quarterly basis. The spectrophotometer will be calibrated on-site using standards every six months. A trained technician will calibrate the mass spectrometer. A calibration of weight percent is done everyday, and a mass calibration is performed every two weeks. Instrument calibration will be recorded in a logbook. Summary data documenting initial calibration and any events requiring recalibration and the corresponding recalibration data will be included with the analytical results.

B8. Inspection/Acceptance of Supplies and Consumables

TPWD and its contractors evaluate items and services received from suppliers upon delivery. These evaluations are based on defined acceptance criteria such as task specifications, product specifications, technical requirements, and quality requirements. The Project Manager or designee determines whether a product or service meets the established acceptance criteria.

TPWD and its contractors will not use items or services that do not meet acceptance criteria. Corrective actions may range from repair or replacement of defective deliverables to re-award of

procurements. State statutes, contract provisions, and TPWD Procurement procedures are the basis for initiating corrective actions.

B9. Non-Direct Measurements

Aerial Imagery and Landscape Analysis

Texas Orthophotography / National Agricultural Imagery Program 2008-2009 data (TNRIS 2009) may be used to estimate the seagrass edge and will be used to sample groundtruth points prior to field work.

No other non-direct measurement sources will be required for this project.

B10. Data Management

Data collected by field staff will be recorded in ink on data sheets or in a field notebook, or may be recorded electronically. In either case, when an electronic file is the primary data source, either a hardcopy printout or a back-up electronic file will be created as soon as possible after sampling. Electronic data provided by vendors will be stored on the TPWD network and backed up on a second drive or on a compact disk.

Original TPWD data sheets, copies of vendors' original data and laboratory bench sheets, and hard-copy printouts, as well as back-up electronic files, will be maintained in Water Quality Program offices. Copies of data sheets will be mailed, or electronic files will be emailed, to the TPWD Project Manager and QAPO. The TPWD QAPO will validate the data and verify that it meets quality requirements as set forth in this QAPP. Any data deemed unacceptable as set forth in this QAPP will not be used. As appropriate, the TPWD Project Manager and QAPO will transfer quality-assured data to the TCEQ in a format compatible with the SWQMIS database, using a data review checklist.

Data Flow

Aerial Imagery and Landscape Analysis
Vendor or TPWD Project Field Staff→ Project Manager→ QAPO→ GIS Analyst → Project Manager

Water and Sediment Quality Indicators, Seagrass Condition Indicators, and Seagrass Epiphyte Fluorescence Measurements
Vendor or TPWD Project Field Staff→ Project Manager→ QAPO→ Data Manager → Project Manager

Data Errors and Loss

Data collectors have primary responsibility for ensuring that any errors in data are corrected or reported as a non-compliance or deficiency at the time of collection. Screening of data for completeness occurs at each step in the data flow process, and incomplete data (such as a date, or time, collector's name) is recovered as quickly as possible. If error or loss renders data

unsuitable for use, a record will be made of the non-compliance or deficiency and the circumstances surrounding this occurrence.

Record Keeping and Data Storage

After quality review, electronic files of orthorectified digital imagery and classified imagery of seagrass sites, along with GPS database files, will be maintained by the Project Manager.

Original TPWD data sheets, copies of vendors' original data and laboratory bench sheets, and hard-copy printouts, as well as back-up electronic files, will be maintained in Water Quality Program offices. Duplicate records of data will be transmitted to the Project Manager and QAPO. Electronic files will be backed up on CDs, DVDs, flash drives, or a common network drive. All records will be maintained for a minimum of 5 years from date of collection.

Data Handling, Hardware, and Software Requirements

All specifications for computer hardware and software selected for TPWD's environmental programs are consistent with agency standards for performing all functions required to calculate, handle and otherwise manipulate data and produce reports, through time-tested applications.

C1. Assessment and Response Actions

The following table presents the types of assessments and response actions for data collection activities applicable to the QAPP.

Table 10. C1 - Assessments and response requirements.

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TPWD—all members of Water Quality Program	Monitoring of the project status and records to ensure requirements are being fulfilled	Update project group periodically
Annual Project Report	Once per year	TPWD Project manager	Brief description of pertinent aspects of the project including status, quality assessments performed, and any quality problems encountered.	Together with other annual quality meeting participants, determine what, if any, response actions are necessary
Contractor Inspection	Dates to be determined by TPWD	TPWD Water Quality Program staff	Monitoring analytical and quality control procedures and records employed at the contract facility	30 days to respond in writing to the TPWD to address corrective actions

Corrective Action
The TPWD Project Manager is responsible for implementing and tracking corrective action procedures as a result of audit findings.

C2. Reports to Management

The Project Manager submits an annual project report, which briefly summarizes pertinent information about the project, to the TPWD QA Officer at the quality assurance annual meeting. The Project Manager also submits new and/or updated QAPPs to the TPWD QA Officer annually for upcoming and ongoing projects. The Project Manager submits regular quarterly reports and a final report to GLO upon completion of the grant.

D1. Data Review Verification and Validation

All field and laboratory data will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the data quality objectives, which are listed in Section A7. Only those data, which are supported by appropriate quality control data and meet the data quality objectives defined for this project, will be considered acceptable.

D2. Verification and Validation Methods

Data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7 of this document.

Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate to the project task. The information to be reviewed, verified, and validated as appropriate (listed by task and responsible party in Table 11**)** is evaluated against technical and project specifications and checked for errors, especially errors in calculations, data reduction, and transcription. Potential errors are identified by examination of documentation and by manual or computer-assisted examination of corollary or unreasonable data. If a question arises or an error is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented. If an issue cannot be corrected, the task manager consults with higher-level project management to establish the appropriate course of action, or the data associated with the issue are rejected. Reviews, verifications, and validations will be documented.

Data validation tasks to be addressed by the TPWD Water Quality Program include, but are not limited to, the confirmation of data review, evaluation of field QC results, additional evaluation of anomalies and outliers, analysis of sampling and analytical gaps, and confirmation that all parameters and sampling sites are included in the QAPP. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data before data validation can be completed. The TPWD Project Manager validates that the data meet the data quality objectives of the project.

Table 11. D2 - Data review, verification, and validation tasks.

Data to be Verified	Field Task	Vendor or Lab Task	Project Manager and/or QAPO
Field documentation complete	√		
Instrument calibration data complete	√	√	
Holding times not exceeded	√	√	
Sample preservation and handling acceptable	√	√	
Standards and reagents traceable	√	√	
Analytical sensitivity consistent with QAPP		√	√
Laboratory bench-level review performed		√	
QC samples analyzed at required frequencies	√	√	√
QC results meet performance and program specifications	√	√	√
Collection, preparation and analysis techniques consistent with SOPs and QAPP	√	√	√
Valid STORET codes			√
Results, calculations, transcriptions checked	√	√	
Corollary data agree	√	√	√
Review of orthorectified digital imagery		√	
Review of landscape classification analysis and results		√	√
Nonconforming occurrences documented	√	√	√
Reasonableness check performed			√

Orthorectified digital imagery and GPS data will be reviewed to ensure they are representative of the study sites, especially for spatial coverage. Completeness and accuracy of the data sets will be verified.

D3. Reconciliation with User Requirements

Data produced in this project will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used by the TPWD for resource management and protection purposes and, as applicable, submitted to the TCEQ.

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Appendix A - Work Plan for Aerial Photography and Digitized Orthophotography

Work Plan

Aerial Photography and Digitized Orthophotography

for

Coastal Management Program Grant for Seagrass Study of Port Bay and East Flats

from

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October 21, 2009

Work Plan
Aerial Photography and Digitized Orthophotography: Coastal Management Program Grant for Seagrass Study of Port Bay and East Flats

Background

This project will investigate the impact of a wastewater discharge on seagrass by study of a potentially-impacted site and a reference site. It will also be a pilot project to test recent recommendations for coastwide seagrass monitoring in Port Bay (Aransas County) and East Flats (Corpus Christi Bay; Nueces County).

Seagrass beds serve as critical nursery habitat for estuarine fisheries and wildlife. Seagrasses provide food for fish, waterfowl and sea turtles, contribute organic material to estuarine and marine food webs, cycle nutrients, and stabilize sediments. They are economically important based on their function in maintaining Gulf fisheries. Increasing coastal development threatens seagrasses.

Project Objectives

The study protocol will follow a recent proposal by Dunton and Pulich to the Seagrass Monitoring Work Group (Landscape Monitoring and Biological Indicators for Seagrass Conservation in Texas Coastal Waters, draft, Dunton *et al.*, 2007). Identical procedures will be used in two areas of the central Texas coast, East Flats in Corpus Christi Bay and Port Bay, where a large development is planned including a wastewater treatment outfall to Port Bay. The study will include three components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) epiphyte analysis. Information from the three components will be integrated to assess the condition of the seagrasses.

Landscape monitoring will be obtained from analysis of 1:9,600 scale true color aerial photography. Seagrass stress indicators obtained include bare patches (number, size), depth limit of seagrass coverage, and macroalgae deposition (areal coverage). Dr. Warren Pulich of Texas State University - San Marcos will assist TPWD staff with analysis of landscape data.

Aerial photography will be conducted in Fall 2009. (Additional photography will be conducted again in Fall 2010 if funds are available. Fall 2010 photography is not addressed in this document.) Seagrass condition monitoring will be conducted three times, once each during the spring, summer, and fall of 2010. A report which summarizes data and findings will be prepared following completion of sampling.

Description of Work

TPWD staff will prepare a Quality Assurance Project Plan (QAPP) that describes procedures that will be followed in data acquisition and analysis for all components of the project.

TPWD staff will prepare a map depicting the areas to be photographed in Port Bay and East Flats.

In November or December 2009 Krawietz Aerial Photography will obtain true-color imagery at 1:9,600 scale of East Flats and Port Bay according to specifications given below.

Prior to acquiring aerial imagery, TPWD staff may place a minimum of four control points within the boundaries of each of the three areas to be photographed, to improve accuracy of the imagery. TPWD staff will retrieve markers after the flight.

TPWD staff will conduct a groundtruthing effort in accordance with the QAPP in conjunction with acquisition of the aerial imagery. The purpose of the groundtruthing will be to identify habitat types (including bare bay bottom, seagrass, and macroalgae) at specified points and compare it with the aerial imagery.

Krawietz Aerial Photography will provide TPWD film diapositives, and scanned and georeferenced imagery in the form of TIFF files within 45 days of acquiring the imagery.

Imagery will be analyzed by TPWD staff.

Specific Requirements

Area to be photographed: Areas to be imaged are outlined in yellow on the map below (Figure 1). In Port Bay, only two of the three yellow-outlined areas will be imaged. Prior to acquiring imagery, TPWD staff will prepare a final map depicting the areas to be photographed in Port Bay and East Flats. Each of the areas outlined in yellow is approximately two square miles. The total project area (Port Bay and East Flats) is approximately six square miles.

Photography:
1. Scale. Aerial photography at a scale 1:9,600.
2. Accuracy. Accuracy of one meter or better is acceptable. We understand the use of control points will improve the accuracy.
3. GPS. Airborne GPS will be used for georeferencing the imagery.
4. Resolution. 0.3 meter high resolution true color georeferenced digital orthophotography.
5. Aerial flight plan with proposed number and location of photos.

Schedule:
1. Imagery will be taken in November or December 2009 and must be obtained prior to December 31, 2009.
2. The exact flight date is weather-dependent and will be determined by mutual agreement between TPWD and the Krawietz Aerial Photography. TPWD point-of-contact will be Ms. Cindy Contreras at (512) 389-8195 (Alternate: Pat Radloff at (512) 389-8730). Flight will be scheduled for a day following the passage of a cold front when skies are clear, and wind is calm, (*i.e.* not exceeding three (3) miles per hour). Preliminary plans must be verified no more than five days prior to flight. Verification that local conditions are acceptable must be made on the morning of the flight and the final fly/no-fly decision will be made by TPWD.

Deliverables:
1. Deliverables will be provided forty-five (45) calendar days following imagery acquisition.

2. One (1) set of 9-inch by 9-inch Color Film Diapositives.
3. One (1) set of scanned, digitized, georeferenced, full frame images from diapositives, on DVD-ROM. All TIFF images should have the projection file associated with them. Preferred projection is UTM NAD83 Zone 14. All file formats shall be compatible with ArcView.
4. Actual flight plan with number and location of photos taken.
5. Metadata set which meets all Federal Geographic Data Committee (FGDC) standards (at least FGDC-STD-001-1998), compatible with ArcView, including: resolution of photos, camera information, projection information and post processing accuracy estimates. Metadata should be HTML, XML, or ASCII text formats.
6. Copy of most recent calibration report for the equipment being used (show serial numbers) if not included with metadata.

Conditions during photography. Photography must be obtained during low tide periods with high angle sun minimum 30 degrees. This usually requires photography be taken between the hours of 9:30 and 11:30 a.m. Photography must not be undertaken when the ground is obscured by cloud cover, haze, fog, or dust. Photography must be taken during minimal wind conditions to preclude wave action or ripple on the waters surface which obscure submerged features in shallow water, less than 3 feet in depth. The photographs cannot contain objectionable shadows caused by relief or low solar altitude.

Flight Plan. Krawietz Aerial Photography will prepare a flight plan that insures coverage of the project area and that meets the following requirements:
1. Forward overlap in the direction of the flight will average 50 percent within 5 percent.
2. Side overlap, if needed, between adjacent flight lines will average 30 percent within 10 percent.
3. Crab will be less than three (3) degrees with respect to the line of flight. Krawietz Aerial Photography acknowledges that crab in excess of three (3) degrees with respect to the line of flight may be cause for rejection of a flight strip or any portion thereof in which the excessive crab occurs. This includes relative crab between any two successive exposures.
4. Tilt of the camera from vertical at the instant of exposure will not exceed three (3) degrees nor shall it exceed five (5) degrees between successive exposure stations.

Aerial Film. Kodak aerial true color film or industry standard equivalent will be used.

Film Processing and Image Quality. All elements of film processing will be done such that the result is a quality image with optimal contrast, tone, balance, resolution, uniformity in range of density, and fine grain exposures.

Film Labeling. Each exposure will have a unique label and exposures in a series will be labeled consecutively. The label area will include at least the clearly legible information: Date of photography; Scale of photography, expressed as a ratio; site name; roll number; flight strip number; exposure number.

Retention of film. Exposed film belongs to TPWD as part of services and product of this work plan. Krawietz Aerial Photography may archive exposed film, but cannot release film or any copies to any entity other than TPWD without the express written consent of TPWD.

Disclaimer: This work is contingent on TPWD receiving funding from the General Land Office (GLO) for a Coastal Management Program grant. The project was approved in early 2009 and a contract is pending between TPWD and GLO. An executed contract is expected in late October 2009. If for any reason the funding is not received, TPWD will not be able to conduct this project and will not be liable for any expenses incurred by Krawietz Aerial Photography. Prior to acquiring imagery, TPWD will advise Krawietz Aerial Photography of the contract status.

Personnel
Work will be conducted by Krawietz Aerial Photography.

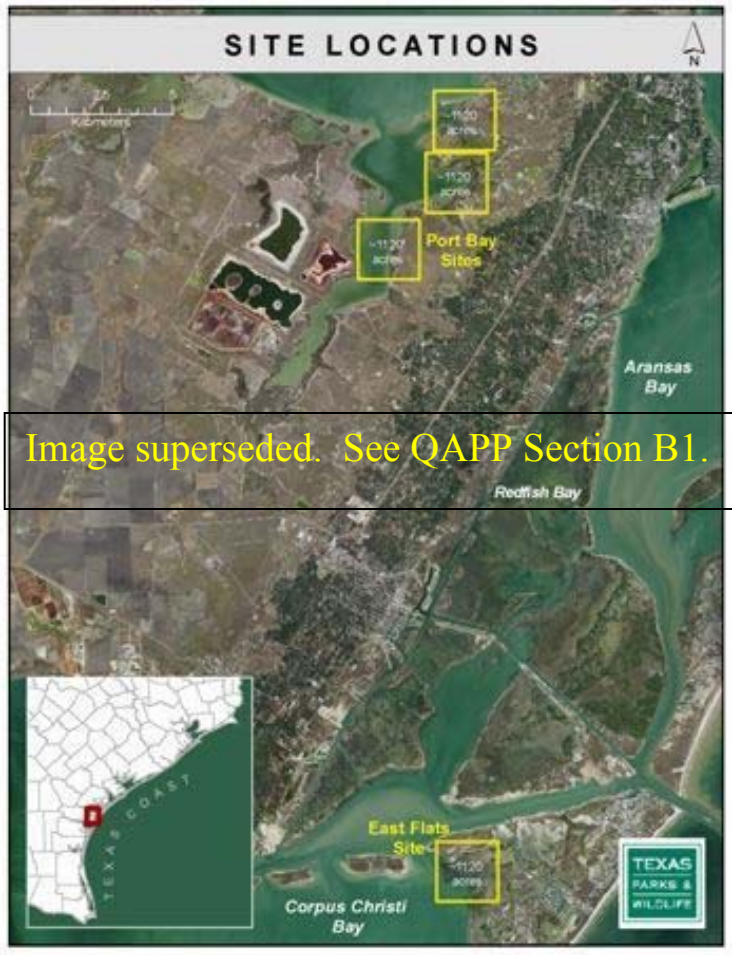

Image superseded. See QAPP Section B1.

Figure 1. Preliminary map depicting areas to be photographed in Port Bay and East Flats. Only two of the three areas indicated in Port Bay will be imaged. A final decision regarding photography sites will be made prior to acquisition of photography.

Appendix B – UTMSI Standard Operating Procedures

Percent Surface Irradiance and Light Attenuation

Ken Dunton and Kim Jackson
Revised December 2009

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:

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

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

Nutrient Methods: Nitrate+Nitrite, Phosphate, Silicate, Ammonia
UTMSI SOP 0201

1. Introduction

1.1 Modern colorimetric assays are based on a limited number of modifications of the same basic chemistry. In the following text, the Lachat protocol will be described with figures included to show detection limits and precision.

1.2 Nutrients will be analyzed using a Lachat Quikchem 8000. The system is fully automated and generates data reports that contain standard curve, sampling statistics and sample concentrations. The latter are reported as both volts and concentration units. The software is capable of ignoring refractive index peaks by gating the integration window to periods where only the sample is in the optical cell. The electronics of this system are so stable that the system can utilize a 1 cm cell and achieve the same detection levels as older 5 cm cells on segmented flow analysis systems.

1.3 The Lachat protocol differs from the methodology described in current EPA methods require the use of segmented flow analysis. The nutrient methods described below use flow injection technology proprietary to Zellweger Analytics. The sensitivity and precision of flow injection is the same as that of segmented flow, however, the data processing and actual plumbing of the sampling manifold is proprietary to the manufacturer (Zellweger Analytics).

2. Field Procedures

2.1 Sample Collection:

2.1.1 At each station, water samples will be collected at the surface.

2.1.2 Two 10 ml sub-samples will be collected and filtered on site using a hand syringe and ~0.7 μm glass fiber filter.

2.2 Sample Preservation:

2.2.1 Samples will be stored in 15 ml capped tubes on dry ice while in the field and frozen on return to the lab.

2.2.2 Samples will be kept frozen until analyzed, no more than 30 days. Analysis is typically within 10 days of sample collection.

3. Sample Handling

3.1 Samples will be thawed prior to analysis. Typically, the sample tube is placed directly into the Lachat manifold. This minimizes sample handling and the introduction of potential error.

4. Analytical Methods

4.1 Standards and Blanks -- The calibration curve for each nutrient species analyzed is checked beyond the linearity criteria using verifiable and traceable second source standards where available. Recoveries of all nutrient analytes from the seawater matrix are tested and documented.

4.1.1 Each sample run consists of 5 standards

4.1.2 Two samples of deionized water (to establish a baseline)

4.1.3 Two samples of Gulf of Mexico water (lowest nutrient seawater available used to check software integration)

4.1.4 Two samples of Gulf of Mexico seawater amended with nitrate to equal the midrange standard (matrix spike to check recovery of nitrate from seawater)

4.1.5 A nitrite sample equal to the midrange standard (to be compared with the nitrate sample in order to verify Cd column efficiency)

4.1.6 The standard addition sample (matrix spike) and the Gulf of Mexico blank are run every 20 sample to verify instrument performance. The matrix spike will include nitrate, nitrite, phosphate, silicate, and/or ammonia as appropriate to the analysis being conducted.

4.2 Nitrate and/or Nitrite in Brackish Waters or Seawater (Lachat Quikchem method 31-107-04-1-A)

4.2.1 Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrate) is then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion is coupled with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting pink dye absorbs at 520 nm. This is the same chemistry used in EPA method 353.4.

4.2.2 Though this method is written for seawater and brackish water, it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of

varying salinity (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 540 nm. The salt effect is less than 2%. The applicable range is 0.03 to 5.0 µM. The method detection limit is 0.03 µM N. The method throughput is 48 injections per hour.

4.2.3 Standard curves are linear (Fig. 1) and will be accepted only when the r^2 $\geq$0.995.

4.2.4 Precision exceeds 1% at the 1.25 µM level in analysis of 10 samples in an optimal laboratory system. Field handling reduces precision to about 5% (Fig. 2). Carryover is negligible.

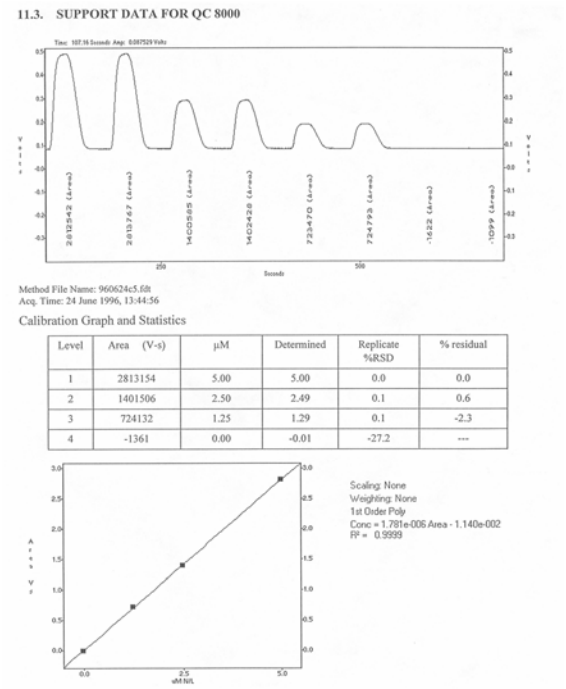

Fig. 1. Standard curve data for nitrate+nitrite analysis using Quikchem method 31-107-04-1-A.

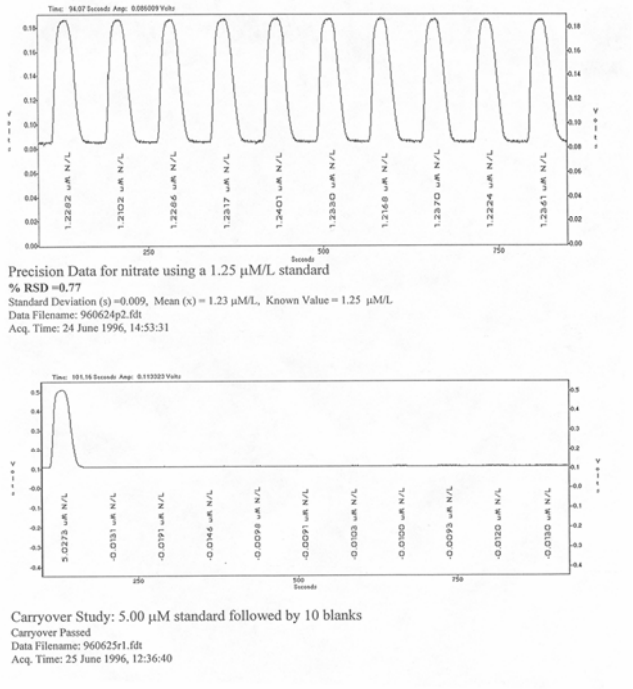

Fig. 2. Precision and carryover of nitrate+nitrite analysis using Quikchem method 31-107-04-1-A.

4.3 Phosphate in Brackish Water or Seawater(Lachat Quikchem method 31-115-01-3-A)

4.3.1 Orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a yellow complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The ascorbic acid and molybdate reagents are merged on the chemistry manifold and the reagent stream is

then merged with the carrier stream. The sample zone appears at the detector less than 10 seconds after injection. The absorbance is proportional to the concentration of orthophosphate in the sample. This method is written for seawater and brackish water but is also applicable to non-saline sample matrixes. The applicable range is 0.03 to 2.00 µM. The method detection limit is 0.03 µM. The method throughput is 48 injections per hour.

4.3.2 The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinity (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 880 nm. The salt effect was less than 2% as measured in Sargasso seawater.

4.3.3 Standard curves are linear (Fig. 1) and will be accepted only when the r^2 $\geq$0.995.

4.3.4 Precision exceeds 1% at the 0.5 µM level in analysis of 10 samples in an optimal laboratory system. Field handling will reduce precision to roughly 5% (Fig. 2). Carryover is negligible.

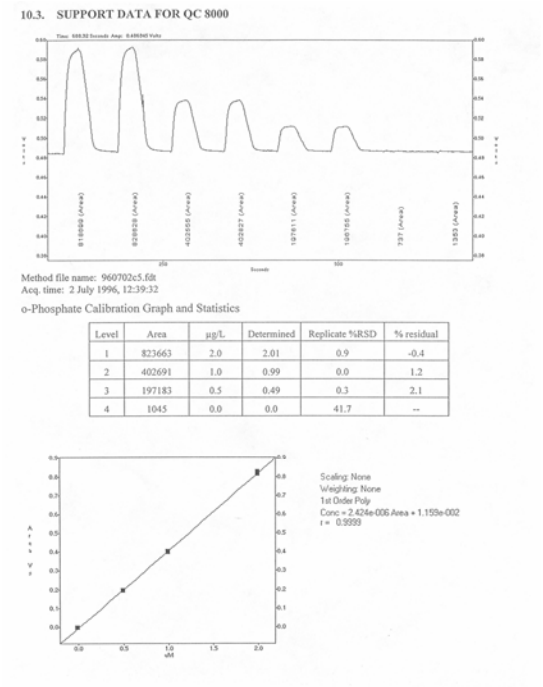

Fig. 1. Standard curve data for orthophosphate analysis using Quikchem method 31-115-01-3-A.

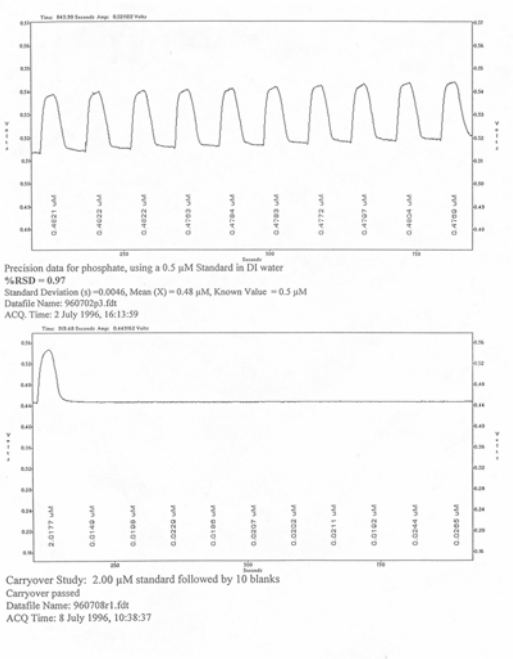

Fig. 2. Precision and carryover of orthophosphate analysis using Quikchem method 31-115-01-3-A.

4.4 Silicate in Brackish or Seawater (Lachat Quikchem method 31-114-27-1-B)

4.4.1 Soluble silica species (silicic acid) react with molybdate at 37* C and pH of 1.2 to form a yellow silicamolybdate complex. This complex is subsequently reduced with stannous chloride to form a heteropoly blue complex which has an absorbance maximum at 820 nm. The intensity of the color is proportional to the concentration of molybdate reactive silica. Though the method is written for Brackish and Seawater, it is also applicable to non-saline sample matrixes.

4.4.2 The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 820 nm. The applicable range is 0.03 to 5.00 µM SiO_2 L^{-1}. The method detection limit is 0.03 µM SiO_2 L^{-1}. The method throughput is 48 injections per hour.

4.4.3 Precision exceeds 1% at the 1.25 µM level in analysis of 10 samples in an optimal laboratory system (Fig. 2). Field handling will reduce to roughly 5%. Carryover is negligible.

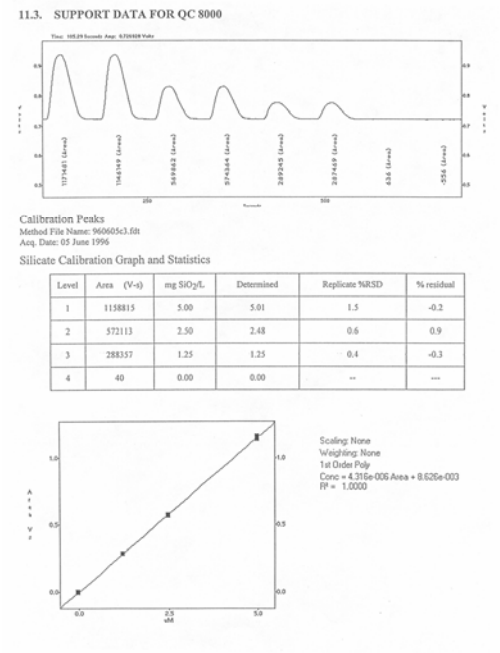

Fig. 1. Standard curve data for silicic acid (soluble silica) analysis using Quikchem method 31-114-27-1-B.

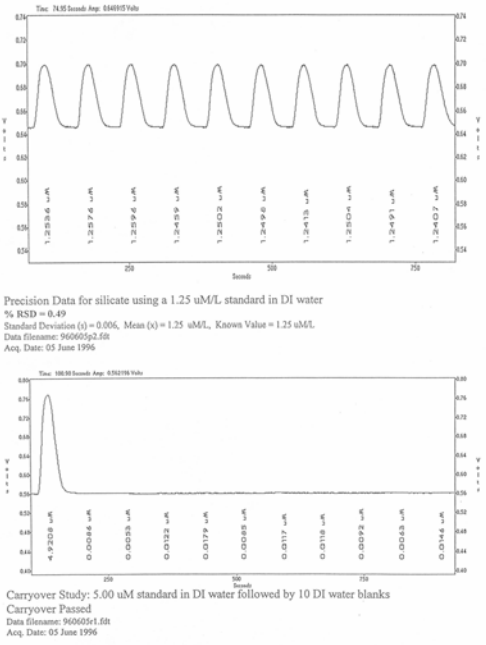

Fig. 2. Precision and carryover of silicic acid (soluble silica) analysis using Quikchem method 31-114-27-1-B.

4.5 Ammonia in Brackish Water or Seawater (Lachat Quikchem method 31-107-06-1-A)

4.5.1 This method is based on the Berthelot reaction. Ammonia reacts in alkaline solution with hypochlorite to form monochloramine which, in the presence of phenol, catalytic amounts of nitroprasside (nitroferricyanide) and excess hypochlorite, gives indophenol blue. The formation of monochloramine requires a pH between 8 and 11.5. At higher pH, ammonia may begin to oxidize to nitrate. At pH greater than 9.6, some precipitation of calcium and magnesium as hydroxides and carbonates occurs in seawater, but these ions may be held in solution by complexing them with EDTA. The indophenol blue measured at 630 nm is proportional to the original ammonia concentration. This is the same chemistry as in EPA method 349.0 modified for flow injection analysis. Though the method is written for Seawater and Brackish water, it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples that have color absorbing at 630 nm. The salt effect was less than 2% as measured in tropical Pacific surface seawater.

4.5.2 At seawater pH, ammonia (NH_3) exists as the monovalent cation ammonium (NH_4^+). The analytical method measures both as ammonium.

4.5.3 Standard curves are linear (Fig. 1) and will be accepted only when the $r^2 \geq 0.995$.

4.5.4 Precision exceeds 1% at the 0.5 µM level in analysis of 10 samples in an optimal laboratory system. Handling in the field will reduce this to a nominal value of 5% (Fig. 2). Carryover is negligible.

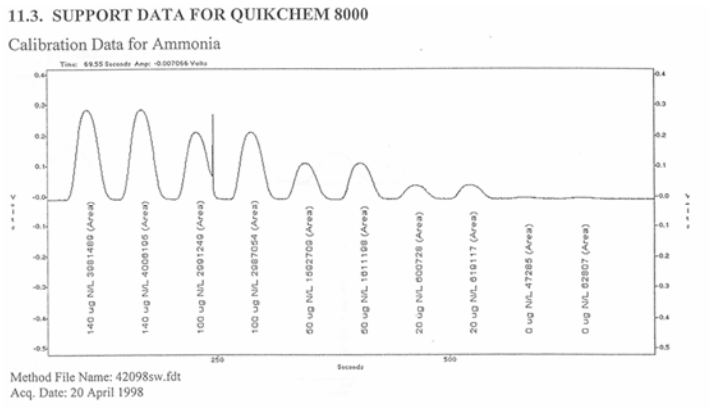

Fig. 1. Standard curve data for ammonium analysis using QuikChem Method 31-107-06-1-A.

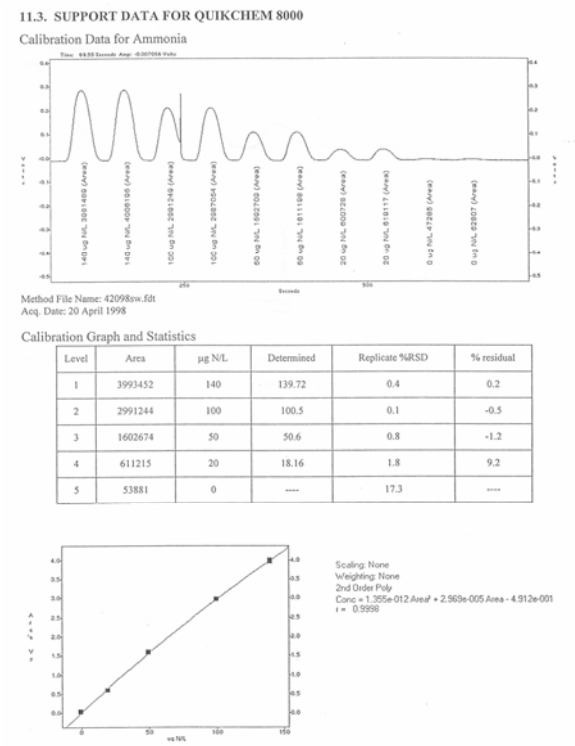

Fig. 2. Precision and carryover of ammonium analysis using QuikChem Method 31-107-06-1-A

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Water Column Chlorophyll *a* Extraction

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Ken Dunton/Kim Jackson

Adapted from: ESS Method 150.1: Chlorophyll – Spectrophotometric, Environmental Sciences Section, Inorganic Chemistry Unit, Wisconsin State Lab of Hygiene, 465 Henry Mall, Madison, WI 53706. Equation for chlorophyll *a* from Jeffrey and Humphrey (1975).

1.0 Introduction

Chlorophyll *a*, a characteristic algal pigment, constitutes approximately 1% to 2% (dry weight) of planktonic algal biomass. This feature makes chlorophyll *a* a convenient indicator of algal biomass. This method is applicable to most surface waters.

2.0 Summary of Method

Algal cells are concentrated by filtering a known volume of water through a membrane filter (25 mm, 0.45 µm pore size nitrocellulose filter). The pigments are extracted from the concentrated algal sample in an aqueous solution of 90% acetone. The chlorophyll *a* concentration is determined spectrophotometrically by measuring the absorbance or optical density (OD) of the extract at various wavelengths. The resulting absorbance measurements are then applied to a standard equation.

3.0 Sample Preservation and Preparation

1) Chlorophyll *a* samples are placed in a dark cooler and packed on ice at the time of collection.
2) All chlorophyll work is carried out in low light conditions (all overhead lights must be off) since light degrades chlorophyll pigments. Arrange the filtering manifold, seawater trap, and vacuum pump (or aspirator) on the lab bench.
3) Using forceps, place a 0.45 µm pore size nitrocellulose filter on each filtering funnel, and filter a known volume (measure with a graduated cylinder) of sample (in the dark), applying vacuum until the sample is dry. The amount of sample required depends on the phytoplankton density in the water sample. For coastal waters, filter in 50 ml increments. When water flow begins to slow, continue to filter small amounts of water until flow almost ceases.
4) Record the volume filtered for each sample.
5) If samples are spectrophotometrically at a later date, fold the filter in half and wrap in pre-labeled aluminum foil or opaque tubes (or wrap test tube wrap with black plastic bag) and freeze. If samples are run immediately, proceed to step 4.0.

4.0 Procedure

1) Place the filter containing the concentrated algal sample in a pre-labeled test tube.
2) Add 5 mL of 90% acetone solution (i.e., 900 ml of acetone mixed with 100 ml of double distilled or ultrapure water).
3) Cap tightly, vortex or shake until filter dissolves.
4) Repeat until the all samples are processed.
5) Create two blanks using 5 ml acetone solution and new unused filter.
6) Wrap test tube rack in a black plastic and place samples in a freezer. Allow extraction to occur overnight (up to 24 hr).

7) Remove samples from freezer. Keep samples covered in low light conditions at all times.
8) Clarify extract by centrifuging samples for 15 minutes at approximately 5000 g. Remember to balance the centrifuge (i.e., put equal number of samples on each side).
9) Turn on spectrophotometer and allow to stabilize while samples are centrifuging.
10) Remove samples from centrifuge. DO NOT SHAKE! Rewrap test tube wrap in black plastic and take samples to spectrophotometer.
11) Carefully transfer the two blanks to the two 1.0 cm cuvettes. Pour using a continuous motion.
12) Set up the spec to measure absorbances at: 750, 664, 647, 630, and 600 nm.
13) Auto zero the spec with the blanks (make sure clear sides of cuvettes are facing away from you when you place in spec).
14) Remove closest cuvette. Empty contents into waste container. Pour first sample into cuvette. Do not shake and only pour once into cuvette after centrifuging.
15) Place cuvette into the slot vacated by the blank. Push Read Sample.
16) Repeat for remaining samples.
17) When finished save output file on computer hard drive and on a floppy disk.

5.0 Calculation

Subtract the absorbance at 750 nm from the 630, 647, and 664 nm values for the turbidity correction, and then use the corrected values in this equation:

$$\mu g \text{ chl } a \text{ } L^{-1} = \frac{S\,[11.85\,(Abs664) - 1.54\,(Abs647) - 0.08\,(Abs630)]}{V}$$

Where S = volume of acetone used for the extraction (mL)
V = volume of water filtered (L)
L = cell path length (cm; this is normally 1 cm for the cuvettes we use)

Total Suspended Solids

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Ken Dunton/Kim Jackson

Adapted from: EPA METHOD #: 160.2

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

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

Assay for Sediment and Water Column Ammonium

Ken Dunton and Kim Jackson
Revised December 2009

Adapted from: Parsons, T.R., Y. Maita and C.M. Lilli. A Manual of Chemical and Biological Methods for Seawater Analysis. Determination of Ammonia (The Alternative Method). Pergammon Press, New York, 1st edition, 173 pp.

Field Procedures

Collection and Storage: Water

Collection bottles are acid washed before use. At the sampling station, bottles and their lids with sample water are rinsed several times prior to filling with actual sample. Leave approximately 1 cm of air space at the top of bottle to prevent water expansion during freezing from cracking the bottle. Immediately place samples on ice in field, and freeze as soon as possible upon return to the lab. DO NOT STORE SAMPLES IN REFRIGERATOR. Allow samples to thaw completely before taking a sub-sample. Bottles can be thawed overnight in a refrigerator. Standard curve and reagents are calculated for 2.5 ml samples. Larger or smaller volumes may be used, but reagent volumes must be multiplied or divided accordingly.

Collection and storage: Sediment

A 2.5 cm core is taken from the top 10 cm of sediment. Extrude the sample into a 50 ml polycarbonate bottle. If using Whirl Pacs, remove the air from the bag, and seal the bag tightly. Immediately place samples on ice in field, and freeze as soon as possible upon return to the lab. DO NOT STORE SAMPLES IN REFRIGERATOR. Allow samples to thaw completely. Samples can be thawed overnight in a refrigerator.

Laboratory Procedures

Once thawed, homogenize samples by stirring with glass rod or squeezing the bag with your hands several times. Fill an individually pre-labeled plastic centrifuge tube ~ ¾ full of sample. Weigh each sample + centrifuge tube to the nearest 0.1 g and match with another sample weighing the same. This assures that the centrifuge is balanced prior to use. Cap each centrifuge tube immediately after weighing to prevent evaporation from the sediment sample. Place the two samples weighing the same opposite each other in the centrifuge. Continue until all eight places are full. Hand tighten the centrifuge lid, shut the door, and set the centrifuge to run for 10-20 min (depending on soil moisture) at 10,000 rpm.

Reagents

1) Ultra pure water: Double deionized reverse osmosis water found in Tracy Villareal's lab.
2) Phenol alcohol: Dissolve 5 g reagent-grade phenol in 50 ml 95% ethanol. Store in dark and in refrigerator.

3) Sodium nitroprusside solution: Dissolve 0.5 g sodium nitroprusside in 100 ml ultra pure water. Store in dark and in refrigerator. Solution is stable for at least one month (a color change to brown indicates that the solution should be re-made).
4) Alkaline solution: Dissolve 80 g sodium citrate and 4.0 g NaOH in 400 ml ultra pure water. Solution is stable indefinitely.
5) Sodium hypochlorite: Use commercially available hypochlorite (e.g. Ultra Clorox-do not use cheap brands) which should be about 1.5 N. The solution decomposes slowly and should be checked periodically (see note 4). A new bottle of bleach should be purchased approximately every month, or before running a new standard curve.
6) Oxidizing solution: Mix 5 ml alkaline solution with 1.25 ml of sodium hypochlorite. This makes enough for ~25 samples. Adjust volumes for larger sample sizes. Keep covered when not in use and prepare fresh each day samples are run (i.e. do not use from previous days).

Standard Curve

Add 133.7 mg NH_4Cl (FW = 53.49 g $mole^{-1}$) to a volumetric flask and bring to 500 ml with ultra pure water (= 5 mM). Add 1 ml of this solution to a volumetric flask and bring to 100 ml with ultra pure water (= 50 μM). Standard curve and reagents are calculated for 2.5 ml samples. Larger or smaller volumes may be used, but reagent volumes must be multiplied or divided accordingly. Dilute stock solution as follows:

Stock solution	Blue Water	μg NH_4^+/2.5 ml	μM NH_4^+
0.0 ml	2.5 ml	0.00	0
0.5 ml	2.0 ml	0.45	10
1.0 ml	1.5 ml	0.90	20
1.5 ml	1.0 ml	1.35	30
2.0 ml	0.5 ml	1.80	40
2.5 ml	0.0 ml	2.26	50

Run at least three replicates (n = 3) for each concentration.

μM concentrations can be calculated from μg NH_4^+/2.5 ml by multiplying by 22.2.

For example, (1 μg NH_4^+/2.5 ml) x (1000 ml/L) x (1 μmole NH_4^+/18 μg NH_4^+) = 22.2 μmole/L

Samples

1) Rinse a test tube rack (use rack with small holes)
2) Set up test tubes in rack (label with site, replicate # if necessary)
3) Add 2.5 mL of water sample or standard (use 5000 μL pipette set to 250; use new pipette tip for each water sample) to corresponding test tube. Dilute as necessary with low-ammonia seawater (i.e. "blue water"). For example, for sediments, dilute 0.5 ml sample with 2.0 ml "blue water". Make sure to run two blue water blanks with your samples and two standards.
4) FROM THIS POINT ON, SAMPLES SHOULD BE PROCESSED IN THE HOOD.
5) Add 0.1 ml phenol alcohol. (Use digital pipette. Hit F, then 2, then make sure it says 1000 μL, hit enter, enter 100 μL, hit enter, enter 10, hit enter, place pipette into phenol alcohol,

hold down button on side until it beeps, put pipette in first sample, push down button until it beeps, then move to next sample and do same, after dispensing in all ten samples, put pipette over phenol and hit the C button to clear any left in pipette, repeat for next set of samples); vortex and wait one minute. (Note: rinse the pipette with the phenol alcohol first to prevent dripping; i.e. withdraw some up into pipette and empty back into phenol container; use the digital pipette.)

6) 0.1 ml sodium nitroprusside solution (use digital pipette); vortex and wait one minute.
7) 0.25 ml oxidizing solution (use digital pipette set to 4 dispenses of 250 μL); vortex and wait one minute.
8) Mix samples thoroughly. Cap or cover with parafilm and allow to develop for 1 hr in the dark and at room temperature.
9) Record absorbance at 640 nm on spectrophotometer. Make sure to auto zero spectrophotometer to blue water blanks that have had chemicals added to them as well. Read standards first and again every 10 samples to ensure spec is running properly.
10) Regress samples absorbance to standard curve (take into account the dilution factor).

Notes

1) Use glassware which has been cleaned with 10% HCl and rinsed thoroughly with DIN free RO water.
2) Use 10 ml disposable test tubes for ease with vortexing and cleaning.
3) Freezing sediment cores may alter the distribution of NH_4^+ because ice crystals may change pore size and as the core freezes, salinity increases in unfrozen porewater and NH_4^+ may be pulled off the sediment and detrital particles.
4) See Parsons et al., 1984. Use only Clorox bleach, no "off" brands. When having difficulties with low readings, check age of bleach.
5) Great care is necessary to reduce contamination from external sources. Latex gloves should be worn at all times and regularly changed during the analyses. No use of ammonia for other purposes should be allowed in the same lab. Do not move back and forth between ammonium and nitrate+nitrite sample processing stations, particularly when using stock solution of ammonium. This can contaminate work areas. Do not transfer scissors, sharpies, etc. between workstations.
6) Sample color is stable for ~ 24 hr after the reaction period, if kept out of direct light.
7) Do not dilute samples with deionized distilled water (use "blue water"); this could upset the ion balance and affect NH_4^+ concentration.
8) If the colorimetric reaction seems somehow not right, check the pH and make sure that it does not exceed 9.8. Also, more than 0.25 ml of oxidizing solution may have to be added.
9) Range: 0.1 to 50 μM. It's important to remember this range when diluting samples, especially sediment porewater.

Sediment Grain Size

Updated December 2009
Ken Dunton/Kim Jackson
(adapted from Rick Kalke, Paul Carangelo and Dr. E.W. Behrens)

Field

Three replicate sediment samples are collected at each station using a plastic syringe (2.5 cm diameter, 10 cm length) driven up to 10 cm into the seafloor (depth is determined by sediment compactness). These samples are placed in pre-labeled Whirl Pak bags and immediately placed on ice for transport to the lab.

Lab

Samples are either immediately frozen or processed upon return to the lab. If samples are frozen, the samples will be thawed overnight in a refrigerator prior to processing.

To determine sediment grain size, sand/silt/clay ratios are determined following the methods of Folk (1964). Percent contribution by weight is measured for four components: rubble, sand, silt, and clay. A 20-ml sediment sample is mixed with 100 ml of 3% hydrogen peroxide and 75 ml of de-ionized water to digest organic material in the sample. The sample is then wet sieved through a 62µm mesh stainless steel screen using a vacuum pump and a Millipore Hydrosol SST filter holder to separate rubble and sand from silt and clay. After drying, the rubble and sand will be separated on a 250µm screen. The silt and clay fractions will be measured using pipette analysis. Briefly, the settling velocity will be used to classify the particles and to determine the percent composition of each fraction, based on weight.

Step by Step Procedure

1. Wash all glassware with detergent (Alconox) and rinse with distilled or deionized water.
2. Extract 20 cc of homogenized sample using a wide-mouth syringe, or fill syringe using a spatula.
3. Place sample in a labeled beaker and add 100 ml 3% hydrogen peroxide. Mix with a rubber policeman and let sit until liquid is clear (several days). This step is to digest the organics in the sample.
4. Weigh labeled aluminum weighing pans and 62µm stainless steel filters; one each for each sediment sample.
5. Decant excess hydrogen peroxide (as much as possible without stirring up the sample). Add approximately 100 ml deionized water to the sample; stir and filter using a vacuum pump and a Millipore Hydrosol SST Filter Holder with 62um screen. Repeat two more times or until water is more or less clear. Dump the remaining sand and rubble onto the filter; rinse beaker and rubber policeman. (Don't use more than 900 ml water in this step).
6. Place sand and screen in weighing pan and dry at 100-130 degrees C for at least 24 hours.
7. Pour the filtrate in a 1-liter graduated cylinder. Add 10 ml 10% Calgon dispersant and dilute to 1 liter. After all samples have sat long enough to be close to room temperature, take water temperature and refer to temperature/fall distance table to determine the length of time between the first and second withdrawals.

8. Weigh and label two beakers (A and B) for each sample.
9. Stir the samples uniformly, working with one sample at a time. Twenty seconds after stirring, insert a pipette to a depth of 20 cm and withdraw 20 ml of the suspension. Put first withdrawal in the pre-weighed A beaker. Rinse pipette with deionized water into A beaker. Proceed with other samples.
10. Second 20 ml withdrawal is taken from a depth of 10 cm at the time indicated on the temperature/fall distance table and placed in the pre-weighed B beaker. Rinse pipette with deionized water.
11. Place A and B beakers into the drying oven and dry at 100-130 degrees C for at least 24 hours.
12. After pans of sand and beakers have dried completely, remove from oven and allow them to cool to room temperature and equilibrate with the humidity in the atmosphere (one to two hours).
13. Weigh each beaker to the nearest 0.001 g.
14. Weigh one aluminum pan (no screen) to the nearest 0.001 g. This pan will be used to weigh the sand fraction of each sample and can be used repeatedly without re-weighing each time. (Just be sure you get all of the sand out).
15. Weigh each pan + screen + sand + rubble to the nearest 0.001 g for each sample. Dump pan contents into a 250µm geological sieve, making sure to get all the sand out of the pan and off the screen. Sieve the sample. Pour the sand into the pre-weighed aluminum pan from step 14; weigh to the nearest 0.001 g.

Notes

1. (Weight of silt/clay in the A beaker) - 0.02 (weight of dispersant/20 ml) x 50 = total weight of silt/clay (call this F) in the total sample. Percent silt equals 100 x (F- weight of clay)/(S + F).

2. (Weight of clay in the B beaker) – 0.02 (weight of dispersant) x 50 = total clay in sample. Percent clay = 100 x weight of clay/(S +F).

3. The percent of sand in the sample is 100S/(S + F), where S is the sand fraction.

Reference

Folk, R.L. 1961. Petrology of Sedimentary Rocks. Hemphills Press. Austin, Texas. 154 p.

Sediment Organic Carbon

Updated December 2009
Ken Dunton and Kim Jackson

Field Procedures

Three replicate sediment samples are acquired at each station using a plastic 60cc syringe (2.5 cm diameter, 10 cm length with end removed) for sampling. Push the syringe into the sediment 10 cm deep. The samples are placed in pre-labeled Whirl Pak bags and immediately placed on ice for transport to the lab.

Lab Procedures

Samples are either immediately frozen or processed upon return to the lab. If samples are frozen, the samples are thawed overnight (in the refrigerator) prior to processing. Samples are homogenized, placed in aluminum weighing tins and dried in a 105 ºC oven (to remove water) for 12-24 hr. Samples are then removed from the oven and placed in a dessicator (to prevent moisture from the air changing the sample weight) to cool to room temperature. Once cooled, samples are weighed to the nearest 0.1 g, and placed in a muffle furnace to combust organic material, at 550 ºC for 4 hr. After cooling samples to room temperature in a dessicator, the samples are reweighed and Loss on Ignition (LOI) calculated using the following formula, where DW is sample dry weight (in grams):

LOI_{550} (as a percentage) = $((DW_{105} - DW_{550}) / DW_{105}) \times 100$

The weight loss is proportional to the amount of organic carbon contained in the sample.

Quantitative Measurements of Seagrass and their Algal Epiphytes

Ken Dunton, Kim Jackson, and Chris Wilson
Revised December 2009

Field Procedures

Transect Lines and Calculation of Seagrass Cover

We can employ three different techniques to assess vegetative percent cover. The first is the Braun-Blanquet method, which can be applied to both seagrasses and benthic macroalgae. At each site a 50-m transect is established by extending a meter tape along the bottom (preferably in an up-current direction in the absence of a depth gradient). Ten quadrats (0.25 m^2) are placed along each transect at pre-determined random distances from the marker rods at the "0 meter" mark. A new set of random sampling positions are chosen before each visit to a site. Each quadrat is examined underwater by a diver. All seagrass species occurring in the quadrat are listed, and a score based on the cover of the species in that quadrat is assigned (Table 1). Cover is defined as the fraction of the total quadrat area that is obscured by a particular species when viewed from directly above.

A second more quantitative measure employs 0.25 m^2 quadrats subdivided into 100 5 x 5 cm cells to estimate percent cover of each seagrass species and bare area along the 50 m transect. The transect line will extend (1) perpendicular to an existing depth gradient from shallower to deeper water, *or in the absence of a discernable depth gradient*, (2) perpendicular from the shoreline outwards into the bay. The "0 meter" mark will be oriented at the shallow edge or at the shoreline and the "50 meter" mark will be positioned toward deeper water or towards the bay.

Leaf Area Index (LAI) is a third measure to assess vegetation cover. It is calculated as a product of blade width measurements, shoot length, and shoot density. These data are retrieved from the analysis of seagrasses in cores collected for biomass (see below) at three or more random locations within 5 m of the transect line.

Table 1. Braun-Blanquet abundance scores (S). Each seagrass species will be scored in each quadrat according to this scale.

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

Seagrass Biomass
Three replicate cores are used for estimates of above- and below-ground biomass. A 15 cm (ID) diameter corer is used to sample *Thalassia,* and a 9 cm ID diameter core is used to sample *Halodule, Syringodium, Ruppia,* and *Halophila*. Samples are taken of each species present within 5 m of the transect line at each site. Species present (i.e. seagrass species composition) will be determined by visual in situ analysis of plants observed within a 25 m radius of each site. Samples are placed in pre-labeled Ziploc bags and immediately placed on ice. A PVC or polycarbonate core is used for the collection of belowground and above ground material. Care is taken to keep only the shoots that actually belong in the core.

Following placement of the large 15-cm core on the seabed, the rubber stopper is removed from the top of the core. For both 9-cm and 15-cm cores, before pressing the corer into the sediment, the diver runs their fingers carefully around the bottom of the core. If grass has been pulled under the core, it is removed. The diver then presses and twists the core down into the sediment 10-15 cm. The stopper is re-installed in the 15-cm core, and the core rocked back and forth. The diver then works their hand under the core and removes it from the grass bed, making sure to keep their hand under the bottom of the core (to prevent loss of sample).

After emptying the core into the sieve, broken shoots are removed since these are likely exterior shoots that were cut by the core tube. Samples are placed in pre-labeled Ziploc bags and immediately placed on ice.

Drift Macroalgal Biomass
Drift macroalgal biomass is determined from the collection of all algal material within ten 0.0625 m^2 quadrats. Material from each replicate is placed in sealed plastic bags and then transported to the laboratory in cooled containers. If algal samples will not be sorted to genus (or species), an additional sample will be obtained for elemental composition (C:N) and stable isotope ratio (^{13}C and ^{15}N) analysis.

Laboratory Procedures

Seagrass and Drift Algal Biomass
Aboveground tissue will include leaves (including sheath material) and floral parts, while below-ground tissues will include root and rhizome material. This process begins with the separation of shoots from non-photosynthetic tissues (where the blade turns white). Leaves are carefully cleaned of all attached biota by scraping with a wet cloth or razor blade prior to analysis. Shoots are carefully counted to obtain accurate estimates of density (number of shoots per square meter). The roots and rhizomes are kept separate from the above-ground tissue and placed in separate aluminum envelopes for drying. Sample labels include information on site, species, date collected, shoots or R/R, and number of shoots. Dead plant material will be discarded. The live tissue (shoots, roots, and rhizomes) is dried to a constant weight (60 °C) and weighed to the nearest milligram. The drying process takes 3-5 days. The biomass values for above- and below-ground biomass are used to calculate a root:shoot ratio.

For benthic macroalgae, samples from each quadrat will be cleaned of debris and non-algal material and may be sorted and identified to genus (and species when possible). Samples (sorted

or unsorted) will be dried to a constant weight, weighed, and archived. If sorted, blade tissues from individual species will be dried, weighed, and archived separately. If not sorted, then cleaned samples will be dried and weighed to obtain a total weight.

C:N:P Ratios

Algal blade tissues and seagrass leaf tissue samples obtained directly adjacent to biomass cores is used for C:N:P determination. Tissue samples for C:N:P ratios must be 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. Algal 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 grounding to a fine powder using a mortar and pestle. Total carbon and nitrogen 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. Phosphorus 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.

Epiphyte Quantification

Estimates of algal epiphytic biomass will be made from separate leaf samples of entire shoots taken directly adjacent to the biomass cores. Leaf samples for epiphytic biomass must be processed within three days of collection. In the laboratory epiphytes are separated from the leaf surface by scraping with a scalpel. Scraped material is then collected and retained on pre-weighed glass fiber filters. The collected epiphytic biomass and scraped seagrass leaves are then dried to a constant weight at 60 °C for determination of dry weight biomass. Algal epiphytic dry weight biomass will be expressed as a percent of total dry weight biomass of seagrass tissue scraped. Estimates of epiphyte biomass made on an areal basis are only possible with *Thalassia* and require accurate measurements of the length and width of the area scraped. This is not a valid procedure for leaves of *Halodule* or *Syringodium*. This is based on the fact that *Halodule* and *Syringodium* leaves are both essentially terete (Fig. 1) and require knowledge of the radius (or diameter) of the leaf for an accurate determination of the surface area, which in turn requires high resolution three-dimensional (e.g. CT) imagery or microscopy.

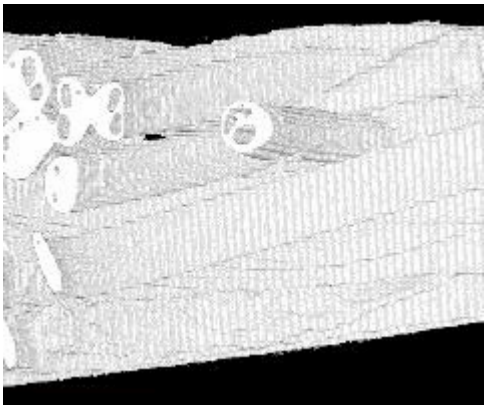

Figure 1. CT imagery of *Halodule* leaves illustrating their tube-like morphology (from Chris Wilson).

Stable Isotope Analysis: Protocol and Procedures

Ken Dunton, Kim Jackson and Patty Garlough
Updated November 2009

General instructions for stable isotope analysis

We analyze excised portions of tissue (for plants) or excised muscle tissue (for animals). When organisms are too small to excise tissues, the entire sample is prepared and combusted for ^{15}N or ^{13}C determination. Gloves are worn at all times for this analysis and organisms must be identified to species or appropriate taxonomic level prior to analysis. Small sample sizes increase the risk of contamination; therefore this process dictates extreme cleanliness. Work areas are kept organized, uncluttered and entirely spotless. Scattered pieces of sample must be removed following each preparation. All utensils are wiped with ethyl alcohol. Field bags, vials, and labels are detailed with organism identity, date, and site. Voucher specimens are often collected to confirm taxonomic identity.

Seagrasses

Samples are collected in well labeled (site, date, rep#, type of sample, species) Whirlpac bags, placed on ice and normally 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 base of the shoot, usually the area above white non-photosynthetic section of the blade sheath. Dead or senescent portions of blades or 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.

Algae

Algal samples are collected, cleaned, and catalogued as described for seagrass leaves. Sample sizes are approximately the size of a dime. Place the clean sample in a 10 ml labeled vial. For calcareous algae, half of the sample is acidified since these algae contain calcium carbonate that must be removed prior to isotopic analysis to obtain an accurate C^{13} measurement (two vials, one containing acidified tissue and one not containing acidified tissue, are needed for each sample replicate). The non-acidified sample vials are placed in a Petri dish and dried in oven. Samples are acidified in a dish containing 3% HCL /90% milli-Q water with just enough 3% acid to cover the tissue. Samples are soaked until bubble formation ceases, then decanted of acid and soaked with milli-Q water for 5 min. After excess water is removed, tissues are dried in an oven for minimum of 24 hours at 60C. A mortar and pestle is used to grind samples with all instruments (i.e. mortar, pestle, and spatula) cleaned with ethyl alcohol using Kimwipes. Ground samples are return to vials 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.

References

Dunton, K.H. and D.M. Schell. 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: ^{13}C evidence. *Marine Biology* 93:615-625

Dunton, K.H. 2001. $\delta^{15}N$ and $\delta^{13}C$ measurements of Antarctic peninsular fauna: trophic relationships and assimilation of benthic seaweeds. *American Zoologist* 41(1):99-112.

Appendix C – TAMU-CC Standard Operating Procedures

Fluorescence Assessment of Seagrass Epiphyte Accumulation

Introduction

This section describes the procedures used to measure abundance and accumulation profiles of epiphytes on seagrasses. The abundance of epiphytes is often considered to be an integrated measure of nutrient conditions in a seagrass bed, but is the result of complex interactions between a variety of factors (discussed in Borum 1985; Lin *et al.* 1986; Frankovich and Fourqurean 1997; Moore and Wetzel 2000; Hays 2005; Heck and Valentine 2007; Peterson *et al.* 2007; Burkholder *et al.* 2007). Eutrophication affects growth of epiphytes and seagrass leaves directly via nutrition and indirectly by stimulation of phytoplankton and changes in top-down control by grazers and predators. The method described here measures fluorescence of photosynthetic accessory pigments as a proxy for epiphyte abundance. This measure achieves significantly greater spatiotemporal resolution compared to traditional measures of epiphyte biomass. The accumulation profile, a plot of incremental epiphyte abundance along the age gradient of the seagrass leaf, will provide an historical record of epiphyte recruitment and growth *relative* to the growth of the seagrass leaf. This relationship is expected to change with increased eutrophication. The relative leaf areas of seagrass samples can also be estimated with the described method. Fluorescence images of epiphytes can be archived for subsequent development of additional analytical tools such as comparisons of the predominant morphotypes of fluorescent epiphytes.

This novel fluorescence method digitally images and analyzes epiphytic organisms which contain photosynthetic accessory pigments absorbing light in the green range of the visible spectrum (532 nm) and emitting fluorescence at wavelengths between 550 nm and 610 nm (Cammarata 2008). These organisms include cyanobacteria, red algae, diatoms, cryptomonads, brown algae and dinoflagellates (Raven *et al.* 2005; Frouin 2006; Robertson 2009). The pigments primarily responsible for this absorption are phycobilins, fucoxanthin and peridinin (French and Young 1952; Dawson *et al.* 1986). The method is based on the preferential excitation and fluorescence emission signatures of the accessory pigments in epiphytes relative to those of the underlying seagrass leaf which contains only chlorophylls and lutein-based carotenoids.

Scanning seagrass leaves by this method does not quantify green algal components of seagrass epiphytes specifically, because the red excitation light needed to excite the chlorophylls of the green algae also excites the leaf pigments. However, if the epiphytes are removed from the seagrass blade by scraping, then removed epiphytes can be fluoresced and quantified using *both* red and green excitation wavelengths. This will provide a measure that includes all of the different types of epiphytic algae, including green (Cammarata *et al.,* 2009). Perhaps more importantly, any changes in the relative contributions of green and red algae to total epiphyte abundance can be captured by comparing the ratio of red-excited fluorescence to green-excited fluorescence. Previous work has documented changes in primary producer composition by nutrient addition (see for example Armitage *et al.* 2005), so it will be useful to monitor for such changes.

The procedures outlined in this section are designed to produce data of consistent quality meeting an objective of not more than 15% total error in fluorescence quantification of a given

sample. In addition, long term comparisons of fluorescence measurements will be facilitated by normalization to measurements of fluorescence standards.

Sample Harvesting

There will be three transects at each of the Port Bay and East Flats study sites. Samples for epiphyte fluorescence measurements will be collected near quadrats representing the shallow end, middle and deep end of each transect. Three replicate samples will be obtained for each significant seagrass species at each quadrat (nine samples per seagrass species for each transect). Seagrass samples will be obtained from locations displaced "up-current" from the transects in order to avoid excessive disturbance caused by other sampling activities. Sampling and fluorescence epiphyte measurements will be performed separately for each seagrass species whose abundance is estimated to exceed 20% of the total seagrass coverage at a quadrat site. Thus, if two species are present at >20% of the total seagrass coverage, separate samples will be obtained for each seagrass species. Single-species seagrass shoot samples (up to 50) will be obtained by gently pinching or cutting off shoots near their base, handling only at the base to avoid disturbing attached epiphytes and transferring to widemouth sample bottles.

Unique identifier numbers/letters will be placed on sample containers and recorded on field sheets. This information will be transferred to a sample log that will accompany samples.

At an appropriate time, samples will be transferred from the field team to a laboratory courier to effect prompt delivery of samples, under the appropriate storage conditions, to the laboratory.

Sample Handling and Storage

Sample processing starts with reception of samples in the laboratory. Upon receipt, each sample identification number will be checked against a copy of the sample log sample identification record. Any discrepancy between sample identification numbers and the sample log, or any missing or damaged samples will be reported to the Project Manager within 24 hours, verbally or in writing.

Within the laboratory, all samples will be carefully tracked by sample number using a laboratory log. It will be the responsibility of the laboratory to keep accurate and timely records of the status of all samples in their custody.

Samples will be stored between 0˚C and 40˚C to avoid freezing and retard evaporative drying. Samples will be stored away from direct sunlight. Samples exposed to environmental extremes or potentially subject to drying due to loosely-fitting or damaged containers will be flagged and described in the sample log. Stored samples must be easily retrieved and protected from environmental extremes.

In the laboratory, samples will be stored at < 20˚C in either an ice chest or a refrigerator. The maximum holding time for sample storage prior to fluorescence scanning will be 72 hours from the time of harvest.

Sample Preparation for Fluorescence Measurements

Seagrass leaves, harvested and stored in sample bottles as described above, will be gently removed from sample bottles and transferred into a shallow tray of distilled water to briefly (< 1 minute) and gently wash off sediments or other unattached debris. Seagrass leaves are to be handled gently and at the base only, to avoid disturbing attached epiphytes. Individual blades will be severed from the shoot at the ligule and transferred to the platen of the scanning fluorescence imager. The number of whole shoots to be scanned will depend on the seagrass species and blade length, but will be at least ten (10) *Halodule* or *Syringodium* shoots, or five (5) *Thalassia* shoots, and not more than 20 of any one species. Only living (green) blades will be scanned. Blades of more than one seagrass species from a single quadrat site may be scanned collectively, but in such case they will be sorted according to species to facilitate separate post-scan analyses. Blades will be positioned lying flat and in a parallel, non-overlapping orientation. Sample washing, transfer to the scanning platen and initiation of scanning will occur within ten minutes to prevent excessive drying. Any blades exhibiting curling will be re-wetted with a few drops of distilled water. The scanning compartment lid will be closed immediately upon loading. The scanned seagrass blades will be scraped to remove epiphytes as described below. The epiphyte-free blades will be dried at 60^{o}C to constant weight for biomass determination.

For measurements capturing the green algal seagrass epiphyte components, and to detect potential shifts in algal family composition, seagrass epiphyte samples removed from the seagrass blades will be transferred into optical 96-well microplates and measured for epiphyte fluorescence. Three (3) to ten (10) whole shoots will be gently washed as described above, transferred to a clean tray containing deionized water (10-50 mL), and gently scraped with the edge of a glass microscope slide to remove the epiphytes from both sides of each blade. Removed epiphytes will be quantitatively transferred to a capped tube and the final volume will be adjusted to a standard volume (typically 14 mL) and stored at 0 – 5 ºC. Fluorescence assays will be made after vigorously vortexing the samples and transferring 50 µL aliquots into the wells of 96-well optical microplates.

Fluorescence Scanning and Quantification

Instrument Settings and Adjustments

For each sample, two types of scans will be obtained. "Green Scans" will utilize green 532 nm laser excitation to excite fluorescence from accessory photosynthetic pigments and "Red Scans" will utilize green 633 nm laser excitation to excite fluorescence from chlorophyll photosynthetic pigments. Standard default instrument settings will be as follows, with any deviation so noted in the laboratory log.

Green Scans

The X:Y coordinates of the platen area to be scanned will be selected.
Acquisition Mode: Fluorescence
Setup Parameters:

- Laser: 532 nm (green) excitation
- Emission Filter: 580 bp 30nm emission filter
- PMT: 360 V
- Sensitivity: Normal

Orientation: "R"
Pixel Size: 200 µm for typical quantification/ 10 µm for high resolution archival images
Press: Yes
Focal Plane: Platen (use +3 mm for samples in 96-well plates)

Red Scans
The X:Y coordinates of the platen area to be scanned will be selected.
Acquisition Mode: Fluorescence
Setup Parameters:
Laser: 633 nm (red) excitation
Emission Filter: 670 bp 30nm emission filter
PMT: 360 V
Sensitivity: Normal
Orientation: "R"
Pixel Size: 200 µm for typical quantification/ 10 µm for high resolution archival images
Press: Yes
Focal Plane: Platen (use +3 mm for samples in 96-well plates)

Measurement Calibration with Reference Fluorophores
To facilitate long term data comparisons, it will be important to understand the sensitivity of the fluorescence detection. Excitation laser power and photomultiplier tube (PMT) sensitivity may change over time, so periodic characterization will enable normalization of quantitative fluorescence data. This characterization will be obtained by recording scans of reference fluorophores. Selected reference fluorophores are B-phycoerythrin or eosin Y. Solutions of these will be prepared in 50 mM Tris-Cl buffer, pH 7.5 and quantified by absorbance measurements ($E_M^{545\ nm} = 2.41 \times 10^6 M^{-1}cm^{-1}$; $E_M^{517\ nm} = 78{,}200\ M^{-1}cm^{-1}$; for phycoerythrin and eosin-Y, respectively).

Initial solution concentrations of the reference fluorophores will be adjusted to final concentrations of 0.1 M (phycoerythrin), 0.1 mM (eosin Y) with buffer, and then used to prepare five, 5-fold serial dilutions to span the *relative concentration* range of 1 X to 1/3,125 X. Standard solutions will be aliquotted and stored appropriately (4 ˚C or -20 ˚C, respectively, dark).

For fluorescence measurement characterization, 50 µL of each reference dilution will be pipetted into the 96-well optical plates. Aliquots of buffer will be included as a control. Both red and green scan images will be obtained using the normal sample scanning parameters outlined above. Total fluorescence signal for each standard concentration will be obtained as described below, and plotted to determine linearity and sensitivity. This data will be entered into the QC Summary Sheet for seasonal sampling.

Sample Measurements
For each sample, two types of scans will be obtained. "Green Scans" will utilize green 532 nm laser excitation to excite fluorescence from accessory photosynthetic pigments, and "Red Scans" will utilize red 633 nm laser excitation to excite fluorescence from chlorophyll photosynthetic pigments. For general quantitative purposes, scans will be obtained using a 200 µm pixel size.

In addition, at least one representative high resolution archival image will be obtained for each major seagrass species from a sample obtained near the middle of each transect. The archival images may be useful to identify epiphyte species morphologies. A 10-25 μm pixel size will be used to obtain high resolution green-excited scans.

Upon sample scan completion, the platen will be rigorously cleaned as follows: All samples are removed and debris is gently blotted away with lint-free laboratory wipers. The platen is washed and wiped clean using, in all cases, non-scratching optical wipers with a defined sequence of solutions: distilled water, 70% ethanol, 10% H_2O_2 and distilled water. (Appropriate safety equipment for use of 10% H_2O_2 includes gloves, eye protection and lab coat).

File Naming, Data Storage and Data Backup

Epiphyte image data obtained at 200 μm resolution will be labeled by the unique sample identification number with "red" or "green" appended as a suffix. Data labels for scans performed at 10 μm resolution will additionally be appended with "10u". Raw image data is obtained in a ".gel" file format. Scan data will be saved in this format, and additionally in ".tif" file format. All scan data will be backed up onto a portable hard drive device following scanning of all samples for an individual sampling trip. Sample data will additionally be backed up onto DVD media as well.

Data Processing

Epiphyte image data obtained at 200 μm resolution will initially be quantified from a ".gel" file format using "ImageQuant" software. The image area(s) to be analyzed is (are) delineated with an object box. Then, for each seagrass species on a scan, the following parameters will be determined:

- Total signal above background from green-excited fluorescence
- Total signal above background from red-excited fluorescence
- Total number of pixels above background from red-excited fluorescence

Numerical values will be recorded into a spreadsheet for calculations and analysis.

Epiphyte image data obtained at 10 μm resolution will be archived in both ".gel" and ".tif" file formats for possible future analyses, if warranted, of epiphyte morphologies.

Accumulation profiles indicative of epiphyte recruitment and growth rates, relative to seagrass leaf growth rate, will be quantified as follows: First, the leaf length of representative full-length undamaged blades will be determined from the red scan image by drawing a line from leaf base to leaf tip and multiplying the number of pixels by 200 μm. This length will be divided to obtain the number of standard sized (1-10 mm) optical slices into which the image will be divided. A single-column grid with this number of rows will then be positioned on the leaf image. This grid will then be copied and pasted onto the corresponding green image (representing epiphytes). A volume report will quantify the total green epiphyte signal in each segment of the image ("total signal above background"). The profile of epiphyte accumulation can be obtained by plotting the fluorescence signal versus the position from the leaf base. Plots of accumulation profiles may, if necessary, be smoothed by the "sliding windows" technique that averages quantitative data for groups of windows.

Data Analysis

Data for the following parameters will be compiled into a spreadsheet which will serve as the data quantification log and compared for each site, sampling event, and seagrass species.

- Total red-excited fluorescence signal above background
- Total number pixels red-excited fluorescence above background (an estimate of scanned leaf area for normalizing epiphyte signal measurements; also a proxy for leaf biomass)
- Total green-excited fluorescence signal above background (a measure of total epiphyte load in shoot samples)
- Total green-excited fluorescence signal above background divided by total number pixels red-excited fluorescence above background (a *normalized* measure of epiphyte accumulation based on scanned leaf area)
- The profile of epiphyte accumulation from the leaf base (a measure of epiphyte recruitment and growth relative to the growth of the seagrass leaf)
- The ratio of the total red-excited fluorescence signal above background divided by the total green-excited fluorescence signal above background (only for microplate assays of removed epiphytes)

For example, data on the analyzed parameters will be plotted by sampling date, by site and by seagrass species to observe apparent trends or differences. Statistical analyses will be applied to determine significance.

Processed data will be transmitted to the Project Manager in electronic format, within 30 days of scanning, for collective analysis of total project data as described elsewhere. All processed data will additionally be compiled in the Final Report.

Quality Assurance and Quality Control

Various quality control (QC) procedures will be implemented to ensure consistent production of high quality data. A minimum of 5% of all samples will be re-scanned to produce a parallel set of data for analysis and comparison. The objective is not more than 15% total error in fluorescence quantification of a given sample. For samples scanned directly on the platen, following the first set of green and red scans, the instrument settings will be returned to those of the first scan (*i.e.* green) and then the whole scanning process will be repeated. Duplicate analyses will be documented and noted in the laboratory log.

The results of sample re-scans may require that certain actions be taken. If results from duplicate scans differ by more than 15%, all of the samples from that particular sampling site or transect (to which that duplicate scan applies) will be re-scanned and a random sample selected again for a duplicate scan. If QC criteria are met, sample residues may be discarded. Re-scan results will be summarized on a QC Summary Sheet.

We anticipate continuation of this project, necessitating long term comparisons of fluorescence measurements. These comparisons will be accompanied by comparison of and normalization to measurements of fluorescence reference samples. We cannot predict if, or how much, instrument sensitivity may change over time. But the reference fluorophore measurements described above will provide a valuable measure of any such changes and permit data normalization for long term comparisons.

Data Management, Reporting and Deliverables

All sample information and numerical data generated in the laboratory are recorded directly onto standardized data forms. Image scans will be archived as described above for future re-analysis. Data forms and sample/file labeling contain all necessary information, so that data is recorded clearly and unambiguously. Completed data forms are kept in notebooks arranged by form type. All sample logs will be digitally recorded by document scanning and saved along with other data. Archived image scans will be stored on an external hard drive and CD/DVD media upon completion of a seasonal sampling event.

The Project Manager will receive electronic copies of this data within 30 days of scanning. Numerical data and forms will be in spreadsheet format while archival pictures will be in ".tif" or ".gel" file formats. A Final Report will be prepared and delivered in electronic format to the Project Manager no later than January 31, 2011.

Data Forms

This section lists example data forms that are used for sample tracking and data recording. Example forms are appended to this document. Examples of data forms are presented in the following order:

Sample Log
Laboratory Log
Quantification Log
QC Summary Sheet
Data Log

References for Fluorescence Assessment of Seagrass Epiphyte Accumulation

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Appendix: Data Forms

Sample Log
Sample ID
Date
Time
Location
Collector
Description
    Site conditions
    Seagrass Species Present
    Collection Container Type
    Observations
Notes
    Damage ?

Laboratory Log
Sample ID
    Checked With Sample Log ?
    Exposure To Environmental Extremes ?
Location, sample type and sample description
Date & Time Received
Receiver
Scan Date & Time
    Scan conditions: Default or Different ?
    Collection Container Type
    Associated Filenames : Default or Different ?
Duplicate Analysis Samples
Notes

Data Quantification Log
Sample ID
    Scan Date & Time
    Associated Filenames
        Total # Pixels Red
        Total Green Fluorescence Above Background
        Total Red Fluorescence Above Background
        Total Green Fluorescence Above Background/ Total # Pixels Red
        Epiphyte Accumulation Profile
        Ratio Total Red Fluorescence Above Background divided by Total Green
    Fluorescence Above Background for microplate assays of removed epiphytes

QC Summary Sheet
Results of Duplicate Scans
    Sample ID & Filenames
        Total # Pixels Red
        Total Green Fluorescence Above Background
        Total Red Fluorescence Above Background
        Total Green Fluorescence Above Background/ Total # Pixels Red