# Supplemental Data Review for Seagrass Response to Wastewater Inputs: Implementation of a Seagrass Monitoring Program in Two Texas Estuaries

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# Supplemental Data Review

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# List of Acronyms

| Acronym | Definition |
|---|---|
| % SI | percent surface irradiance |
| µg | microgram |
| µM | micromoles per liter |
| AOI | area of interest |
| CBOD | carbonaceous biochemical oxygen demand (5-day) |
| GIS | geographic information systems |
| GLO | General Land Office |
| $K_d$ | light attenuation coefficient |
| km | kilometer |
| lbs/day | pounds per day |
| MANERR | Mission Aransas National Estuarine Research Reserve |
| mg/L | milligrams per liter |
| MGD | million gallons per day |
| mL | milliliter |
| NOAA | National Oceanic and Atmospheric Administration |
| NTU | nephelometric turbidity units |
| PAR | photosynthetically active radiation |
| ppt | parts per thousand |
| RSR | root-to-shoot ratio |
| s.d. | standard deviation |
| SI | surface irradiance |
| SWQM | Surface Water Quality Monitoring |
| SWQMIS | Surface Water Quality Monitoring Information System |
| TAMU | Texas A & M University |
| TCEQ | Texas Commission on Environmental Quality |
| TCOON | Texas Coastal Ocean Observation Network |
| TOC | total organic carbon |
| TPWD | Texas Park and Wildlife Department |
| TSS | total suspended solids |
| UTMSI | University of Texas Marine Science Institute |
| VSS | volatile suspended solids |
| WCID | Water Control and Improvement District |

# Introduction

The Coastal Management Program of the General Land Office (GLO) has funded Texas Parks and Wildlife Department (TPWD) to conduct a seagrass monitoring project in two Texas estuaries. This project will investigate the impact of a domestic 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.

Seagrass, a type of submerged aquatic vegetation, has been identified as a critical habitat under the Coastal Coordination Act. 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 also economically important based on their function in maintaining Gulf fisheries. Growing coastal populations and increasing coastal development threaten seagrass habitat.

Three state agencies with primary responsibility for conserving coastal natural resources, Texas General Land Office, the Texas Commission on Environmental Quality (TCEQ), and the Texas Parks and Wildlife Department, signed the Seagrass Conservation Plan for Texas in 1999 (TPWD 1999). Currently, TPWD facilitates quarterly meetings of a Seagrass Monitoring Work Group comprised of experts from academics and government. A major objective of the group is to develop a state-wide seagrass monitoring plan.

This eighteen-month project will evaluate seagrass condition in two areas of the central Texas coast using protocols recommended to the Seagrass Monitoring Work Group by Dunton and Pulich (2007). One area is more or less “pristine” (East Flats in Corpus Christi Bay) and the other area has a planned domestic wastewater treatment discharge into a seagrass bed (Port Bay). Identical procedures will be used in East Flats and Port Bay and includes three components: 1) landscape monitoring using high resolution color aerial photography, 2) seagrass condition and water quality indicators, and 3) epiphyte analysis.

The landscape monitoring portion includes aerial photography that will be obtained at least once during the study at each site. Each site or area of interest (AOI) will be photographed at a 1:9,600 scale using color imagery. The photography scale provides the needed accuracy for the analysis of three seagrass stress indicators: bare patches (number, shape), depth limit of seagrass coverage, and macroalgae deposition (areal coverage).

For seagrass condition and water quality indicators, this project will use recommendations of Dunton *et al.* (2007), which identified indicators of stress on seagrasses. Water quality data that will be collected include nutrients, chlorophyll-*a*, suspended solids, light attenuation, salinity, dissolved oxygen, and temperature. Sediment will be analyzed for pore water ammonium, total organic carbon (TOC), and grain size. 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 be working with Dr. Kenneth Dunton of the

University of Texas Marine Science Institute (UTMSI) to collect information regarding seagrass condition in addition to relevant water quality indicators.

The final component of this study includes a novel technique developed by Dr. Kirk Cammarata of Texas A&M University (TAMU) - Corpus Christi to quantify and analyze epiphytic algae growth on seagrass leaves. In nutrient-enriched waters, epiphytic algae growth may increase, which can at some point interfere with photosynthesis and cause seagrass loss. Measurements of epiphytic algal density are 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).

Data collected at East Flats and Port Bay will be used to test the Dunton and Pulich (2007) seagrass monitoring protocol as well as help determine whether the effluent limitations that TCEQ has permitted are effective in protecting seagrasses in the vicinity of the planned discharge in Port Bay. Seagrass condition, water quality indicators and epiphytic algae analysis will be conducted along three transects at Port Bay and East Flats during the spring, summer, and fall of 2010. As of April 2010, the wastewater facility on Port Bay has not begun discharging. If the facility 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 facility is not yet operational, the sampling will continue as described above with Port Bay data being used as baseline data which can be compared with data obtained after the plant is operational. A second phase of sampling is not included in this project, which concludes March 2011.

A review summarizing previous studies of the East Flats and Port Bay areas is presented here. Existing data from near the two study sites will provide valuable background when interpreting the data collected for this project. This review focuses on the recommended seagrass condition and stressor indicators listed above, local human influences, and natural features. Sources, including peer-reviewed articles, natural resource agency reports and databases, and information from coastal conservation organizations, were reviewed for this report.

## Study Area

East Flats and Port Bay are in the coastal bend of the Texas coast. East Flats is a small shallow embayment within Corpus Christi Bay, TCEQ Segment 2481, approximately 5 km west of the City of Port Aransas (Figure 2). Port Bay lies within the Copano Bay system, TCEQ Segment 2472, of the Mission and Aransas Rivers. It is a narrow bay with a small watershed that enters the southern most corner of Copano Bay approximately 9 km west of the City of Rockport (Figure 3).

Areas of interest (AOIs) have been defined for both East Flats and Port Bay. The AOIs represent a subsample of the bays containing seagrass (Figure 2 and Figure 4). The AOIs will be the focus for landscape analysis of aerial imagery and are where water quality and seagrass condition measures will be sampled at three 50-m transects. The AOIs for East Flats and Port Bay are approximately 154 ha and 109 ha, respectively. Transects have been strategically placed to contain the deep edge of the seagrass beds to help determine which environmental factors limit seagrass growth. Transects in Port Bay are also placed in relationship to the planned domestic discharge in order to measure any potential impacts to the AOI (Figure 2 and Figure 4).

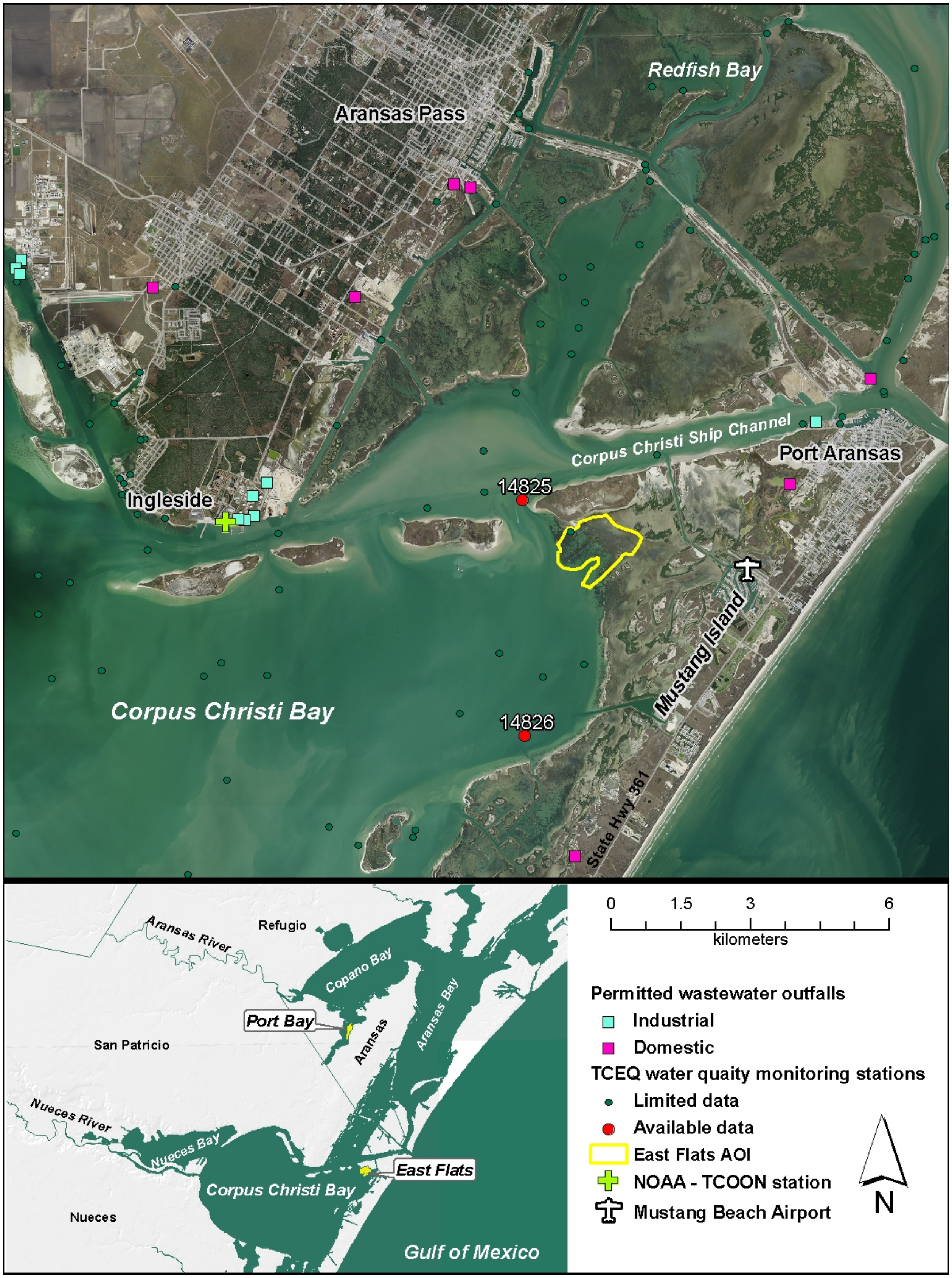


**Figure 1. East Flats study area.**

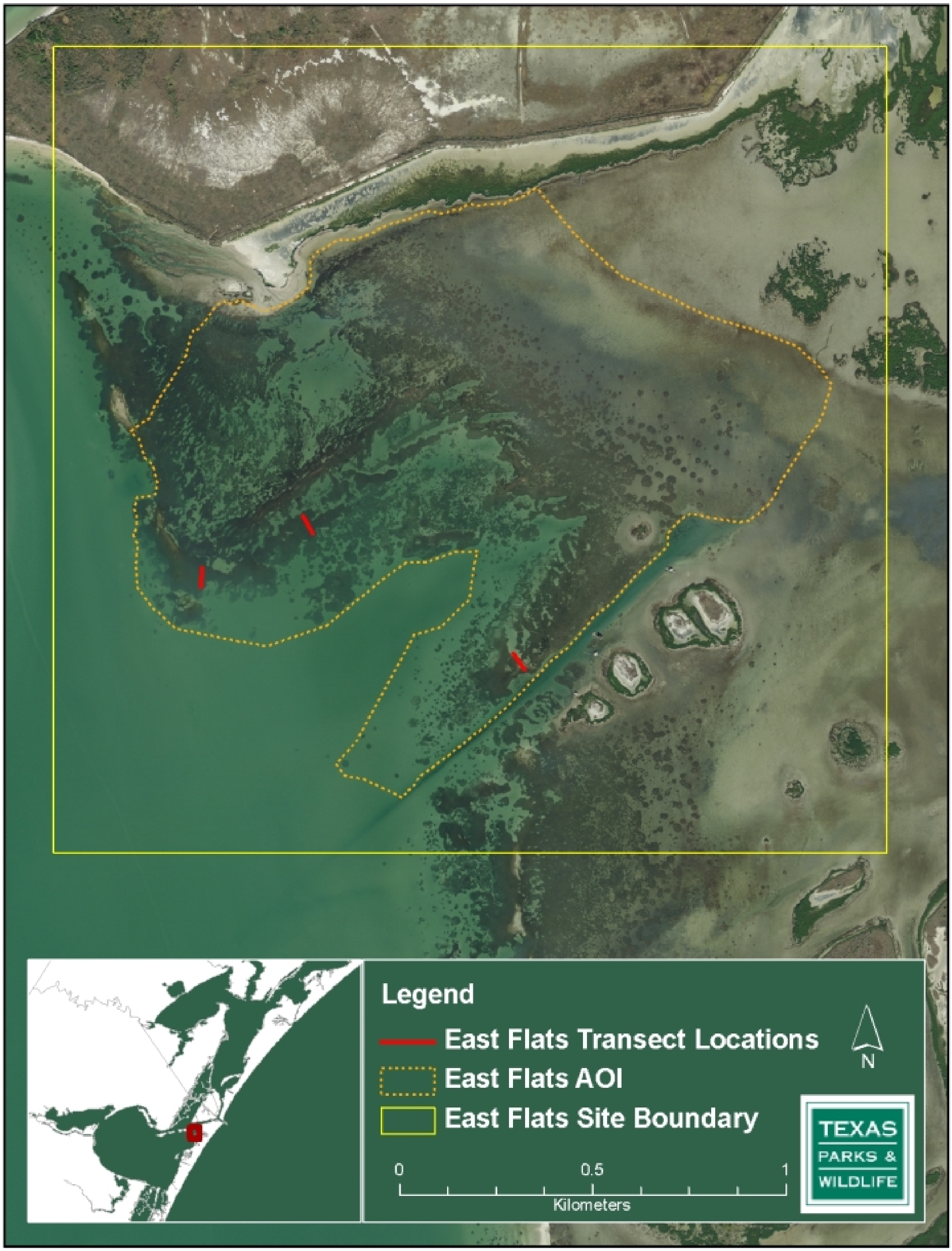


**Figure 2. East Flats study area, area of interest (AOI), and transects.**

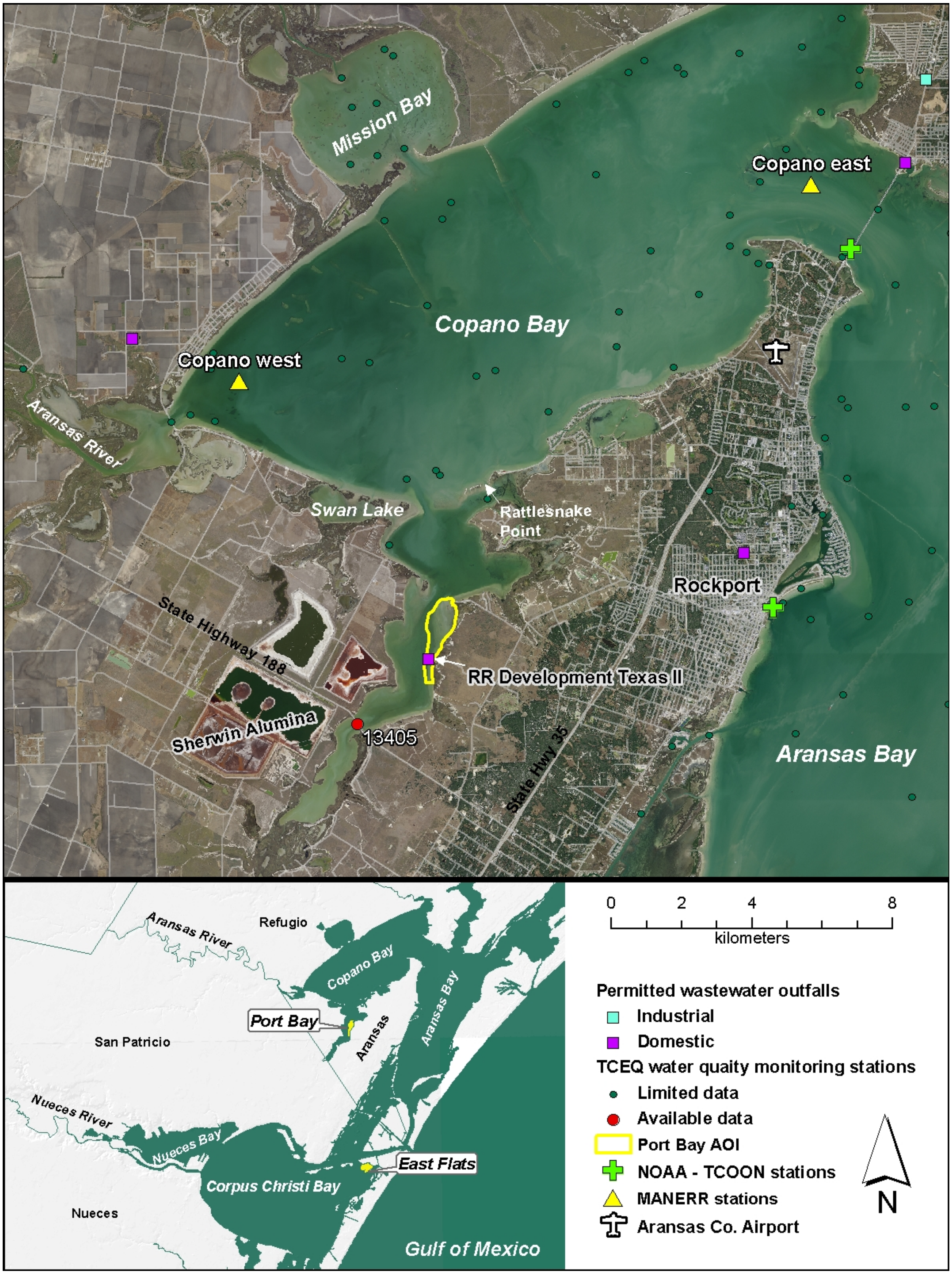


**Figure 3. Port Bay study area.**

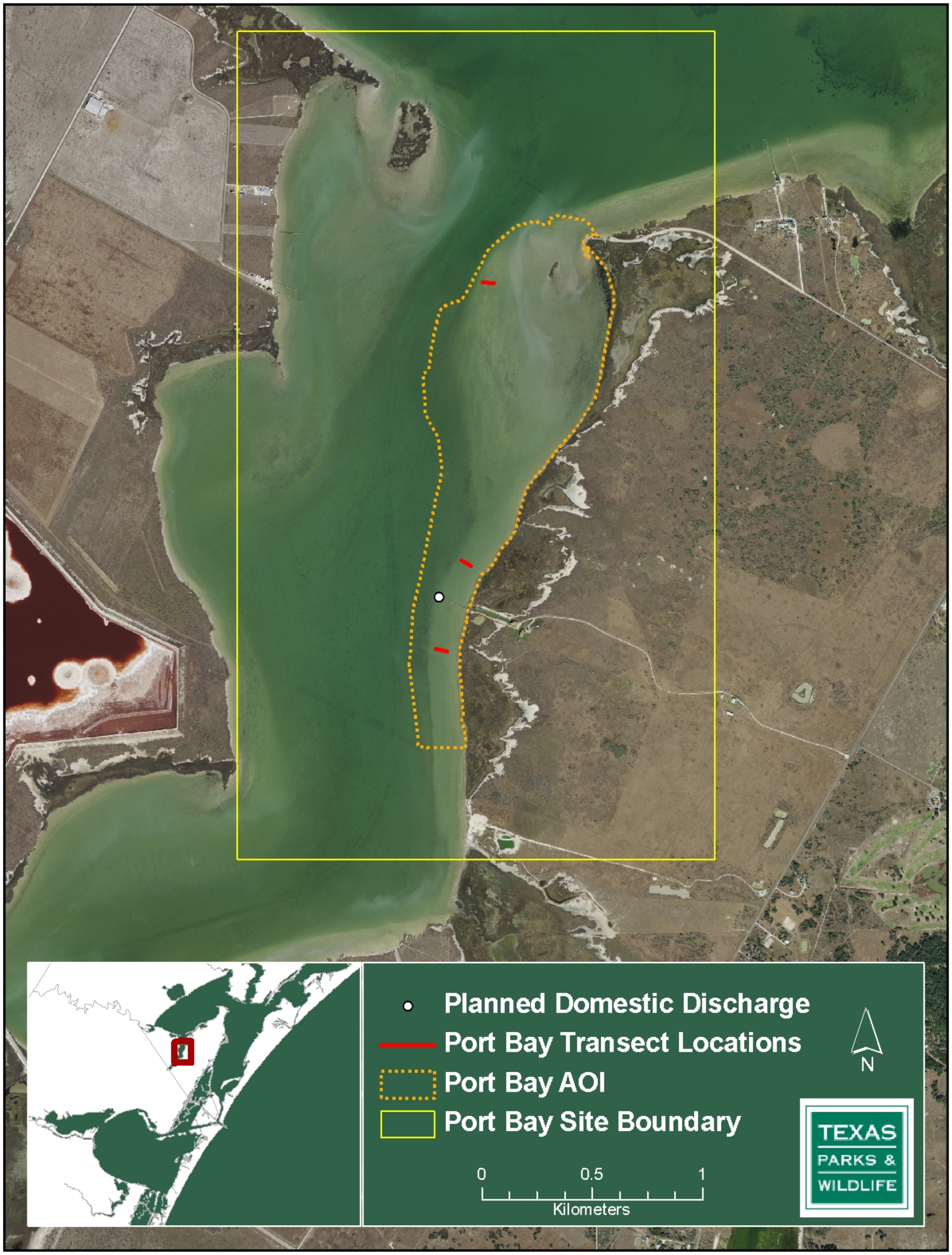


**Figure 4. Port Bay study area, area of interest (AOI), and transects.**

# Natural Features and Human Activities

Seagrass beds provide habitat for fish and invertebrates, food for waterfowl, help reduce sediment suspension and act as water quality indicators. Many scientists consider them ecological indicators and, in fact, Orth *et al.* (2006) refers to seagrasses as "coastal canaries." Unfortunately, seagrasses worldwide are under direct threat from a variety of human influences (Orth *et al.* 2006).

Worldwide human influences in coastal regions include increased nutrient and sediment runoff, introduction of invasive species, hydrological alterations and commercial fishing practices (Orth *et al.* 2006). Along the Texas coastal bays and estuaries human influences include increased commercial and recreational use of the bays, increased nutrient and contaminant loading, and growing populations (Pulich and Onuf 2007).

## *East Flats*

East Flats contains all five species of seagrass, *Halodule wrightii*, *Thalassia testudinum*, *Ruppia maritima*, *Syringodium filiforme*, and *Halophila engelmannii*[1]. The seagrasses provide habitat for juvenile fish and invertebrates, food for waterfowl and prey for larger fish. The East Flats area is protected on all except the western side by dredge spoil islands and is considered to have had minimal human influence since the 1950s when oil and gas exploration occurred in the area. One study hypothesized that salinity fluctuations and higher epiphyte loads at East Flats may act as continuing disturbances, stalling seagrass succession and thus accounting for the ongoing persistence of multiple seagrass species (Gutierrez *et al.* 2010).

The human activities most likely to affect the seagrass beds in East Flats are dredging operations and boating. The northern border of East Flats is delineated by the Corpus Christi Channel, which must be periodically dredged so that large ships can use the channel. Other dredging activity occurred in the process of oil and gas exploration, which created cuts and channels in the shallow flats and marshes. Dredging activities re-suspend sediment into the water column, affecting water clarity. The dredged sediments are often deposited near or on top of seagrass beds. Current human activities in East Flats include hunting, fishing and boating. Both boating and dredging can disturb the seagrass roots and suspend sediments which block sunlight needed for growth.

The western portion of East Flats is bordered by Corpus Christi Bay and the rest of the surrounding lands are flats and marshes. Due to the relatively inaccessible location of East Flats, there is a "noticeable lack of residential and waterfront development" (Pulich 2007).

There are 22 permitted industrial and municipal wastewater outfalls to Corpus Christi Bay; all are more than 6.4 km from East Flats. The closest permitted discharges are two domestic wastewater discharges from Nueces County WCID 4, which are located in protected areas without a direct hydrologic connection to East Flats. The industrial dischargers are located on

[1] Taxonomic designation of seagrasses is under review. *Halodule wrightii* is sometimes designated *Halodule beaudettei* and *Syringodium filiforme* as *Cymodocea filiformis*.

the northern shore of Corpus Christi Bay; the Corpus Christi Ship Channel runs between the dischargers and East Flats. Due to the locations of the permitted discharges, East Flats is not directly affected by these dischargers.

### Port Bay

Two seagrass species are predominant in Port Bay; *H. wrightii* and *R. maritima*. The seagrasses provide habitat for juvenile fish and invertebrates, food for waterfowl and prey for larger fish. The Port Bay area also has estuarine and freshwater marshes that attract migratory birds.

In contrast to East Flats, Port Bay supports several types of human activity. The Port Bay Hunting and Fishing Club was established in 1909 and continues to host hunting and fishing excursions. Commercial crabbing is also found in Port Bay. TPWD stocked 333 *Centropomus undecimalis* common snook into the bay in 1985, which is the only time that TPWD stocked the area.

Residential development around Port Bay has been minimal, however much of the undeveloped land is available or has been purchased and is in the planning stages of development. The majority of existing development is located in the northern portions near Copano Bay. The northeastern shore (just south of Rattlesnake Point) has an established housing development, Cape Valero. The western portion, east of Swan Lake, is currently for sale as Port Bay Point. Between Port Bay Point and the Sherwin Alumina holding ponds, on the western shore, sits a small housing tract. The eastern and southern edge of Port Bay has not yet been developed.

RR Development Texas II, Inc. has a 144 unit housing area planned for construction on the eastern shore of Port Bay. They have received a domestic wastewater discharge permit (WQ0014925-001) that authorizes a discharge to the eastern part of Port Bay of 0.183 MGD during the first phase and 0.55 MGD in the final phase. Discharge limits are 5 mg/L for carbonaceous biochemical oxygen demand (CBOD) (5-day), 5 mg/L for total suspended solids (TSS), 2 mg/L for ammonia-nitrogen, 6 mg/L for total nitrogen and 35 cfu/100mL for *Enterococci* (colonies per 100 mL). Total phosphorus limits are 3 mg/L for total phosphorous in the first phase and 1.0 mg/L in the final phase. At the time this was written, the wastewater plant has not been built.

A large portion of the western shore of Port Bay is owned by Sherwin Alumina Company and contains their secondary holding tanks for their bauxite smelting operations. The bauxite is manufactured approximately 13 km south of the holding tanks. There is no discharge permit for the holding ponds as there is no discharge to the bay. The ponds take up an estimated 25 $km^2$ of the land on the west side of Port Bay.

### Weather, tide, and freshwater inflow information

Environmental conditions such as weather, sea level, and freshwater inflow can play a significant role in seagrass condition. Each study site has weather, tide, and water quality data available from nearby sources. These sources can be used to obtain information such as wind velocity and direction, water level, salinity, precipitation, and air and water temperature, which can be used to help interpret seagrass condition data. Data sources include monitoring stations maintained by the National Oceanic and Atmospheric Administration (NOAA), the Texas Coastal Ocean Observation Network (TCOON), and the Mission Aransas National Estuarine Research Reserve (MANERR).

East Flats and Port Bay are situated in a semi-arid region with a subtropical climate. Average annual precipitation for this area is 81.9 cm with September being the wettest month. Average high and low temperatures for January are 19 ºC and 8 ºC, and average high and low temperatures for August are 34 ºC and 24 ºC. Annual average wind velocity is approximately 8 knots and predominantly from the southeast with periodic winds from the north in the fall and winter (NWS 2010; TCEQ 2010).

Both sites are shallow and water levels are influenced by tidal and weather patterns, and can fluctuate between high and low tide as much as twice daily. The Rockport tide gage has been used for observing trends in sea level for the Texas coastal bend since 1948. On an annual basis average mean sea level is highest in October (0.138 m) and lowest in January (-0.111 m). While there are daily and seasonal water level changes, there has been a steady rise in mean sea level which is equivalent to 5.16 ± 0.67 mm/yr from 1948 to 2006 (NOAA 2010). This is equivalent to a change of 0.52 m in 100 years.

## Seagrass Coverage and Species Composition

Aerial photography and geographic information system (GIS) technology are useful tools for measuring seagrass coverage and have potential for detecting changes in seagrass beds. Aerial photography at 1:24,000 scale has been commonly used to look at historical changes in coastal habitats, including seagrass. At this scale, it is also possible to differentiate between continuous and patchy seagrass beds. Continuous seagrass beds are undisturbed homogenous areas of plant shoots, while patchy seagrass beds, generally considered disturbed or stressed, are a mixture of seagrass and bare areas (Pulich *et al.* 1997). Recent studies have integrated higher resolution aerial photography (1:9,600 scale) and GIS technology to detect, assess, and monitor changes and landscape disturbances to seagrass beds (Pulich *et al.* 2007). With this finer resolution, analysis of continuous and patchy seagrass beds can be more accurately delineated which in return can help identify the disturbances affecting seagrass health. Studies have also identified seagrass species composition in East Flats, and to some extent in Port Bay (Pulich *et al.* 1997, Pulich 2007).

### *East Flats*

Current seagrass distribution and historical trends for the Corpus Christi Bay side of Mustang Island (including East Flats) are discussed in Pulich *et al.* (1997) and Pulich (2007). Aerial photography (1:24,000 scale) from the late 1950s, 1974, and 1994 was used to review the trends in seagrass distribution. Two seagrass bed types were mapped in East Flats, continuous and patchy. These landscape features could be clearly distinguished at sizes above 0.05 ha. Patchy seagrass beds were groupings of small patches of seagrass, between 0.05 and 0.10 ha in dimension, with equally small, open bare areas separating them. These projects found seagrass increased by 98 ha between 1958 and 1974 and 375 ha between 1974 and 1994 for a total of 1503 ha on the bay side of Mustang Island in 1994. Of this total, 917 ha were continuous seagrass beds and 586 ha were patchy seagrass beds. The probable causes for the increase of seagrass included minimal shoreline developments, increasing water depth (subsidence and sea level rise), and fetch protection. These factors allowed seagrass to be established in previously disturbed areas and newly-inundated tidal flats.

Both analyses identified seagrass composition for the bay side of Mustang Island. Frequency of seagrass species were calculated based on groundtruthing surveys from 1995 – 1996. The bay side of Mustang Island is dominated by *H. wrightii* (86%), followed by *T. testudinum* (15.8%), *R. maritima* (15.0%), *S. filiforme* (1.9%), and *H. engelmannii* (0.4%). Total percentage is more than 100% because of mixed assemblages of species in many samples. A separate study (Czerny and Dunton 1995) characterized East Flats as having mixed and pure strands of *H. wrightii* and *T. testudinum* to depths of 1.2m, and deeper areas were dominated by *H. wrightii and H. engelmannii*. *T. testudinum* was also found to be the most common seagrass in East Flats (Mutchler and Dunton 2007).

Pulich *et al.* (2007) conducted landscape analysis in Corpus Christi Bay (East Flats) and Redfish Bay in support of developing techniques to detect and monitor changes in seagrass beds. The techniques involved classifying seagrass bed characteristics into landscape indicators using 1:24,000 and 1:9,600 scale aerial photography taken in 2004 and 2005, and GIS analysis. The study identified five seagrass bed polygon classes for the East Flats study area (approximately 120,000 m²): bare bottom, dense seagrass, patchy seagrass, macroalgae, and mixed seagrass/algae (Table 1). They found that bare and patchy polygons less than 2-3 m² in size are significantly underestimated at the 1:24,000 scale compared to 1:9,600 scale. Aerial photography at 1:9,600 scale was shown to have potential in detecting seagrass bed changes over time. Comparison of changes in vegetated and bare areas at East Flats between 2004 and 2005 has not been completed.

**Table 1. East Flats 2004 landscape indicator classes by acreage (Pulich *et al.* 2007).**

| Polygon class (m²) | 1:9,600 | 1:24,000 |
|---|---|---|
| Bare | 29,653 | 29,122 |
| Patchy | 20,583 | 20,092 |
| Dense seagrass | 57,703 | 62,070 |
| Macroalgae | 8,038 | 7,567 |
| Mixed seagrass/algae | 4,362 | 1,489 |

## Port Bay

Pulich *et al.* (1997) and Pulich 2007 identified 724 ha of seagrass in Port Bay and Swan Lake from 1994 aerial photography, 188 ha of which was classified as patchy, 440 ha as continuous, and 96 ha as a mixture of rooted seagrass and wrack (dead seagrass, macroalgae, and detritus). Neither looked at historical trends for Port Bay. Seagrass composition for the Aransas and Copano Bay system, including Port Bay, was calculated based on groundtruthing surveys from 1995 – 1996. The Aransas and Copano Bay system is dominated by *H. wrightii* (90.0%), followed by *T. testudinum* (15.3%), *R. maritima* (9.2%), *H. engelmannii* (6.0%), and *S. filiforme*

(2.8%). Total percentage is more than 100% because of mixed assemblages of species in many samples. It was noted that *H. engelmannii* and *T. testudinum* were not found in Port Bay. No mention was made regarding presence or absence of *S. filiforme*.

Tremblay *et al.* (2008) used color infrared aerial photography from 2004, as well as historical aerial photography from the 1950s and 1979, to identify the current and historical distribution of wetland and aquatic habitat in the Corpus Christi Bay area (including Port Bay). Wetland and aquatic habitats were delineated at a scale of 1:5,000 using color infrared aerial photography. In the 1950s, Port Bay had relatively few seagrass beds mapped (136 ha). Seagrasses flourished between the 1950s and 1979, spreading along the shoreline of Port Bay and into Swan Lake, reaching their height in 1979, when 811 ha were mapped. In 2004, 606 ha of seagrass were mapped, representing a long-term increase, but a 25% decrease from 1979. By 2004, seagrasses had invaded previous tidal flat areas as sea level rise inundated flats, resulting in a net long-term gain of seagrass.

### *Additional seagrass coverage information*

Seagrass coverage shapefiles created from aerial photography from 1994 and 2004 can be viewed at http://gis-apps.tpwd.state.tx.us/website/Seagrass/viewer.htm. Both data sets include seagrass coverage for East Flats and Port Bay. The 1994 seagrass coverage data is from Pulich (1997). The 2004 seagrass coverage data is from a NOAA benthic mapping project for the Texas Coastal Bend (NOAA 2007).

The Texas Strategic Mapping Program, Texas Natural Resources Information System and the Texas Water Development Board published 0.5-meter aerial photography from January 2009. The imagery included 18 coastal counties. Seagrass coverage data has not yet been developed from this imagery.

## Water Quality Indicators

Water quality data can be used to interpret seagrass condition measurements and to detect human influence. However, the correlation between seagrass condition and water quality indicators is not well-represented in the literature, suggesting the need for further study. Seagrass condition is influenced by a number of natural stressors as well as human disturbances, and it can be difficult to distinguish those variables. Seagrass condition and water quality are also variable through time and space. Long-term data sets of routine monitoring at strategic locations will be necessary to interpret the complex relationship between seagrass condition and water quality stressors (Mutchler and Dunton 2007). Available water quality indicators in East Flats and Port Bay include instantaneous and long-term physicochemical measurements as well as water chemistry data.

## *East Flats instantaneous physicochemical measurements*

### Dissolved oxygen

Dissolved oxygen concentration is typically measured to assess impacts to aquatic life. Impacts include the onset of eutrophication-induced periods of anoxia that can have devastating effects on benthic communities (Mutchler and Dunton 2007). Anoxia can also negatively impact seagrass photosynthesis, metabolism, and growth (Burkholder 2007).

Mutchler and Dunton (2007) found dissolved oxygen measured monthly at 10 sites in East Flats to be highly variable and poorly related to seagrass condition indicators. Some difficulty arose in interpreting dissolved oxygen concentrations, as hourly and daily variation was high due to effects of time of day and daily irradiance levels. To capture the range of conditions that are likely to affect seagrass condition, the study recommends measuring dissolved oxygen continuously at select sites as part of a coastwide seagrass monitoring program.

A search of the TCEQ Surface Water Quality Monitoring Information System (SWQMIS) database for instantaneous dissolved oxygen data near East Flats identified stations in Corpus Christi Bay, however no data currently exist for the region near East Flats.

### Salinity and specific conductance

Salinity measurements can be used to determine influences on seagrass condition from weather events such as drought and precipitation, and upstream water uses. However, to date, a correlation between salinity and seagrass condition in East Flats has not been observed. One reason may be seagrasses' ability to tolerate short-term salinity fluctuations. Another factor is that the site is relatively remote from freshwater inflow sources (Touchette 2007).

Salinity rather than specific conductance is typically reported in estuarine systems. A few studies in East Flats have reported instantaneous salinity values, which ranged from 12.1 to 39.4 ppt (Dunton 1990; Dunton 1996; Herzka and Dunton 1997; Mutchler and Dunton 2007). Salinity was one of several parameters measured by Dunton (1990) to explore correlations with leaf elongation rates and shoot production of *R. maritima* and *H. wrightii* in the Guadalupe Estuary and the Nueces Estuary, which included East Flats. Statistical analysis showed that changes in salinity from freshwater inflow had little to no effect on the growth dynamics of seagrass.

Dunton (1996) measured several abiotic parameters, including salinity, to characterize seasonal patterns of above- and below-ground biomass, leaf chlorophyll content, and *in situ* differences in photosynthetic parameters for *H. wrightii* along an estuarine gradient in three estuarine systems, including East Flats. Salinity varied considerably over both temporal and spatial time scales while high productivity was found in *H. wrightii*. This is not surprising, since the species is widespread and successful at colonizing new habitats.

Salinity was measured to supplement several parameters used to examine seasonal patterns in photosynthesis and irradiance in Herzka and Dunton (1997). A relationship between salinity and photosynthesis and irradiance was not identified.

In Mutchler and Dunton (2007), salinity was one of several parameters measured to identify habitat quality indicators for monitoring seagrass health. Salinity was measured monthly at 10 sites in East Flats. The highest values were recorded in the deepest sites where Corpus Christi Bay is most influential. Strong seasonal differences in salinity were also evident with salinity higher in the summer and lower in the winter. The study recommends salinity as a key parameter to detect local climatological influences and suggests measuring salinity monthly at all study sites and continuously at select permanent sites in a coastwide seagrass monitoring program.

Two TCEQ Surface Water Quality Monitoring (SWQM) stations in Corpus Christi Bay near East Flats have historical salinity data (Table 2).

## Temperature

Water temperature is a primary factor controlling seasonal growth of seagrasses (Lee and Dunton 1996; Lee *et al*. 2007; Mutchler and Dunton 2007). Optimal growth temperature for tropical/subtropical seagrass species is between 23 °C and 32 °C (Lee *et al*. 2007).

Several studies in East Flats have measured temperature with seasonal values ranging from 9 ºC in the winter to 33 ºC in the summer (Dunton 1990; Dunton 1996; Lee and Dunton 1996; Herzka and Dunton 1997; Mutchler and Dunton 2007). Temperature was one of several parameters found by Dunton (1990) to correlate with leaf elongation rates and shoot production of *R. maritima* and *H. wrightii* in the Guadalupe Estuary and the Nueces Estuary including East Flats. The study found the period of maximum leaf growth in *H. wrightii* coincided with a noticeable increase in seasonal water temperatures.

Dunton (1996) measured several abiotic parameters, including temperature, to characterize seasonal patterns of above-ground and below-ground biomass, leaf chlorophyll content, and *in situ* differences in photosynthetic parameters for *H. wrightii* along an estuarine gradient in three estuarine systems including East Flats. There were no significant differences in water temperature among sites; distinct seasonal variation was noted for all three systems. However, a consistent correlation could not be established between *H. wrightii* plant parameters and temperature.

Lee and Dunton (1996) studied seasonal changes in *T. testudinum* plant biomass, carbohydrate carbon content, leaf chlorophyll, and leaf productivity in relation to coincident measurements of temperature and continuous measurements of underwater photosynthetically active radiation. Leaf production was closely correlated with water temperature changes throughout the year. Productivity increased rapidly with increasing temperature during spring and summer and decreased rapidly with falling temperatures during fall and winter. The study concluded that annual productivity of *T. testudinum* appears to be primarily regulated by temperature.

Herzka and Dunton (1997) examined seasonal patterns in photosynthesis and irradiance for *T. testudinum* as related to annual fluctuations in temperature (in the field and laboratory). The study found *T. testudinum* displays higher light and respiratory requirements when *in situ* temperatures are high.

Temperature was one of the parameters measured to identify habitat quality indicators for monitoring seagrass health in Mutchler and Dunton (2007). Temperature was measured monthly at 10 sites in East Flats where values exhibited a strong seasonal pattern with average temperature in the summer approximately twice the average temperature in winter. The study recommended temperature as a key parameter to measure monthly at all study sites and continuously at select permanent sites of interest in a coastwide seagrass monitoring program.

Two TCEQ SWQM stations in Corpus Christi Bay near East Flats have historical temperature data (Table 2). Temperature and salinity were the only water quality parameters identified at TCEQ SWQMS stations near East Flats.

**Table 2. TCEQ SWQM instantaneous salinity and temperature data near East Flats (TCEQ 2009).**

| Station ID | Parameter Description | Period of Record | | n | Min | Max | Average | Median |
|---|---|---|---|---|---|---|---|---|
| 14825 | Salinity (ppt) | 31 Jul 1985 | 17 Dec 2002 | 74 | 10.8 | 34.1 | 25.3 | 25.7 |
| | Temperature (ºC) | 11 Mar 1996 | 17 Dec 2002 | 32 | 13.9 | 25.6 | 20.1 | 20.6 |
| 14826 | Salinity (ppt) | 05 Dec 1988 | 17 Dec 2002 | 73 | 10.0 | 34.5 | 25.7 | 26.5 |
| | Temperature (ºC) | 11 Mar 1996 | 17 Dec 2002 | 32 | 13.3 | 25.6 | 20.1 | 20.6 |

## pH

Studies conducted at East Flats have not reported pH nor have the nearby TCEQ SWQMIS stations. No studies were found that attempted to correlate pH measurements and seagrass condition.

## Secchi depth

Secchi depth is an easy and inexpensive way to measure water transparency and has been correlated with seagrass depth limits (Vicente and Rivera 1982). Secchi depth is a recommended field method for a coastwide seagrass monitoring plan (Dunton and Pulich 2007). Secchi depth data were not available for East Flats.

## Light availability

The distribution and primary productivity of seagrass is largely regulated by light attenuation within the water column (Dunton 1994). Both instantaneous and long-term light levels have been measured in previous studies of seagrass condition (Czerny and Dunton 1995; Dunton 1994; Mutchler and Dunton 2007). Typically, photosynthetically active radiation (PAR; 400 to 700 nm wavelength) was measured and light attenuation coefficients ($K_d$) and percent surface irradiance (% SI) were calculated.

Percent surface irradiance is 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.

Light attenuation is calculated using the transformed Beer Lambert equation:

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

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

Dunton (1994) stated that *H. wrightii* light demand is 18% SI while other seagrasses require 11% SI. Mutchler and Dunton (2007) collected data from East Flats and Redfish Bay, and found in East Flats the average % SI at deep sites (>1.7m) was 13.3 ± 15.5% and at shallow sites (<1.7m) was 59.5 ± 18.0%. Data ranged from 0.1% - 95%. The mean light attenuation coefficient measured at East Flats was 0.6 ± 0.5 $m^{-1}$ and ranged from 0.3-2.5 $m^{-1}$ (Mutchler and Dunton 2007).

Czerny and Dunton (1995) found that *T. testudinum* and *H. wrightii* require PAR levels that are equal to or greater than 14 to 16% of surface irradiance, especially in the fall. When the surface irradiance is reduced below 14 to 16%, the seagrass growth rates slow down due to decreases in photosynthesis. Czerny and Dunton also stated that *T. testudinum* and *H. wrightii* need more light during the spring and summer months when increasing water temperatures increase metabolic demands for growth and maintenance.

## *East Flats long-term physicochemical measurements*

### Water quality

An investigation of UTMSI studies, TCOON sites and TCEQ SWQM stations revealed no long-term dissolved oxygen, salinity, or specific conductance data near East Flats.

Dunton and Pulich (2007) recommended measuring light availability, dissolved oxygen, salinity, and temperature on a continuous basis with permanently deployed sensors to capture the range of conditions that are likely to affect seagrass. (Dunton and Pulich 2007).

### Additional long-term water quality information

A TCOON real-time monitoring station in Corpus Christi Bay near Ingleside, Texas, approximately 7 km west of East Flats, collects water temperature data. Current and historical data beginning in 1992 can be accessed from the Division of Nearshore Research, Texas A&M University-Corpus Christi website, http://lighthouse.tamucc.edu/datum/006.

## Light availability

Light penetration of the water column tends to vary over short time scales due to seasonal winds that stir up sediments from the bottom of the shallow bays, total suspended solids, chlorophyll-*a*, human activities, and other factors.

Dunton (1994) collected long-term measurements of PAR in three different bay systems along the south Texas coast, one being East Flats. The study showed that chronic long-term reductions in underwater PAR were reflected in total plant biomass rather than leaf elongation. Dunton also stated that annual light levels at East Flats collected between 1990 and 1992 ranged from 3300 to 5100 mol $m^{-2}$ $yr^{-1}$. He also found that ideal growth conditions for *H. wrightii* were an average depth of 1.25 m with an the annual light dose ranging from 5110 to 5700 mol $m^{-2}$ $yr^{-1}$ or 41 to 46 % SI based on an average surface irradiance of 12,506 mol $m^{-2}$ $yr^{-1}$.

The most effective use of light attenuation and % SI appears to be in identifying distribution limits at which seagrass ceases to occur (Mutchler and Dunton 2007). Mutchler and Dunton found a good correlation between % SI and depth. They suggested measuring light availability at the deep edge of seagrass beds to track light-mediated changes in seagrass distribution.

# *East Flats water chemistry*

## Inorganic nitrogen

Inorganic nutrients are considered a major factor in controlling seagrass growth (Lee *et al*. 2007). Seagrasses derive nutrients from the sediment and the water column (Burkholder *et al*. 2007; Lee and Dunton 1999b), and can assimilate nutrients through both leaf and root tissues, often in roughly equal proportions (Lee *et al*. 2007). On the other hand, increasing levels of nutrients (eutrophication) is a primary cause of decline of seagrasses worldwide (Burkholder *et al.* 2007). Damage to seagrass beds by excess nutrients is usually mediated indirectly by overgrowth of phytoplankton, epiphyte, and/or drift macroalgae, which can decrease the amount of light available to seagrasses (Dunton and Pulich 2007). Although inorganic nutrients are vital to seagrass growth, nutrient concentrations in estuaries are variable over time and space and human activities can increase the amount of nutrients available in the water column. Collection and analysis of water samples for dissolved nutrients can be time-consuming and costly, which puts a constraint on obtaining a large data set. The limited data that are available consist of instantaneous measurements that may not be representative of typical conditions in seagrass beds and must be interpreted with caution.

Several studies in East Flats have measured dissolved inorganic nitrogen as ammonium ($NH_4^+$) and nitrate + nitrite ($NO_3^-$ + $NO_2^-$) (Dunton 1996; Herzka and Dunton 1997; Lee and Dunton 1999a; Lee and Dunton 2000a; Dunton and Pulich 2007). Lee and Dunton (2000a) summarized data since 1990 and found that values ranged from 0.02 to 5.1 μM for $NH_4^+$ and 0.20 to 1.19 μM for $NO_3^-$ + $NO_2^-$. Mean values are 1.16 and 0.84 μM for $NH_4^+$ and $NO_3^-$ + $NO_2^-$, respectively.

Mutchler and Dunton (2007) collected dissolved inorganic nitrogen as $NH_4^+$ and $NO_3^-$ + $NO_2^-$ at 10 sites in East Flats to study water quality stress indicators for seagrass. Water column nutrient concentrations were generally low throughout the study, for example, $NO_3^-$ + $NO_2^-$ rarely exceeded 1 µM. Lee and Dunton (1996) also found nitrate plus nitrite almost always less than 1 µM. Herzka and Dunton (1997) found nitrate plus nitrite concentrations at East Flats to average 0.9 ± 0.1 (standard error) µM and ammonium at 1.3 ± 0.2 µM. Mutchler and Dunton (2007) found $NH_4^+$ was negatively correlated with *T. testudinum* blade length and above-ground biomass, indicating the potential for this variable to serve as an indicator of seagrass condition. Additionally, concentrations of $NH_4^+$ varied temporally with higher concentration in the winter and lower in the summer. Dunton (1996) also found that $NO_3^- + NO_2^-$ concentrations varied over both temporal and spatial scales. Another study found no distinct seasonal trend in nitrate plus nitrite (Lee and Dunton 1996).

## Ortho-phosphate

The main phosphorus source for seagrasses is ortho-phosphate ($PO_4^{-3}$), which occurs in the water column as well as in sediments (Lee *et al.* 2007). A limited amount of phosphorus data was identified for the East Flats area. Mutchler and Dunton (2007) collected $PO_4^{-3}$ monthly at 10 sites in East Flats in their study of water quality stress indicators for seagrass. The maximum annual average $PO_4^{-3}$ concentration was 1.35 ± 0.01 µM, while the minimum was 0.05 ± 0.02 µM. Analysis showed that $PO_4^{-3}$ concentration was positively correlated with cover of *H. engelmannii* and the tissue N:P ratios of *T. testudinum* leaves, and it was negatively correlated with *T. testudinum* blade width. The correlations suggest the potential exists for these variables to serve as possible indicators of seagrass condition.

## Chlorophyll-*a*

Chlorophyll-*a* is commonly used as a measure of the amount of phytoplankton in the water column. High levels of phytoplankton can decrease light reaching seagrasses and contribute to anoxia associated with nutrient enrichment (Dunton and Pulich 2007).

Mutchler and Dunton (2007) collected chlorophyll-*a* at 10 sites in East Flats in their study of water quality stress indicators for seagrass. Chlorophyll-*a* concentrations ranged from the detection limit of the method used, 0.2 µg $L^{-1}$, to 20.7 ± 4.1 µg $L^{-1}$, with higher values occurring in winter 2003, winter 2005 and summer 2005. Chlorophyll-*a* was not strongly correlated with nutrient concentrations, light attenuation, percent surface irradiance, or seagrass condition indicators. However, previous work in the Laguna Madre has demonstrated the effect that phytoplankton blooms can have on the light regime and consequently on the seagrass community (Onuf 1996). Herzka and Dunton (1997) also collected chlorophyll-*a* as supplemental data and reported mean, minimum, and maximum of 6.2, 2.1, and 14.5 µg $L^{-1}$, respectively.

## Total suspended solids and turbidity

Total suspended solids (TSS) and turbidity, measures of the amount of suspended inorganic and organic material in the water column, are linked to light attenuation and light availability for seagrass growth. Suspended solids absorb and scatter light as it travels through the water column. As a result, measurements of TSS and turbidity have clear relevance to seagrass condition (Dunton and Pulich 2007). Turbidity measurements were not identified near East Flats.

Mutchler and Dunton (2007) collected TSS monthly at 10 sites in East Flats in their study of water quality stress indicators for seagrass. Average TSS values ranged from 0.6 ± 0.6 to 90.7 ± 9.9 mg $L^{-1}$. The highest TSS values occurred during the summer 2003, summer 2005 and winter 2004. TSS was positively correlated with light attenuation, but the strength of association was weak. Many environmental factors can make TSS variable in both space and time and can weaken the usefulness of instantaneous TSS measurements. Mutchler and Dunton suggest, rather than collecting TSS samples at various sites on different days, a more effective approach may be to collect samples at strategic locations in one day. Alternatively, they suggest that continuous measurements with a turbidity meter would permit estimation of the duration of turbid conditions associated with high TSS. Combining these measurements with light recordings at the seagrass canopy would allow estimation of the effect of high TSS values on the light reaching the seagrass.

## *Port Bay instantaneous physicochemical measurements*

Instantaneous water quality data in Port Bay is limited to one TCEQ SWQM station. Station 13405 is located at State Highway 188 in Port Bay approximately 2.7 km from the center of the Port Bay study area. This station has several years of instantaneous measurements (Table 3). A search of the literature revealed no additional data, including light measurements, for Port Bay.

**Table 3. Port Bay instantaneous water quality data, TCEQ SWQM Station 13405 (TCEQ 2009).**

| Parameter | Period of record | | n | Min | Max | Average | Median |
|---|---|---|---|---|---|---|---|
| Temperature (ºC) | 18 Sep 1973 | 25 Mar 2009 | 159 | 6.8 | 32.5 | 22.6 | 23.9 |
| Secchi depth (m) | 27 Feb 1990 | 25 Mar 2009 | 66 | 0.1 | 1.0 | 0.4 | 0.3 |
| Specific conductance (μmhos $cm^{-1}$) | 18 Sep 1973 | 25 Mar 2009 | 158 | 1098 | 51300 | 20676 | 19119 |
| Dissolved oxygen (mg $L^{-1}$) | 18 Sep 1973 | 25 Mar 2009 | 155 | 4.2 | 14.3 | 8.2 | 7.9 |
| pH | 18 Sep 1973 | 25 Mar 2009 | 156 | 7.2 | 9.3 | 8.2 | 8.2 |
| Salinity (ppt) | 05 Nov 1985 | 25 Mar 2009 | 87 | 1.7 | 33.8 | 14.5 | 12.8 |

## *Port Bay long-term physicochemical measurements*

Long-term physicochemical data from Port Bay is limited to TCEQ SWQM Station 13405. This station has a few years of diel measurements (Table 4). A search of the literature has identified some data sources in Copano Bay outside Port Bay, which are not summarized here. When considering the spatial variability in physicochemical measurements, the Copano Bay data was determined to be too far away to be representative of the Port Bay study site. A search of the literature revealed no light measurements for Port Bay.

**Table 4. Port Bay diel physicochemical data, TCEQ SWQM Station 13405 (TCEQ 2009).**
**10 sets of 24-hr data collected (30 Jul 2002 - 27 Aug 2004).**

| Parameter | Min | Max | Average | Median |
|---|---|---|---|---|
| Temperature (ºC), min | 22.5 | 29.5 | 26.9 | 27.5 |
| Temperature (ºC), max | 25.6 | 32.1 | 29.8 | 30.4 |
| Temperature (ºC), average | 23.8 | 30.6 | 28.3 | 29.0 |

| Parameter | Min | Max | Average | Median |
|---|---|---|---|---|
| Specific conductance (μmhos $cm^{-1}$), min | 4840 | 20857 | 10652 | 9556 |
| Specific conductance (μmhos $cm^{-1}$), max | 5380 | 22431 | 12367 | 10697 |
| Specific conductance (μmhos $cm^{-1}$), average | 5290 | 21403 | 11506 | 10415 |
| pH, min | 8.1 | 9.0 | 8.4 | 8.2 |
| pH, max | 8.3 | 9.2 | 8.7 | 8.7 |
| Dissolved oxygen (mg $L^{-1}$), min | 4.1 | 6.9 | 5.8 | 5.8 |
| Dissolved oxygen (mg $L^{-1}$), max | 7.2 | 11.5 | 9.3 | 9.1 |
| Dissolved oxygen (mg $L^{-1}$), average | 6.2 | 8.0 | 7.2 | 7.2 |

## Additional long-term water quality information

The TCOON monitors temperature continuously in Copano Bay at Hwy 35 near the confluence with Aransas Bay. This is 16 km northeast of the Port Bay study area. Current and historical data beginning in 1992 can be accessed from the Division of Nearshore Research, TAMU-Corpus Christi website, http://lighthouse.tamucc.edu/overview/036.

The MANERR maintains two continuous monitoring stations in Copano Bay. Copano East is near Hwy 35 and Aransas Bay, 17 km northeast of the Port Bay study area, and Copano West is near the mouth of the Aransas River in Copano Bay, 9.5 km northwest of the Port Bay study area.

Copano East continuously collects chlorophyll concentration, dissolved oxygen, temperature, photosynthetically active radiation, pH, salinity, specific conductance, turbidity, and water depth. Current and historical data beginning in 2007 can be viewed or queried from the Division of Nearshore Research, TAMU-Corpus Christi website, http://lighthouse.tamucc.edu/overview/146.

Copano West continuously collects chlorophyll concentration, dissolved oxygen, temperature, pH, salinity, specific conductance, turbidity, and water depth. Current and historical data beginning in 2007 can be viewed or queried from the Division of Nearshore Research, TAMU-Corpus Christi website, http://lighthouse.tamucc.edu/overview/147.

## *Port Bay water chemistry*

Water chemistry data for Port Bay are limited to TCEQ SWQM Station 13405 (Table 5).

**Table 5. Port Bay water chemistry data, TCEQ SWQM Station 13405 (TCEQ 2009).**

| Parameter | Period of record | | n | Min | Max | Average | Median |
|---|---|---|---|---|---|---|---|
| Ammonia, nitrogen (mg $L^{-1}$) | 29 Oct 1973 | 25 Mar 2009 | 130 | 0.01 | 1.60 | 0.07 | 0.05 |
| Nitrite, nitrogen (mg $L^{-1}$) | 29 Oct 1976 | 12 Jul 2000 | 45 | 0.01 | 0.10 | 0.03 | 0.03 |
| Nitrate, nitrogen (mg $L^{-1}$) | 29 Oct 1973 | 12 Jul 2000 | 77 | 0.01 | 1.75 | 0.08 | 0.03 |
| Nitrate + nitrite, nitrogen (mg $L^{-1}$) | 29 Apr 1976 | 25 Mar 2009 | 84 | 0.00 | 0.15 | 0.04 | 0.02 |
| Ortho-phosphate, phosphorus (mg $L^{-1}$) | 29 Oct 1973 | 19 Aug 2003 | 147 | 0.01 | 5.90 | 0.10 | 0.03 |
| Chlorophyll-*a* (μg $L^{-1}$), spectrophotometric | 29 Oct 1973 | 25 Mar 2009 | 90 | 1.0 | 60.0 | 7.4 | 5.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Chlorophyll-*a* ($\mu$g $L^{-1}$), fluorometric | 03 Nov 1998 | 16 Aug 2005 | 23 | 0.5 | 61.9 | 9.4 | 4.4 |
| Total suspended solids (mg $L^{-1}$) | 29 Oct 1973 | 04 Aug 2009 | 131 | 1 | 1690 | 89 | 39 |
| Turbidity (NTU) | 26 Oct 1999 | 04 Aug 2009 | 37 | 2.5 | 83.9 | 28.5 | 20.5 |

# Sediment Chemistry

Since seagrasses are rooted in sediment and take up nutrients through below-ground tissues, it is important to characterize the sediments in seagrass beds. Grain size analysis provides a general categorization of the type of sediment present in a seagrass bed. Total organic carbon reflects the amount of organic material present in the sediments. Seagrass beds themselves are a source of some of this material, since some dead seagrass tissues will sink to the bottom and decompose. Organic material may also be transported from outside the seagrass bed in the water column, then settle into the sediments in the seagrass bed. Porewater ammonium reflects an important source of nutrients to the seagrass plants in the bed, but can be toxic to seagrasses at high levels.

## *Grain size*

Grain size analysis divides sediment samples into categories based on the diameter of the particles making up the sediment. Sandier sediment may hold less organic material than sediment composed of smaller grains, such as mud or clay (Mutchler and Dunton 2007). Some seagrass species may grow better in certain types of sediment, for example *T. testudinum* shoot density was negatively correlated with percent sand in a study of Redfish Bay and East Flats (Mutchler and Dunton 2007). However, the presence of seagrass causes particles to drop out of the water column, modifying the sedimentation rate and composition of sediment. This two-way interaction between sediment grain size and the presence, species composition, and abundance of seagrass must be considered in interpreting sediment grain size data. *T. testudinum* and *H. wrightii* were found in "a broad range of sediment types" in Redfish Bay and East Flats (Mutchler and Dunton 2007).

Results from several studies on East Flats, including some unpublished data, were summarized in Lee and Dunton (2000a). East Flats was characterized as having 91.19 ± 0.47% sand content and 8.81 ± 0.47% clay and silt content. In an earlier study, sand was the main grain size category (81.5% ± 1.9), followed by mud (9.5%) and rubble (9.0%) (Dunton 1996). In another study Dunton (1990) characterized the sediments as 78% sand and shell, 14% clay, and 8% silt. In a more recent study, average sand constituted at least 70 ± 5.2% at 8 of 10 sites (Dunton *et al* 2007). Sediment porosity can be calculated from percent of water in sediments and grain density. Average sediment porosity at East Flats was higher in seagrass beds than in bare areas (Lee and Dunton 2000b). Porosity also decreased with sediment depth below 3 cm.

## *Total organic carbon*

Total organic carbon (TOC) represents organic material in sediment, both fine particles settling from the water column and accumulation of detritus from the seagrass habitat community

(Mutchler and Dunton 2007). There are a variety of methods for estimating the organic content of sediments. Sediment TOC, measured by percent loss on ignition, did not correlate strongly with any seagrass condition indicators in a recent study in Redfish Bay and East Flats (Mutchler and Dunton 2007).

Average TOC (measured by percent loss on ignition) at Redfish Bay and East Flats ranged from 0.6 ± 0.1% to 3.8 ± 0.4% (Mutchler and Dunton 2007). TOC was strongly correlated with sediment composition; there was less TOC in sandier sediment samples than in muds and clays. Sediment TOC was positively correlated with silt, clay and rubble. In another study, sediment organic carbon was measured using elemental analysis. That study, conducted in East Flats and the lower Laguna Madre, found average sediment organic carbon to be 0.78% in seagrass beds and 0.092% in adjacent bare areas (Lee and Dunton 2000b).

## *Porewater ammonium*

Seagrasses can take up nutrients through both leaves and roots (Romero *et al.* 2006). Most of the nitrogen in sediment porewater is in the form of ammonium, which typically occurs at concentrations many times higher than that of all forms of inorganic nitrogen (nitrite, nitrate, and ammonia) in the water column combined. The acquisition of nitrogen as nitrate costs seagrass plants more energy than the acquisition of nitrogen as ammonium (Lee *et al.* 2007). One might assume that the sediment is the most important source of nitrogen for seagrasses, however, nitrogen dynamics in seagrasses are more complex than that and not completely understood. For example, Dunton and Lee (1999a) found that seagrass leaf tissue has higher nitrogen uptake affinities than root tissue. The relative allocation of biomass between above-ground and below-ground portions also affects the dynamics of nitrogen uptake. That said, the addition of nitrogen to the sediments will typically cause an increase in leaf biomass for seagrass in nutrient-limited environments (Lee and Dunton 1999b, 2000a). Rapid remineralization and turnover of nitrogen must be kept in mind when evaluating porewater ammonium levels as an indicator of nutrient availability (Lee *et al.* 2007; Lee and Dunton 1999a). In a recent study of Redfish Bay and East Flats, porewater ammonium did not correlate strongly with seagrass condition indicators (Mutchler and Dunton 2007). Shoot density was the only indicator that was higher at the low-ammonium site (located in the lower Laguna Madre). This occurs because plants in the lower Laguna Madre produce fewer, but larger, shoots after long-term nitrogen fertilization, a strategy that helps avoid self-shading to a greater degree than more numerous shoots. It is important to note that porewater ammonium can be toxic to seagrasses at high concentrations (Burkholder *et al.* 2007).

Results from several studies, including some unpublished data, were summarized in Lee and Dunton (2000a). Mean porewater ammonium for East Flats since 1990 averaged 115.08 ± 11.12 µM. Czerny and Dunton (1995) measured average porewater ammonium between 41.6 and 224.0 µM. Dunton (1996) found porewater ammonium to be variable at East Flats; the mean and standard error were 128 µM +/- 14. From 1996 - 1997, porewater ammonium ranged from 37 to 180 µM (mean = 87 µM) at East Flats in a *T. testudinum* bed (Lee and Dunton 1999a). Lee and Dunton conducted a nutrient-addition experiment and measured porewater ammonia at fertilized plots and control plots (Lee and Dunton 1999b). Porewater ammonium at control plots ranged from 37.4 to 109.3 µM, while values were significantly higher in experimentally-shaded areas.

In a more recent study of Redfish Bay and East Flats, Mutchler and Dunton (2007) found porewater ammonium not strongly correlated with sediment grain size or TOC.

# Seagrass Condition Indicators

Seagrass research has been conducted for over two decades in East Flats. A search of the literature did not identify any published seagrass studies for Port Bay.

## *Shoot density*

Higher shoot density values generally reflect favorable environmental conditions for maintenance and growth of seagrass beds. Shoot density is obtained by counting the shoots growing within a known area and dividing to obtain number of shoots per square meter. Shoot density can be variable due to the patchy nature of seagrass beds (Mutchler and Dunton 2007). Studies over the past 15 years in East Flats have found shoot densities ranging from about 300 to 1700 $m^{-2}$ for *T. testudinum* (Mutchler and Dunton 2007; Lee and Dunton 2000a, 1997, 1996), and from about 850 to about 11,000 $m^{-2}$ for *H. wrightii* (Czerny and Dunton 1995; Dunton 1996, 1990; Gutierrez *et al.* 2010). Shoot density for *T. testudinum* and *H. wrightii* in East Flats showed seasonal trends, with higher shoot density near the end of the growing season in the fall than near the beginning in the spring (Lee and Dunton 1997; Dunton 1990). Lee and Dunton (1997) also found that *T. testudinum* shoot densities decreased when plants were experimentally shaded.

## *Leaf morphometrics (blade length and width)*

Leaf morphometrics can respond to environmental conditions, and have shown promise as indicators of seagrass condition (Mutchler and Dunton 2007). Seagrass samples are collected and the length and width of the longest blade of a shoot are measured. Measured this way, leaf length can also be interpreted as "shoot height." Leaf blade length for *T. testudinum* at East Flats ranged from about 4 to over 45 cm. Blade length showed a seasonal trend, ranging from about 30 cm in May to 50 cm in August (Mutchler and Dunton 2007; Lee and Dunton 2000a). Average blade width ranged from about 2 to over 9 mm (Lee and Dunton 2000a, 1997). Seasonal variation is less pronounced for blade width than for blade length (Mutchler and Dunton 2007). *T. testudinum* blade width decreased when plants were experimentally shaded (Lee and Dunton 1997). Blade length and width were positively correlated with each other ($r^2$ = 0.55; Mutchler and Dunton 2007).

## *Biomass and root-to-shoot ratio*

Biomass is a reflection of productivity of seagrasses, which is affected by environmental conditions. The relative allocation of biomass between below-ground and above-ground components can sometimes be used to infer availability of nutrients or light to the plant. Total biomass of seagrass plants can be obtained by removing cores from seagrass beds, removing sediment and epiphytes from the core and plants, and obtaining a dry weight for the plant parts.

Biomass is expressed in grams dry weight of plant material per square meter by dividing by the cross-sectional area of the coring device. Separate weights can also be obtained for the above-ground and below-ground parts of the plants, to calculate the "root-to-shoot ratio" or RSR. RSR is a measure of the relative allocation of the plant to above-ground and below-ground parts, and may reflect plant response to nutrient concentrations in the water column and/or sediment porewater.

For *T. testudinum* in East Flats, below-ground tissues comprised about half of the total biomass on an annual basis (Lee and Dunton 1996). Various studies of *T. testudinum* at East Flats have estimated total biomass ranging from about 450 to about 900 g dry wt $m^{-2}$ (Lee and Dunton 2000a, 1996) and above-ground biomass ranging from about 50 to about 395 g dry wt $m^{-2}$. (Mutchler and Dunton 2007; Lee and Dunton 2000a, 1999a, 1996). Both above-ground and below-ground biomass have shown seasonal trends attributed to changes in water temperature and underwater irradiance, with highest biomass values near the end of the growing season (Lee and Dunton 1999a, 1996). In Lee and Dunton (2000a) the RSR was 1.63.

For *H. wrightii* in East Flats, biomass was quite variable (Dunton 1996). Total biomass ranged from about 150 to above 500 g dry wt $m^{-2}$ (Dunton 1994). Above-ground biomass ranged from about 70 to about 300, and below-ground biomass from about 160 to over 200 g dry wt $m^{-2}$ (Dunton 1996, 1990). Above-ground biomass exhibited a seasonal pattern similar to that of *T. testudinum*, but below-ground biomass did not show much seasonal change (Dunton 1996). RSR has been estimated from about 1.0 to about 5.0 (Dunton 1996, 1994; Gutierrez *et al.* 2010). RSR was higher in winter and lower in summer, reflecting seasonal changes in above-ground biomass while below-ground biomass held steady (Dunton 1996).

## *Tissue nutrient content*

Seagrass tissue nutrient content is a function of nutrient loading to the seagrass bed (Mutchler and Dunton 2007). C:N and N:P ratios are expected to decline with increasing loading of nitrogen and phosphorus, respectively. Carbon and nitrogen content are measured using elemental analysis, and the molar ratio of carbon to nitrogen is then calculated. Phosphorus content is estimated by analyzing for particulate total phosphorus (Fourqurean and Zieman 1992). Carbon content for *T. testudinum* leaves at East Flats averaged 35.51% and nitrogen content averaged 2.64% (Lee and Dunton 1999b). C:N was thus 15.97. Rhizome tissue had 35.21% carbon and 1.08% nitrogen content. Experimental nitrogen fertilization resulted in increases to the leaf carbon and nitrogen content, and the rhizome nitrogen content. As a result, C:N declined in both leaf and rhizome tissues after fertilization. Tissue carbon and nitrogen content and C:N showed strong seasonal trends. Tissue nitrogen content was higher during periods of slow growth in winter (Lee and Dunton 1999b).

## *Stable isotope ratios*

Stable isotope ratios can be used to determine the source of nutrients and carbon that is assimilated by seagrass. Nitrogen is found in nature in two dominant forms, $^{14}N$ and $^{15}N$, varying at the atomic level by the number of neutrons present. The ratio of the heavier $^{15}N$ to $^{14}N$ is symbolized by $\delta^{15}N$, and presented as parts per thousand relative to the composition of atmospheric nitrogen. Nitrogen compounds in domestic wastewater tend to have elevated $\delta^{15}N$ relative to natural sources. Likewise for carbon, $\delta^{13}C$ represents a ratio of the heavier isotope $^{13}C$ to $^{12}C$. There are currently no data in the literature reporting stable isotope ratios for East Flats. In a study of a seagrass bed on the Caribbean coast of Mexico, $\delta^{15}N$ was used to try to identify wastewater impacts to macrophytes (Mutchler *et al.* 2007). Confounding factors, including submarine groundwater discharges into the seagrass area, made it difficult to quantify the effects of wastewater on the seagrasses.

### *Macroalgae biomass*

Drift macroalgae take up nutrients from the water column, so may be an indicator of eutrophication. Increases in drift macroalgae density have been associated with seagrass decline (Mutchler and Dunton 2007). Macroalgae may affect seagrass by shading and/or changing water and sediment quality characteristics in the seagrass beds. For example, drift macroalgal abundance can be associated with higher porewater ammonium concentrations in seagrass beds (Kopecky and Dunton 2006). Extensive macroalgal accumulations are known to occur in East Flats. Drift macroalgae biomass in East Flats ranged from zero to >600 g $m^{-2}$ (Mutchler and Dunton 2007).

## Epiphyte Load

Epiphytes are bacteria, algae and invertebrates that grow attached to seagrass leaves. Epiphyte load can increase when nutrients become elevated in the water column (Mutchler and Dunton 2007). As nutrient levels increase through eutrophication, grazing by crustaceans, gastropods and other organisms may counter-balance the increased growth of epiphytes up to some threshold level when epiphyte growth overwhelms the grazing rate (Burkholder *et al.* 2007). Epiphyte load is traditionally measured by scraping epiphytes from leaves, drying and weighing the epiphytes and dividing by the flat surface area of the leaf scraped to obtain mg dry wt $cm^{-2}$. On seagrass species with a more terete or cylindrical leaf cross-section (e.g. *H. wrightii*), epiphyte biomass is expressed as the percentage of the dry weight of the seagrass. Epiphyte load in general tends to decrease near the deep edge of seagrass beds, where light may limit their growth (Dunton 1996). Epiphyte loads on *T. testudinum* ranged from near zero to about 20 mg dry wt $cm^{-2}$. Epiphyte load may be higher during winter in East Flats, possibly due to reduced leaf elongation and reduced grazer activity during the colder months (Mutchler and Dunton 2007).

A novel technique has been developed by Cammarata (Cammarata *et al.* 2009) for estimating epiphyte load using fluorometry of seagrass blades to identify and quantify epiphytic algae. Preliminary efforts have been made using *H. wrightii* leaves from Redfish Bay and an area adjacent to East Flats. Fluorescence of photosynthetic accessory pigments is measured as a proxy for epiphyte abundance. This measure provides greater spatiotemporal resolution of epiphyte biomass than the traditional leaf-scraping method. The accumulation profile, a plot of incremental epiphyte abundance along the age gradient of the seagrass leaf, provides a 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 leaf area for a seagrass sample can also be determined with the described method, at least for species with more or less flat leaves. Fluorescence images of epiphytes can be archived for subsequent development of additional analytical tools such as comparisons of the predominant morphotypes of fluorescent epiphytes. The literature did not reveal data on estimating epiphyte load with fluorometry for seagrass beds in East Flats.

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