Estuaries and Coasts
https://doi.org/10.1007/s12237-023-01170-5

SPECIAL ISSUE: LOW INFLOW ESTUARIES

Impacts of Episodic Freshwater Inflow Pulses on Seagrass Dynamics in the Lower Laguna Madre, Texas, 1998–2017

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Received: 5 August 2022 / Revised: 29 December 2022 / Accepted: 1 January 2023

Abstract

The Lower Laguna Madre of Texas (LLM) is a subtropical, hypersaline lagoon dominated by lush seagrass beds and characterized by a historically low freshwater inflow (FWI) regime. From 1998 to 2012, a net 8.8% seagrass decrease occurred in the LLM, primarily *Syringodium filiforme* and *Thalassia testudinum*, despite the concomitant expansion of *Halodule wrightii*. In the middle region of the LLM affected by a major FWI source, the Arroyo Colorado, the decrease was > 20%. We proposed that *Thalassia* and *Syringodium* decrease was largely due to high FWI pulses and N loading from an increasingly modified LLM watershed. Salinity modeling studies showed that FWI pulses lowered salinities for months in this region potentially associated with non-point source nutrient (viz. ungauged) inputs. At a site near the Arroyo Colorado, *Thalassia* biomass and shoot density were lower 319.3 g/m^2 and 567 shoots/m^2, respectively versus 1296 g/m^2 and 3382 shoots/m^2 for the most productive LLM site. Targeted sampling in four subwatersheds adjacent to the LLM demonstrated high dissolved N loadings (nitrate and organic nitrogen) to the lagoon. Nitrogen isotope analysis of LLM seagrass further indicated that nitrogen was likely from inland wastewater runoff from the Arroyo Colorado. We concluded that freshwater pulses to the lagoon produce hyposaline conditions and high nutrient loading, stressing seagrasses and resulting in diminished resilience and reduced distribution of *Thalassia* and *Syringodium*. With climate change, coastal population growth, and enhanced watershed drainage, episodes of hyposalinity, and enriched nutrient runoff are likely to increase in frequency and duration, altering seagrass resilience and diversity in the LLM.

Keywords Seagrasses · Subtropical lagoon · Freshwater pulses · Hyposalinity · Nutrient loading · Ungauged runoff

Introduction

The Lower Laguna Madre of Texas (LLM) is unique as a highly productive, hypersaline lagoon dominated by seagrass beds and characterized by historically low freshwater inflow (FWI) regimes. Low FWI contrasts with most other estuaries where moderate to large amounts of FWI are considered essential to maintain estuarine productivity. Increasing seagrass production and changes in seagrass species distribution in LLM since the 1950s have been documented by Quammen and Onuf (1993) and Onuf (2007) based on analysis of mapping data from surveys between the 1950s and 1998 and amelioration of the lagoon's historical hypersalinity. As of 1998, the LLM contained about 52% by area of the seagrasses along the Texas coast (Pulich and Calnan 1998). However, since 1998, a significant seagrass decrease has been observed, especially *Syringodium filiforme* and *Thalassia testudinum* in the northern half of the LLM, with concomitant expansion of *Halodule wrightii* mostly in the vicinity of the confluence with Arroyo Colorado, a Rio Grande distributary and drainage channel. Since salinities in LLM had stabilized by the late 1970s, seagrass trends since 1998 represent an example of unexplained ecological change.

Cause(s) of these seagrass dynamics were the subject of speculation in a study for the Texas Environmental Flows Program (BBEST 2012) which completed a critical assessment of freshwater inflow impacts on the LLM. While the Lower Rio Grande Valley (LRGV) has only two natural,

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Communicated by Holly Greening

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major drainage systems, the Arroyo Colorado and Brownsville subwatersheds, this area is overlaid with a complex irrigation and drainage system, that contributes significant urban and agricultural runoff to the LLM (BBEST 2012). Due to flooding events and increasing urban development, the LRGV drainage system has been modified to improve stormwater drainage, thus potentially increasing the amount of freshwater inflow in recent years to the LLM from non-point source runoff.

Land use and land cover (LULC) are linked to watershed export of freshwater, organic matter, and nutrients to coastal waters. Changes in LULC such as agriculture gain or loss and urbanization, septic systems, etc. are known to lead to changes in water quantity and quality, organic matter, and nutrient input to estuarine ecosystems (Short and Wyllie-Echeverria 1996; Nixon et al. 2001). Changing land use in LLM watersheds and population increases in LRGV in the past 30 years have been hypothesized to alter the quantity and quality of FWI to the lagoon (Quammen and Onuf 1993; Onuf 1996; BBEST 2012).

Despite a surface area of 1308 km^2, LLM has a rather small watershed area of 13,165 km^2 with a watershed to estuary area ratio of 10:1, one of Texas' smallest. Due to its shallow depth, LLM has a small estimated volume of 994×10^6 m^3 ($35{,}124 \times 10^6$ ft^3) at low tide and $2{,}317 \times 10^6$ m^3 ($81, 872 \times 10^6$ ft^3) at high tide, leading to a potentially long water residence time and low turnover rate. In fact, the average annual combined freshwater inflow of *ca* 524,000 ac-ft ($22{,}869 \times 10^6$ ft^3) per year from 1977 to 2010 (Texas Water Development Board, TWDB) would cause the residence time to be very long, when coupled with the large negative water balance of the LLM due to high evaporation to precipitation ratio. An estimation by George Ward of the University of Texas (pers. comm.) indicated that the residence time or freshwater flushing time is on the order of 284 days, or a turnover time of less than 1.28 times per year.

With the population increase in south Texas, there will be increased nutrient loading to the lagoon from point and non-point sources and an increase in impermeable surfaces increasing runoff. As a low-flow estuary with a large negative water balance, the LLM would be especially susceptible to excessive nutrient loading stresses due to nutrient retention. In general, wastewater treatment plant discharge is one of the main sources of point source nitrogen and particularly dissolved organic nitrogen (DON) in surface water (Sipler and Bronk 2015; Czerwionka 2016). In a metadata analysis, Sipler and Bronk (2015) found that the % DON in total dissolved nitrogen in estuarine water varied from 17 to 98% with the average being 72% ± 23 ($n = 21$). DON can be used by phytoplankton (Li et al. 2010), seaweeds (Tarutani et al. 2004) as well seagrasses (Vonk et al. 2008) but is seldom monitored, so its impact on coastal ecosystems is not known. Knowing the sources and quantity of nitrogen loading and the forms of nitrogen is important in managing coastal ecosystems.

Based on results of the State of Texas Environmental Flows Program (BBEST 2012), a hypothesis was proposed that seagrass trends since the late 1990s have resulted from higher freshwater inflows into the LLM ecosystem, producing lowered LLM salinities concomitantly with high non-point source nutrient (viz. ungauged FWI) inputs. To assess this hypothesis, we reviewed published studies and utilized the authors' unpublished research and herein summarize the following analyses:

1) Quantification of changes in seagrass acreage and species composition in the Lower Laguna Madre over the period 1998–2017 based on aerial photography analysis and ground transect surveys;
2) Comparison of salinity regime maps from hydrodynamic modeling with seagrass distribution maps, thus providing presumptive evidence for zones of elevated nutrients and hyposalinity that impacted sensitive seagrass species;
3) Comparison of *Thalassia* production and hydrologic monitoring data from sampling sites differing in proximity to FWI sources to the Lower Laguna Madre.
4) Determination of nitrogen isotope values for components in the Arroyo Colorado (dissolved N and periphyton) and in the LLM (seagrass and drift algae) which documents that nitrogen runoff from inland sources is utilized by primary producers in the Lower Laguna Madre;
5) Demonstration of nutrient runoff dynamics from four ungauged subwatersheds showing that episodic freshwater pulses produce high nutrient loadings, especially nitrogen, into the LLM, which could potentially impact seagrass in combination with lowered salinity stress.

Materials and Methods

This paper is organized into three sections. Table 1 provides a reference to studies for each section, with time periods, data sources, and geographic coverage for datasets used in this study.

1. We review species and abundance changes for seagrasses based on mapping surveys over the period from 1998 to 2017, mostly focused on results for the middle region of the LLM designated as the "mid-LLM Study Area", directly affected by the Arroyo Colorado (AC) (Table 1 and Fig. 1). *Thalassia* production and hydrologic monitoring data were also compared for the 2005–2006 period at four sample sites in the LLM differing in proximity to the AC freshwater inflow (FWI) source.
2. Salinity output from the TWDB TxBLEND hydrodynamic model for the LLM was reviewed, demonstrat-

Table 1 Summary of case studies and data sets reviewed. Hurricane Alex occurred in 2010

Studies reviewed	Date of coverage	Study area	Data source
Seagrass mapping projects			
Seagrass transect survey	1998	LLM, entire	Onuf 2007
NAIP photoimagery analysis	2009	LLM, middle region	BBEST 2012
Seagrass transect survey	2012	LLM, entire	DeYoe & Kowalski 2013
Hexagonal grid survey	2017	LLM, entire	Dunton 2019
Plant Phenology Projects			
Thalassia biomass & growth	2005 – 2006	LLM, 4 sites	DeYoe & Kowalski, unpublished
Halodule δ^{15}N isotope	2011	LLM, middle region, N-S & E-W transects	BBEST 2012
Hydrology projects			
Salinity/hydrodynamic modeling	2010	LLM, entire	Texas Water Development Board
Nutrient loading project	2014 – 2015	Brownsville & Arroyo subwatersheds	DeYoe & Pulich, unpublished
Water quality monitoring	2006 – 2010	LLM, 4 sites	DeYoe, unpublished

ing salinity plume dynamics under inflow regimes for 2010, a wet year due to the occurrence of Hurricane Alex. Field surveys and methods used as part of a LLM seagrass monitoring program are also described showing the potential effects of hyposaline/high-nutrient regimes and δ^{15}N isotope source loading from the Arroyo Colorado on LLM seagrass.

3. Results of a case study performed in 2014–2015 are presented which document real-time nutrient loading dynamics from four subwatersheds to LLM under known episodic inflow (viz. rainfall) events. These data are presented as examples of the amounts of dissolved N and P capable of entering the LLM in FWI pulses from subwatersheds, although the study time period was not associated with actual seagrass changes observed in this study.

Seagrass Mapping and Field Surveys

LLM Seagrass Survey (Onuf 1998)

Field survey methods and mapping data originally collected in 1998 by Onuf have been reported in the Onuf (2007) publication on LLM seagrasses. Surveys were based on point samples along line transects, followed by input of GPS point data into ArcGIS, and GIS maps were produced by kriging. The digital GIS map files developed from this original Onuf 1998 data were obtained from the USGS National Wetlands Research Center Lab at Stennis Space Center, Miss., and used for our comparative analysis.

Seagrass Map Data for 2009 from BBEST (2012) Report

USDA National Agricultural Inventory Program (NAIP Jan. 2009) natural color photoimagery of LLM at 1-m pixel resolution was analyzed by photo delineation techniques to classify seagrasses in the mid-LLM Study Area shown in Fig. 1. The source digital orthophotography was obtained from Texas Natural Resources Information System (TNRIS) in Austin, Texas. On-screen digitizing was performed in ArcGIS to classify seagrass into Sparse and Dense seagrass polygons. Sparse describes patchy seagrass polygons with greater than 50% area of bare patches of < 10 m in size. Dense describes continuous seagrass polygons with lesser amounts (< 50% area) of such bare patches. Areas of 10 m or more visually lacking seagrass were mapped as Bare. Additional details on verifying and ground-truthing these maps during 2010/2011 are reported in the BBEST (2012) report.

LLM Seagrass Survey (DeYoe and Kowalski 2013)

A 2012 seagrass survey of LLM was conducted by DeYoe and Kowalski (2013) replicating the field methods and a majority of transects surveyed by Onuf (2007). This survey included 167 sites using 25 east–west transects in the LLM that were a subset of the Onuf 1998 study sites. Additional sites near the mouth of the Arroyo Colorado were included because of interest in making comparisons with past studies. Sampling was done in June and July of 2012. The geographic position was determined at all stations using a handheld GPS unit (Garmin) at a resolution of at least 10 m.

At a site, the four cardinal compass points around the boat were sampled using a 50-cm by 50-cm quadrat haphazardly positioned on the bottom. The presence or absence of seagrass and associated macroalgae were noted and, where seagrass and/or macroalgae were present, percent cover assessed within the quadrat and scored using a Braun-Blanquet index for seagrass, macroalgae, and unvegetated bottom.

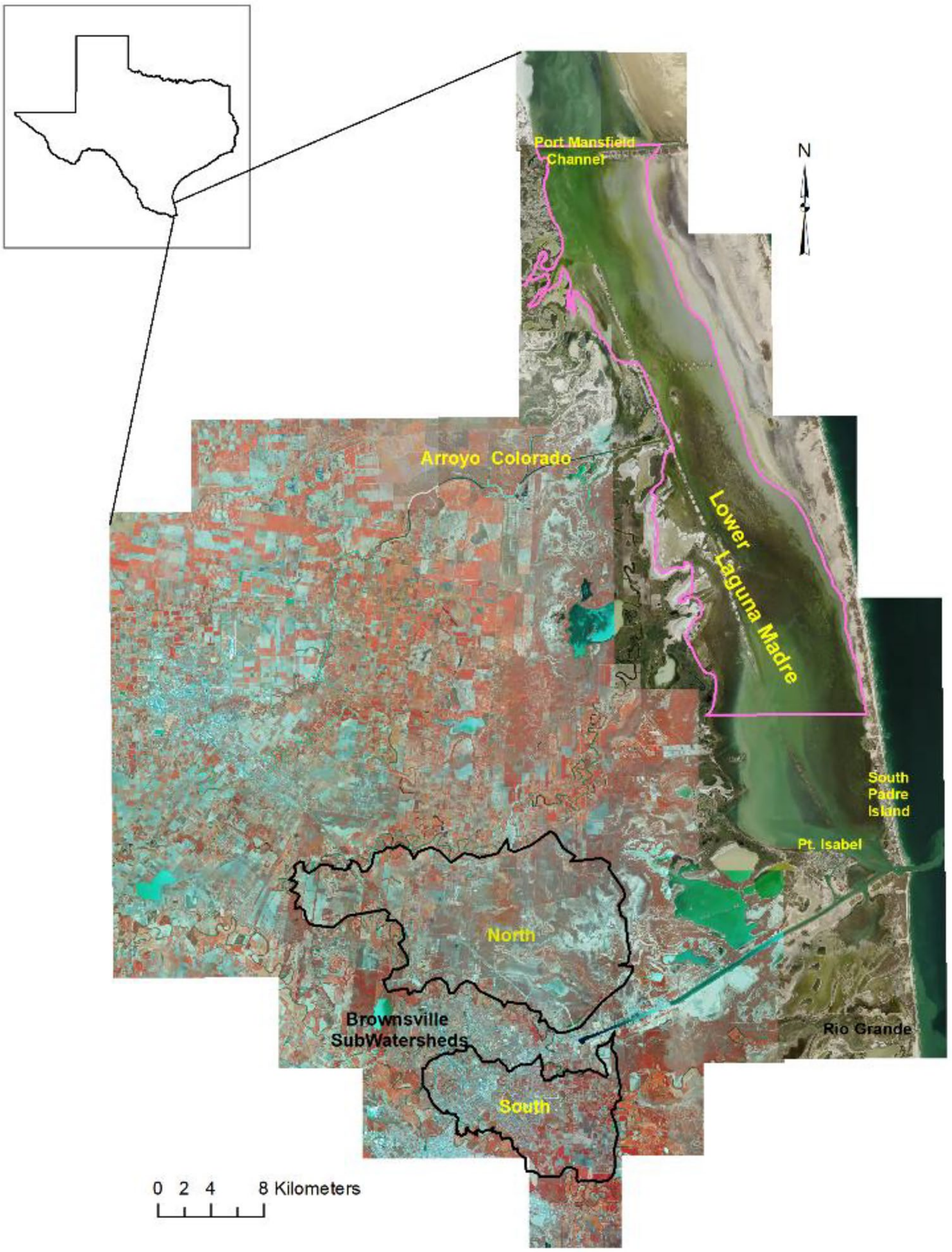


Fig. 1 Lower Laguna Madre (LLM) and Rio Grande Valley (LRGV) regions shown with 2009 NAIP natural color (LLM) and 2012 color infrared (LRGV) photography. Purple outlined polygon shows the mid-LLM Study Area focused on for seagrass trends analysis. Black outlined polygons in the photo delineate two Brownsville sub-watersheds (North and South)

Maps of percent cover were made from the *X–Y* GPS points in ArcGIS Pro for *Halodule wrightii*, *Syringodium filiforme*, *Thalassia testudinum*, and *Ruppia maritima*. To achieve this goal, a kriging geostatistical interpolation method was used to estimate the percent cover for each species at all locations in the study region. Kriging is an interpolation method to estimate values at an unknown location based on the values at known points by a weighted averaging of nearby samples. Kriging is similar to IDW (Inverse Distance Weighted) in that it weights the surrounding measured values to derive a prediction for an unmeasured location. The general formula for the kriging interpolation is as follows:

$$\hat{Z}(s_0) = \sum_{i=1}^{N} \lambda_i Z(s_i)$$

where $\boldsymbol{Z(s_i)}$ = the measured value at the *i*th location, $\boldsymbol{\lambda_i}$ = an unknown weight for the measured value at the *i*th location, $\boldsymbol{s_0}$ = the prediction location, and $\boldsymbol{N}$ = the number of measured values. Settings used for our analysis were as follows: Ordinary model; Search radius, spherical; and Variable point distribution with 12 as the number of points averaged.

Next, the Reclassify tool, a raster analysis tool in ArcGIS Pro, was used to change the values in the rasters of percent cover into three different classes: bare, sparse, and dense, respectively. The tool produced a final raster image with three classes shown in different colors on seagrass maps. The cutoff between Sparse and Dense in the maps was: *Thalassia* (Sparse = 5–30% cov, Dense > 30%); *Syringodium* (Sparse = 5–15% cov, Dense > 15%); and *Halodule* (Sparse = 5–50% cov, Dense > 50%).

LLM 2017 Seagrass Survey

A seagrass survey dataset for 2017 was obtained from the Texas Statewide Seagrass Monitoring Program website (https://www.texasseagrass.org/results.html) coordinated by K. Dunton at the University of Texas Marine Science Institute, Port Aransas, TX. The 2012 survey comprised 167 sampling points in our mid-LLM Study Area, where data had been collected by a hexagonal grid sampling scheme developed by the EPA-REMAP program. GIS kriging was not performed on the 167 LLM sampling points. Instead, percent cover maps for *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* were produced in ArcMap showing the distribution of dense and sparse seagrass cover by species at the 167 sampling points. We classified Dense and Sparse cover as described for the 2012 dataset.

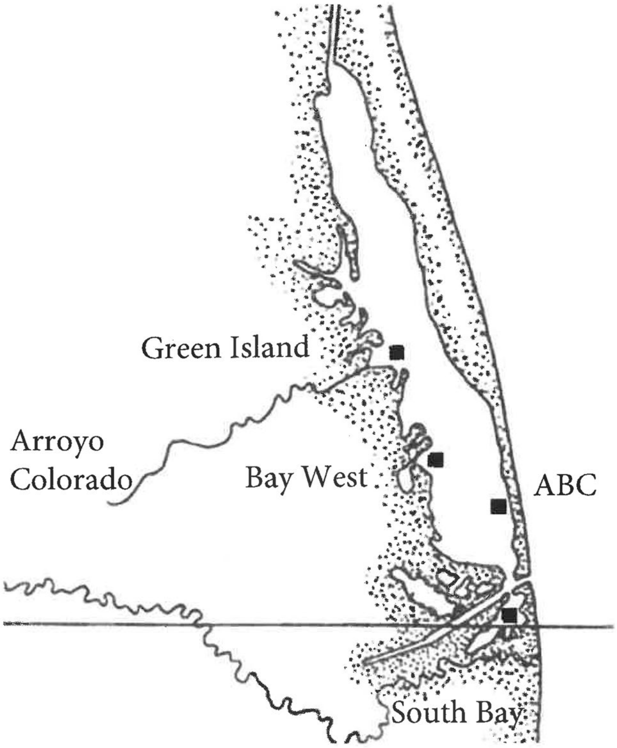


Fig. 2 *Thalassia* monitoring sample sites in the LLM for 2005–6

Thalassia Monitoring Study

Seasonal performance of the seagrass *Thalassia testudinum* was measured five times (March and May 2005, March, August, October 2006) at four LLM sites (Green Island, ABC, Bay West, and South Bay) (Fig. 2). These sites were selected based on differences in water quality that were related to their distance from the Arroyo Colorado. The Green Island site was 2.9 km northeast from the confluence of the Arroyo Colorado and the Lower Laguna Madre. Prevailing winds are from the southeast (April – Oct) so Arroyo Colorado water is usually directed to this site resulting in generally elevated nutrient levels and biogenic (phytoplankton) turbidity (DeYoe pers obs.). Site Bay West (BW) was 18.9 km south of the Arroyo Colorado on the west side of the Gulf Intracoastal Waterway (GIWW) spoil islands. It was selected as an "average" LLM site with moderate nutrient levels and largely abiogenic turbidity. Site ABC (Andy Bowie Control) was 25.9 km south of the Arroyo Colorado and a clear water site with low nutrients. The South Bay (SB) was the most distant from the Arroyo Colorado and closest to the Gulf of Mexico due its proximity to the Brazos-Santiago Pass.

During each collecting trip, the sites were visited to measure *Thalassia* shoot growth rate, total biomass, and shoot density. At each site, four 15-cm-diameter cores (0.018 m^2) were collected to determine seagrass biomass, leaf length, and shoot density. Biomass cores were rinsed of sediment, sorted into above- and belowground parts, dried, and weighed. Shoot growth rates were determined by the leaf-marking method (Zieman 1974). Ten shoots at each site were marked and 2 to 4 weeks later the shoots were harvested for measurement of the length and weight of new leaf tissue.

Quarterly water samples were collected from 2006 to 2010 during seagrass monitoring studies at Green Island, Bay West, ABC, and South Bay. Within 24 h of collection, water samples were filtered through Whatman GF/C filters and frozen for later analysis. Water samples were analyzed for dissolved orthophosphate, ammonia nitrogen, nitrate-nitrite nitrogen, total dissolved nitrogen (subset of samples), and ^{15}N isotope (subset of samples). Water samples were analyzed for nitrate-nitrogen, ammonium-nitrogen, and soluble reactive phosphorus using the following EPA Methods (USEPA 2022): nitrate-EPA Method 0353.2, ammonium- EPA Method 0350.2, dissolved orthophosphate- EPA Method 0365.2. Filters were retained and frozen for chlorophyll a analysis to estimate phytoplankton abundance. Chlorophyll a was quantified by acetone extraction followed by fluorometric quantitation (Baird and Bridgewater, 2017).

ANOVA (one-way) was used to determine if there were significant differences among the sites for *Thalassia* parameters (biomass, shoot density, leaf length, areal production) and for water column chlorophyll and nitrate data using SysStat 11.0.

Nitrogen Stable Isotope Tracing

In August 2011, 27 sites in LLM arrayed along a North–South transect and an East–West transect (Fig. 3) were visited for the collection of samples of seagrass leaves (*Halodule wrightii*), and macroalgae (*Palisada poiteaui*). In April and August 2011, periphyton samples from the Arroyo Colorado, a main source of FWI into the LLM, were collected from polyethylene plastic strip substrates that were deployed for 2–3 weeks at two sites—Thomae Park (6.4 km upstream from the confluence of the Arroyo Colorado with the LLM) and River Ranch (35.6 km upstream from the confluence of the Arroyo Colorado with the LLM).

Fig. 3 Sampling sites (green dots) in the mid-LLM Study Area visited on August 18, 2011, for collection of seagrasses and drift algae analyzed for stable N isotopes. This sampling design of North–South and East–West transects was based on the August 2008 salinity plumes (red polygons) from TxBLEND hydrodynamic modeling. The East–West transect shown by the blue arrow begins opposite the confluence of the Arroyo Colorado with the LLM

After collection, the various samples were rinsed, cleaned of epiphytes (if any) (Dauby and Poulicek 1995), dried at 80 deg C, ground and then analyzed for C and N content and stable C and N isotope ratios (δ^{13}C and δ^{15}N) by the University of Alaska- Fairbanks, Stable Isotope Laboratory.

Analyses of Freshwater Inflows to LLM

Contributions of Gauged and Ungauged Flows to Total LLM Inflows

There are eight subwatersheds that comprise the watershed of the LLM as identified and numbered by TWDB and USGS (Fig. 4). Two of these subwatersheds (Arroyo Colorado #22,603 and North Floodway #22,604) have flow gauging stations (IBWC #08,470,400 at Harlingen on the Arroyo Colorado and IBWC #08,470,200 at Sebastian on the North Floodway). The other six subwatersheds are ungauged. Ungauged flows were calculated from the TxRR runoff model (Matsumoto 1992). The total combined gauged and ungauged monthly inflows from 2000 to 2020 derived from the ungauged models plus the two IBWC flow gauges are given in Fig. 5 below. Due to operational and quality assurance issues with the Sebastian gauge, only the Arroyo Colorado gauged station data could be used for the 2000–2011 period. From 2012 to 2020, flow data from the Sebastian/N. Floodway stations were considered reliable and were then added to the Harlingen station flow to obtain a total combined gauged flow value. Ungauged monthly inflows for the entire 20-year period were derived from TxRR modeling of all eight subwatersheds to the LLM.

TWDB staff uses the Texas Rainfall-Runoff model (TxRR) to estimate daily stream flows in ungauged watersheds following precipitation events. The original model was calibrated for representative watersheds using gauged stream flow records and precipitation records and by adjusting parameters for soil type and land use. TWDB staff has verified the performance of the TxRR model for ungauged subwatersheds (Matsumoto 1992; Schoenbaechler et al. 2011) reflecting land use-land cover in 20 coastal Texas subwatersheds that establish relationships between runoff curve numbers and maximum soil moisture.

TWDB Hydrodynamic Modeling

The Texas Water Development Board, Bays and Estuaries Program (TWDB, Austin, Tx), performs hydrodynamic simulation modeling of water circulation and salinity in Texas bays and estuaries such as Lower Laguna Madre, using the TxBLEND hydrodynamic and salinity transport model. TxBLEND is a two-dimensional, depth-averaged

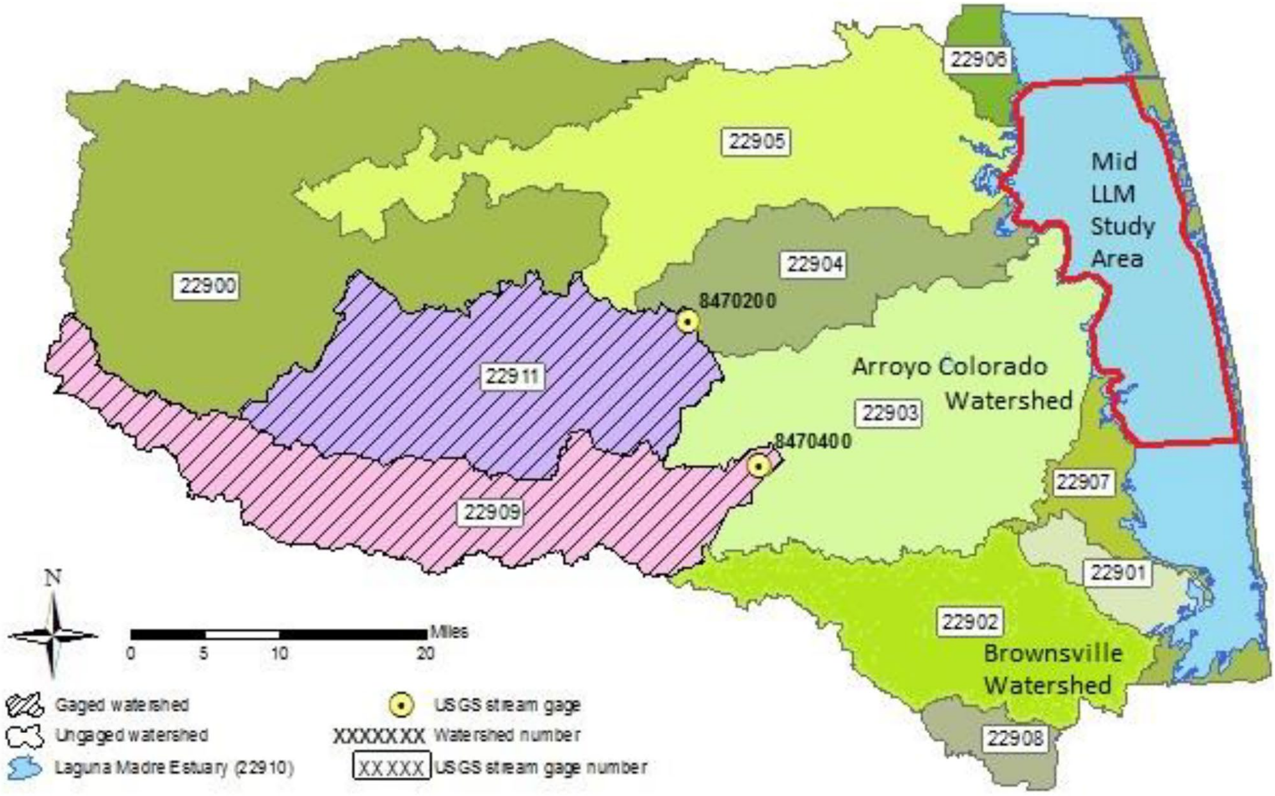
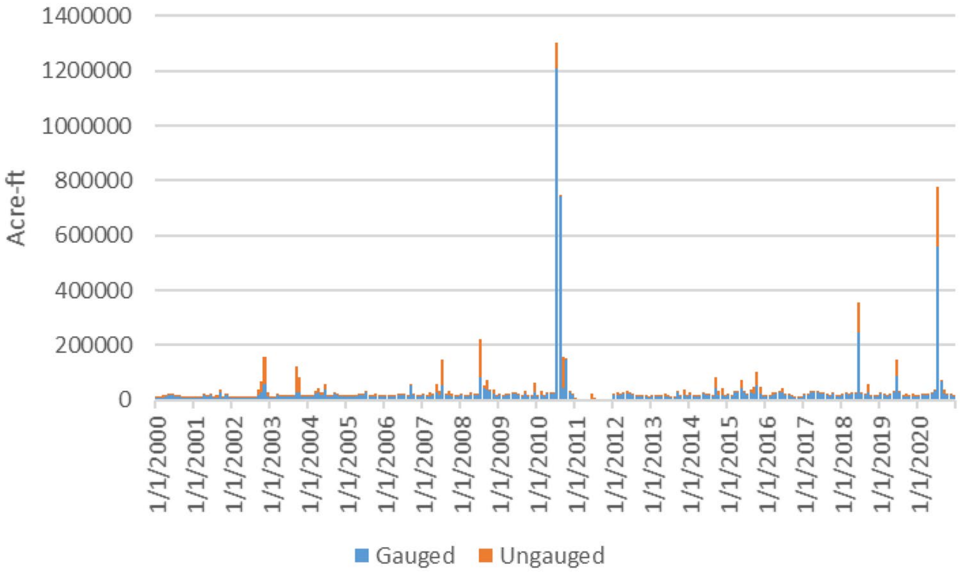


Fig. 4 Map of Lower Laguna Madre subwatersheds in relationship to the mid-LLM study area

hydrodynamic and salinity transport model designed to simulate water circulation (currents) and salinity conditions within Texas bays (Schoenbachler et al. 2011). Salinity conditions reflect the input of total gauged and ungauged inflows to the Lower Laguna Madre as computed above. Model simulations allow for a quantitative depiction of the effects of volume and timing of freshwater inflows and concomitant meteorological and tidal processes on the distribution and persistence of water circulation and salinity within the LLM.

TxBLEND produces high-resolution, dynamic simulations of estuarine conditions over daily to long-term periods, using a model grid mesh (Fig. 6). For the LLM study, TWDB incorporated finer resolution grid nodes and greater detail focusing on the area adjacent to the mouth

Fig. 5 Sum of gauged and ungauged monthly inflows to Lower Laguna Madre, 2000–2020. Note high flows from Hurricane Alex (2010) and a tropical depression in 2018. From 2000 to 2020, the average ratio of gaged to ungauged inflow was 13.4 (SD 16.0), while the median ratio was 8. The highest ratios occurred during periods of extreme precipitation events, such as tropical storms conditions in 2010, 2018, and 2020



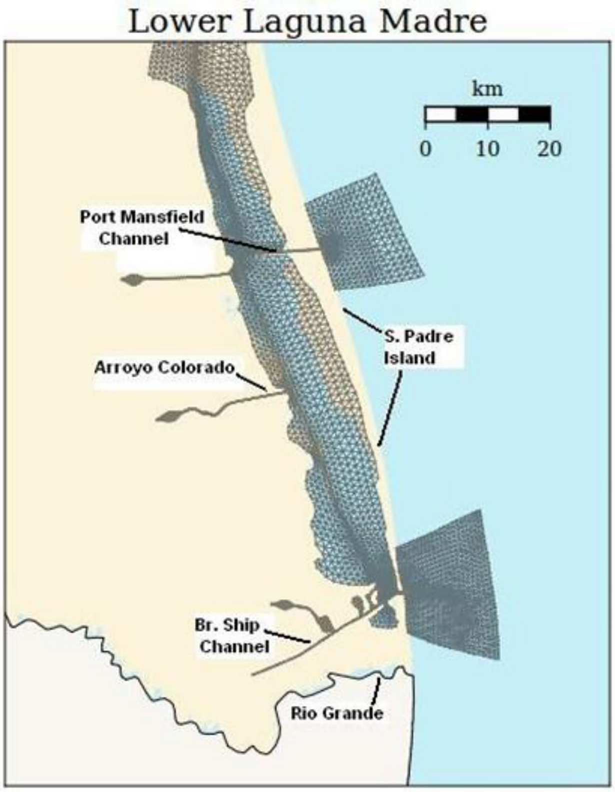


Fig. 6 TxBLEND hydrodynamic model grid network applied to Lower Laguna Madre

of the Arroyo Colorado, Gulf passes, and deeper channels (e.g., Gulf Intracoastal Waterway). For a full description of the TxBLEND model refer to Schoenbaechler et al. (2011) and the 2012 BBEST report (BBEST 2012). To generate the salinity concentrations, a TxBLEND simulation was run with the Laguna Madre Model for the entire model grid (Schoenbaechler et al. 2011). The year 2010 was pulled from a full model run (1987–2018). The months of interest (July, August, October, November, and December) were extracted from the daily salinity model output using Python. The extracted daily output was averaged across the month, formatted in Python, and transferred to ArcGIS Pro where it was converted from a table to point data (x = easting, y = northing, coordinate system NAD_1983_UTM_Zone_14N).

Once the data was in ArcGIS Pro, the kriging procedure was performed with the kriging tool (Ordinary, model: Spherical, search radius: Variable point distribution) on the point data to create a raster. The raster was then clipped to the area of the TxBLEND grid using the clip raster tool. The clipped raster was colored using the classify symbology-defined interval method with an interval size of 2. Finally, contour lines were added using the contour tool with an interval of 2.

Case Study of Nutrient Loading in Brownsville and Arroyo Colorado Ungauged Subwatersheds

Two ungauged subwatersheds in the Brownsville area—Port of Brownsville North subwatershed, (POB North) and Port of Brownsville South, (POB South) (see Figs. 1 and 4) were selected for sampling. These were selected because they captured a large portion of the drainage from the city of Brownsville, including nine wastewater outfalls. The sampling sites were positioned as far downstream as possible to capture ungauged sources; however, the sites were near the seawater interface so were affected by minimal tidal action. Two additional ungauged subwatersheds in the Harlingen-Arroyo Colorado region (see Fig. 4), plus the Arroyo Colorado itself (AC at Port of Harlingen), were also chosen for study. Arroyo Colorado sites were selected because AC-East covered largely agricultural land (95%) and the other (AC-West) drained largely urban land (26%). GIS LU/LC maps comparing the land use types in the four subwatersheds were developed from Ecosystems Mapping LU/LC Classification Survey data compiled by Texas Parks and Wildlife Dept. during their statewide mapping project (TPWD 2014).

The two Port of Brownsville (POB) subwatersheds were different in size and LU/LC acreage (Table 2). POB North (area 25,510 ha) had approx. 16% tidal marsh/open flats, 69% salty grasslands or woodlands, and 8% agriculture, but only 6.5% rural, low urban area (Table 2). POB South (area 11,871 ha) had approx. 34% salty grasslands, 14% shrublands, 4% agriculture, but 36% rural and low urban and 8% highly urban area. POB South was three times more urbanized than POB North, but with 80% less woodlands/brushlands, as well as 50% less grasslands. The two Arroyo Colorado subwatersheds were also different in size (AC-West, 6,541 ha and AC-East, 1,987 ha) and LU/LC acreage (Table 2). Both AC East and AC West were smaller than the Brownsville sites (< 8–25% area) and had very little tidal brackish marsh as they were located well inland from the LLM proper. AC East had approx. 61% salty grasslands or woodlands, 31% agriculture, and < 2.5% rural, low urban area (Table 2). However, AC West (about 3 times larger than AC East) was approx. 25% salty grasslands, 46% agriculture, but 26% rural, low urban, and high urban area. Thus, AC West was ten times more urbanized than AC East and had 10% more agriculture.

Automated stormwater samplers (ISCO©) and Acoustic Doppler Current Profilers (ADCP) were installed in each subwatershed drain to collect water samples and collect flow data during baseline periods (no rain) and rainfall (runoff) events, respectively. Rainfall events were generally sampled if there had been no significant rainfall in the previous seven days. Rainfall data for the sample sites were obtained from

Table 2 Land cover/land use acreage and percentages for Brownsville and Arroyo Colorado subwatersheds (classified LU/LC data from TPWD Ecosystems Mapping Project, 2014)

Common name	Descriptive landcover	Port BrownsvilleNorth	Port BrownsvilleSouth	Arroyo Colorado East	Arroyo Colorado West
Coastal: tidal flats or salt/brackish low/high tidal marsh	Tidal flats or salty marsh	10,208.6 ac 16.2%	941.3 ac 3.21%	–	83.1 ac 0.51%
South Texas: floodplain/riparian (evergreen/deciduous forest, oak woodlands, shrublands)	Floodplain riparian shrubland and forest	5033 ac 7.98%	4224.1 ac 14.46%	83.2 ac 1.7%	361.15 ac 2.43%
Gulf coast: salty prairie, disturbed grassland, or mixed shrubland	Salty grassland/shrubland	38,064.2 ac 60.4%	9929 ac 33.9%	2993.1 ac 60.9%	4037.6 ac 25%
Row crops/orchard	Agriculture	5048.8 ac 8.0%	1269.7 ac 4.3%	1716.3 ac 35.0%	7337.8 ac 45.6%
Urban high intensity	Urban high	632.9 ac 1.0%	2,262.2 ac 7.7%	17.9 ac 0.36%	215.8 ac 1.34%
Urban low intensity	Urban low	3510.6 ac 5.54%	10,651.8 ac 36.32%	95.4 ac 1.94%	4017.1 ac 24.86%
Open water (rivers, canals, and ponds)	Open water	510.9 ac 0.81%	152.2 ac 0.52%	–	72 ac 0.45%
Total acreage		63,009 ac 25,510 ha	29,322 ac 11,871 ha	4,908 ac 1987 ha	16,154 ac 6541 ha

the National Weather Service office at the Brownsville, TX airport. For each rainfall event, composite water samples (two 400-ml samples every 2 h) or hourly samples (800-ml samples every hour) were collected for 24–48 h using automated samplers equipped with temperature, conductivity, and water level sensors. The collection of water samples was triggered either by increasing water depth in channel and low-conductivity water (to avoid seawater sampling) or triggered remotely by the PI who monitored weather conditions. The water sampling period ran from June 2014 to November 2015.

Within 12 h of collection, water samples were filtered through Whatman GF/C filters and frozen for later analysis. Water samples were analyzed for dissolved orthophosphate, ammonia nitrogen, nitrate-nitrite nitrogen, total dissolved nitrogen (subset of samples), and δ^{15}N isotope values (^{15}N / ^{14}N ratio) of seston (subset of samples). Water samples were analyzed for nitrate-nitrogen, ammonium-nitrogen, and soluble reactive phosphate using the following EPA Methods (USEPA 2022): nitrate- EPA Method 0353.2, ammonium- EPA Method 0350.2, dissolved orthophosphate- EPA Method 0365.2. Total dissolved nitrogen analysis was performed by Texas A&M University-Corpus Christi using the combustion oxidation and chemiluminescence detection method (ASTM). To characterize the nitrogen source of drains, a subset of seston samples were analyzed by the University of Texas Marine Science Institute for ^{15}N/^{14}N isotope ratios of seston.

To estimate the total ungauged nutrient loading from the drains, nutrient-flow relationships were developed from hydrologic and water quality data collected at the sampling sites. Nutrient loading for precipitation events was calculated by multiplying the measured nutrient concentration (g/m^3) by the average instantaneous discharge (m^3/h) for each hour recorded by ADCP sampling. The total nutrient load for each event was calculated by summing the hourly loading rates over the course of the sampling period.

Results

Seagrass Status and Trends Between 1998 and 2017

Because the Arroyo Colorado (AC) is the dominant source of freshwater inflow into LLM, we focused our study on impacts of inflows from the Arroyo and surrounding ungauged subwatersheds on adjacent seagrasses in the LLM. As shown previously in Fig. 1 and Fig. 4, this Middle LLM Study Area extends from Mansfield Pass south to the area just south of Stover Point, a distance of 34.5 km (21.4 mi), and comprises an area of *ca* 39,050 ha.

Seagrass Decreases in Mid-LLM Region Between 1998 and 2012

The seagrass distribution map in 1998 produced by Onuf (2007) was initially compared using GIS analysis with a photo-delineated 2009 NAIP seagrass map for the mid-LLM Study Area (Fig. 7) (BBEST 2012). This 1998 to 2009

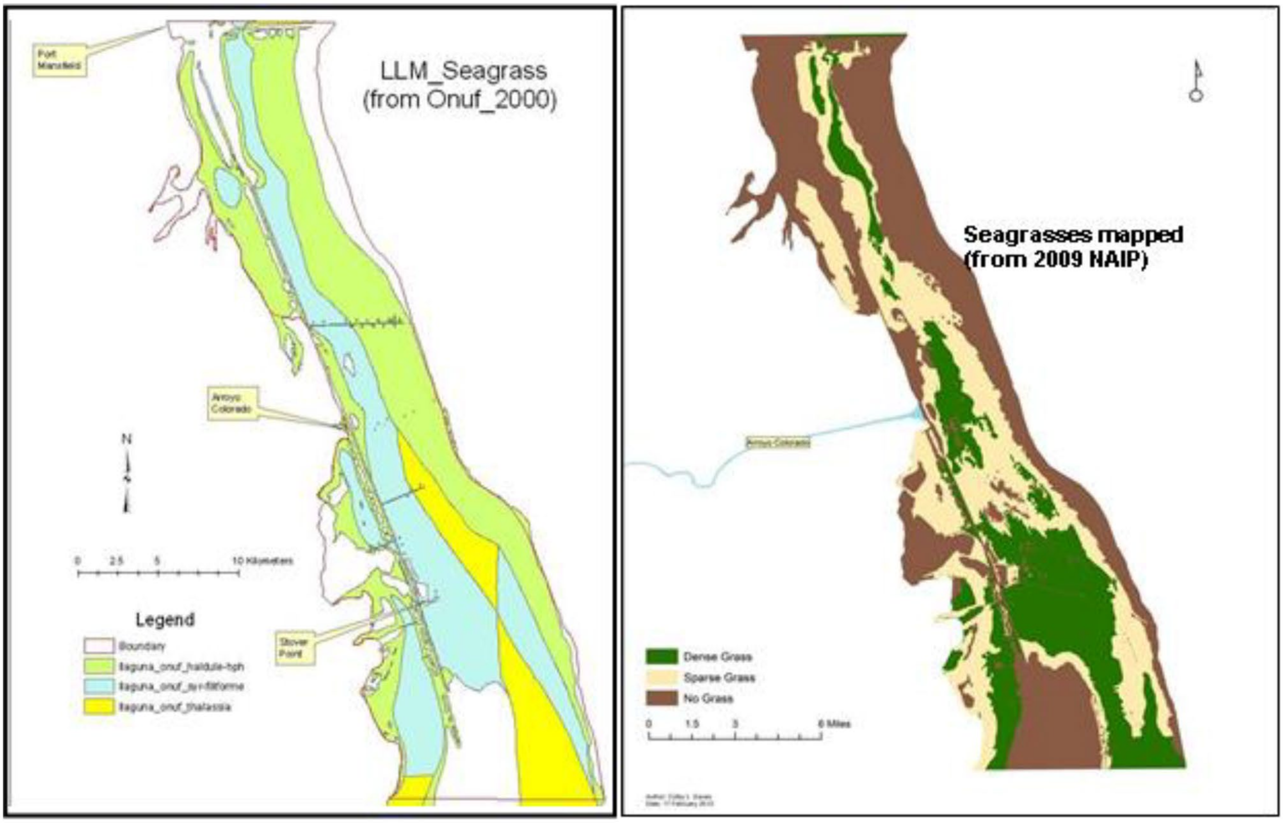


Fig. 7 Seagrass maps of the mid-Lower Laguna Madre Study Area from Onuf 1998 survey (Onuf 2007) (left) and classified 2009 NAIP photo-imagery (BBEST 2012) (right)

comparative analysis confirmed the time period and progression of the mid-LLM seagrass decline in the earlier BBEST study (BBEST 2012). The total seagrass area summed for all species in the Onuf 1998 map (37,211 ha) compared to the NAIP 2009 total seagrass area (28,398 ha) (Fig. 7) shows that total seagrass acreage in 2009 had decreased *ca* 24% from 1998. Although some of this decrease may be due to technical differences between the 2 mapping methods used

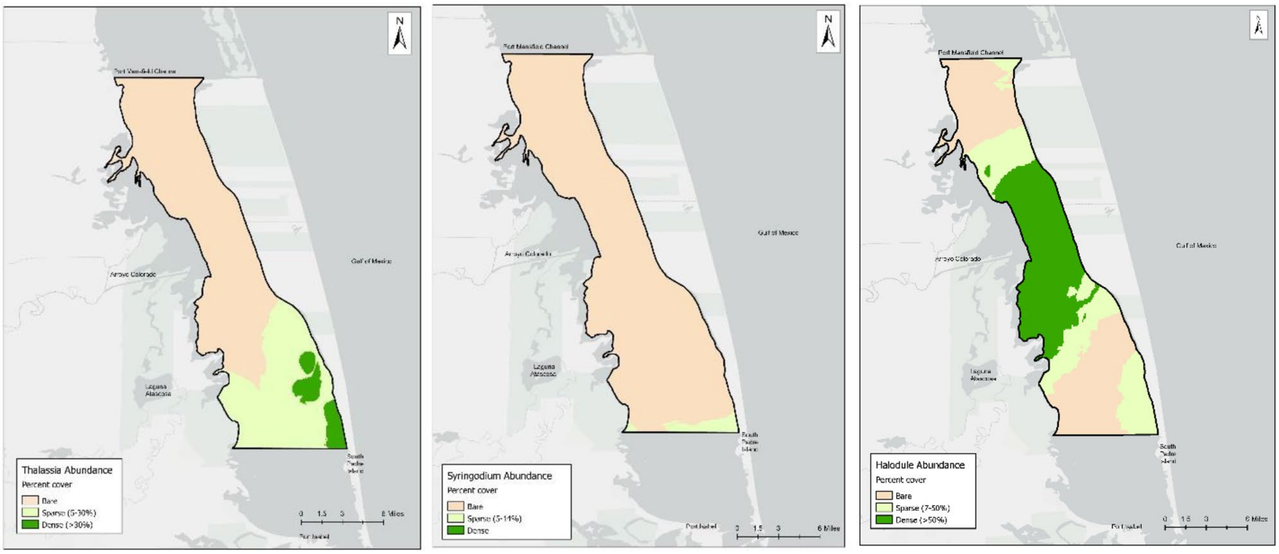


Fig. 8 *Thalassia*, *Syringodium*, and *Halodule* percent cover maps for the mid-LLM Study Area based on the 2012 seagrass survey of DeYoe and Kowalski (2013)

Table 3 Seagrass acreage and percent cover by species for the mid-LLM Study Area in 1998 (from Onuf 2007) and 2012 (from DeYoe and Kowalski 2013). *nd*, not detected

Species	1998 (ha)	1998	2012 (ha)	2012
Thalassia	8267	21.2%	6,975	17.9%
Syringodium	11,737	30.0%	1,126	2.9%
Halodule	16,858	43.2%	21,398	54.8%
Halophila	349	0.9%	nd	–
Ruppia	nd	–	2,967	–
Bare	1840	4.7%	9,543	24.4%
Total vegetated	*37,211*	*95.3%*	*29,507*	*75.6%*
Total area	39,051 ha		39,050 ha	

(spatial interpolation between transect points in 1998 vs. photo-delineation of polygons in 2009), these results provide a reasonable estimate of seagrass changes intervening between 1998 and before 2012 and indicate that significant reduction in seagrass cover was already underway by 2009. No unusually high-inflow events had occurred between 1998 and 2009 to create drastic hyposaline conditions, as happened later in 2010 from Hurricane Alex. Based on ground-truthing in 2009–2010 by DeYoe and colleagues, most of this seagrass decline in the mid-LLM Study Area consisted of *Syringodium* and *Thalassia* in shallow intertidal flats northwards from Stover Point towards Port Mansfield.

After Hurricane Alex in 2012, DeYoe and Kowalski (2013) conducted their LLM seagrass survey replicating Onuf's 1998 survey. As shown in Fig. 8, the distributions of *Thalassia* and *Syringodium* had shifted from being widely distributed in 1998 to being restricted to the lowest portion of the mid-LLM Study Area. By this time, dense *Halodule* (interspersed with the oligohaline SAV, *Ruppia maritima*) had become widely established in the middle reach of the LLM Study Area (Fig. 8). Quantitative comparison of the 1998 Onuf data with the 2012 survey (DeYoe and Kowalski 2013) is shown in Table 3. Large decreases of *Thalassia* (21% down to 17.9%) and *Syringodium* (30% down to 2.9%) had occurred, while there was a significant increase of *Halodule* (43.2% up to 54.8%). *Halophila engelmannii*, which was reported at 0.9% cover in the 1998 survey, was not recorded in the 2012 survey. The opposite, however, was true for *Ruppia* (not reported in the 1998 survey) but found mixed in with *Halodule* at 4.3% cover in the 2012 survey. The total bare area had expanded greatly from 4.7 to 24.4% despite the increase in *Halodule*.

Seagrass Change Showing Recovery from 2012 to 2017

Seagrass dynamics over the 5 years between 2012 and 2017 were evaluated by examining maps made from the Texas Statewide Seagrass Monitoring Program data (Dunton 2019) for *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia*

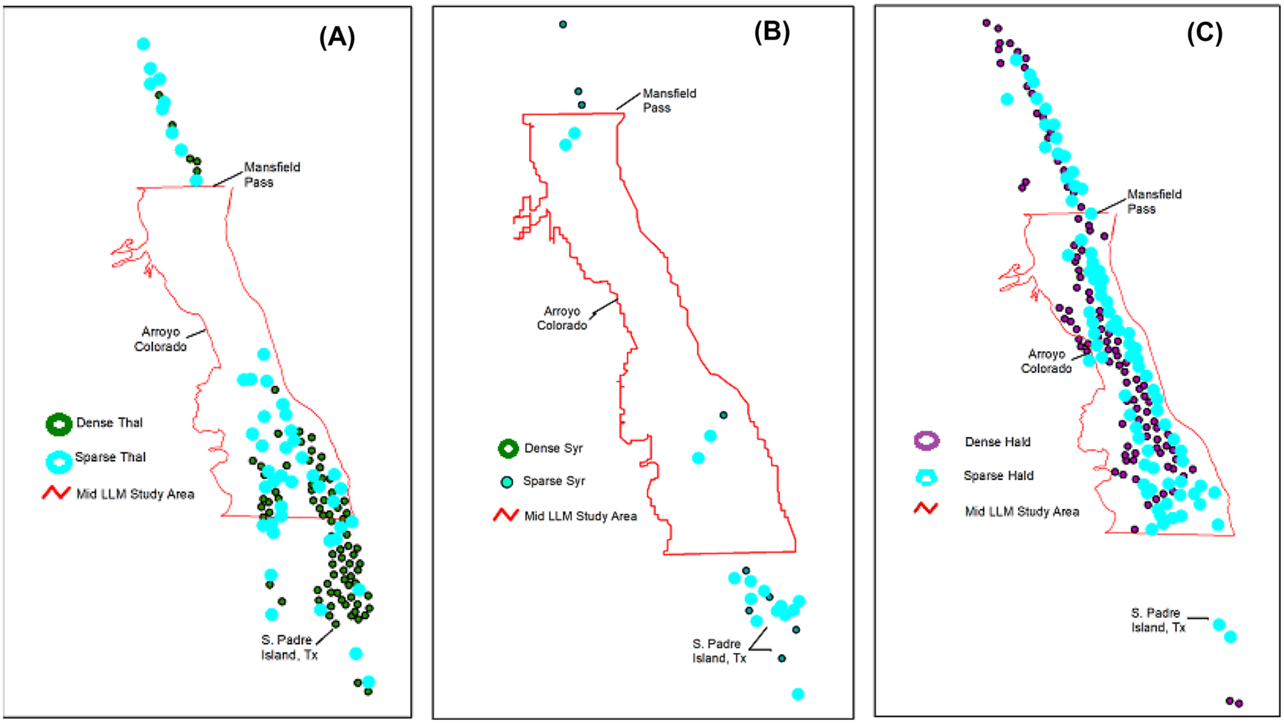


Fig. 9 *Thalassia* (**A**), *Syringodium* (**B**), and *Halodule* (**C**) percent cover maps for the entire LLM and the LLM Study Area (red polygon) based on the 2017 seagrass survey of Dunton (2019)

Table 4 Average *Thalassia* parameters for four Lower Laguna Madre sites for the period March 2005 to October 2006. Average (Std deviation)

	ABC	Bay West	Green Island	South Bay
Total biomass (g/m^2)	1296.7 (332.7)	1110.7 (522.8)	319.3 (296.0)	450.3 (250.7)
Shoot density (shoots/m^2)	3382 (1252)	1620 (838)	567 (583)	1134 (559)
Leaf length (mm)	43 (16)	51 (18)	71 (22)	68 (30)
Areal production (g/m^2/day)	1.98 (0.78)	3.40 (1.76)	1.19 (0.43)	1.84 (1.16)

testudinum (Fig. 9) and comparing to the previous 2012 maps (Fig. 8). *Thalassia* occurrence in 2017 (Fig. 9A) showed a similar distribution as in 2012, although sparse *Thalassia* ($<40\%$ cover at sites) had expanded northward several miles into the mid-LLM study area towards the Arroyo Colorado. This represents an estimated increase from our 17.9% coverage in 2012 to *ca* 34%. Essentially no recovery since 2012 was exhibited for the mid-LLM Study Area by *Syringodium* (Fig. 9B) (2.9% vs. 3.6%), as well as the entire LLM (8.4% 2017 vs. 7.9% 2012). *Halodule* was still the dominant species in the mid-LLM area (Fig. 9C) where dense coverage had expanded since 2012 from 54.8% to *ca* 80%. Additionally, no *Ruppia* was recorded during the 2017 monitoring. Overall, this large increase in *Halodule* in 5 years had replaced the two climax species in the LLM Study Area.

Thalassia Monitoring Study (2005–2006)

Average *Thalassia* total biomass was significantly lower at the Green Island site (319.3 g/m^2, $n=21$) (closest to the inflow of the Arroyo Colorado) and South Bay site (450.3 g/m^2, $n=25$) compared to sites ABC (1296.7 g/m^2, $n=22$) and Bay West (1110.7 g/m^2, $n=17$) ($p<0.001$)(Table 4). Seasonal areal production at Green Island was generally lower than the other sites but not significantly. Average leaf length was greater at Green Island (71 mm, $n=16$) and South Bay (68 mm, $n=32$) compared to ABC (43 mm, $n=26$), and Bay West (51 mm, $n=24$)($p<0.001$). Shoot density was significantly lower at Green Island (742 shoots/m^2, SD = 618, $n=14$) and South Bay (1134 shoots/ m^2, SD = 559, $n=22$) compared to ABC (3207 shoots/m^2, SD = 1375, $n=18$) and Bay West (1620 shoots/m^2, SD = 838, $n=13$)($p<0.001$).

Nutrient and chlorophyll levels were higher for the Arroyo Colorado compared to the LLM sites (Table 5). Excluding the Arroyo Colorado, the Green Island site, closest to the Arroyo Colorado, had nitrate levels three or more times higher ($p=0.079$) and chlorophyll levels two or more times higher than the other LLM study sites ($p<0.001$) (Table 5). Ammonia and dissolved phosphate levels were similar at all LLM sites.

TxBLEND Modeling of Salinity Plumes During 2010

Hurricane Alex in 2010 produced an extended period of low salinity in the LLM starting in early August (Fig. 10). The extent of freshwater intrusion into the LLM was sufficient to depress salinities to 0 to 5 at stations 10 km north and south of the Arroyo Colorado for more than 1 month (Kowalski et al. 2018). Depressed salinities were noted in the northern half of the lagoon through October 2010 and the normal salinity regime for the lagoon did not return until December 2010. The LLM middle region from near the AC entrance north to Pt. Mansfield experienced oligohaline salinity conditions (5 or less) throughout August 2010 in the heat of summer (Avg. water temp. 31 °C), and salinity stayed at 10 to 15 throughout September into October (water temp. range 28–30 °C)(unpublished data). This area corresponds to the LLM regions of major *Syringodium* and *Thalassia* loss in this period (Fig. 8).

The salinity output of the TxBLEND model was verified by field surveys after Hurricane Alex and results are published in Kowalski et al. (2018). During that study, turbidity data (as Secchi disk depth) was also collected along a North–South transect from north of the Arroyo Colorado south to Brazos-Santiago Pass starting three days after landfall of the hurricane

Table 5 Average water column chlorophyll a and nutrient data for the Arroyo Colorado and four LLM sites (Fig. 2). Green Island is nearest the Arroyo Colorado with South Bay being the furthest south. Bay West and ABC are also south of the Arroyo at intermediate distances. Period of record is from 2006 to 2010. *SD*, standard deviation, *n*, sample size

	Chlorophyll a, µg/L		*n*	Ammonia, mg N/L		*n*	Nitrate, mg N/L		*n*	Diss.Phosphate, mg P/L		*n*
	Avg	SD		Avg	SD		Avg	SD		Avg	SD	
Arroyo Colorado	20.8	21.4	13	0.17	0.16	22	0.87	1.15	25	0.16	0.11	21
Green Island	5.88	6.58	7	0.12	0.15	29	0.11	0.29	27	0.02	0.03	28
Bay West	2.94	2.94	6	0.16	0.25	19	0.01	0.01	9	0.05	0.12	13
ABC	0.86	0.82	7	0.08	0.13	26	0.01	0.01	26	0.02	0.03	27
South Bay	1.16	0.36	6	0.12	0.16	15	0.03	0.06	21	0.03	0.04	18

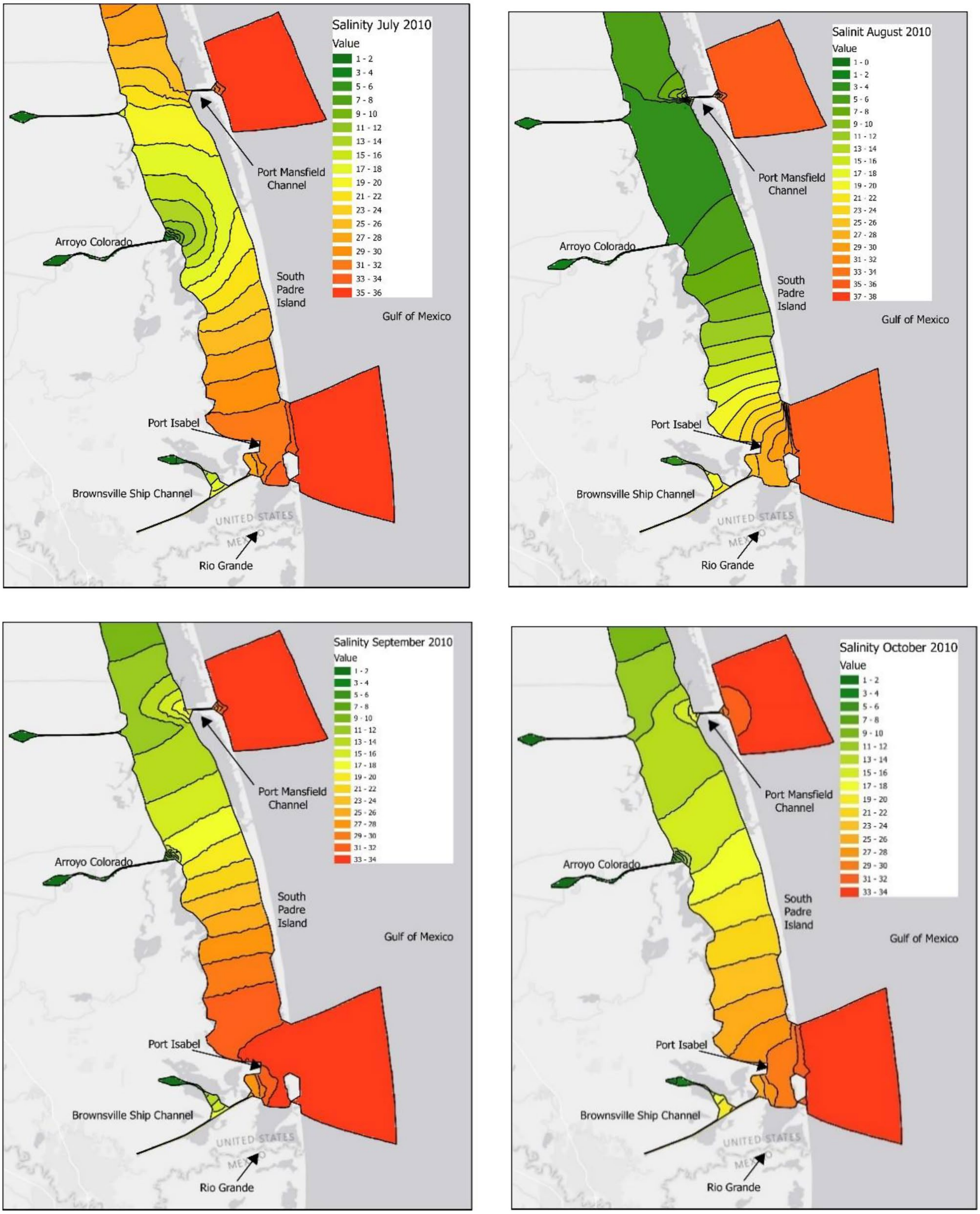


Fig. 10 Monthly LLM salinity plume maps derived from the TxBLEND model for July–October 2010

(2 July 2010) through 25 September 2010 (unpublished data) for three segments in the mid-reach Study Area, centered on the Arroyo Colorado: North segment, Middle segment (near Arroyo Colorado confluence) and South segment (Table 6). The average Secchi disk depth for the middle segment area did not appear appreciably lower than the other segments in the 3 months following the hurricane.

Nitrogen Source Tracing (Stable Isotope Analysis)

δ^{15}N values of periphyton collected on two dates were consistently high ($> 10°/_{oo}$) in the tidal segment of the Arroyo Colorado (Table 7). Average δ^{15}N values for AC water column nitrate in April and August 2011 were $13.0°/_{oo}$ for River Ranch near AC West. Seagrass (*Halodule wrightii*) collected along an east–west transect were elevated nearest the confluence of the Arroyo Colorado and showed a decline in δ^{15}N values and were negative at the two sites furthest east from the AC confluence (Fig. 11). The drift macroalgae (*Palisada poiteaui*) collected at three of the five sites on the transect were similarly elevated nearest the Arroyo Colorado but did not show as dramatic of a decline to the east as did *Halodule* (Fig. 11).

Potential Nutrient Loading from Ungauged Subwatersheds

As examples of ungauged nutrient loading, data are presented for sites in the Brownsville and Arroyo Colorado subwatersheds for one or two events per site (Table 8). Data represent estimates of the potential magnitude of nutrient loading coming from representative LLM subwatersheds and are not intended to correlate with any particular sites of 2010 seagrass impacts.

The tidal influence did affect the POB north and POB south sites but the data for the events presented had minimal tidal influence. The tidal influence did not affect the Arroyo Colorado or Arroyo Colorado subwatershed drains (AC East and AC West).

The discharge values for a rainfall event are a function of the subwatershed size, rainfall, and landuse/landcover. The POB South subwatershed was about half the size of the POB North subwatershed while the AC East and AC West subwatersheds both considerably smaller than the POB subwatersheds (Table 2). There are also differences in the number of point sources i.e., wastewater treatment plants (WWTP) i.e., there are 3 permitted WWTPs in the POB North and 1 in the POB South. The Arroyo Colorado watershed sample site was located upstream of AC East and AC West subwatersheds and other small subwatersheds so data for that site does not reflect all the FWI for the Arroyo Colorado.

Table 6 Average Secchi disk depth (m) for three segments of the lagoon (North, Middle, and South) for July, August, and September 2010 (SD)

	July	August	September
South	0.54 (0.22)	0.43 (0.12)	0.63 (0.07)
Middle	0.51 (0.07)	0.34 (0.03)	0.43 (0.22)
North	0.69 (0.04)	0.37 (0.01)	0.40 (0.14)

Table 7 δ^{15}N values of periphyton collected in 2011 from two sites in the tidal segment of the Arroyo Colorado. April $n = 2$, August $n = 1$

Collection date	Site	Type	δ^{15}N ($°/_{oo}$)
April 2011	River Ranch	Periphyton	10.48
April 2011	Thomae Park	Periphyton	12.47
August 2011	River Ranch	Periphyton	16.90
August 2011	Thomae Park	Periphyton	16.87

Brownsville and Arroyo Colorado Subwatershed Loading Events

POB North June 2014 baseline event (no rainfall)—Sampling for this event captured a period with no rainfall and outgoing tide conditions. Discharge ranged from 0 to 7458 m^3/h. The loading rate was highest for nitrate with an average value of 7.94 kg/h. Average loading rates for ammonia and phosphate were both less than 0.5 kg/h. The total nutrient load for the sampling period was highest for nitrate at 159.2 kg.

POB North November 2014 rainfall event (7.26 in.)—Discharge increased through the sampling period and was still increasing at end of the sampling period so the completion of the event was not captured. The highest discharge for the last sampling was 40,000 m^3/h while the average discharge was 12,108 m^3/h. The loading rate was highest for nitrate with two peaks (Fig. 12) and an average of 27.4 kg/. Loading rates for ammonia and phosphate were less than 1.5 kg/h. The total nutrient load for the portion of the event captured was highest for nitrate at 468.1 kg, at least 16 times greater than the total loads for ammonia and phosphate.

POB South August rainfall event (4.32 in.)—A modest rainfall event was captured with a peak discharge of 4,228 m^3/h and an average discharge of 2217 m^3/h. The mean nutrient loading rate was highest for nitrate 0.57 kg/h with a peak 2.4 kg/h while mean loading rates for ammonia and phosphate were less than 0.2 kg/h (Fig. 12). Total nutrient loads for this event were approximately 10, 0.8, and 4 kg for nitrate, ammonia, and phosphate, respectively (Table 8).

Three rainfall events were captured for the Arroyo Colorado—one for the Arroyo Colorado and two for the Arroyo Colorado subwatersheds (East and West) between August and October 2015. Since the two Arroyo Colorado subwatersheds were relatively small compared to the POB

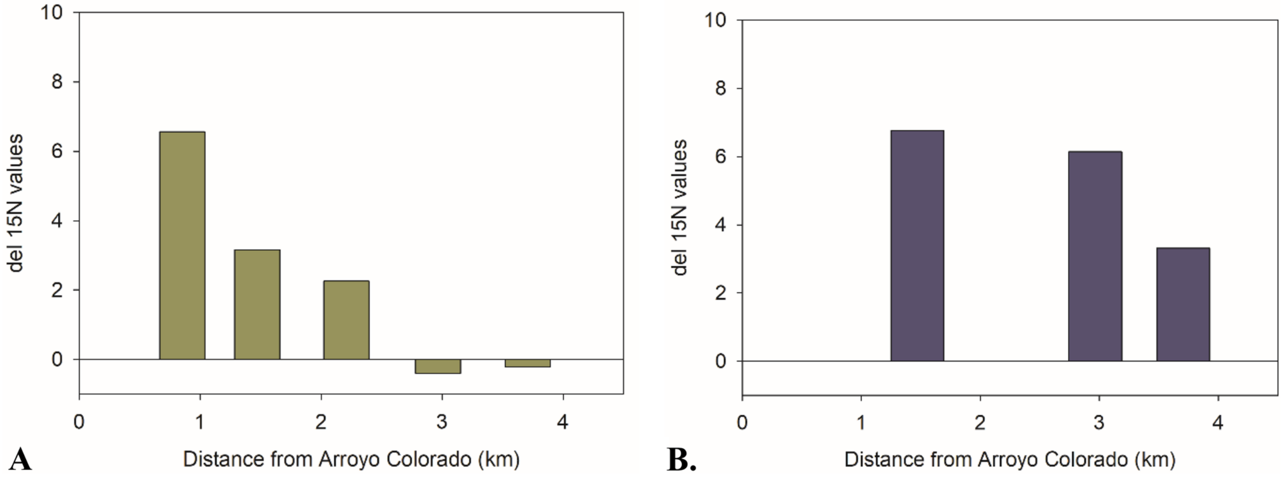


Fig. 11 δ^{15}N values for composite samples of the seagrass leaves of *Halodule wrightii* (**A**) and the macroalgae *Palisada poiteauii* (**B**) collected on August 2011 in the LLM along the west to east transect starting near the Arroyo Colorado confluence

subwatersheds, the total nutrient loads for nitrate, ammonia, and phosphate were lower than those of the POB subwatersheds despite rainfall being between 6 to 9 cm per event (Table 8). One exception is the total ammonia load for the AC West which was higher than the other subwatersheds (Table 9). Excluding the Arroyo Colorado, we normalized the total nutrient loads by dividing the load by subwatershed area. The normalized POB North nitrate value was substantially higher than the other subwatersheds (Table 8).

Nitrogen Composition and Sources

In all the rainfall events captured, nitrate was generally more abundant than ammonia with the exception of the AC West event (Table 8). Including dissolved organic nitrogen (DON), it appears that DON is a substantial component of the total dissolved nitrogen (TDN) accounting for 10 to 67% of the TDN. For example, in the POB North November rainfall event, the DON varied from 16.3 to 55.6 µM with an average of 27.7 µM and was 34.2% of total dissolved nitrogen (Table 9). δ ^{15} N values for these select FWI events were all positive and elevated varying from 5.1 to 13.3°/$_{oo}$.

Relationship Between Ungauged Inflow and Nutrient Loading

The correlation of the 2010 salinity plume dynamics to 2012 seagrass loss indicates that impacts of extreme inflows were pronounced when episodic ungauged flows to LLM were highest (Fig. 5). When the eight subwatersheds (Fig. 4) that contribute to the LLM are considered, ungauged flow dominates in these subwatersheds during large inflow events. The ungauged flows of these eight subwatersheds are presented for the period of 2001–2020 in Fig. 13. It is noteworthy that three of these eight subwatersheds (including Arroyo Colorado #22,903) show the highest inflows in the last 15 years, with possibly an increasing trend; and these regions drain directly into the middle portion of LLM where most seagrass decline was documented in 2012. These three subwatersheds (22,903–22,905) are the largest, most heavily developed, mostly inland subwatersheds in south Texas, whereas 22,901 and 22,906–908 are smaller, less populated, coastal subwatersheds. From our nutrient loading case study of representative ungauged drainages, this would indicate that nutrient loading is also higher in these subwatersheds when runoff is higher.

Table 8 Estimated total nutrient loadings and rainfall for select precipitation events by subwatershed site

Subwatershed site	Event date	Rainfall (cm)	Nitrate (kg)	Ammonia (kg)	Phosphate (kg)	Nitrate (kg/10^4 ha)
POB North	Jun. 2014	0	159.2	4.1	10.6	62.4
POB North	Nov. 2014	7.26	468.2	17.7	25.5	183.5
POB South	Aug. 2014	4.32	10.3	0.8	4.0	8.7
AC-West	Oct. 2015	6.27	11.8	18.5	11.4	1.8
AC-East	Sept. 2015	9.27	0.7	1.5	0.2	3.3
Arroyo Colorado	Aug. 2015	5.61	19.8	6.9	12.4	-
Arroyo Colorado	Sept. 2015	9.27	65.0	32.6	28.6	-

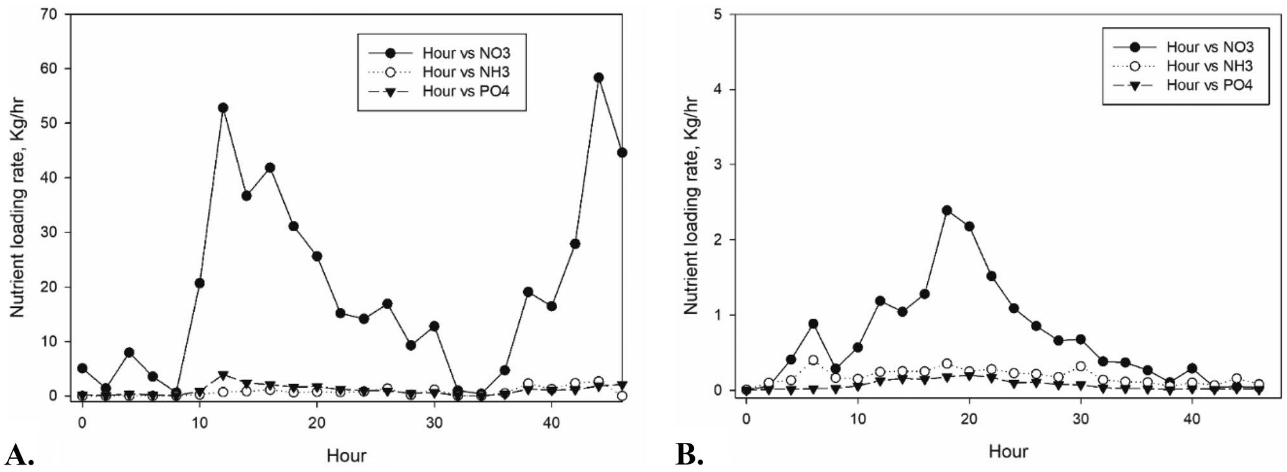


Fig. 12 **A** Nutrient loading for Port of Brownsville North drain for November 5–7, 2014, rainfall event. **B** Nutrient loading for Port of Brownsville South drain for August 30–September 1, 2014, rainfall event. Note differences in *Y* axis scale of the two graphs

Discussion

Overview of Seagrass Trends

Seagrass acreage and composition in the LLM have changed significantly in the past 50+ years, as documented by McMahan (1968b), Merkord (1978), Quammen and Onuf (1993), Onuf (2007), and DeYoe and Kowalski (2013). Onuf (2007) summarized these changes and reported the acreages as follows. From the 1960s until 1988, total acreage decreased from approx. 59,150 ha to 46,624 ha, and then dropped slightly to 46,174 ha in 1998. However, species composition changed dramatically. In the mid-1970s, *Syringodium filiforme* (manatee grass) and *Halodule wrightii* (shoal grass) were the most common species (25.9% and 70.0% cover, respectively), and *Thalassia testudinum* (turtle grass) was only 3.0% cover. By 1988, *Halodule* had decreased to 46.3% cover, *Syringodium* had increased to 37.7% cover, and *Thalassia* had increased to 8.5% cover. This mixed community continued into the 1990s, when beds were dominated by *Syringodium* and *Thalassia*. By 1998, *Thalassia* (24.1%) had expanded and replaced some of the *Syringodium* (27.8%), while *Halodule* remained stable at 45.7% cover. Because *Halodule* is considered a pioneer, euryhaline species, while *Syringodium* and *Thalassia* are considered polyhaline, climax species, these changes up to the early 1990s are generally viewed as the result of amelioration of salinities in the historically hypersaline lagoon over time and in line with salinity tolerances of the species (Pulich 1980; Quammen and Onuf 1993).

One deviation from this trend occurred during the 1980s and into the 1990s, however, when significant shifts in species' composition took place in localized, mid-reaches of the LLM, south of the Arroyo Colorado. Onuf (1994, 1996) concluded that maintenance dredging along the Gulf Intracoastal Waterway through this region produced excessive turbidity which in turn caused decline of *Halodule* from 32,600 ha in the late 1970s, to 21,594 ha by 1988. Despite the loss of *Halodule* in this localized areas, total seagrass cover in the entire LLM remained remarkably constant over the period from 1970s (at 46,558 ha) to 1998 (at 46,174 ha). Apparently, the expansion of and replacement by *Thalassia* and *Syringodium* in other areas compensated for this *Halodule* loss.

Beginning in 2000, anecdotal reports indicated that localized reduction in seagrasses had started to occur (recreational bay users; DeYoe, pers. obs.). Following Onuf's methodology, DeYoe and Kowalski (2013) repeated a survey of the entire LLM in 2012. This survey documented an 8.8% overall decrease (*circa* 5396 hectares) in total seagrass that occurred between 1998 and 2012, primarily in *Syringodium* and *Thalassia*, while there was a 10% increase in *Halodule*

Table 9 Average dissolved organic nitrogen (DON) in µmoles N/L, DON % of total dissolved nitrogen, and δ^{15}N values of seston for the study sites in 2015. *SD*, standard deviation. $n = 3$ or 4

Site	Date	DON (µM N)	DON (SD)	DON (%)	δ^{15}N (‰)
POB North	November 2014	27.7	15.0	34.2	8.0
POB South	August 2014	42.3	30.4	67.4	8.6
AC-east	September 2015	72.0	6.2	21.8	5.1
AC-west	October 2015	37.9	8.1	54.0	13.3
AC	August 2015	66.5	24.0	9.6	8.1

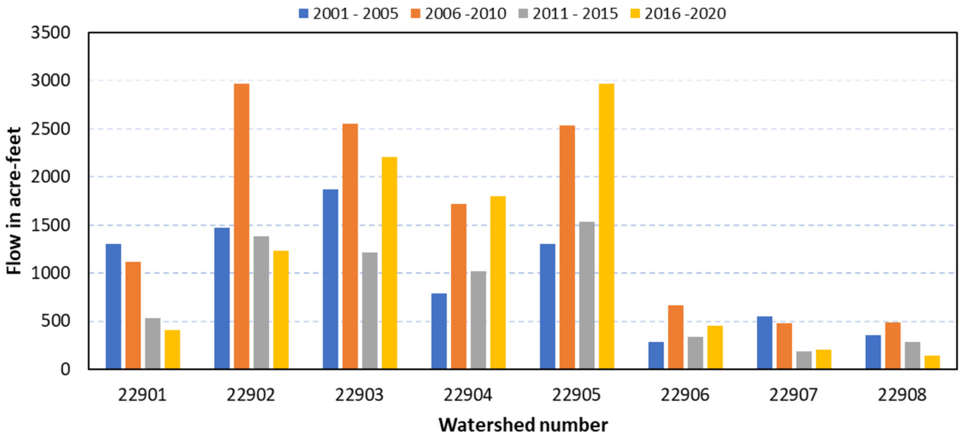


Fig. 13 Five-year increments of ungauged monthly mean flow values calculated from four different periods of record (time periods from 2001–2005, 2006–2010, 2011–2015, and 2016–2020) for the eight different LLM sub-watersheds. Watershed numbers and respective surface areas (sq. km): 22,901–118.7 (Pt Isabel); 22,902–586.8 (Brownsville); 22,903–1032.5 (Arroyo Colorado); 22,904–417.4 (North Floodway); 22,905–976.8 (Raymondville); 22,906–73.9 (Pt. Mansfield); 22,907–130.1 (Laguna Atascosa); and 22,908–90.1 (Rio Grande)

(Table 10). *Halophila engelmannii* was found by Onuf in 1998, but no *Ruppia maritima* was reported. The opposite result was observed in 2012 by DeYoe and Kowalski (2013).

Using the 1998 data provided by Onuf as a baseline, we examined the sequence of species changes over the past 20+ years and focused on the middle LLM region near the entrance of the Arroyo Colorado channel. A hypothesis was developed that centered on hydrologic impacts due to episodic freshwater pulses that cause hyposalinity, coupled with high nutrient loading (BBEST 2012). Based on seagrass mapping analysis of 2009 NAIP imagery and results from a special *Thalassia* productivity monitoring study in 2005-6, along with FWI records from the early 2000s period, the BBEST study concluded that from 1998 to 2009, very significant declines (> 20%) of *Thalassia* and *Syringodium* were already occurring in this middle LLM region. Then in August 2010, Hurricane Alex inflows occurred (mostly via the Arroyo Colorado), flooding the LLM and producing freshwater conditions (0–2 salinity) lasting for 2 months (Kowalski et al. 2018). The correspondence between low-salinity plumes (Fig. 10) and areas of complete die-off of climax seagrass species that ensued in the middle region of LLM, and some re-colonization by the low-salinity tolerant species, *Halodule* and *Ruppia* in the same areas, provides evidence that extreme hyposalinity was involved in this die-off.

Since the 2010 die-off, seagrass recovery in the middle LLM reach several miles above and below the Arroyo Colorado confluence appears to reflect the stabilization of salinity regimes and ensuing subtropical species succession. As of 2017, the low-mesohaline tolerant species *Halodule* has almost completely re-colonized, possibly from a large seed bank existing in the sediments. No *Syringodium*, and only modest amounts of *Thalassia,* have re-established, however. It will be important to watch the future time course of climax species re-colonization in this die-off area, concurrently with additional monitoring of ecological factors such as nutrient loading dynamics.

It is notable that *Halophila engelmannii,* a halophilic seagrass with maximal growth at 23–40 salinity regimes, was recorded in all surveys up to 1998, but not in the 2012 survey, however, some *Halophila* had returned by 2017. *Ruppia maritima*, freshwater- to hypersaline-tolerant SAV species, with maximal growth below a salinity of 20 (Koch et al. 2007), was recorded only in the 2012 survey, but not in earlier or in the 2017 survey.

Factors Related to Seagrass Decline in the Middle Laguna Madre Region

Salinity Response

A review of salinity tolerance reveals the complexity of seagrass responses to FWI events. The subtropical seagrass species found in LLM have strict salinity requirements as

Table 10 Seagrass percent cover by species compared for the entire LLM in 1998 (Onuf 2007) and 2012 (DeYoe and Kowalski 2013). *nr*, not recorded

	1998	1998	2012	2012
Thalassia	11,132 ha	16%	4692 ha	6.8%
Syringodium	12,861 ha	19%	4830 ha	7.0%
Halodule	21,118 ha	31%	28,290 ha	41%
Halophila	1063 ha	1.5%	nr	
Ruppia	nr		2,967 ha	4.3%
Bare	22,761 ha	33%	29,256 ha	42%
Total vegetated	*46,174 ha*	*67%*	*40,779 ha*	*59%*
Total area	68,935 ha	99%	69,000 ha	101%

documented by numerous investigators (Phillips 1960; McMillan and Mosely 1967; Zieman 1974; Pulich 1985). For these obligatory saltwater plants, salinity requirements are a function of exposure time, time of year (growing season), and temperature. Their growth tolerance limits also depend on root exposure to lower or higher salinity porewater, but this relationship has rarely been investigated. While tolerance to maximum salinities varies among the four species, all species are tolerant of salinities above 35; thus, hypersaline conditions in the LLM up to 45 are not problematic for short times (Table 11). All four species can tolerate low salinity only briefly down in the 6 to 13 range (*Halophila* withstands salinity of 13, while *Halodule* withstands 6, and *Thalassia* or *Syringodium* tolerate salinity of 10) (McMillan and Mosely 1967). These low salinity levels will kill seagrass leaves when exposed directly for more than a few hours, and roots/rhizomes in the sediments after longer exposures (e.g., several days). Less severe salinity reductions will cause inhibition of metabolic and physiological processes. At a minimum, the dead leaves are shed, which temporarily stunts their growth (Zieman et al. 1999). Otherwise, all four species have minimum salinity tolerances for sustained growth in the polyhaline range between 20 and 24, with *ca* 24 considered to be a lower salinity threshold for sustained growth of *Thalassia* and *Syringodium* (Phillips 1960; Zieman et al. 1999).

When rooted seagrasses are considered, the lethal effects of unfavorable low salinity conditions from FWI, can be long-lasting with root-rhizome destruction. The effect of low salinities produced by high inflows may also lead to long-term deleterious effects on seagrass beds from sediment erosion and resuspension, much like mechanical damage from motorboat propeller scarring in Texas (Dunton and Schonberg 2002) or hurricane damage in Florida (Steward et al. 2006).

Nutrient Responses

Nutrient additions (nitrogen and phosphorus) can have positive and negative as well as direct and indirect impacts on seagrass. Lee (1998) showed that *Thalassia testudinum* at one site in the LLM responded positively to ammonia additions with increased growth. In an example of a direct negative effect, Burkholder et al. (1994) found that nitrate in excessive amounts had a detrimental effect on the growth of *Zostera marina*, a temperate zone species, due to its physiology; while the same nitrate treatment produced modest to substantial growth increase in *Halodule* and *Ruppia*, respectively. However, *H. wrightii* is inhibited at high nitrate levels (100 µM) in sediments (Pulich 1980), while *Ruppia maritima* is inhibited by high ammonia levels (Burkholder et al. 1994). Long-term experimental fertilization of a *Thalassia testudinum* bed eventually led to its replacement by *Halodule wrightii* (Fourqurean et al. 1995). Direct responses by *Thalassia* and *Syringodium* to elevated nitrogen loadings in situ have generally not been well-characterized, although Tomasko et al. (1996) documented negative impacts of anthropogenic nutrient enrichments on *Thalassia* beds in Sarasota Bay, Florida. Indirect effects of nutrient enrichment included stimulation of the growth of phytoplankton, macroalgae, and seagrass epiphytes. Such conditions can lead to reduced seagrass productivity due to light reduction (LaPointe et al. 1994).

The interactive effects of low salinity waters enriched with nutrients has not been intensively studied for LLM seagrass species. As noted above, low salinities have detrimental effects by themselves on seagrass, however, when large quantities of low-salinity water also carry significant amounts of nutrients, the potential exists for impacts due to synergistic effects. Although Quammen and Onuf (1993), Onuf (1996), and others have discussed the impact of high nutrient loading, this was routinely in the context of nutrients only, without salinity stress. Studies by Burkholder et al. (2007) on the temperate zone seagrass *Zostera marina* indicate that synergistic effects can occur between temperature and nitrate enrichment, an example of how interactions between multiple factors can occur. For *Thalassia testudinum* seedlings at lower salinities (10–20), increasing the level of ammonium negatively impacted seedling growth (Kahn and Durako 2006). A complex synergistic effect of nutrient concentration, salinity, and seagrass ecotype has been shown for *Z. marina* (van Katwijk et al. 1999).

Prior to 2010, *Thalassia testudinum* was a widespread seagrass species in the LLM but as noted above *Thalassia* distribution had contracted, perhaps due to a combination of hyposalinity stress and perhaps excessive nutrient loading. Our *Thalassia* monitoring study gives an example of a

Table 11 Salinity tolerance ranges of LLM seagrasses. Data from McMillan and Mosely 1967; McMahan 1968a; Phillips 1960; Zieman 1974; Verhoeven 1979; Pulich 1980, 1985; Koch et al. 2007. *Not considered a seagrass by some researchers

Seagrass species	Optimal growth salinity range	Lethal salinity range
Shoal grass (*Halodule wrightii*)	20–44	6 or <; 70 or >
Clover or star grass (*Halophila engelmannii*)	23–40	13 or <; 50 or >
Turtle grass (*Thalassia testudinum*)	24–38	10 or <; 48 or >
Manatee grass (*Syringodium filiforme*)	24–38	10 or <; 44 or >
Widgeon grass (*Ruppia maritima*)*	0–20	55 or >

site (Green Island) that was routinely exposed to hyposalinity events and elevated nutrient loading. *Thalassia* at the Green Island site had lower biomass and lower productivity compared to the sites further from the Arroyo Colorado, a major FWI source for the lagoon. In addition, *Thalassia* leaves were longest at Green Island compared to the other sites. Although light intensity was not measured at the four monitoring sites, the low productivity and biomass combined with the longer leaves at Green Island suggest that light intensity was not adequate to achieve maximal growth rates of *Thalassia*. As noted earlier, of the four monitoring sites, water column nitrate and chl a were highest at Green Island. Phytoplankton growth was likely stimulated in the Green Island area due to high nitrate levels, although DON may have contributed but was not routinely measured. Onuf (1996) also reported that macroalgae biomass was three times greater near the Arroyo Colorado confluence than other areas in LLM, thus suggesting that nutrient enrichment was occurring stimulating macroalgal growth. With higher phytoplankton or macroalgal density, there is likely a reduction in light intensity for seagrass. Although not quantified in this study, seagrass in the vicinity of the Arroyo Colorado had substantial seagrass epiphyte loads (pers. obs.) which would further depress seagrass growth due to light limitation.

Considering the condition of *Thalassia* at the Green Island site in 2005–2006, the seagrass could already be considered stressed. With tropical storms and hurricanes like Hurricane Alex in 2010, hyposalinity events would further stress *Thalassia* as well as *Syringodium*. As noted above, both species were absent in the 2012 seagrass survey for the middle region of the LLM 2 years after Hurricane Alex.

Nutrient loading with emphasis on nitrogen inputs

As noted above, excessive nutrient loading is not beneficial to LLM seagrass; therefore, characterization of the quantity and quality of nitrogen sources entering the LLM is important. As a low-inflow estuary with a high residence time, nutrient inputs to the LLM are not readily flushed out by tidal exchange. Because nitrogen is the limiting nutrient for portions of the LLM (Whitledge and Stockwell 1995; Kaldy and Dunton 2000), the nitrogen will be utilized by primary producers. Although phytoplankton density may not be excessive, drifting macroalgae can produce substantial mats and wrack that is retained in ion seagrass flats. Deposition of such mats and wrack has been observed to kill seagrass in the lagoon (pers. obs.).

This is to our knowledge the first study to identify DON input to the LLM. The amount of DON entering the LLM system was substantial (34–67% of TDN) during two episodic pulses; however, we could not estimate DON loading rates. Results from Mooney and McClelland (2012) indicated that DON was a major constituent of normal and pulsed inflows for Copano and Mission Bays in south Texas. In that system, DON was in the 20–30 µmol N/L range, similar to the values for the two Brownsville subwatershed drains. Considering that WWTP discharge is one of the main sources of DON in surface water (Sipler and Bronk 2015; Czerwionka 2016), a more complete characterization of DON loading to the LLM is needed since population growth will likely increase WWTP discharges in south Texas, thereby potentially increasing DON inputs to the lagoon.

δ^{15}N values for dissolved nitrate and periphyton in the Arroyo Colorado were elevated (up to $+14^{\circ}/_{oo}$), suggesting that the nitrogen source for the Arroyo Colorado was treated wastewater (Xue et al. 2009). In the LLM East–West transect which started at the Arroyo Colorado confluence, δ^{15}N values of the seagrass *Halodule* and the seaweed *Palisada* were both elevated, starting at $+6^{\circ}/_{oo}$. There was a distinct decline in *Halodule* δ^{15}N values to as low as $-0.3^{\circ}/_{oo}$ as distance increased from the Arroyo Colorado. δ^{15}N values for seston from the two Brownville subwatersheds were also elevated ($+8.0$ and $+8.6^{\circ}/_{oo}$), suggesting that the source of nitrogen in this drainage was treated wastewater discharge and/or septic tanks. Cuddy (2018) over a 5-year period (2011–2015) reported elevated δ^{15}N values ($+6$ to $+10^{\circ}/_{oo}$) in the LLM for the area adjacent to the confluence of the Arroyo Colorado.

Nutrient (esp. nitrate) loading data for the two Brownsville subwatersheds in our study did not seem to reflect the difference in land use despite *circa* 7 times more urban area in the South subwatershed drain than the North subwatershed drain. The North subwatershed produced 25 times higher loading rate of nitrogen than the South; however, the magnitude of rainfall event for the North drain event was 1.5 times that for the South drain event. Although land use correlation with nutrient loading was rather inconclusive in our study, the δ^{15}N data for the Arroyo Colorado was especially consistent with nitrogen input from sewage (including septic tanks) and/or possibly lawn fertilizer from urban runoff. Future land use changes should be expected to alter FWI quality and quantity, due to more impervious land cover and increasing nonpoint source loadings.

Future of LLM seagrass

Nutrient loading will continue to be a stressor for the low inflow Lower Laguna Madre and its seagrass-based ecosystem unless there is a concerted effort to upgrade existing WWTPs, implement green infrastructure practices, and increase regional capacity to keep up with population growth. Controlling FWI discharges by means of inland-constructed wetlands should be considered.

Each time a hyposalinity event occurs that diminishes or eliminates salinity-sensitive seagrass species i.e., *Thalassia* and *Syringodium*, this re-sets typical subtropical seagrass succession (Bare > *Halodule* > *Syringodium* > *Thalassia*) (Robbins and Bell 2000). This situation may favor pioneer seagrass species like *Halodule wrightii*. If the frequency and magnitude of hyposalinity pulses increase over time, this may result in *Halodule* becoming more common in Gulf coast embayments. Loss of *Syringodium* and *Thalassia* due to hyposalinity pulses may also result in destabilization of sediments which could reduce water clarity, thereby limiting the depths at which *Halodule* can grow. The ecological roles of *Halodule*, *Syringodium*, and *Thalassia* should not be assumed to be identical (Horinouchi 2007; Gullstrom et al. 2008). Seagrass changes in LLM have substantial potential to negatively impact estuarine-based fisheries and recreational fishing, major sources of income for south Texas.

Changes in precipitation are a consequence of anthropogenic climate change due to higher atmospheric levels of moisture associated with increasing global temperatures (Trenberth and Fasullo 2012). Although annual precipitation may decline, extreme precipitation events may increase as in Europe (Christensen and Christensen 2004). This scenario is expected to continue (IPCC 2022). For south Texas, extreme precipitation events are problematic since the LLM has long been a hypersaline lagoon with the biota acclimated to high salinity. As noted above, seagrass species differ in tolerance to salinity change (increase or decrease). Species with wider salinity tolerance like *Halodule wrightii* and *Ruppia* could become more common in the future in the lagoon. Such a change has potential implications for seagrass-dependent species in the LLM.

Climate change is likely to result in more frequent and severe precipitation events (IPCC 2014) which will result in more FWI pulses to the LLM. If climate change predictions come true, the impact of episodic extreme FWI events on the biota of the LLM is likely to become unpredictable and deleterious. Combining climate change with population growth (more impermeable surfaces) and an enhanced drainage system for south Texas, freshwater pulses are likely to become more frequent and intense. The synergistic effect of two stressors—salinity change and nutrient loading may continue to exacerbate the decline of LLM seagrass, especially for climax species.

The focus of this article has been on seagrass impacts. However, there has been little attention on assessment of direct and indirect impacts of hyposalinity and nutrient loading stressors on macroalgae and sessile LLM biota like shellfish and benthic invertebrates. Declines of seagrasses, especially the climax species, could have cascading effects for the entire LLM food web.

For other low-inflow estuaries worldwide, climate change should be an issue of concern if precipitation patterns change, resulting in increased estuarine FWI regimes. If humans alter coastal hydrology as in south Texas, predicting how simultaneous changes in precipitation and hydrology will affect these low-inflow estuarine systems will be a challenge.

Acknowledgements Funding was provided via contracts with the Texas Commission on Environmental Quality and the Texas General Land Office Coastal Management Program grant 14–085-000–7953. The Texas Water Development Board contributed equipment and personnel for the project. Special thanks to Tom Eubanks who made significant contributions to the fieldwork.

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