FINAL REPORT

Flora and Fauna Responses to Freshwater Inflows in Galveston Bay

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List of Abbreviations and Acronyms

ACFT	acre feet
BBEST	Basin and Bays Expert Science Team
cfs	cubic feet per sec
chl *a*	chlorophyll *a*
CL	Clear Lake
d	monthly diversions
EB	East Bay
FWI	fresh water inflows
g	daily gaged flow
GBEP	Galveston Bay Estuary Program
GERG	Geochemical and Environmental Research Group
HPLC	High Performance Liquid Chromatography
HB	House Bill
m	modeled daily ungaged flow
MDS	multi-dimensional scaling
NGB	Northern Galveston Bay (also Trinity Bay area)
PSU	practical salinity unit
QAPP	Quality Assurance Protection Plan
QA/QC	Quality Assurance/Quality Control
r	monthly return flows
RD	Trinity River and delta area
RLA	Resource Limitation Assay
SAV	submerged aquatic vegetation
SB	Senate Bill
TAMU	Texas A&M University (College Station)
TAMUG	Texas A&M University at Galveston
TCEQ	Texas Commission on Environmental Quality
TGLO-CMP	Texas General Land Office-Coastal Management Plan
TPWD	Texas Parks and Wildlife Department

TN	Total particulate nitrogen
TNRIS	Texas Natural Resources Information System
TP	Total particulate phosphorus
TWDB	Texas Water Development Board
TxRR	Texas Rainfall – Runoff model, computer program developed by the TWDB and used to simulate ungaged flows discharging directly into Texas bays and estuaries
USGS	United States Geological Service

1. Abstract

The Galveston Bay Estuary Program (GBEP) of the Texas Commission on Environmental Quality (TCEQ) identified an "examination of the impacts of freshwater inflow (FWI) and bay circulation" as priority areas in its comprehensive conservation management action plans. The program's goals are specifically to ensure *beneficial* FWI necessary for salinity, nutrient and sediment loading regimes adequate to maintain productivity of economically important and ecologically characteristic species in Galveston Bay. The major gap in the present knowledge is a clear understanding of the downstream ecological impacts of changes to FWI on estuaries, specifically phytoplankton, *Vallisneria* and *Rangia* communities. The collection of new data for the project spanned a range of inflow conditions into the Galveston Bay estuary between March 2010 and December 2012, with the "exceptional" drought running through 2011. Whilst we found that reduced FWI lowered both sediment and nutrient loading, salinities progressively increased throughout the Bay until spring 2012. Consequences to the phytoplankton were three fold. We observed changes in the community composition but not particularly to the overall biomass. We also found they were nitrogen limited in the bioassays performed throughout the Bay regardless of season or the amount of FWI. Phytoplankton communities experienced a decline in taxonomic diversity during the drought which was driven by a change in salinity. *Vallisneria americana* (wild celery) was completely absent from the Bay during the entire study period. The paucity of historical data makes it impossible to know if there was a significant decline as a result of the drought conditions or if its absence was due to other factors. For example, being out competed by another species such as *Ruppia* whose meadows we found or perhaps some abiotic factor yet to be identified. *Rangia cuneata* (Atlantic *Rangia*) responses to FWI were examined on two time scales. Long term we observed a significant decline in the number and biomass of *Rangia* across all of Galveston Bay. We hypothesize that the decrease in clam populations since the 1980s may be due to a concurrent decline in food (chlorophyll *a*) as a result of nutrient decreases since the 1970s and the introduction of the Clean Water Act. Further studies are needed to test this hypothesis. On shorter time scales (years), we found shell length, parasite load and ratios of males:females was salinity dependent suggesting freshwater inflows are important to the health of these freshwater clams. A comparison of TxBLEND and Dataflow generated salinity maps suggests that the modeling approach provides a good estimation of the spatial and temporal variability in salinity. The ongoing challenge is to understand the linkages

between the magnitude of FWI and the flora and fauna in Galveston Bay. Long term data sets are required in order to distinguish between the effects of short term extremes (annual drought) versus long term natural oscillations in this ecosystem.

2. Introduction

Galveston Bay, the second largest estuary in the Gulf of Mexico, is home to more than 4 million people and a billion dollar commercial and recreational fishery. Water quality will be mapped on fine spatial and temporal scales. We will examine phytoplankton responses to perturbations in nutrient loads from FWI and to different ratios of nutrients (particularly nitrate and ammonium) to stimulate the impact of returned flows (from industry and sewage treatment). The presence of algal (harmful) blooms will also be monitored; the occurrence of which could be exacerbated by shifts in nutrient loading patterns. The scientific data collected will be used to develop an understanding of nutrient cycling dynamics (sources, fate, transport, effects) from rivers into estuaries and between nutrients and ecosystem response(s). Long term, the project outputs will support establishment of appropriate and protective nutrient criteria for the Galveston Bay estuary, which can be considered a representative system for modeling efforts. In this way, best management approaches to sustaining 'beneficial flows' in this and other systems in the Gulf of Mexico can be developed. Throughout the project period there will be close coordination with the Governors' Gulf of Mexico Alliance Nutrient Reduction Team.

2.1 Background to the issues

The GBEP of the TCEQ is charged with implementing the Galveston Bay Plan *(The Plan)*, a comprehensive conservation management plan for Galveston Bay. Balancing human needs for water and the FWI necessary for Galveston Bay is a key element of *The Plan.* In support of this Plan element, this project will assess the seasonality, frequency and magnitude of inflows required to maintain the existing ecological structure and integrity of the Bay to assist resource management decisions regarding inflow regime needs of the Bay.

Created by the 80th Texas Legislature, 2007, in recognition of the importance that the ecological soundness of our riverine, bay, and estuary systems and riparian lands has on the economy, health, and well-being of our state, House Bill (HB) 3 and Senate Bill (SB) 3, require the TCEQ to adopt by rule appropriate environmental flow standards for each river basin and bay system in the state. Senate Bill 3 begins the implementation of the state's 50-year water plan. Details of the process can be found at:

http://www.tceq.state.tx.us/permitting/water_supply/water_rights/eflows/group.html. SB 3 empowers the TCEQ to set aside freshwater to inflow into the state's bays and estuaries in an effort to maintain the health of inter-coastal waterways. The science behind this flow management is being developed by a Texas Environmental Flows Science Advisory Committee, made up by hydrologists and other earth-scientists who advise TCEQ on the best way to ensure the viability of bays and estuaries. This plan would be suspended in the event of a natural emergency, like a drought, where water resources would be diverted to help human services. The Texas Water Development Board (TWDB) would be directed to create a state-wide conservation awareness program under SB 3. The Project Investigator (Quigg) for this project served on the Trinity-San Jacinto Basin and Galveston Bay Basin and Bays Expert Science Team (BBEST) and the GBEP's Monitoring and Research Subcommittee.

Flora and fauna - some of which have been identified by the BBEST members making FWI recommendations for Galveston Bay - known to respond to FWI in Galveston Bay will be investigated to characterize their viability as bio-indicators. Specifically, the BBEST identified *Vallisneria americana* (Wild Celery) and *Rangia cuneata* (Atlantic *Rangia*) responses as being important to understanding the role of FWI in maintaining the health of Galveston Bay. For details on the committee's findings with respect to these bio-indicators, plus additional species which were identified, refer to Espey et al. (2009).

2.2 Background on Galveston Bay

Galveston Bay (Fig. 1) is also the most productive of all Texas' estuaries with an oyster production that is unsurpassed in the U.S. (ca. 1800 metric tons with a value of $8 million), a commercial fishery industry that is one third of the state's commercial fishing income (Galveston Bay contributed ca. $99 million from 1994-1998), and a recreational fishery that made a gross direct contribution to the local economy of $171.5 million in 1986 (GBEP 2001; Lester and Gonzalez 2002; Pinckney 2006; TWDB 2007). Galveston Bay is home to important recreational and commercial fisheries consisting of oysters (2 species), shrimp (13 species), crab (17 species) and fish (over 150 finfish species; Lester and Gonzalez 2002). Nonetheless, the Galveston Bay watershed is the focus of conservation issues due to the high density industrialization and urbanization which starts in the Dallas-Fort Worth area and extends to the Gulf of Mexico. The value of Galveston Bay was recognized in the early 1980s with the establishment of an EPA national estuary program for this watershed (one of only 28 in the U.S.). The GBEP (www.gbep.state.tx.us) is a non-regulatory program administered by the TCEQ whose mission is to preserve Galveston Bay for generations to come**.**

Changes in the characteristic hydrological and physio-chemical nature of estuaries worldwide are occurring as a result of increased nutrient inputs (e.g., wastewater treatment facilities, anthropogenic inputs) associated with urbanization and industrialization, alterations in the magnitude and frequency of FWI, changes in water circulation patterns (e.g., dredging programs for ship channels) and other human-induced changes including but not limited to tourism. Of these, the most frequently investigated phenomena are eutrophication (Howarth 1988; Howarth and Marino 2006) and harmful algal blooms (Granéli and Turner 2006*)*, which may lead to fish kills (Thronson and Quigg 2008; McInnes and Quigg 2010) and the loss of other fauna, flora, and/or habitats (e.g., mangroves - Phillips and Kevekordes 2008; seagrasses - Quigg et al. 2008). Reduced water quality in the Galveston Bay estuary in Texas is no exception. Changing land use patterns, largely driven by rapid coastal development, has increased pressure to develop management strategies to reduce nutrient loads and protect marine flora, fauna and habitats whilst providing for human activities. To achieve this we need to further characterize how Galveston Bay and other estuaries along the Gulf of Mexico respond to environmental perturbations driven by nutrient loading.

In Texas, studies have shown that changes in FWI affect productivity of juvenile brown shrimp, macrophyte productivity, root:shoot ratios and species diversity, and benthic macrofaunal and meiofaunal densities and diversity (Montagna and Kalke 1992; Dunton et al. 1995; Heilman et al. 1999; Riera et al. 2000; Ward et al. 2002). Coastal wetland loss in Louisiana has also been attributed to a reduction in sediment loading as a result of freshwater diversion (Boesch et al. 1984). The magnitude of flushing and nutrient loading, mode of nutrient loading, and ratios of potentially limiting nutrients within the load (Malone et al. 1988; Chan and Hamilton 2001) are additional factors important to a productive bay ecosystem. Observing and assessing how the present Galveston Bay ecosystem responds to nutrient and sediment loading from FWI can provide a basis for better understanding potential impacts of future water management strategies affecting FWI to the Bay.

Figure 1 *Galveston Bay sampling campaign map. Water quality parameters were examined along a tightly gridded transect shown by the black line. The northern part of the Bay would typically take a day to complete, and the southern part a second day. Six fixed stations were sampled in order to check the calibration on the Dataflow. Ancillary measurements were also collected at stations in red as part of this project.*

While there is consensus among the scientific community that FWI are needed to maintain the unique biological communities and ecosystems characteristic of a “healthy” estuary (Longley 1994; Nixon 1995), there are varying opinions regarding the appropriate delivery of these flows. However, by combining findings of multi-year studies, and multi-agencies, patterns and/or trends can be identified and relationships between observations can be defined. Patterns which are seasonal can be de-convoluted from those that are responses to changes in the magnitude and duration of a FWI event.

2.2.1 Phytoplankton responses

At the base of the food web, phytoplankton are a sensitive bio-indicator of the state of the Bay. The appearance of new and/or harmful phytoplankton species has been used to identify potential environmental stressors in places such as the Neuse River Estuary (Paerl et al. 2003), Chesapeake Bay (Lane et al. 2001) and the Canadian Rocky Mountains and ponds (Vinebrooke and Leavitt 1999). Recent studies by Texas A&M researchers have shown that the phytoplankton community in Galveston Bay is either N-limited and/or N and P co-limited (Quigg et al. 2007, 2009a). There is also a spatial and temporal variability in phytoplankton community composition (Quigg et al. 2009b) which is related to nutrient dynamics (Quigg et al. 2009a) and FWI (Quigg et al. 2007, 2009a).

2.2.2 *Vallisneria americana* (Wild Celery) responses

Vallisneria plants are localized in the Trinity River basin of Galveston Bay as germination and establishment of plants (spring) requires salinities of < 5 ppt while adult plants tolerate up to 10 ppt (Espey et al. 2009). Germination and establishment of these plants can occur in 30 days, and survival is diminished by unsuitable salinities of duration longer than 30 days. The BBEST determined that the salinity range for germination should be met for one month in the spring and the salinity range for survival should be met for 2 of 3 months in summer and fall (Espey et al. 2009).

Potter and Lovett-Dousti (2001) found that leaf-to-root surface area ratios in *Vallisneria* provide a simple and inexpensive relative measure of overall site quality in stressed aquatic ecosystems. In the review of Touchette (2007), parameters such as chlorophyll content and photosynthetic rates were diminished in plants responding to salinity stress. The uptake of nutrients is thought to be strongly influenced by salinity in submerged aquatic vegetation such as *Vallisneria* but while our basic understanding of how these plants survive in saline environments is increasing, it still lags well behind marine algae and terrestrial halophytes (Touchette 2007). Hence, it is unknown what the interactive effects of salinity and nutrients will be on *Vallisneria.*

2.2.3 *Rangia cuneata* (Atlantic *Rangia*) responses

BBEST (Espey et al. 2009) proposed that *Rangia* clams (*Rangia cuneata*) would be a good bio-indicator species of FWI into Galveston Bay because they can live only within a narrow range of salinities (0 to 18 ppt) and further, can only spawn within an even narrower range of salinities (2 to 10 ppt). While not discussed by the BBEST, it is also known that spawning is initiated by a rapid increase or decrease in salinity. Fertilization occurs in the water column and larvae become shelled within 24 hours after fertilization. Most larvae settle on the bottom between September and March and a second settling can occur in midsummer. How the juveniles disperse is uncertain but it is known that the adult clams rarely move. The specific details of the reproductive cycle and environmental conditions necessary for spawning are still not well known. The life span of the brackish water clam has not been confirmed but its average life span is thought to be between 4 and 5 years with a maximum of 15 years (Anderson and Bedford 1973; Hopkins et al. 1973; Dauer 1993; http://www.dnr.state.md.us/bay/cblife/).

Texas Parks and Wildlife Department (TPWD) has examined *Rangia* distributions in Galveston Bay from July 1983 to December 2009. The data were summarized by the BBEST. Greatest *Rangia* counts are associated with the area closest to the mouth of the Trinity River (Fig. 2). In some cases *Rangia* were found in unexpected locations (West Bay and East Bay) but this requires further examination of the data for *Rangia* as well as the water quality data in these basins. Knowing the location and distribution of *Rangia* clam beds in Galveston Bay provides important information on the influence of salinity on the presence (and absence) of *Rangia* clams and the subsequent use of this species as a potential bio-indicator.

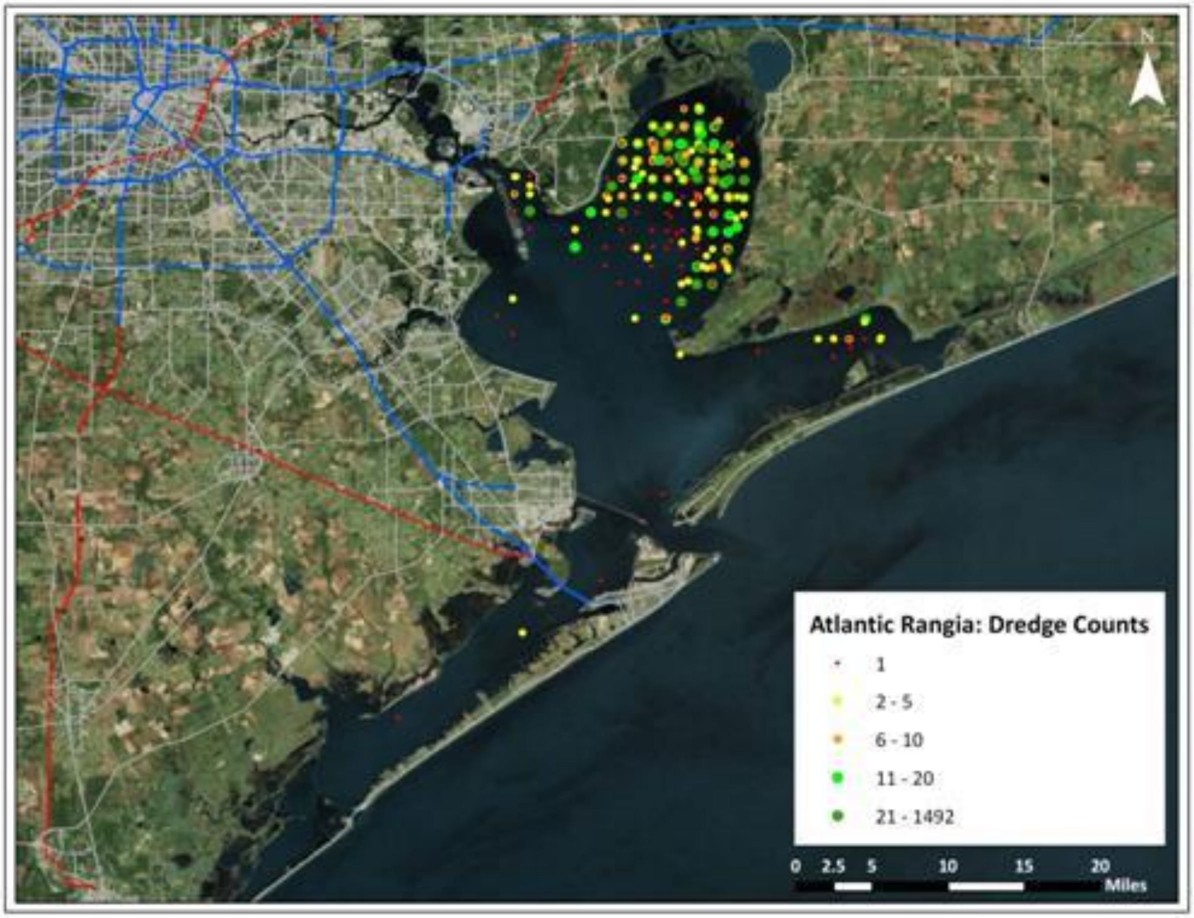


Figure 2
Map of the collections of Atlantic *Rangia* by TPWD in dredge samples and the abundance of clams in each collection. (Figure 30 from Espey et al. 2009).

Additionally, *Vallisneria* - whose distributions are intimately associated with Trinity River flows - was identified as a potential bio-indicator for purposes of developing flow recommendations (Espey et al. 2009). However, there are seasonal variations in salinity niche conditions. Work conducted on this project will provide additional data to better characterize the utility of *Vallisneria* and *Rangia* as bio-indicators for assessing the amount of FWI required for Galveston Bay.

2.2.4 TxBLEND – a two-dimensional hydrodynamic model

The TxBLEND two-dimensional hydrodynamic model was developed for Galveston Bay by the TWDB to simulate water circulation and salinity condition within the bays. (TWDB 1992; Fig. 3). The challenges of accurately modeling salinity transport within estuarine systems using a 2D model are well documented (Monismith 2005; Ward 1993). The least tractable of these is the inherent inability of 2D models to describe physical processes which occur in three dimensions. With respect to salinity this issue is manifest in the vertical effect of density currents of hydrodynamics. Salinity is both driven by and a driver of hydrodynamics. To address this issue the TxBLEND model includes a dispersion term in the salinity balance equation. Since this dispersion term cannot be measured directly, it effectively becomes a calibration term and "at best, such a model should be regarded as a means for extrapolating salinity beyond the configuration used for calibration." (Ward in Jensen et al. 1993). Important parameters and features of the model are explained in Table 1.

When the Trinity-San Jacinto Basin and Galveston Bay BBEST began to develop their recommendations, the TxBLEND model for Galveston Bay had been calibrated but not validated. It was calibrated for both hydrodynamic and salinity transport performance by using water velocity and surface elevation data from intensive field studies and long-term time-series salinity data for period from 1987-1996 by adjusting parameters such as the dispersion coefficient and Manning's n efforts to improve model performance. In a model validation exercise, a model is executed using a dataset that is independent from the set for which it was calibrated. Model performance is determined by comparing model predictions with observed values without making any adjustments to model calibration parameters. A draft report on the model validation was produced for the Galveston Bay model by the TWDB in 2009. To produce this report, a model run was conducted to simulate salinities for the period 1997-2005. These salinity values were then compared to observed salinities obtained from the TWDB Data sonde Program for four monitoring sites: Bolivar Roads, Redbluff, Dollar Point and Trinity Bay (Fig. 4).

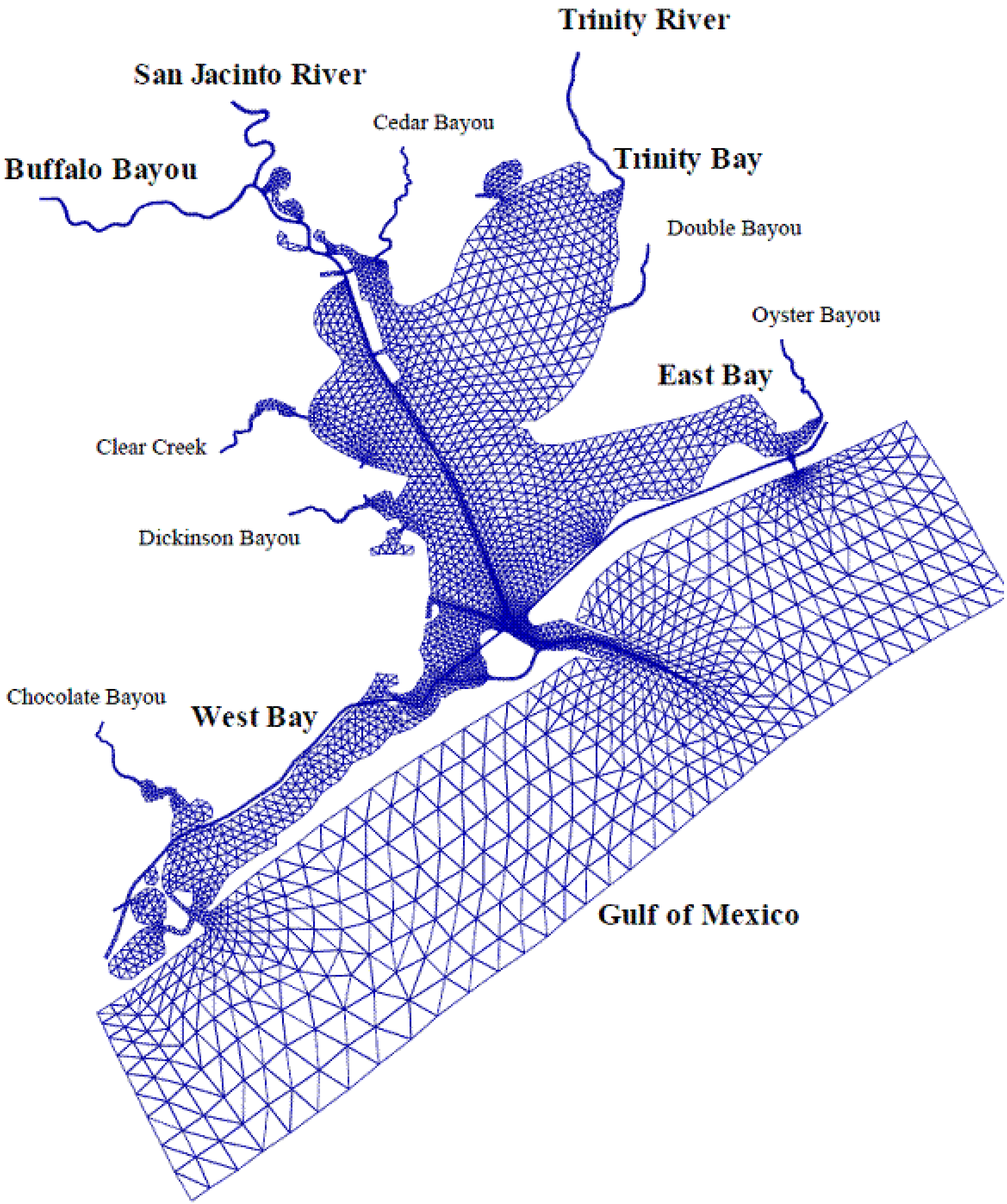


Figure 3 Computational grid for the Galveston Bay TxBLEND model.

Table 1. Description of TxBLEND model parameters, features and inputs (from Guthrie 2012).

Feature	*Description*
Generalized Wave Continuity Equation (GWCE)	A special form of the continuity equation designed to avoid spurious oscillation encountered when solving the primitive continuity equation using the finite element method. Solved by an implicit scheme prior to solving the momentum equation. The GWCE is an established equation used to solve mass-balance or flow continuity in 2-D finite element hydrodynamic models (Kinnmark and Gray 1984).
Momentum Equation	2-D, Depth Integrated Momentum Equation is solved for most applications. Non-linear terms are neglected most of the time.
Advection-Diffusion Equation	Used to calculate salinity transport.
BigG	A parameter in the generalized wave continuity equation. Larger values of BigG reduce mass balance errors by increasing the enforcement of the continuity equation at the price of increased numerical difficulty (TWDB 1999). Typically, set at 0.01 – 0.05.
Manning's n Roughness Coefficient	Used to represent bottom friction stress. For TxBLEND, 0.015 to 0.02 is a reasonable default value, but can be increased to 0.03 or higher for a seabed with thick grasses or debris or lowered to 0.01 or less to represent a smooth bay bottom.
Turbulent Diffusion Term	A diffusion factor, representing horizontal diffusion, used to diffuse momentum as a result of the non-linear term in the momentum equation.
Boundary Conditions	Three types of boundaries form the edge of the model domain. (1) River Boundary – portion of river entering the bay; (2) Tidal Boundary – the limited portion of Gulf of Mexico included where salinity and tidal boundary conditions are set; and (3) Shoreline Boundary – enclosing boundary of the bay.
Wind Stress	Used to impose the effect of wind on circulation.
Dispersion Coefficient	Uses a modified version of the dispersion constant (DIFCON) that can be varied depending on expectations for mixing rates and to better simulate salinity conditions. Due to variable velocities, the dispersion coefficient is updated in 30-minute intervals during simulation. For most applications, constant dispersion coefficients are used.
Coriolis Term	Used to impose the Coriolis Effect on the hydrodynamics
Tide Data	Water surface elevations at the ocean boundary are specified by input tides.
River Inflow Data	Daily river inflows are introduced at identified inflow points. The data are obtained from TWDB Coastal Hydrology estimates based on gaged and ungaged inflows.
Meteorological Data	Includes evaporation, precipitation, wind speed, and wind direction. Wind data may be input as daily average, 3-hour average, or as hourly data. Evaporation data is used to reflect the effect of evaporation on salinity (Masch 1971). Evaporation rate is a modification of the Harbeck equation to estimate daily evaporation from estuaries developed by Brandes and Masch (1972). Precipitation is input as daily values.

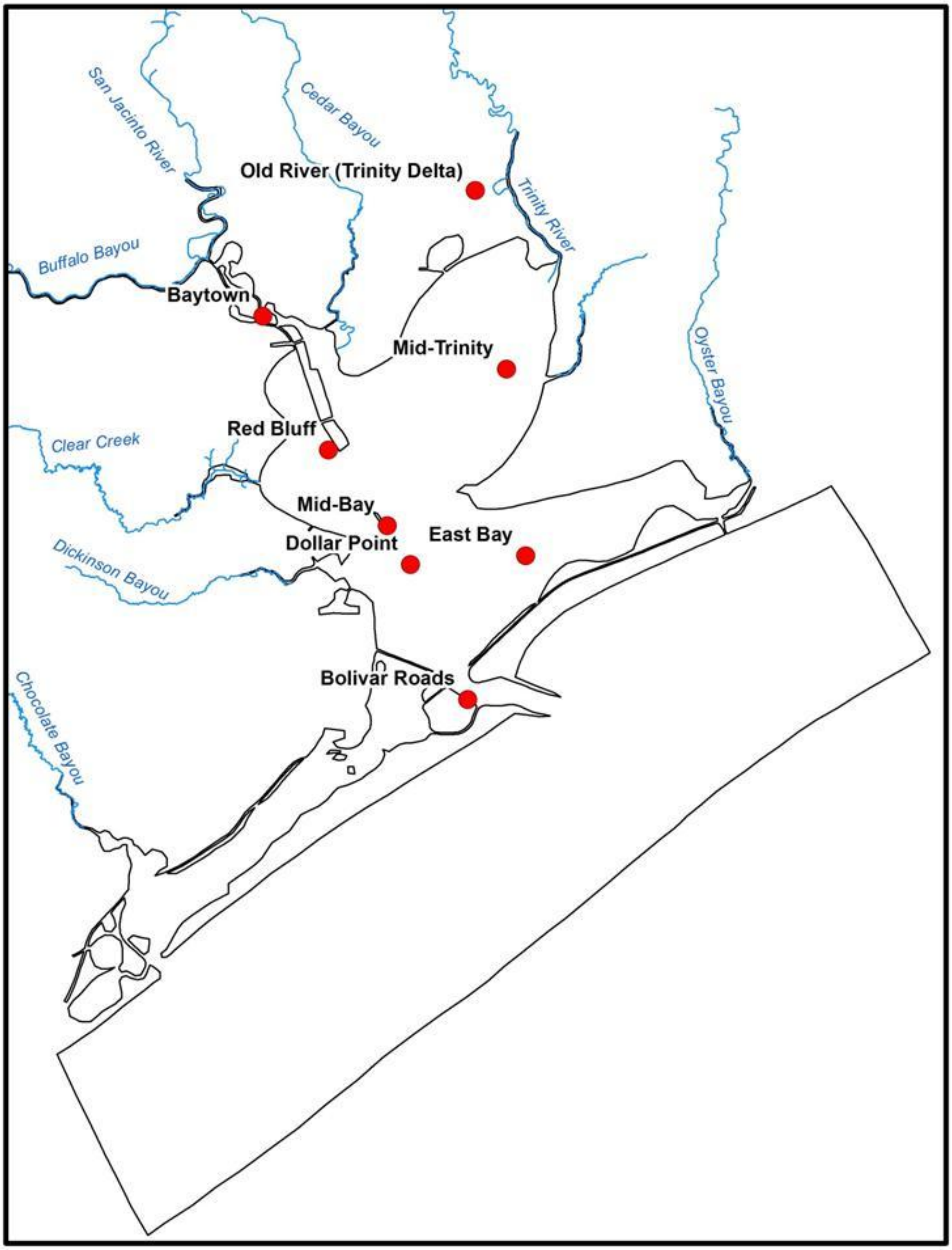


Figure 4 TWDB Datasonde sites (long-term water quality data collection sites) in Galveston Bay.

Since the completion of the Trinity-San Jacinto Basin and Galveston Bay BBEST report, other BBESTs have employed the TxBLEND model to conduct salinity zonation analysis similar to that produced for Galveston Bay. For each of these the TWDB has produced more formal calibration and validation reports specific to these other estuaries. These reports include figures of time series and of scatter plots comparing observed versus predicted salinities, and table of summary statistics for comparisons of simulated to observed salinities. Statistical analysis included Pearson product moment correlation coefficient (r^2), root mean square error and the Nash-Sutcliffe Efficiency Criterion. The TWDB validation reports have generally concluded that "TxBLEND captures major salinity trends in the system reasonably, but high frequency fluctuations are more difficult to simulate." (TWDB 2010) This suggests that while model may not be suitable for tracking hourly or daily fluctuations, it is useful for course time steps (monthly) changes in salinity over a broad area across the Bay.

The model validation approach employed by TWDB in the other estuaries and begun in Galveston Bay as part of the BBEST process, was replicated and updated based on the input data currently available for the Galveston TxBLEND model. Long term salinity monitoring data is available for eight sites in this study area (Table 2).

Table 2 TWDB datasondes in the Trinity San Jacinto Estuary.

Site Name	Description	Start	End	Years
Old River (Trinity Delta)	Trinity River Delta at I10 Bridge	3/2/1997	11/29/2012	15.8
Mid-Trinity	Trinity Bay NW Double Bayou Channel	12/17/1986	11/29/2012	26.0
Baytown	Houston Ship Channel at SH146, Baytown	4/18/2001	11/29/2012	11.6
Red Bluff	Upper Galveston Bay @range marker near HSC 71/72	5/14/1990	5/5/1999	9.0
Mid-Bay	Mid Galveston Bay near Red54 @range marker	2/8/2001	12/6/2012	11.8
Dollar Point	Galveston Bay off Dollar Point @range marker	1/30/1987	9/14/2000	13.6
East Bay	East Bay at Hannah Reef exp oyster platform	5/16/1990	7/31/1996	6.2
Bolivar Roads	Houston Ship Channel, @Pelican Is	5/15/1990	12/6/2012	22.6

For each of the series with long term salinity monitoring data the validation report includes:

(i) Time series plots of simulated versus observed salinity

(ii) Scatter plots of simulated versus observed salinity

(iii) Tables of summary statistics including

a. Pearson product moment correlation coefficient (r^2),
b. Root mean square error and
c. Nash-Sutcliffe Efficiency Criterion

In this study, we determined that specific areas of the Bay, flow ranges or seasonal responses are of significant importance and it may be possible to refine the calibration of the TxBLEND model to focus on these particular issues. In such a case the procedures documented in the TWDB calibration and validation reports will be used to recalibrate and validate the model.

2.3 Project/Task Description

With funding from GBEP, Sea Grant, TGLO-CMP, TWDB and other programs, we have collected four years (2005-2006 and 2008-2009) of water quality, nutrient and plankton samples. Recently, we have secured additional funds from the EPA and TWDB to continue collecting such data through to the end of 2012 (but both these programs only provide annual funds and provide no guarantee of the long term (3 years) support). The focus of the current program will be to complement the EPA program and its goals by collecting complementary data on phytoplankton responses to FWI as indicators of estuarine health. Further, the additional funding provided by the current program will also allow the needs of the GBEP initiatives to be addressed.

2.4 Project objectives, tasks, and schedule of deliverables

Project objectives, tasks, and schedule of deliverables described are:

(i) Examine the phytoplankton samples collected in 2005-2006 and 2008-2012. These will be identified to genera level, enumerated and the biovolume calculated. A digital photo library will also be established and made available on the PI's website,

(ii) High spatial and temporal resolution mapping of Galveston Bay water quality parameters from March 2010 to December 2012,

(iii) Define influence of nutrient and sediment load on the phytoplankton in Galveston Bay from March 2010 to December 2012,

(iv) Determine the distribution of *Vallisneria* plants in relation to salinity gradients in Galveston Bay from March 2010 to December 2012,

(v) Determine the distribution of *Rangia* clams in relation to salinity gradients in Galveston Bay from March 2010 to December 2012, and

(vi) Using the data collected, develop a better understanding of the use of these flora and fauna as biological indicators of the effects of freshwater inflows in Galveston Bay, specifically by looking at the role of salinity.

2.5 Roles and Responsibilities of Key Personnel and Organizations

Key personnel and organizations are listed below. The reporting structure is outlined in Fig. 5 below.

Texas A&M University (TAMU) is a land-grant, sea-grant and space-grant institution located in College Station, TX. TAMU at Galveston (TAMUG) is a branch campus, located on the Gulf of Mexico, specializing in marine related studies (biology, science, engineering, administration). TAMU is dedicated to the discovery, development, communication, and application of knowledge in a wide range of academic and professional fields. Its mission of providing the highest quality undergraduate and graduate programs is inseparable from its mission of developing new understandings through research and creativity.

Dr. Antonietta Quigg (TAMUG Project coordinator/principal investigator) (herein referred to as Project Manager) worked with the sponsor to ensure that the technical quality requirements were met in accordance with contract and grant specifications. The TAMUG Project Manager determined the priorities for the data collection and analysis for the project, and oversaw the work of the TAMUG Research Team. The TAMUG Project Manager drafted work plans, wrote quarterly progress reports and communicated with the GBEP Project Manager. She worked to ensure the project was accomplished on schedule, prepared grant reporting documents and coordinated technical reviews. She supervised personnel directly involved with this study. The TAMUG Project Manager oversaw the final project report deliverables.

Dr. Anja Schulze from TAMUG oversaw the *Rangia* studies with support from the staff at Texas Parks and Wildlife (Dickinson Office). The TAMUG co-PI (Schulze) worked with the Project Manager to determine priorities for data collection, analysis and the activities of the Research Team. This co-PI contributed to the development of the draft work plans and quarterly progress reports, and worked with the TAMUG Project Manager in the development of the final project report deliverables. This co-PI assisted the Project Quality Assurance/Quality Control (QA/QC) Officer in performing routine QA/QC checks of data and staff supervision.

Mrs. Tyra Booe (TAMUG Project QA/QC Officer) worked directly with the Project Manager. The TAMUG Project QA/QC Officer is responsible for implementing the quality system as defined by the contract and in the Quality Assurance Protection Plan (QAPP). The TAMUG Project QA/QC Officer worked with the Project Manager to write, maintain, and distribute the QAPP and ensure the quality of data submitted to GBEP. The TAMUG Project QA/QC Officer was responsible for maintaining records of the QAPP distribution, including appendices and amendments. The TAMUG Project QA/QC Officer provided oversight of sampling events, collected samples, the chain of custody for samples, sample analysis, and data validation through systematic and routine paper and field audits. The TAMUG Project QA/QC Officer was responsible for compiling audit reviews, findings, and corrective actions taken for submission in reports to the TCEQ.

Ms. Rachel Windham (TAMUG Phytoplankton Analyst) worked under the supervision of the TAMUG Project Manager in the collection and analysis of plankton data collected for the project. In particular, she was responsible for training students in the PI's laboratories in these techniques. The TAMUG Phytoplankton Analyst followed QA procedures in addition to providing oversight of all sampling events, chain of custody, sample analysis, and data validation through systematic and routine paper and field audits.

Mr. Lance Robinson (TPWD Coastal Fisheries) allocated staff and resources so that the *Vallisneria americana* (Wild Celery) and *Rangia cuneata* (Atlantic *Rangia*) studies could be conducted. He and his staff provided expertise from previous years conducting similar such activities, boat time, and other kinds of hands on assistance. In addition, Mr. Robinson and his staff provided information from the TPWD archives which was used to develop an appropriate sampling strategy. These data were also used to provide context to the current study.

Mr. Joe Trungale (Trungale Engineering & Science) was contracted to help develop a salinity model and if possible, one for chlorophyll *a* (this was not performed). For the salinity model, he was responsible for producing isohaline maps with TxBLEND for period of record. The TxBLEND predictions were then compared to observed salinities measured at fixed stations in Galveston Bay (TWDB, TPWD, etc...) and potentially adjusting TxBLEND outputs to account for discrepancies.

The Galveston Bay Estuary Program (GBEP), a program of the Texas Commission on Environmental Quality (TCEQ), is comprised of an advisory group which provides a link between scientists, regulators and the general public.

Mr. Steven Johnston (GBEP Project Manager) is the regional Monitoring and Research Coordinator functioned as Project Manager for the Loadings, Water Quality Mapping and Phytoplankton Project, which fell under the Regional Monitoring and Research program areas. The term Project Manager was used interchangeably with "TCEQ Project Representative", "GBEP Project Representative" and "Contract Manager". The Project Manager was responsible for:

- Maintaining necessary lines of communication and good working relationships between lead division staff, personnel of other divisions and organizations participating in a project;
- Ensuring the lead division administrative services coordinator or grant budget coordinator, and the TCEQ federal funds coordinator were informed of changes, revisions, or additions to the project;
- Elevating problems and issues requiring resolution to the Division Director or designee(s) for disposition, when appropriate; assist in preparing contracts and intergovernmental agreements;
- Reviewing the contractor's performance, including quality and timeliness of deliverables, reasonableness of expenditures, progress on meeting objectives/goals of the contract and enforce corrective action measures to assist contractors in meeting deadlines and scheduled commitments.

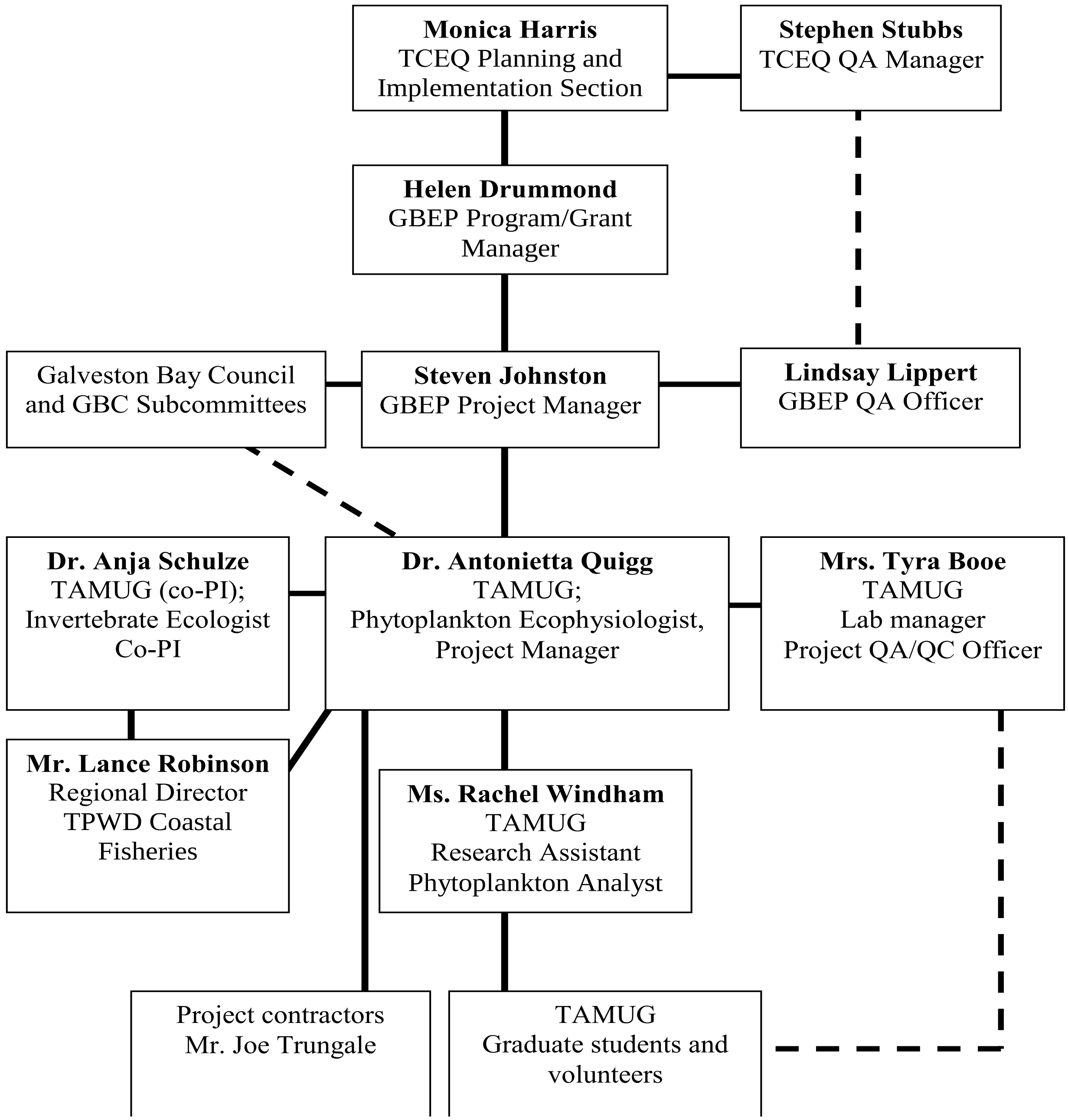


Figure 5 *Organizational chart of project personnel.*

3. Methods

Detailed procedures for each of the methods used can be found in the QAPP associated with this project – this is available from either one of the Project Managers or the Project QA/QC Officer. The QAPP includes standard operation procedures for each of the major procedures including phytoplankton identification, enumeration and biovolume calculations (Appendix A), the Dataflow (Appendix B), the collection and assessment of *Vallisneria* plants and related physical parameters in Galveston Bay, Texas (Appendix C), the collection and assessment of *Rangia* clams and related physical parameters in Galveston Bay, Texas (Appendix D), nutrient analysis (Appendix E), the HPLC METHOD - Technical Description and QA/QC Protocol (Appendix F). Below we include a summary of the methods used to address each of the objectives.

3.1 Objective 1:

Phytoplankton collection, identification, enumeration and biovolume calculation

Samples were collected at six fixed stations in Galveston Bay from 2005 to 2006 and from Jan 2008 to March 2010 from stations in Table 3 and from April 2010 to December 2012 at stations in Table 4. These were analyzed to identify, enumerate and calculate phytoplankton biovolume. Phytoplankton collection involved towing a 67 μm net in the water for no less than five minutes. This was used to concentrate plankton into a 50 mL sample which is preserved in an acid cleaned HDPE rectangular bottle (125 mL; Nalgene) using Glutaraldehyde (final 5%). Total phytoplankton community composition was assayed by collecting 100 mL water from each station, and storing it in an acid cleaned HDPE rectangular bottle (125 mL; Nalgene) containing a 25% Glutaraldehyde (15 mL) solution.

Samples will be examined microscopically for identification to genera level, and species when possible, with the assistance of Tomas (1997). Digital photographs of representatives of each species were recorded along with the magnification, sizes and any other distinguishing detail (Fig. 6). A digital photo library is available from the Project Manager or by going to the project website:

http://www.tamug.edu/phytoplankton/Research/Galveston%20Bay%20Phytoplankton.html.

Utermöhl Chambers (Utermöhl 1958) were employed for enumeration of plankton according to the protocol suggested by Wetzel and Likens (1991). A small volume of the 'total' phytoplankton sample was allowed to settle overnight (no less than 24 hrs). All the cells in the field of view and/or at least 200 cells in each sample were counted in order to obtain a representative cross section of the sample community (Fig. 6). We used descriptions in Table 5 to determine cell dimensions and ultimately biovolumes. The detailed protocol is included in the QAPP (Appendix A).

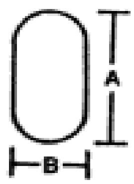
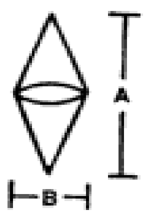
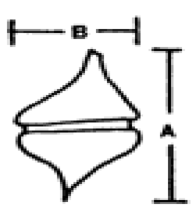
Table 3 Latitude and longitude of fixed sampling stations in Galveston Bay (see Fig.1) from which discrete samples were collected from 2005 to 2006 and from January 2008 to February 2010. *The map number corresponds to numbers in Fig. 1. Bold numbers indicate at which samples for phytoplankton identification, enumeration and biovolume calculations have been completed and will be included in this report.

Station	**Map number**	**Latitude**	**Longitude**
1	**29**	29°21.18'	94°45.18'
2	27	29°18.38'	94°52.11'
3	34	29°32.28'	94°34.44'
4	**6**	29°30.56'	94°51.35'
5	4	29°36.39'	94°55.48'
6	**12**	29°42.9'	94°44.29'

Table 4 Latitude and longitude of fixed sampling stations in Galveston Bay (see Fig.1) from which discrete samples were collected from March 2010 to December 2012. *The map number corresponds to numbers in Fig. 1. Bold numbers indicate at which samples for phytoplankton identification, enumeration and biovolume calculations haven been completed and will be included in this report.

Station	**Map number**	**Latitude**	**Longitude**
1	**12**	29°42.9'	94°44.29'
2	**6**	29°30.56'	94°51.35'
3	4	29°36.39'	94°55.48'
4	17	29°36.59'	94°49.44'
5	25	29°24.17'	94°52.7'
6	**29**	29°21.18'	94°45.18'

Table 5 In order to calculate the volume for each of the phytoplankton cells, the following measurements and formula were used (from Wetzel and Likens 1991). Formulas used assume a basic kind of cell shape; we used the closest corresponding formula for each genera.

Shape	Diagram	Formula	Representative species
Sphere		$\pi A^3/6$	*Sphaerocystis schroeteri*
Ellipsoid		$\pi AB^2/6$	*Scenedesmus bijuga* *Crypomonas* *Euglena*
Rod		$\pi AB^2/4$	*Melosira granulata* *Cyclotella* *Asterionella*
Two cones		$\pi AB^2/12$	*Ankistrodesmus falcatus*
One cone		$\pi AB^2/12$	(Horn of *Ceratium*)
Ellipsoid/cone	[1/2 EL + CN] 	$\frac{\pi B^2(A+B/2)}{12}$	*Rhodomonas minuta* *Gymnodinium helveticum* *Mallomonas* *Synura*
Irregular		$\pi AB^2/9$	*Peridinium*

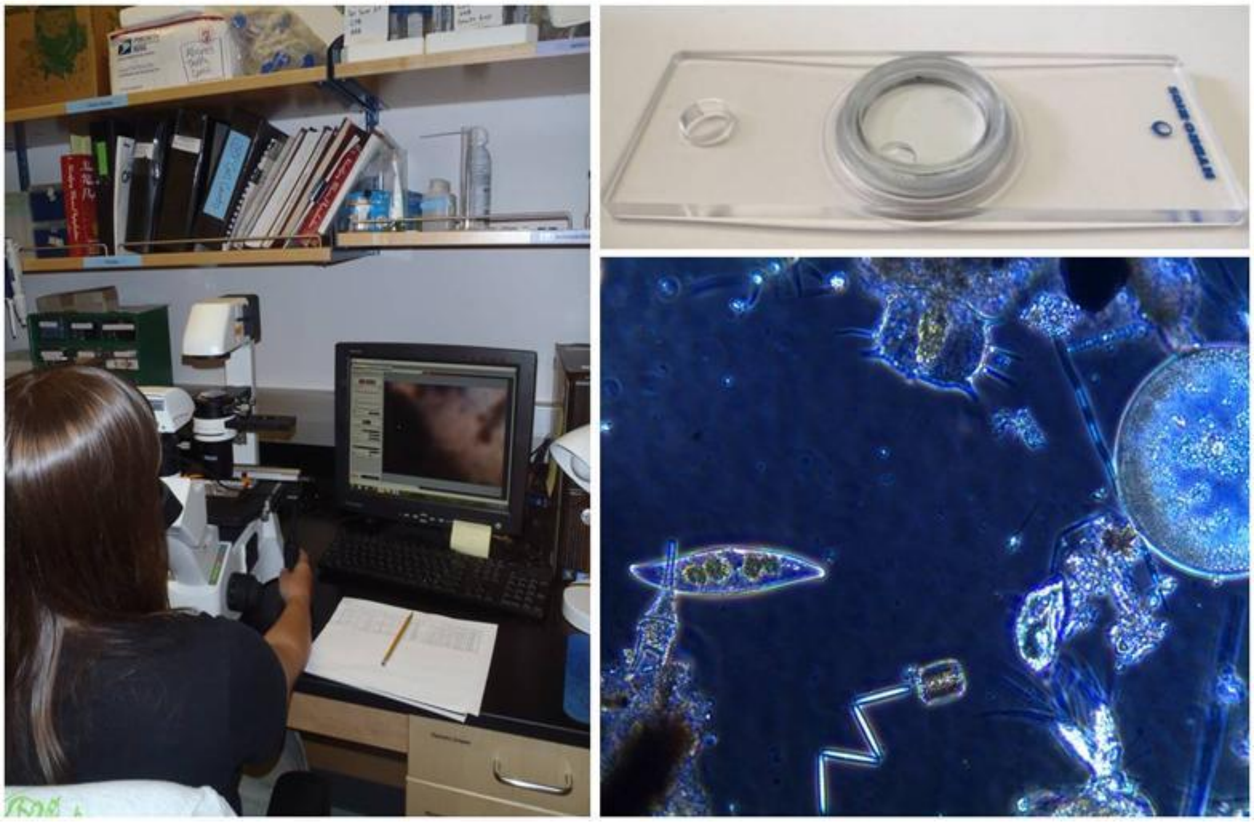


Figure 6 An inverted microscope in conjunction with photography software is used for cell identification, enumeration and biovolume calculations. The Utermöhl chamber is used to hold a known volume of sample for enumeration and biovolume calculations. The most common cells in the samples are typically dinoflagellates and diatoms.

3.2 Objective 2: High spatial and temporal resolution mapping of Galveston Bay water quality parameters

Water quality was measured twelve times per year with a *Dataflow*: a high-speed, flow-through measurement apparatus developed for mapping physico-chemical parameters in shallow aquatic systems (Madden and Day 1992) from a boat, running tight transects across Galveston Bay (see transect line in Fig. 1) between March 2010 and December 2012. Water quality measurements were taken at 4-sec intervals (every 2–8 m depending on boat speed) from about 10 cm below the surface. An integrated GPS was used to simultaneously plot sample positions, allowing geo-referencing of all measurements for each variable. This integrated instrument system was used to concurrently measure water temperature, pH, salinity, water clarity (beam transmittance), chlorophyll *a* (chl *a*; *in situ* fluorescence), dissolved organic matter (DOM; *in situ* fluorescence), phycocyanin and phycoerythrin. It took two eight hour days to physically map Galveston Bay. Detailed procedures can be found in Appendix B of the QAPP.

After each field trip, the data were checked by the QA/QC officer and then used to generate high resolution maps using the program Surfer (Version 8.0). Data were cross checked with water samples taken from fixed stations throughout the Bay (see Table 4 above). At these fixed stations, discrete waters are also collected to measure:

- nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_3),
- total particulate nitrogen (TN) and total particulate phosphorus (TP),
- phytoplankton biomass (chlorophyll) and
- phytoplankton community composition.

Upon returning to the lab, samples from the discrete stations were processed immediately and frozen until analysis. For nutrient (dissolved and total) analysis, water samples from each station were filtered (GF/F; Whatman) onto a filter under low vacuum (< 130 kPa) pressure. The filtrate was stored in an acid cleaned HDPE rectangular bottle (125 mL; Nalgene) which was triple rinsed with extra filtrate before keeping the final sample for analysis. Samples for nutrient analysis were frozen immediately until analysis was performed by Geochemical and Environmental Research Group (GERG) located at Texas A&M University (College Station). Specific details on these procedures can be also found in Appendix E of the QAPP.

Water from each station was also filtered (GF/F; Whatman) under low vacuum (< 130 kPa) pressure for chl *a* analysis which we use as a proxy to estimate phytoplankton biomass. Filters were folded and frozen at -20°C for chlorophyll analysis and at -80°C for pigment analysis. Chl *a* concentrations were measured using a Turner 10-AU fluorometer. Calibration and measurement techniques were according to Arar and Collins (1997) with some modifications. Filters were extracted with a 60/40 solution of 90% acetone/DMSO and kept overnight in the dark at 4°C. Filters were removed and samples centrifuged for 5 min to pellet any particulates. After measuring the initial fluorescence, samples were acidified with 10% HCl and the fluorescence measured a second time.

3.3 Objective 3:

Define influence of nutrient and sediment load on the phytoplankton in Galveston Bay

Resource limitation assays (RLAs) were performed to identify which resource: nutrient(s) and/or sediment (light), is most limiting to primary productivity. These were performed essentially as previously described (Fisher et al. 1999; Örnólfsdóttir et al. 2004; Quigg 2009, 2010). These assays are also referred to as nutrient addition assays in the literature but given as we are interested in nutrient and sediment loading effects, we refer to them herein as RLAs. The evaluation criteria detailed in Fisher et al. (1999) was used to determine the outcome of each nutrient assay. RLAs and corresponding measurements were performed at six stations (Table 4) along transects from the San Jacinto and Trinity River basins to the Gulf of Mexico during periods of high (November-May) and low (June to October) FWI. This facilitates the examination of the role of nutrient loads in controlling primary production and phytoplankton community composition throughout the Galveston Bay. Given the arrangement of sampling stations, the aim of the sampling strategy was to determine the importance of nutrient loading on downstream ecological processes.

Surface (0 - 0.5 m) water was collected from each site in acid washed carboys and dispensed into 4L cubitainers (thirty per site; total of 180 per RLA). Triplicate cubitainers were then randomly selected out of the 30 for each site and treated as follows (nutrient concentrations reflect the final concentrations in each treatment) for a complete multi-factorial experimental design:

(i) a control (no addition),

(ii) +N (30 μmol L^{-1} NO_3^-),

(iii) +A (50 μmol L^{-1} NH_4),

(iv) + Si (100 μmol L^{-1} SiO_3)

(v) +P (2 μmol L^{-1} PO_4^{3-}),

(vi) +NA (30 μmol L^{-1} NO_3^-,50 μmol L^{-1} NH_4)

(vii) +NP (30 μmol L^{-1} NO_3^-, 2 μmol L^{-1} PO_4^{3-})

(viii) +ALL (30 μmol L^{-1} NO_3^-,50 μmol L^{-1} NH_4, 2 μmol L^{-1} PO_4^{3-} , and 100 μmol L^{-1} SiO_3)

(ix) +GC – a “grazing” control (no nutrients were added but water was pre-filtered using a 380 μm filter before filling each cubitainer), and

(x) a “light” treatment (cubitainers were covered with shade cloth resulting in a 50% reduction in light penetration).

Treatments were incubated outdoors at ambient water temperature and turbulence and 50% ambient sunlight in a free floating corral outdoors as shown in Fig. 7. Each corral was designed to handle 30 cubitainers.

Additional water samples (1 L) were collected from each site and returned to the laboratory – this water was used to measure the initial (Day 0) water quality and phytoplankton characteristics of the sample. All containers and bottles were triple rinsed prior to filling. We measured the hydrological (temperature, salinity, light availability), chemical (nutrients - NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_3), and biological (phytoplankton biomass and community composition) characteristics of the water at Day 0. At the end of the incubation period (~ seven days), we measured changes in phytoplankton biomass and community composition using procedures described above. Cubitainers were collected and processed as quickly as possible either in the laboratory or outdoors in a low light (shaded) environment (Fig. 7). Each cubitainer was shaken vigorously to mix contents.

Figure 7 Floating “corrals” used for resource limitation assays (RLAs) deployed in Galveston Bay. Cubitainers were spiked with nutrients or covered in shade cloth (see treatments above) and then set out to float under ambient conditions of temperature and turbulence and 50% of ambient light. After seven days, cubitainers were returned to the lab so that water samples could be processed. There were 30 cubitainers for each site, six sites, such that 180 cubitainers were incubated for each RLA.

3.4 Objective 4: Determine the distribution of *Vallisneria* plants in relation to salinity gradients in Galveston Bay

The BBEST concluded that "*Vallisneria americana* in the Trinity River delta should be mapped, ground-truthed, assessed and then monitored with salinity and other water quality parameters to enhance the understanding of its distribution within the delta and its relationship to freshwater inflow" (Espey et al. 2009). It was proposed that sampling transects would be conducted in the spring with the assistance of Texas Parks and Wildlife (Dickinson Marine Lab) with additional sampling trips planned for the fall depending on availability of funds and of TPWD personnel. Standard EPA/TCEQ protocols for mapping and ground-truthing submerged aquatic vegetation have been developed by Dr. Warren Pulich Jr. (River Systems Institute, Texas State University - San Marcos). A modified version of his protocol was included in the QAPP in Appendix C. This protocol was developed for seagrass mapping and utilizes high resolution aerial photography that provides sub-meter ground feature resolution across a large field of view (ca 4.8 sq km photo footprint). The ground-truthing would distinguish *Vallisneria* from other species like *Ruppia* which cannot be distinguished in photos. Similar such protocols have been used elsewhere (e.g., Siciliano et al. 2008). In addition, the protocols published by Adair et al. (1994) were followed to allow historical comparisons. Adair et al. (1994) investigated the distribution and status of submerged vegetation in estuaries of the upper Texas coast, including Galveston Bay. *Vallisneria americana* were only found in the shallow (<60 cm), oligohaline (<10 ppt) waters of Trinity Bay.

Based on above, personnel scanned the upper Trinity River basin as well as the areas surrounding the mouth of the Trinity River monthly each spring (2010-2013) but were not able to find *Vallisneria* (Fig. 8). The following approaches were used in an effort to find this plant material: As the turbid waters of the Trinity River Delta often obscured the view of any sub-aquatic vegetation, a rake was used to check for the presence or absence of *Vallisneria americana* in the study area (Fig. 8). At each sample site, a 14-tine bowhead rake measuring 0.33 m wide was pulled along a 3 m transect and checked for any plant material. This process was repeated three times per site. In the event that *Vallisneria americana* was found in the tines of the rake, a 1x1 m quadrat would be used to assess characteristics of the population such as abundance, distribution and age profile. Clippers would be used to collect a sample of the vegetation for laboratory analysis concerning the health and reproductive potential of the plant. These samples would be placed in bags labeled with the site name and stored over ice in a cooler while being transported. We had anticipated making the measurements in Table 6 if *Vallisneria* was present. This sampling strategy will provide spatial information on the extent of *Vallisneria*, its health and reproductive potential in relation to FWI into Galveston Bay.

Table 6 Distribution and health metrics for *Vallisneria* in Galveston Bay.

Metric	**Information**	**Measurement**	**Reference**
"salinity zonation" [1]	distribution	presence/absence of plants	Adair et al. 1994; Siciliano et al. 2008; Pulich 2006
	abundance	#/area	Adair et al. 1994; Siciliano et al. 2008; Pulich 2006
	age profile	heights of plants [2]	Adair et al. 1994; Pulich 2006
	primary nutrient source	15N/14N ratios [3]	Armitage et al. 2006
	geological properties of the sediment	grain size, porosity [4]	Folk, 1980; Peng et al. 2005
"health" or "reproductive potential"	health	leaf-to-root surface area ratios [4]	Shinano et al. 1996
	health	Chlorophyll content [5]	Shinano et al. 1996
	reproductive potential	Seed/flower production [5]	

[1] water column water quality characteristics: salinity, temperature, dissolved oxygen, pH, water depth
[2] this is dependent on access to plants and sediment consolidation; hence this work may be limited to feasible locations
[3] Protocols for 15N/14N ratios can be found in Armitage et al. (2006)
[4] quarterly sampling will be done in Year 2 during high and low flow periods – this information will be used to examine additional characteristics which may provide further insights into the zonation and health of the *Vallisneria*. Protocols for sediment samples (grain size and porosity) can be found in Folk (1980) and Peng et al. (2005). Grain-size distribution of the samples was determined with a Malvern Mastersizer 2000 analyzer with a measurement range of 0.02–2000 µm. Samples were pretreated with 10–20 ml of 30% H_20_2 to remove organic matter and then with 10 ml of 10% HCl to remove carbonates. About 2000 ml of deionized water was added, and the sample solution was kept for ca 24 h to rinse acidic ions. The sample residue was finally treated with 10 ml of 0.05 M $(NaPO_3)_6$ on an ultrasonic vibrator for 10 min to facilitate dispersion before grain-size analysis. The Mastersizer 2000 automatically yields the median diameter and the percentages of the related size fractions of a sample with a relative error of less than 1%.
[5] as part of the quarterly sampling we may also measure traditional and non-traditional proxies of plant health using documented protocols. Protocols for leaf-to-root surface area ratios can be found in Shinano et al. (1996). Protocols for chlorophyll content can be found in Shinano et al. (1996).

Figure 8 *Ruppia maritima* meadow at a study site near the Trinity River Delta where *Vallisneria americana* has historically been sighted (29˚45.85’, 94˚43.86’). The rake used to test for presence or absence of sub-aquatic vegetation is depicted at the bottom right.

3.5 Objective 5: Determine the distribution of *Rangia* clams in relation to salinity gradients in Galveston Bay

The BBEST found that "monitoring of *Rangia cuneata* distribution and its response to salinity regimes is important to gain increased knowledge between the inflow recommendation and its response to different salinity conditions. Current monitoring of this species may not be temporally or spatially resolute to ascertain relationships with salinity" (Espey et al. 2009).

3.5.1 Determine the distribution of *Rangia* clams using historical datasets

Working with TPWD Officers Lance Robinson and Bill Balboa, we have obtained historical datasets of *Rangia* distributions from surveys conducted by TPWD in Galveston Bay. The data base contained information on the presence of the clams, latitude, longitude, dates, salinity, temperature and other parameters. Real-time flow data from a USGS monitoring station (Trinity River at Romayor) near the river's mouth was used to determine the FWI into Galveston Bay from January 2005 to December 2012. The data presented is that previously checked by the USGS's QAPP. These two data sets were combined to examine relationships between *Rangia* distributions and FWI which will be used as a proxy for salinity.

3.5.2 New surveys performed during 2010-2012 with concurrent salinity measurements

Working with TPWD Officers Lance Robinson and Bill Balboa, we conducted surveys in 2010 along the regularly visited stations on the TPWD transect lines. Additional collection trips were conducted in 2011-2012 to coincide with the reproductive life-stages of the *Rangia*. All field work was performed according to standard procedures provided in the TPWD Marine Resource Monitoring Operations Manual (Martinez-Andrade and Fisher 2010) so that new findings will be directly comparable to previous efforts. These are described in detail in Appendix D of the QAPP.

At each sample site, a metal quadrat measuring 0.33 m by 0.54 m was tossed haphazardly and allowed to sink. The area within the borders of the quadrat was excavated to a critical depth of 0.3 m with trowels and by hand. Any clams found within that space were placed in a bag labeled with the site name and replicate number and stored over ice in a cooler for laboratory analysis. This process was repeated four times per site. In the event that less than 10 clams were recovered from the quadrat areas after four throws, the area around the quadrats was searched by hand until at least 10 clams were available for laboratory analysis. Any clams found outside the quadrat were placed in bags labeled with the site name and a designation that they were not from the quadrats. Water quality at each site was assessed using a Hydrolab MS5 water quality multiprobe to measure temperature, salinity and dissolved oxygen content. Water and sediment samples from each site were collected for laboratory analysis.

Back in the laboratory, on no fewer than 10 *Rangia* from each site, were assessed according to the following:

1. Measure the shell width,
2. Measure meat index of wet meat tissue and whole clam mass (including valve), and
3. Determine gender and therefore sex ratio of clams from each site.

3.5.3 Conduct new surveys during 2010-2012 with concurrent salinity measurements. Focus will be Spring and Fall periods and assessment of adult gonadal condition as indicator of reproductive potential and spat settlement as indicator of larval survival.

During surveys conducted as part of 3.5.2, we collected *Rangia* clams to assess both adult gonadal condition as indicator of reproductive potential and spat settlement as indicator of larval survival. This involved:

1. Gonad tissue removal (Fig. 9) to examine gonad development using simple visual staging (0%, 25%, 50%, 75%, 100% gonad development) according to Dr. Sammy Ray (TAMUG). Given the large degree of variability, this simple system provided the best possible information without the need to have to develop complex protocols.
2. We also checked microscopically for gender and gamete development to determine the ratio of males to females (Fig. 9); this is also a possible metric for reproductive potential according to Dr. Sammy Ray (TAMUG).

Multiple sampling events were conducted with and without TPWD (Dickinson Marine Lab). These measurements were used to examine *Rangia* clam salinity zonation and health (or reproductive potential).

Further, the sampling strategy was designed to obtain information on the spatial and temporal extent of *Rangia* in relation to FWI into Galveston Bay as well as role of salinity on the clam health (see Table 7). For example, measurements of clam size will provide information on the antecedent salinity levels in the estuary. Given the life span of these clams, this aspect will provide information on how the salinity has fluctuated over this period. Hence, this will give us information on *Rangia* clam exposures to different salinities prior to the start of our sampling events. Defining the "health" of clams was more difficult and currently poorly defined in the literature. Based on oyster studies, we examined several metrics which are known to apply to oysters but which may also be applicable to clams (Table 7).

Table 7 Distribution and health metrics for *Rangia* clams in Galveston Bay.

Metric	**Information**	**Measurement**
"salinity zonation" [1]	Distribution	presence/absence of Rangia
	Abundance	#/area of Rangia
	age profile	size of clam shells – width [2]
"health" or "reproductive potential"	Health	ratio of clam meat to shell size
	reproductive potential	gonad content [3]
	health	fat content [3]
	reproductive potential	calorie content [3]
	health	DNA:RNA content [3]

[1]water column water quality characteristics: salinity, temperature, dissolved oxygen, pH, water depth
[2]determination of size/class cohorts could be used to determine (estimate) secondary productivity
[3]these are parameters we may or may not measure depending on time, resources and availability of appropriate instruments.

Figure 9 *Rangia* spp. collection and laboratory analysis. In the field, *Rangia* were collected and quantified using a metal quadrat and water quality was assessed with a Hydrolab MS5. In the laboratory, *Rangia* spp. health and reproductive potential were assessed using various analyses of the wet tissue and gonads.

3.6 Objective 6:

Using the data collected, develop a better understanding of the use of these flora and fauna as biological indicators of the effects of freshwater inflows in Galveston Bay. Role of Salinity.

This objective is designed to help resolve issues that arose as part of the BBEST deliberations to develop recommendation for FWI into Galveston Bay (see Espey et al. 2009). For example, the BBEST found a weakness of salinity–abundance relationships for most common species in the estuary. This led the work group to seek relationships with life history stages that were more sensitive than adults, e.g. seed germination in *Vallisneria* and larval survival in *Rangia cuneata.* "While sessile organisms were preliminarily selected for their utility in identifying freshwater inflow targets, the Trinity-San Jacinto Basin and Galveston Bay BBEST indicated it is necessary to ensure these results work within the context of the needs of the remaining indicator organisms identified" (Espey et al. 2009). However, the BBEST found that no such work has been performed so that the assertion that the "flows incorporated within the proposed recommendation are necessary for a sound ecological environment would be limited to only those organisms studied (i.e. *Vallisneria*, *Rangia*), and not suggested as representing a healthy Galveston Bay ecosystem in its entirety". Given the scope of the current program, the team will endeavor to address these issues and others raised in the report, time permitting.

Specifically, this study builds on previous work conducted by the SB3 BBEST extending the period of record available for modeling from 2005 to 2012 and including a comparison to recently collected salinity data. TxBLEND, a two-dimensional, depth-averaged hydrodynamic and salinity transport model, to simulate water circulation and salinity condition within bays was used in this study. Detailed documentation of the model and the most recent calibration and validation work can be found in TWDB (1999) and Guthrie et al. (2012), respectively.

Herein the aim was to perform an analysis of the available information collected in Galveston Bay (USGS, TCEQ, TWDB, TPWD, NOAA, PIs), specifically:

(i) produced isohaline maps from monthly average salinities predicted by Texas Water Development Board's TxBLEND model for period of record, 1983 to 2012.

(ii) compared the TxBLEND predictions with the measured salinities at fixed stations in Galveston Bay measured by the state agencies and discussed, with the TWDB, potential adjustments TxBLEND calibration to account for discrepancies.

(iii) compared the TxBLEND predictions with the observed salinities measured on fine spatial scales by Quigg (2008 to 2012) in Galveston Bay and potentially adjust TxBLEND calibration to account for discrepancies, and

(iv) determined if model outputs match known salinities in Galveston Bay, and if not, where the greatest source of errors occur.

3.6.1 Updated TxBLEND inputs

Prior to the initiation of this current study, the TWDB had updated TxBLEND input files through 2009. For this study the model was updated to 2012. This included updating metrological data including wind, tide, evaporation and offshore (gulf boundary) salinity, and discharges and returns directly to and from the Bay by two power plants. These updates were made by TWDB following their established procedures. It also included updates to daily surface water inflows from eight rivers and streams that drain into Galveston Bay (Fig. 10).

The river flow data was provided by the TWDB data disaggregated by sub watershed (Fig. 10) and as either daily gaged flow (g), daily ungaged flow, which is modeled using the TWDB's TxRR computer program; a rainfall-runoff model used to simulate ungaged flows discharging directly into Texas bays and estuaries (m), monthly diversions (d) or monthly return flows (r).

Surface water inputs for each of the rivers and streams were calculated by the following equation:

$$\text{Inflow} = \text{Gage inflows (g)} + \text{Ungaged Inflows (m)} - \text{Diversions (d)} + \text{Returns (r)}$$

Diversions and returns were disaggregated to constant levels within each month. While the data from the gage and ungaged sources are complete through 2012, the diversion data from TCEQ is only available through 2011 and the return flow data is only available through 2009. For the diversion data, values reported in 2011 were used to fill in data from 2012. Similarly return flow data from 2007-09 were used to fill in missing data from 2010-2012. In reviewing previous estimates of diversion data produced by the TWDB, it appears that recent diversion data obtained from TCEQ may be missing a water diversion report for subwatershed 7070, a small correction was applied in attempt be consistent with earlier data. Diversions and returns from these coastal watersheds are small relative to total inflow and it is an expected error incorporated as a result of these fill-ins and modifications would have only small effect on Bay salinity.

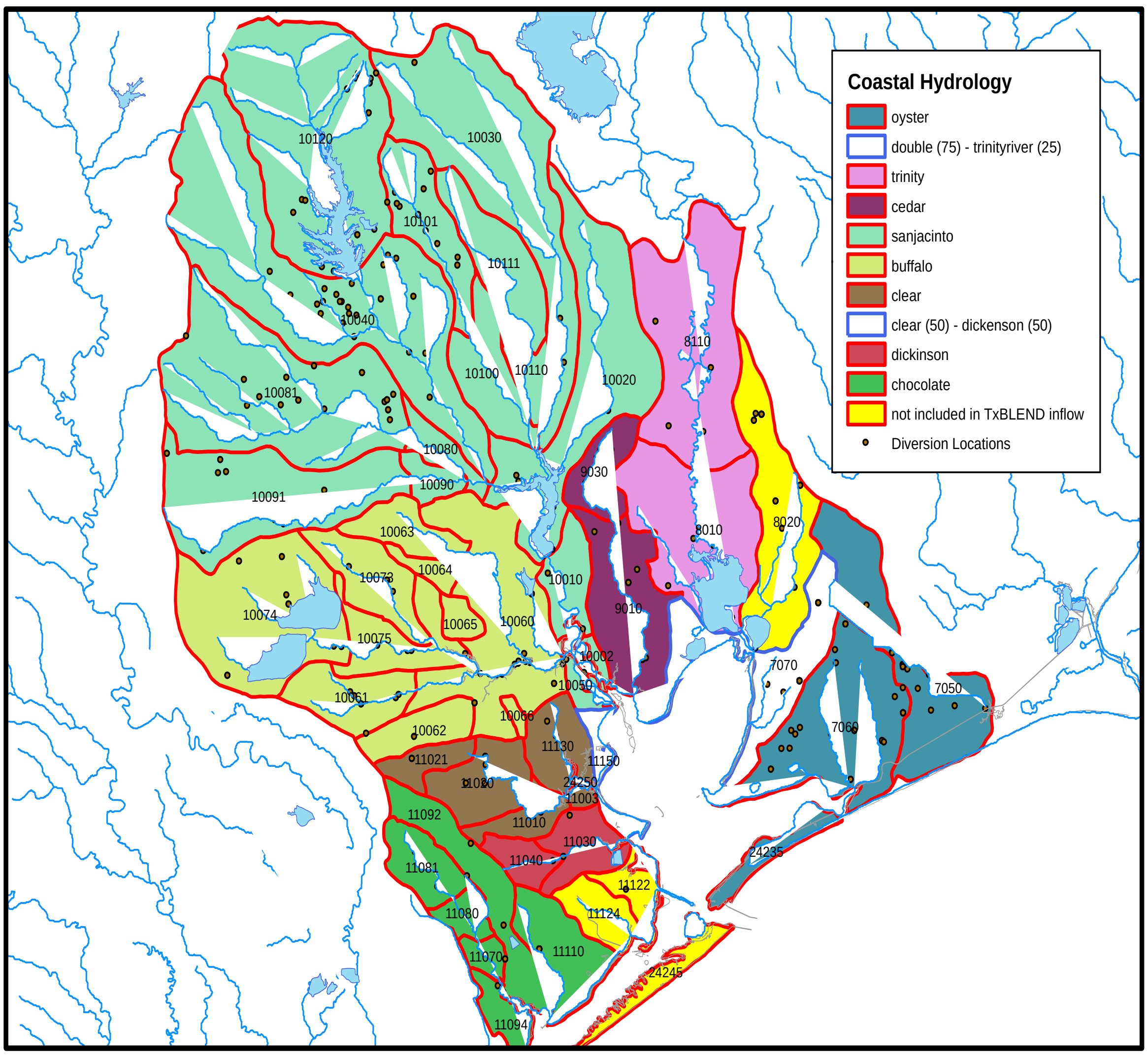


Figure 10 Coastal subwatersheds used to develop surface water inflows (gaged flows, ungaged flows, diversions and returns) for TxBLEND model.

3.6.2 Executed model simulation from period from 1983 – 2012

The TxBLEND model was executed by the TWDB for the period from 1983-2012 using the UNIX version of the program. A PC version is available, however, a 30 year simulation of Galveston Bay takes approximately 7-10 days on a PC versus about 21 hours on the TWDB UNIX sun stations. The primary model output of concern in the study is salinity which is output for all nodes as monthly average values and for up to 50 select 'checknodes' on an hourly time step. Hourly outputs were produced for 41 sites corresponding to dataflow station locations and 8 sites corresponding to TWDB long term data sonde monitoring locations.

3.6.3 Produce isohaline maps including shape files to compute percentages of Bay areas within salinity ranges

Model predictions of monthly average salinity are all 5070 nodes within the model domain were linked to an ESRI ArcGIS point shape file of the model nodes. This shape file and bounding polygon representing the model area were used as inputs to a custom python script in ArcGIS which stepped through all 360 months in the period of record and performed the following tasks:

1. Applied an inverse distance weighted interpolation scheme to produce a grid representing model salinities based on the node values.
2. Applied a legend with 5 PSU increments to the grid, which was then overlain on a background map of the Bay area and river inflows to produce an image file (Fig. 11).
3. Reclassified the grid into 1 PSU increments and converted to a polygon shape file.
4. Finally, calculated the area for each polygon and added this value to the polygon shape file database table.

A custom visual basic macro in Microsoft Excel was then used import each of the 360 polygon shape file database tables into excel and calculate the percent of the overall bay area for which salinities were within each 1 practical salinity unit (PSU) increment from 1 to 30 (areas with salinities greater than 30 PSU were grouped together). These data were then used to produce Fig. 12 which presents a time series of monthly average salinities as percentage of the total Bay area that falls within 5 increment PSU salinity ranges. The figure in the lower panel of Fig. 12 shows the monthly inflow into the Bay in acre feet (ACFT)/month.

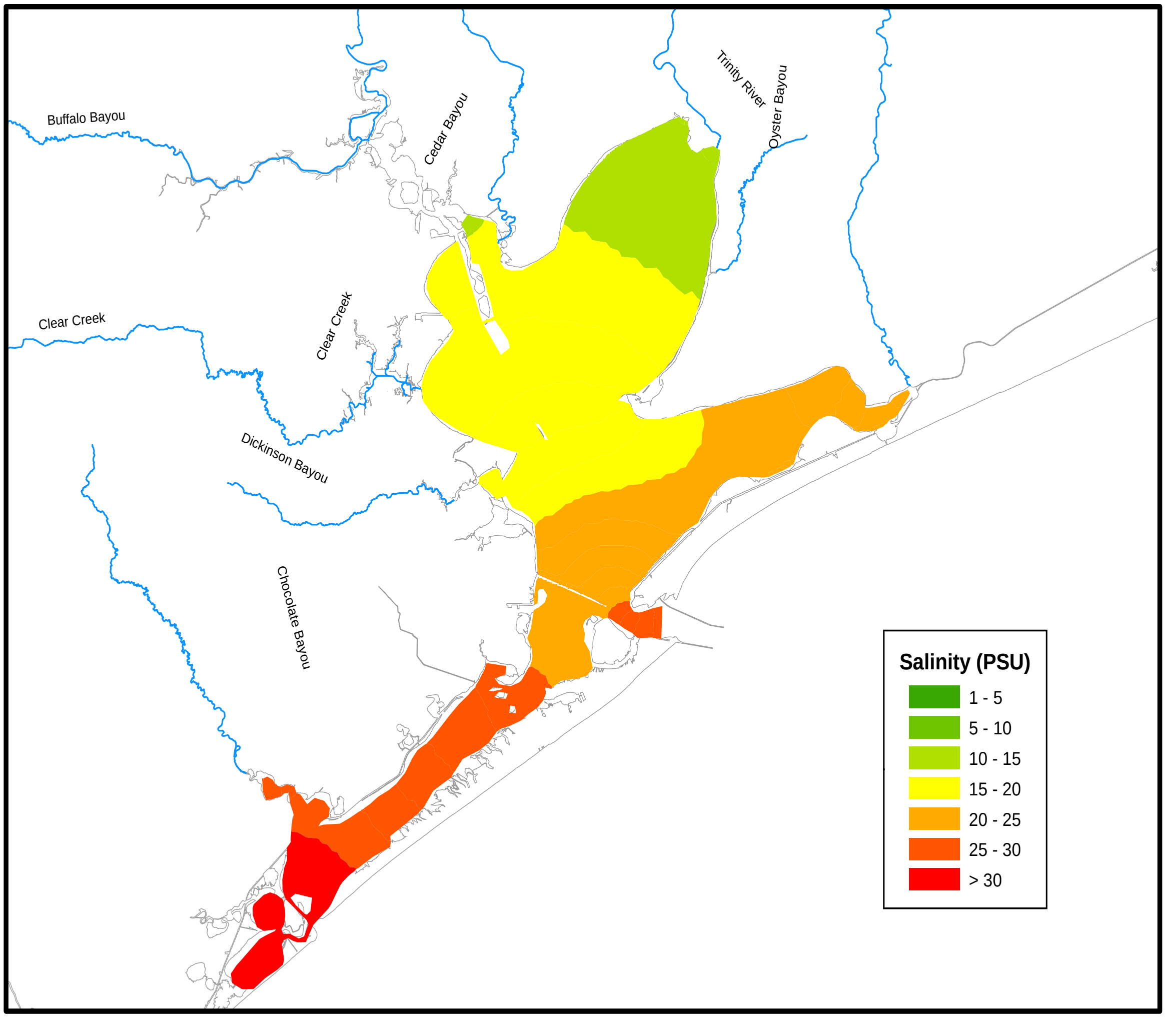


Figure 11 Monthly average salinity as predicted by TxBLEND model for August 2010.

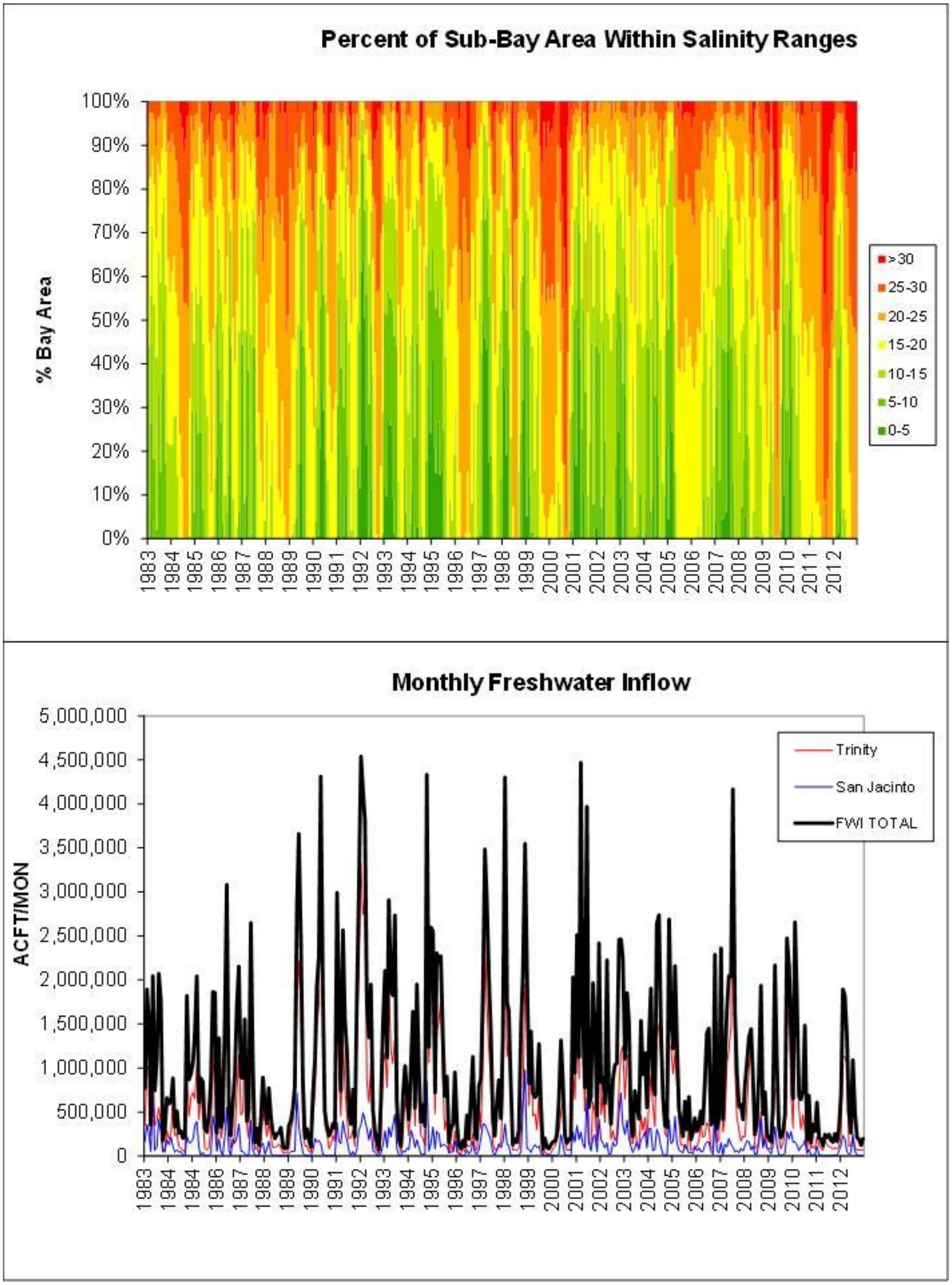


Figure 12 Time series of percent of total Bay area within salinity ranges.

3.6.4 Produced maps and hydrographs to visually compare with observed and simulated data

TxBLEND reports salinity on an hourly time interval for up to 50 user defined 'checknodes'. These nodes were defined prior to executing the model by selecting the TxBLEND nodes that are located closest to the 41 salinity monitoring stations used in this study (Fig. 1) and the eight long term data sondes maintained by the TWDB (Fig. 4). A custom query was developed in using Microsoft Access to calculate daily average salinities at these locations based on the hourly predictions reported by the TxBLEND simulation. Daily salinities for the days when the dataflow measurements were collected were linked to an ArcGIS shapefile of these 41 dataflow checknodes. Fig. 13 shows observed salinities as measured by the TAMU dataflow boat (which appears as a colored track but is actually individual point measurements) as compared with the salinities predicted by TxBLEND (labeled larger circles) for one of the sampling events (August 16, 2010).

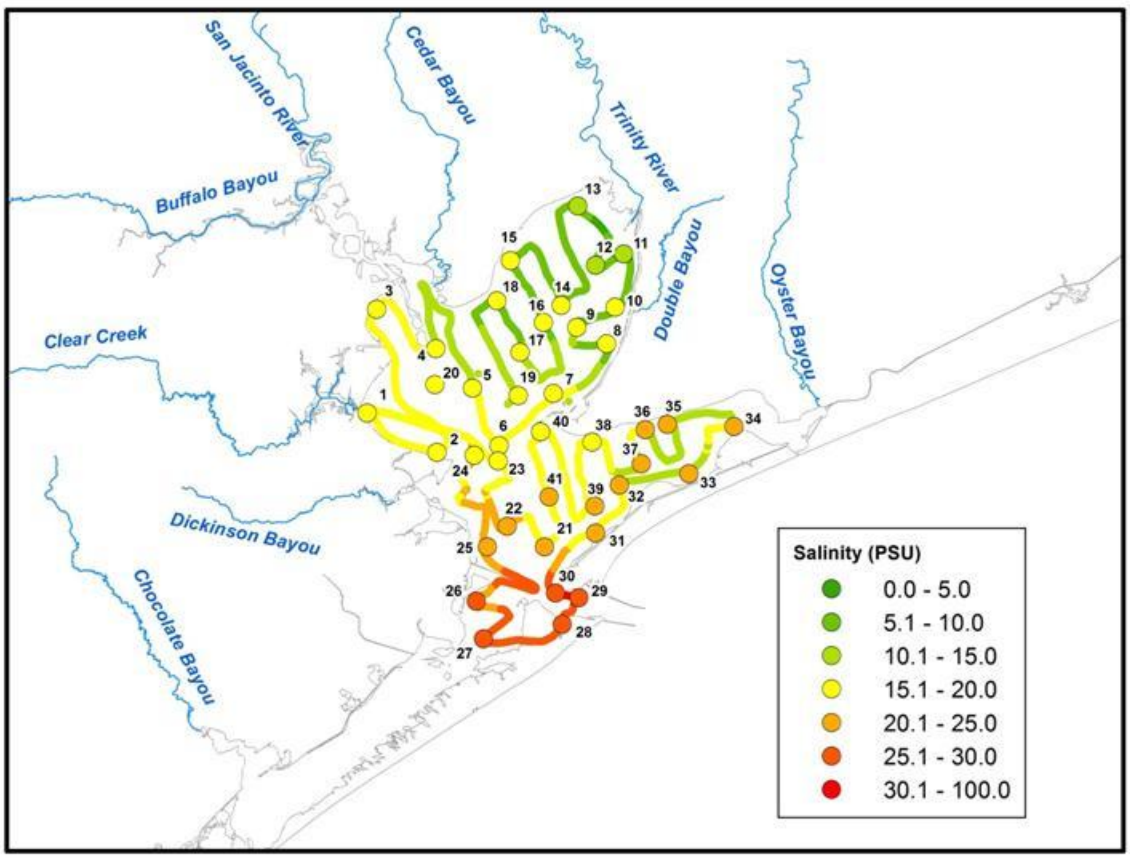


Figure 13 Daily average salinities predicted by TxBLEND compared with dataflow observed salinities on August 16, 2010.

The modeled and observed salinities for each sampling were also used to produce time series graphs for each of the checknode stations (including the 8 TWDB long term datasonde stations). Figure 14 shows a time series of predicted (TxBLEND) vs. observed (Dataflow) salinities at dataflow station 21.

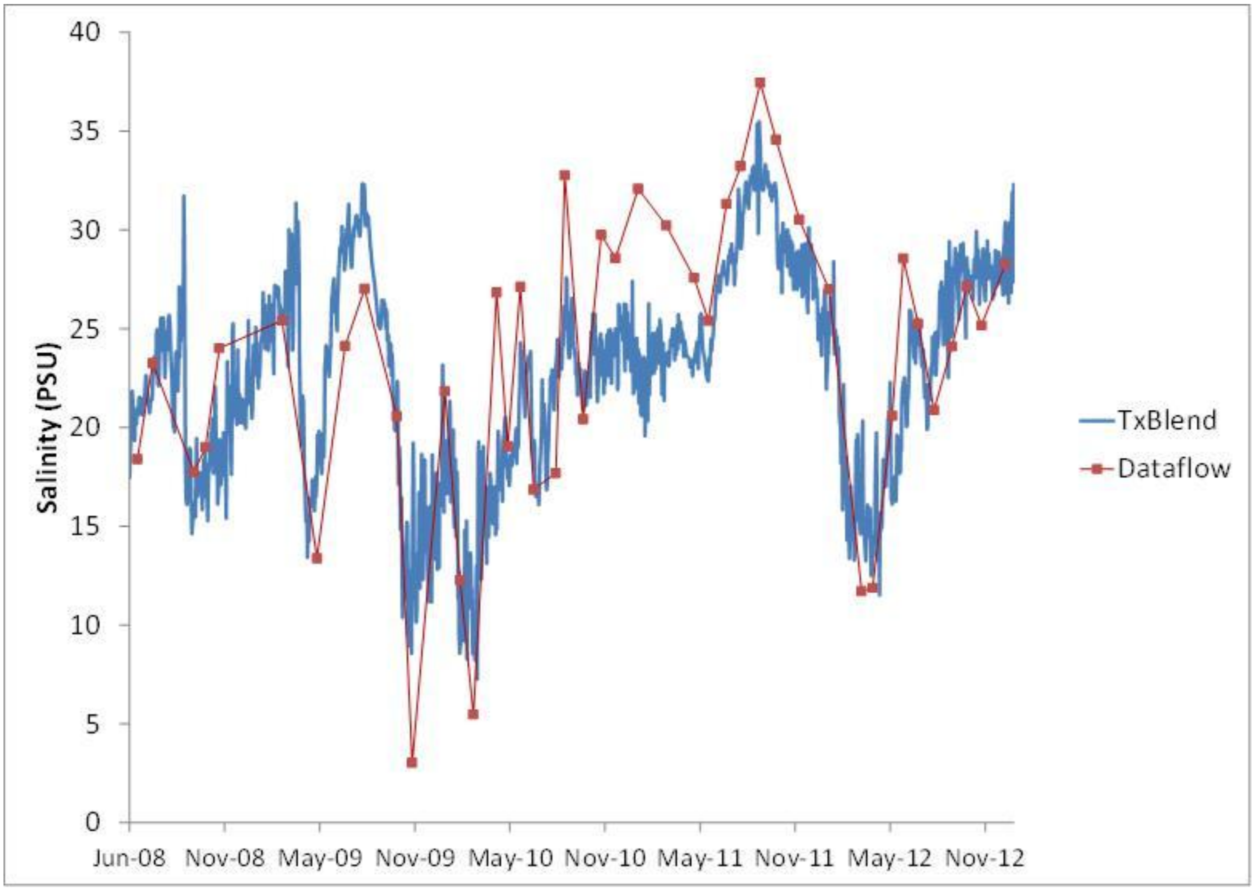


Figure 14 Time series (June 2008 to December 2012) of salinity measured with the dataflow at station 21 (red) compared to modeled salinity at the nearest TxBLEND Node (1833).

3.6.5 Calculated statistics to compare observed vs. predicted (modeled) salinities

Statistical analysis was conducted to compare predicted (modeled) salinities with observed salinities. Observed data included point measurements from the TAMUG dataflow system for approximately 50 months from June 2008 to December 2012 as well as continuous monitoring from 8 long-term fixed datasondes maintained by the TWDB.

Statistics include

1. Mean observed and simulated salinities.
2. Coefficient of determination (RSQ) which provides an estimate of proportion of variation explained by the model.
3. Nash-Sutcliffe Efficiency Criterion (NSEC) which describes model performance, where $E = 1.0$ represents a match between model output and observed data, and $E < 0$ suggests the model is a poor predictor.
4. Root Mean Squared Error (RMSE) which is a measure of model accuracy but is scale dependent.
5. RMSE-observations standard deviation ratio (RSR) which normalizes the RMSE based on the standard deviation in the observed data.
6. Percent bias (PBIAS) which measures the average tendency of the simulated data to be larger or small than their observed counterparts (Positive values indicate model underestimation and negative values indicate model overestimation).

All but the last two of these statistics were presented in the TWDB's model calibration and validation report (TWDB 2012).

Table 8 Summary statistics for observed and simulated salinity for 1 TAMUG dataflow stations and 8 TWDB datasondes in the Trinity-San Jacinto Estuary. Colors indicate more performance where red indicates poorer performance and green indicates better performance. Abbreviations for statistics are n (number of observations), ObsMean (observed mean based on dataflow or datasonde monitoring) SimMean (simulated mean in TxBLEND model), DifMean (observed mean minus simulated mean), RSQ (coefficient of determination), RMSE (root mean squared error), STDEV (standard deviation in observed salinities, used to calculate RSR), NSEC (Nash-Sutcliffe Efficiency Criterion), RSR (RMSE-observations standard deviation ratio) and PBIAS (percent bias).

Group	Code	Description	n	ObsMean	SimMean	DifMean	RSQ	RMSE	STDEV	NSEC	RSR	PBIAS
TAMUG Dataflow Stations	1		47	16.79	16.72	0.08	0.66	4.16	7.23	0.66	0.58	0.47
	2		48	18.75	17.76	0.99	0.55	5.04	7.41	0.53	0.68	5.30
	3	Transect	50	18.39	16.67	1.72	0.44	5.59	6.92	0.34	0.81	9.37
	4	Fixed	51	17.44	16.62	0.82	0.56	5.43	8.20	0.55	0.66	4.70
	5		48	18.52	16.99	1.53	0.64	5.31	8.49	0.60	0.63	8.26
	6	Fixed	50	20.21	18.53	1.68	0.67	4.83	7.96	0.62	0.61	8.34
	7		50	16.51	17.09	-0.58	0.72	4.50	8.39	0.71	0.54	-3.53
	8		49	14.09	15.56	-1.47	0.61	5.67	8.84	0.58	0.64	-10.45
	9		50	14.68	15.71	-1.03	0.66	5.47	9.17	0.64	0.60	-7.02
	10		50	13.55	15.10	-1.55	0.60	6.11	9.35	0.57	0.65	-11.47
	11	Transect	51	11.43	14.44	-3.01	0.59	6.59	9.20	0.48	0.72	-26.35
	12	Fixed	50	11.78	14.58	-2.79	0.61	6.16	8.84	0.50	0.70	-23.70
	13	Transect	50	10.48	14.57	-4.09	0.62	6.84	8.92	0.40	0.77	-39.03
	14	Transect	51	14.51	15.50	-0.99	0.65	5.49	9.11	0.63	0.60	-6.84
	15		43	13.93	15.27	-1.34	0.64	5.40	8.71	0.61	0.62	-9.60
	16	Transect	50	15.67	15.92	-0.24	0.66	5.36	9.13	0.65	0.59	-1.55
	17	Fixed	48	16.61	16.43	0.18	0.69	5.17	9.11	0.67	0.57	1.08
	18		40	14.63	15.92	-1.30	0.64	5.08	8.29	0.62	0.61	-8.86
	19	Transect	41	16.90	16.92	-0.03	0.73	4.42	8.47	0.72	0.52	-0.16
	20	Transect	42	18.77	16.50	2.27	0.55	5.55	7.59	0.45	0.73	12.10
	21		43	23.43	22.30	1.13	0.66	4.49	7.55	0.64	0.60	4.83
	22	Transect	43	22.60	20.92	1.68	0.70	4.16	7.01	0.64	0.59	7.43
	23		42	19.90	18.95	0.95	0.76	4.01	7.91	0.74	0.51	4.80
	24	Transect	43	19.88	18.43	1.45	0.66	4.30	7.01	0.61	0.61	7.29
	25	Fixed	42	21.86	21.45	0.41	0.64	3.82	6.18	0.61	0.62	1.86
	26		41	25.59	23.97	1.62	0.48	4.44	5.41	0.31	0.82	6.32
	27		43	25.92	24.13	1.79	0.47	4.77	5.69	0.28	0.84	6.90
	28		37	26.87	26.29	0.58	0.44	4.43	5.63	0.36	0.79	2.16
	29	Fixed	44	26.88	26.53	0.35	0.36	4.56	5.28	0.24	0.86	1.29
	30	Transect	43	25.93	25.00	0.93	0.49	4.39	5.75	0.40	0.76	3.57
	31		42	21.89	21.40	0.49	0.63	4.51	7.50	0.63	0.60	2.25
	32		41	20.14	20.60	-0.46	0.60	4.65	7.38	0.59	0.63	-2.29
	33		42	19.79	21.07	-1.28	0.47	5.41	7.28	0.44	0.74	-6.45
	34		42	18.94	21.83	-2.89	0.47	6.01	7.33	0.31	0.82	-15.28
	35		41	18.77	20.97	-2.20	0.56	5.07	6.98	0.46	0.73	-11.74
	36		42	18.88	20.80	-1.92	0.58	4.82	6.89	0.50	0.70	-10.17
	37	Transect	42	20.00	20.67	-0.67	0.59	4.65	7.24	0.58	0.64	-3.35
	38		40	18.76	20.09	-1.33	0.71	4.18	7.50	0.68	0.56	-7.08
	39		40	20.88	20.87	0.01	0.73	3.97	7.66	0.72	0.52	0.05
	40		39	16.79	18.27	-1.48	0.80	4.14	8.31	0.75	0.50	-8.79
	41	Transect	42	20.65	20.50	0.15	0.72	3.90	7.43	0.72	0.52	0.74
TWDB Datasoundes	BOLI	Bolivar Roads	4854	22.50	22.80	-0.31	0.62	3.76	6.02	0.61	0.62	-1.36
	EAST	East Bay	1659	13.50	17.13	-3.64	0.68	5.09	6.32	0.35	0.81	-26.94
	DOLLAR	Dollar Point	3530	17.56	17.05	0.51	0.74	3.55	6.86	0.73	0.52	2.93
	MIDG	Mid-Bay	2811	18.08	16.50	1.58	0.66	3.94	5.87	0.55	0.67	8.74
	TRIN	Mid-Trinity	5313	10.90	13.59	-2.69	0.67	5.14	7.62	0.54	0.67	-24.70
	RED	Red Bluff	2165	11.80	12.42	-0.62	0.69	3.60	6.24	0.67	0.58	-5.23
	BAYT	Baytown	2880	12.13	9.91	2.21	0.71	3.77	5.56	0.54	0.68	18.24
	OLDR	Old River (Trinity Delta)	3216	2.55	1.68	0.87	0.33	3.44	4.01	0.26	0.86	34.00

Results were color coded to quickly, visually assess model performance. The NSEC, RSR and PBIAS, along with graphical techniques similar to those presented above, were recommended by Moriasi et. al. (2007) to evaluate simulation models. Moriasi et. al. (2007) also proposed guidelines performance ratings. Since Moriasi et. al. (2007) specifically discusses watershed simulation models these guidelines should not be viewed as hard and fast rules acceptance or rejection of model performance in this study. In fact, any evaluation of model performance should consider specific objectives of the particular study. However, these guidelines were used to color code Table 8 for these three statistics. The coding for differences between observed and simulated means (DiffMean) and the RSQ values were based on the professional opinion of Joe Trungale: Red indicates poorer performance while, green indicates better performance.

4.0 Results

4.1 Climatology

Most of Texas, including the Gulf of Mexico coastal ecoregion, experienced an “exceptional drought” or D4, the most severe classification by the U.S. Drought Monitor (Tinker et al. 2011) which is equivalent to less than -5 on the Palmer Drought Severity Index (http://droughtmonitor.unl.edu/classify.htm) for the duration of 2011. By October of 2011, almost 88% of Texas was classified as experiencing exceptional drought (Nielsen-Gammon 2011). This classification is defined by exceptional and widespread crop/pasture losses, shortages of water in reservoirs, streams and wells creating water emergencies. Some areas would have ranked even higher if the U.S. Drought Monitor range was extended past D4 (Travis County 2011).

In terms of rainfall, 2011 was one of the top five driest years on record for the Galveston Bay watershed on record since records started in 1871 in Texas (www.nws.noaa.gov). The City of Houston received ~25 inches of rain in 2011 making this the third driest year on record (Table 9) while the City of Galveston received ~ 23 inches of rain in 2011 (Table 9). This is at about 30 to 50 percent of the expected normal rainfall for the City of Houston, Houston Hobby and City of Galveston which typically receive 49.77, 54.65 and 50.76 inches of rain respectively (www.nws.noaa.gov).

Table 9 Rainfall (inches) recorded for five driest years (listed in order of lowest to highest) on record for cites adjacent to the Trinity-San Jacinto Estuary.

	City of Houston		Houston Hobby		City of Galveston	
1	17.66	1917	**25.41**	**2011**	21.40	1948
2	22.93	1988	26.65	1988	21.43	1917
3	**24.57**	**2011**	28.32	1956	21.84	1956
4	27.09	1901	28.76	1954	22.29	1954
5	27.23	1951	31.11	1931	**22.95**	**2011**

The City of Houston experienced the warmest year on record (Table 10), matching the previous record set in 1962 (www.nws.noaa.gov). The City of Galveston recorded its second warmest year on record, with 2006 established as the warmest year since record keeping started. For comparison, the five warmest years on record for cites adjacent to the Trinity-San Jacinto Estuary are listed in Table 10 (data from www.nws.noaa.gov).

Table 10 Five warmest years (listed in order of highest to lowest) on record for cites adjacent to the Trinity-San Jacinto Estuary.

	City of Houston		Houston Hobby		City of Galveston	
1	71.9°F	1962	**72.4**°F	**2011**	72.6°F	2006
2	**71.9**°F	**2011**	72.3°F	1998	**72.5**°F	**2011**
3	71.7°F	1933	71.4°F	2006	72.3°F	2005
4	71.5°F	1965	71.3°F	2008	72.3°F	1994
5	71.5°F	1927	71.1°F	2009	72.3°F	1999

We also examined records collected at Hobby Airport which is adjacent to Galveston Bay to examine rainfall and ambient temperatures on a monthly basis from 2010-2012 (Fig. 15 and 16 respectively). While the 30 year average (1981-2010) rainfall is 54.65 inches in this location (www.nws.noaa.gov), less than half this amount fell in 2011 (25.41 inches). The year before and after had closer to average rainfalls: 47.02 and 51.58 inches in 2010 and 2012 respectively. While 2012 rainfalls were variable between months, 2010 was a more typical year for the region with highest rainfalls in the summer.

The ambient temperatures were less variable between months at this location (Fig. 16). The mean annual temperature in 2010 was 69.7 ºF but in 2011 and 2012 it was higher, 72.4 ºF and 72.6 ºF respectively. The 30 year average (1981-2010) temperature is 70.2 ºF in this location (www.nws.noaa.gov), making the latter two years of the study warm than typical for this location. Nielsen-Gammon (2011) reported that average temperatures from June through August several degrees over the long-term average.

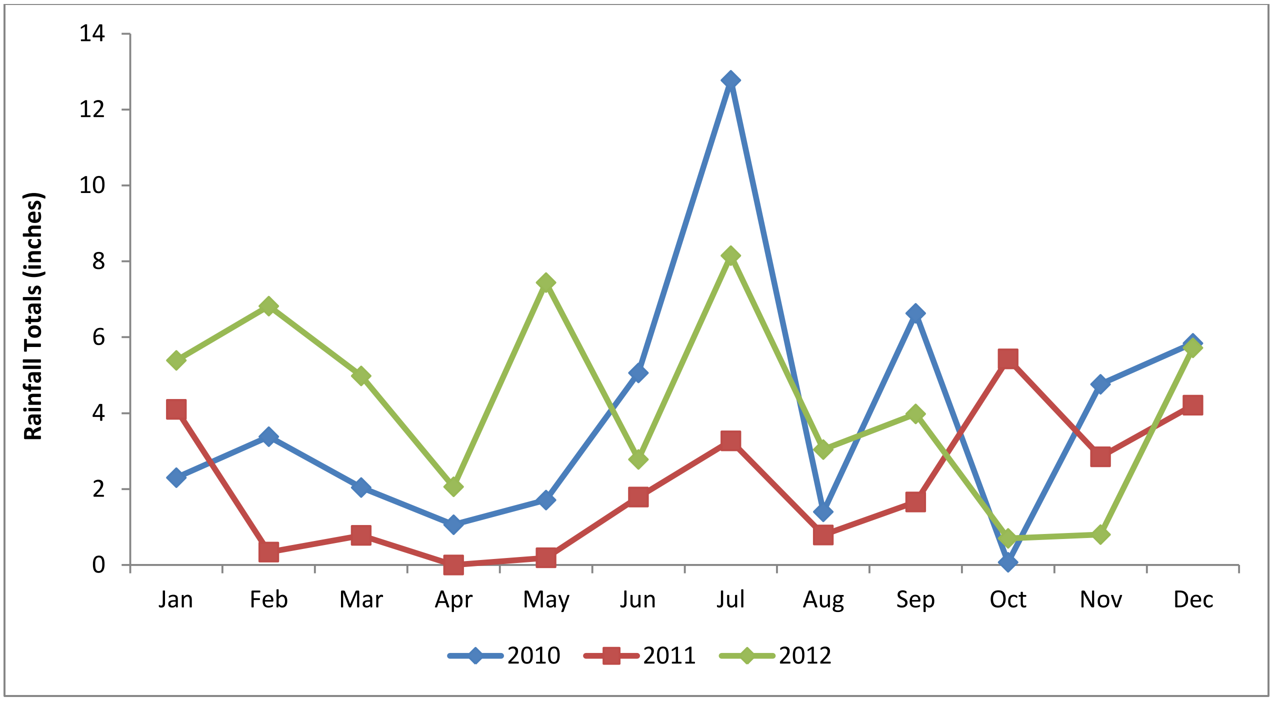


Figure 15 Rainfall total per month (inches) measured at Hobby Airport (http://www.srh.noaa.gov/hgx/?n=climate_hobby_normals_summary) from 2010 to 2012.

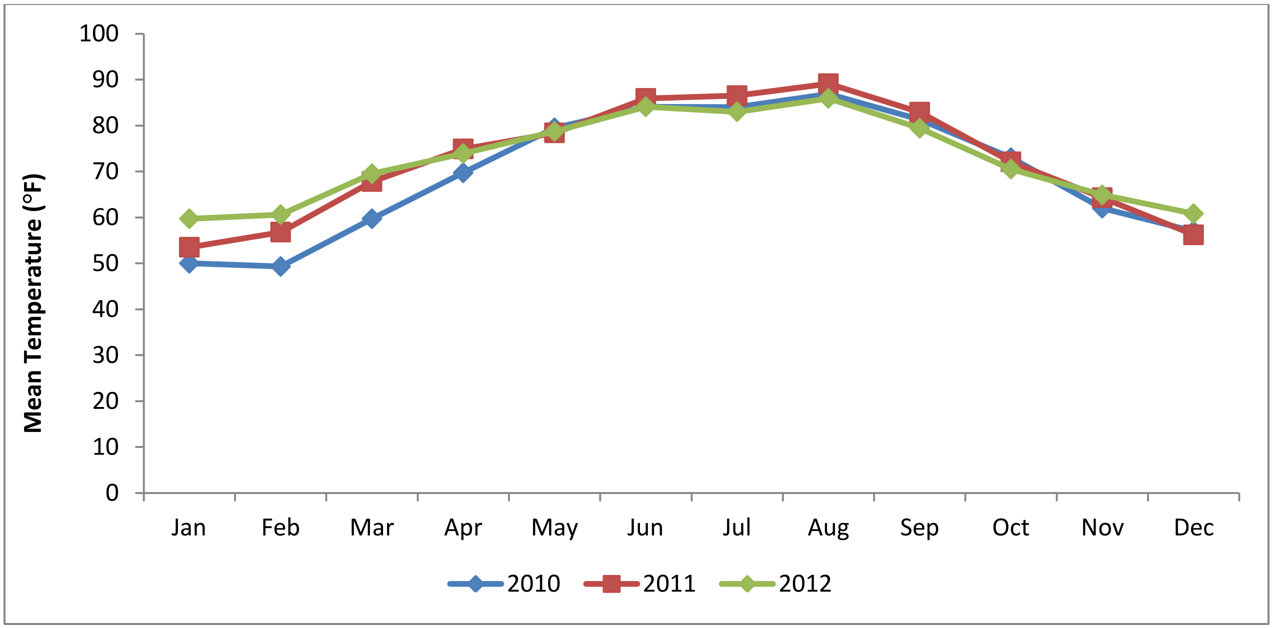


Figure 16 Temperature per month (ºF) measured at Hobby Airport (http://www.srh.noaa.gov/hgx/?n=climate_hobby_normals_summary) from 2010 to 2012.

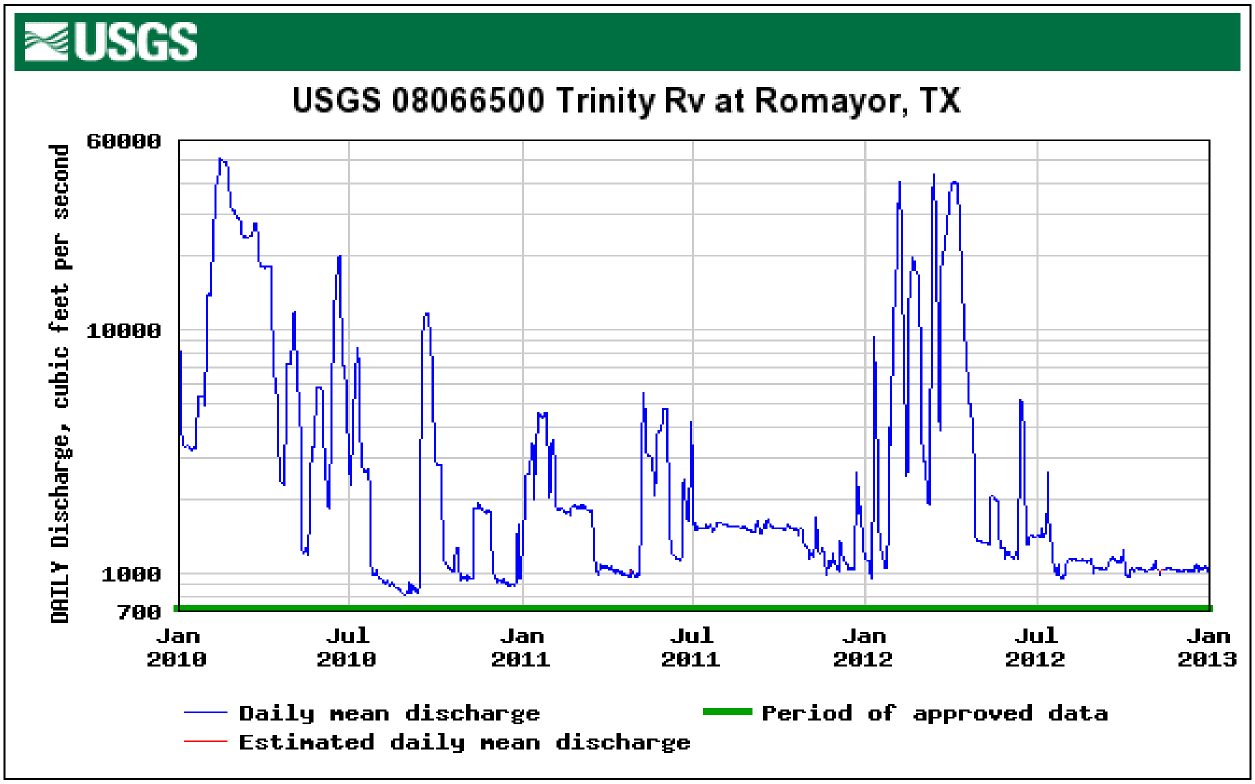
4.2 Freshwater Inflow

Real-time freshwater inflow measured as daily discharge (www.waterdata.usgs.gov) in cubic feet per second (cfs) to Galveston Bay for January 01 2010 to December 31 2012 was downloaded from the USGS monitoring gauge located on the Trinity River at Romayor (08066500) as well as the corresponding gage height (feet; Fig. 17).

Consistent with 2011 having little rainfall, there was little freshwater inflow into Galveston Bay from the Trinity River (Fig. 17) relative to that measured in the year before or after. Looking at the log y-axis, peak discharge events (freshets) of >10,000 cfs in magnitude typically occur except during the spring (2010 and 2012 respectively) while in 2011, the largest freshets were ~5,000 cfs. Freshets also occur during the fall, but this was only observed in 2010 (Fig. 17). The annual (total) discharge in 2011 was 656,466 cfs (~1.3 million acre-feet), about 20% of the total discharge (2,973,821 cfs; ~5.9 million acre-feet) recorded in 2010 (Fig. 17). There were also relatively lower flows in 2012 relative to 2010. In addition, river levels fell significantly during 2011 (Fig. 17) compared to what was observed in 2010 and 2012. This is consistent with suppressed flows due to drought conditions in 2011.

Table 11 Annual Mean Flow (cfs) measured at the Trinity River at Romayor (08066500) from 2000 to 2012.

Year	**Annual Mean Flow (cfs)**
2000	2957
2001	14900
2002	8193
2003	9113
2004	9757
2005	8858
2006	1828
2007	14480
2008	6214
2009	3531
2010	12840
2011	1791
2012	5284



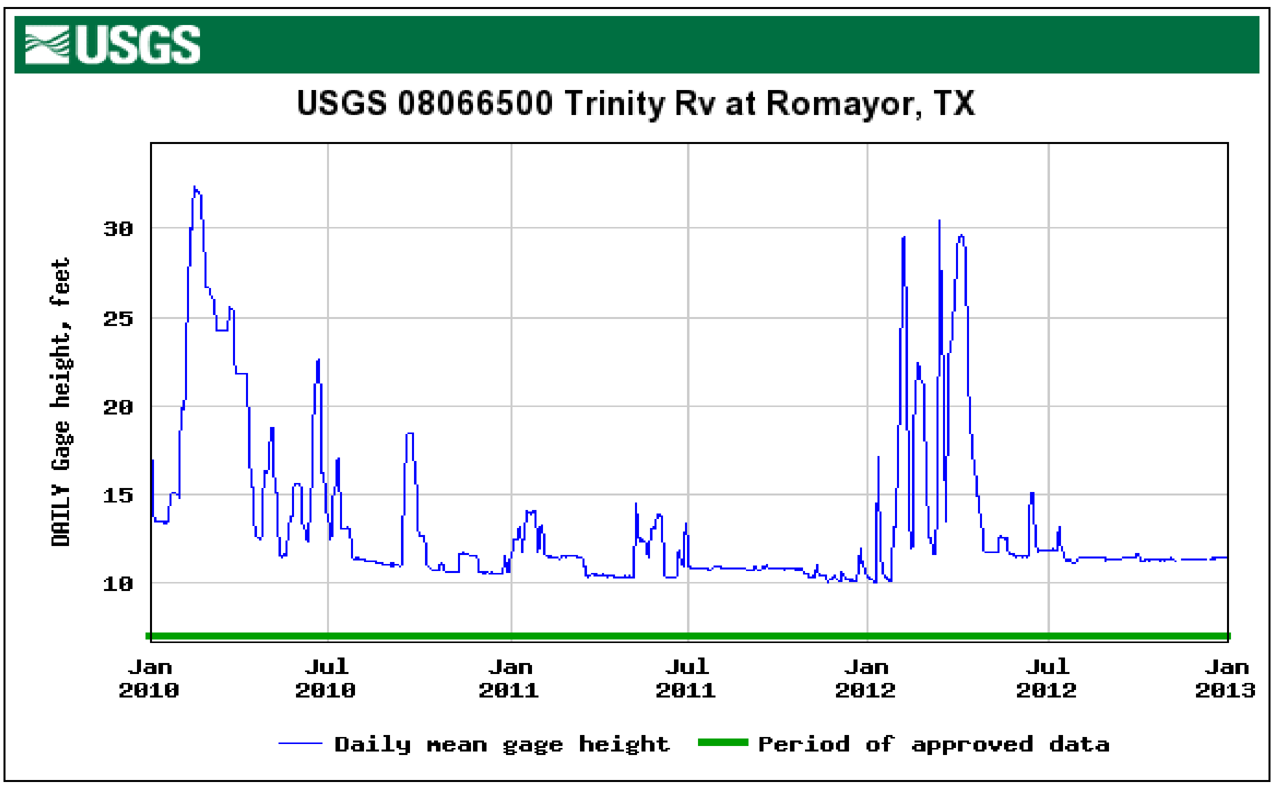


Figure 17 Daily discharge (cfs) and gage height (feet) flowing into Galveston Bay from the Trinity River in 2010 to 2012 (www.waterdata.usgs.gov).

Relative to previous years this decade, flows in 2011 were the lowest recorded, closely followed by those during the drought in 2006 (Table 11). By comparison, flows in 2010 were relatively high, similar to those measured during 2001 and 2007 while those in 2012, were relatively low, similar to those from 2008 and 2009 (Table 11).

4.3 Phytoplankton collections

Samples were collected at six fixed stations in Galveston Bay from 2005 to 2006 and from January 2008 to December 2012 as part of this and previously funded programs (no funding was available during 2007). Samples from Station 1, 2 and 6 (see Tables 3 and 4, Fig. 1) were examined microscopically to assess phytoplankton cell numbers, identification and biovolume at points along a transect running from the Trinity River mouth to mid-Galveston Bay and ending near the entrance to the Gulf of Mexico.

Cell counts and identifications were conducted on a 5 mL aliquot of sample from each of the three stations with a goal of identifying and measuring 200 objects for biovolume calculations. The conditions for some of the months were unfavorable in that the water sampled was very turbid and chamber slides were heavily filled with debris after settling which obscured plankton, if present. In these samples, multiple aliquots were settled in an effort to improve the counting efficiency. Nonetheless, this resulted in low total counts at some stations during some parts of the study. Months with inclement weather or technical obstacles that prevented sampling will not have any data – these were left blank in figures below.

We found diatoms to be the dominant algal group for all three stations over the course of this study (Fig. 18, 19 and 20). As shown in Figure 18, Station 1 had the most diverse algal community composition throughout the years. Chlorophytes (green algae) became important at this station in the spring of 2009 as well as the spring of 2012. Cyanobacteria (blue-green algae) were also abundant in the spring of 2012 at this station (Fig. 18). Dinoflagellates had a distinct presence at Station 1 in warmer months throughout the study period especially in 2005, 2009 and 2012 (Fig. 18). The TPWD Harmful Algal Bloom Red Tide status website indicated that harmful algal blooms did occur in these years, but those reported were all prevalent at places further south along the Texas coast (http://www.tpwd.state.tx.us/).

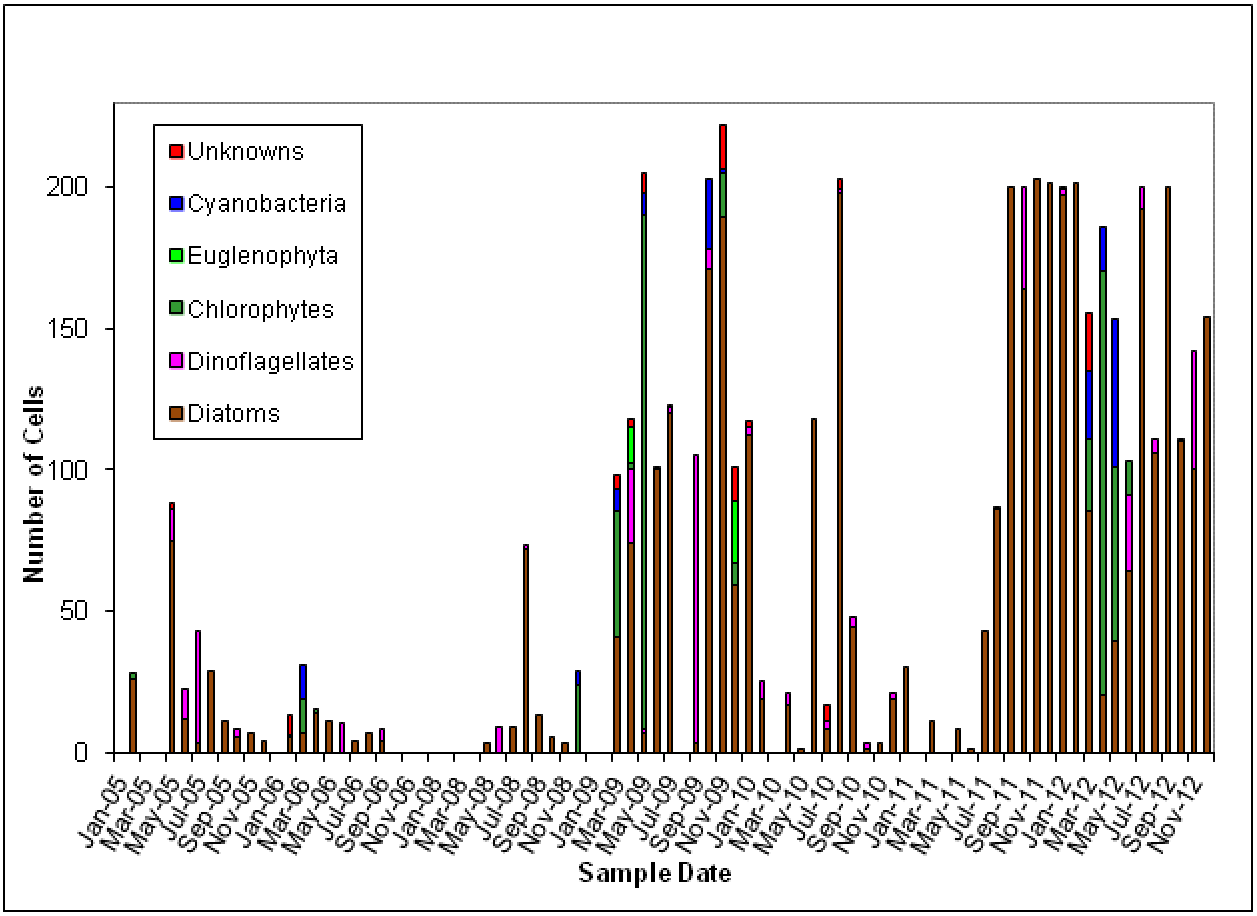


Figure 18 Dominant phytoplankton groups from January 2005 to December 2012 collected from Station 1 located in the upper Trinity Basin (see Fig. 1).

Figure 19 below depicts the results of microscope analysis for Station 2 which was more obviously diatom dominated than the other two stations examined. Dinoflagellates were consistently present from March 2009 to March 2010 and euglenoids were abundant in the spring of 2012 (Fig. 19). This station is adjacent to the most productive oyster reefs in Galveston Bay. Oysters are known to preferentially consume diatoms as a food source (Sammy Ray, pers. comm.).

Station 6 is represented in Figure 20, and again, depicts a diatom dominated community. Of the three stations, Station 6 had the highest prevalence of dinoflagellates especially in 2005, 2008-10 and 2012 (Fig. 20). This result is not a likely indicator for dinoflagellate blooms but rather an effect of the station's proximity to the Gulf of Mexico in which marine dinoflagellates are more common.

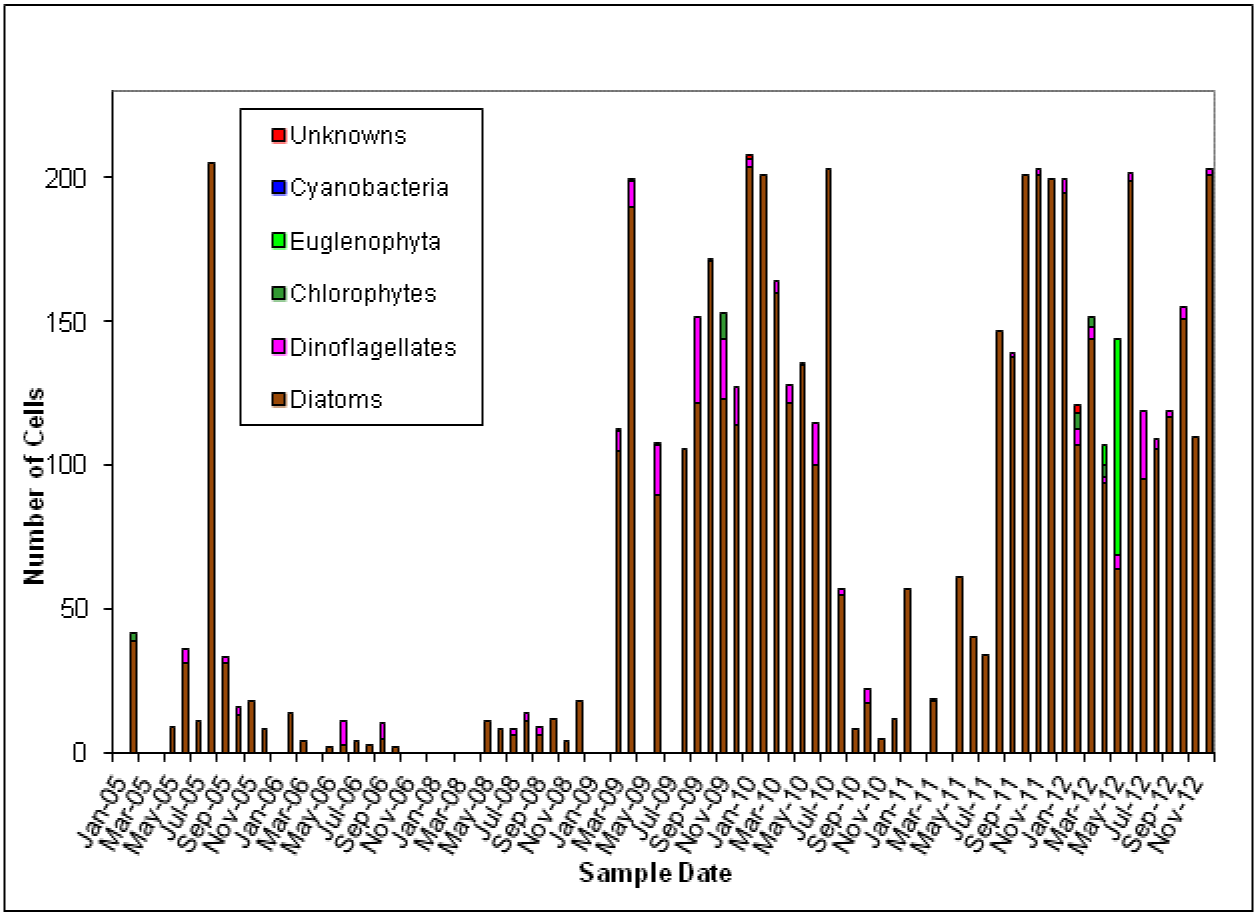


Figure 19 Dominant phytoplankton groups from January 2005 to December 2012 collected from Station 2 located in the upper Trinity Basin (see Fig. 1).

Phytoplankton genera found at each of the three stations throughout the course of the study are represented in Tables 12, 13 and 14 below. Plus signs indicate that the genus was present in the cell counts for the designated station and year, blank spaces indicate an absence. For all three stations, diatoms such as *Coscinodiscus*, *Navicula*, *Nitzschia* and *Pleurosigma* were common. Dinoflagellates were less consistent across the stations and study period, however, *Alexandrium*, *Ceratium* and *Prorocentrum* were among the more frequently identified genera. Euglenophytes were rare, occurring sporadically throughout the study period and only at Stations 1 and 2.

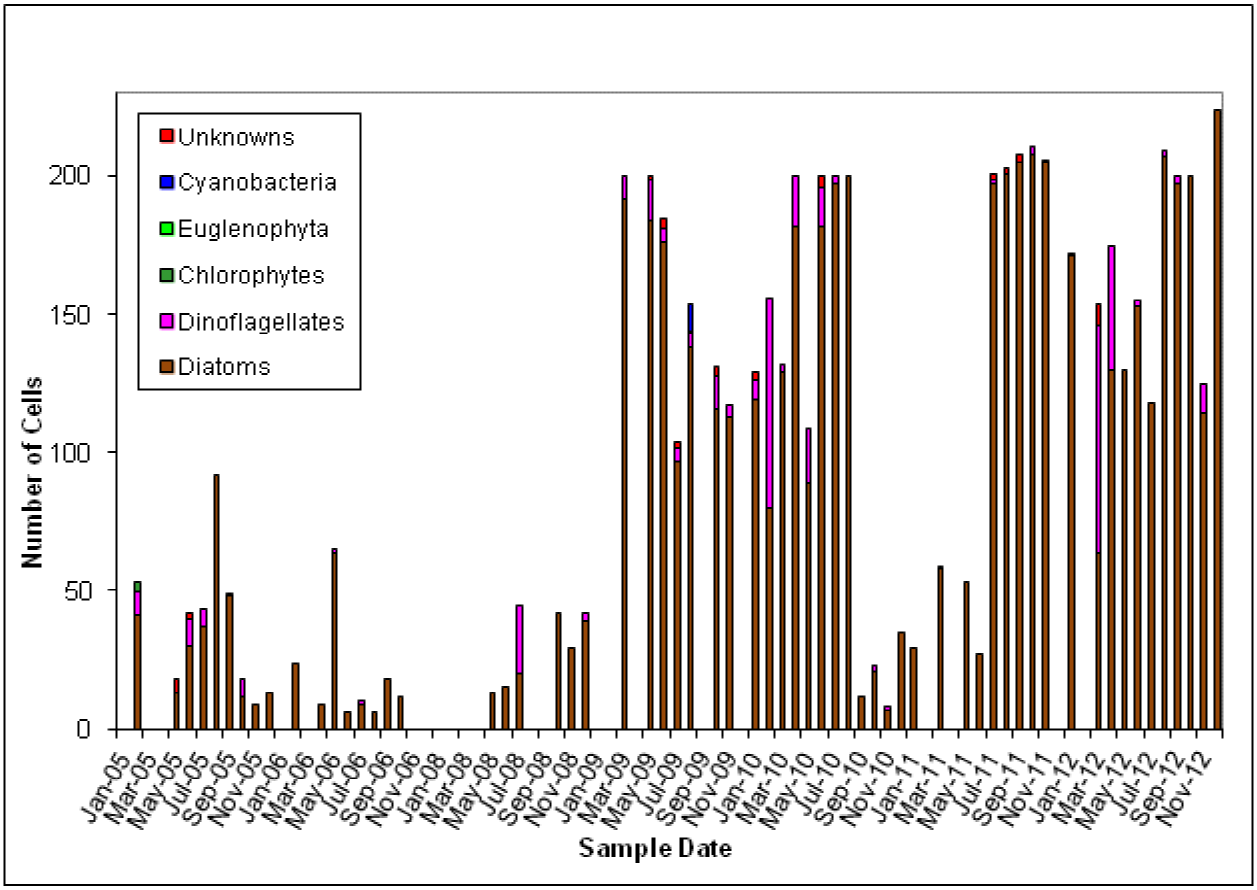


Figure 20 Dominant phytoplankton groups from January 2005 to December 2012 collected from Station 6 located in the upper Trinity Basin (see Fig. 1).

Chlorophytes were also uncommon in cell counts, but the most frequently identified genus was *Ankistrodesmus* which was observed at all three stations. *Scenedesmus* and *Pediastrum* regularly occurred at Station 1 which was closest to the Trinity River mouth. Cyanobacteria were rare at Stations 2 and 6, but *Merismopoedia* was found in several Station 1 samples. These results indicate that the community composition along the sampling transect from Station 1 to 6 follows a salinity gradient. Groups such as Chlorophytes and Cyanobacteria which are more common in freshwater influenced systems were more abundant at Station 1 near the river mouth while dinoflagellates which are more common in marine environments were sighted more frequently at Station 6 near the Gulf of Mexico.

Table 12 Presence-absence of phytoplankton genera at Station 1, 2005-06 and 2008-12.

Station 1		**2005**	**2006**	**2008**	**2009**	**2010**	**2011**	**2012**
DIATOMS	*Achnanthes*				+			+
	Actinoptychus							
	Amphora							
	Asterionellopsis				+	+	+	+
	Azpeitia						+	+
	Bacteriastrum				+			
	Chaetoceros				+	+	+	+
	Coscinodiscus	+	+		+	+	+	+
	Cylindrotheca	+	+	+	+		+	+
	Ditylum	+					+	+
	Entomoneis							
	Eucampia							
	Fragilariopsis							
	Grammatophora						+	+
	Guinardia				+		+	+
	Hemiaulus						+	
	Leptocylindrus				+	+	+	+
	Mastogloia							
	Navicula	+	+	+	+	+	+	+
	Nitzschia				+	+	+	+
	Odontella	+	+			+	+	
	Pinnularia							+
	Pleurosigma	+	+	+	+	+	+	+
	Pseudo-nitzschia				+	+	+	+
	Rhizosolenia				+	+	+	+
	Roperia				+			
	Skeletonema				+	+	+	
	Stephanopyxis				+			
	Thalassionema				+		+	+
	Thalassiosira	+	+	+	+	+	+	+
	Trachyneis				+			
DINOFLAGELLATES	*Akashiwo*	+	+		+	+		
	Alexandrium		+	+	+		+	+
	Ceratium	+	+	+		+	+	
	Dinophysis							
	Gonyaulx		+					
	Gymnodinium							
	Noctiluca	+			+	+		+
	Oxyphysis				+		+	+

	Polykrikos				+			
	Prorocentrum				+	+	+	+
	Protoperidinium	+	+		+	+		
CHLOROPHYTES	*Actinastrum*							+
	Ankistrodesmus	+	+		+			+
	Crucigenia							+
	Scenedesmus		+	+	+			+
	Pediastrum		+		+			+
EUGLENOPHYTA	*Euglenoids*				+			
CYANOBACTERIA	*Merismopoedia*		+		+			+
	Oscillatoria				+			
	Spirulina			+				
UNKNOWNS		+	+		+	+	+	

Table 13 Presence-absence of phytoplankton genera at Station 2, 2005-06 and 2008-12.

Station 2		**2005**	**2006**	**2008**	**2009**	**2010**	**2011**	**2012**
DIATOMS	*Achnanthes*				+			
	Actinoptychus			+				
	Amphora							
	Asterionellopsis			+	+	+	+	+
	Azpeitia						+	
	Bacteriasrtum							
	Chaetoceros					+	+	+
	Coscinodiscus	+	+	+	+	+	+	+
	Cylindrotheca	+	+		+	+	+	+
	Ditylum	+	+			+	+	+
	Entomoneis						+	
	Eucampia							
	Fragilariopsis							+
	Grammatophora						+	+
	Guinardia		+		+	+	+	+
	Hemiaulus				+			
	Leptocylindrus				+	+	+	+
	Mastogloia						+	
	Navicula	+		+	+	+	+	+
	Nitzschia	+	+		+	+	+	+
	Odontella	+			+	+	+	+
	Pinnularia							
	Pleurosigma	+	+	+	+	+	+	+
	Pseudo-nitzschia				+	+	+	+
	Rhizosolenia				+	+	+	+

	Roperia				+			
	Skeletonema				+		+	+
	Stephanopyxis							
	Thalassionema	+			+	+	+	+
	Thalassiosira	+	+	+	+	+	+	+
	Trachyneis				+			
DINOFLAGELLATES	*Akashiwo*	+	+	+				+
	Alexandrium				+			+
	Ceratium	+	+	+	+	+		
	Dinophysis	+						
	Gonyaulax							
	Gymnodinium							
	Noctiluca				+	+		+
	Oxyphysis		+		+	+	+	+
	Polykrikos							
	Prorocentrum	+			+	+	+	+
	Protoperidinium				+			
CHLOROPHYTES	*Actinastrum*							
	Ankistrodesmus	+			+			+
	Crucigenia							
	Scenedesmus							+
	Pediastrum							
EUGLENOPHYTA	*Euglenoids*				+			+
CYANOBACTERIA	*Merismopoedia*							
	Oscillatoria							
	Spirulina							
UNKNOWNS					+	+		

Table 14 Presence-absence of phytoplankton genera at Station 6, 2005-06 and 2008-12.

Station 6		**2005**	**2006**	**2008**	**2009**	**2010**	**2011**	**2012**
DIATOMS	*Achnanthes*							+
	Actinoptychus				+	+		
	Amphora				+			
	Asterionellopsis			+	+	+	+	+
	Azpeitia					+		+
	Bacteriasrtum							
	Chaetoceros	+				+	+	+
	Coscinodiscus	+	+	+	+	+	+	+
	Cylindrotheca	+	+		+	+	+	+
	Ditylum					+	+	+
	Entomoneis							

	Eucampia						+	
	Fragilariopsis							+
	Grammatophora				+		+	
	Guinardia	+	+	+	+	+	+	+
	Hemiaulus							+
	Leptocylindrus				+	+	+	+
	Mastogloia							
	Navicula	+	+	+	+	+	+	+
	Nitzschia	+	+		+	+	+	+
	Odontella	+	+	+	+	+	+	+
	Pinnularia	-	+					
	Pleurosigma	+	+	+	+	+	+	+
	Pseudo-nitzschia	+			+	+	+	+
	Rhizosolenia				+	+	+	+
	Roperia							
	Skeletonema				+	+	+	+
	Stephanopyxis							
	Thalassionema	+	+		+	+	+	+
	Thalassiosira	+	+	+	+	+	+	+
	Trachyneis				+			
DINOFLAGELLATES	*Akashiwo*	+						
	Alexandrium			+	+			+
	Ceratium	+			+	+	+	
	Dinophysis	+						
	Gonyaulax							
	Gymnodinium				+			
	Noctiluca							
	Oxyphysis					+	+	+
	Polykrikos							
	Prorocentrum	+			+	+		+
	Protoperidinium		+		+			+
CHLOROPHYTES	*Actinastrum*							
	Ankistrodesmus	+			+			
	Crucigenia							
	Scenedesmus							
	Pediastrum							
EUGLENOPHYTA	*Euglenoids*							
CYANOBACTERIA	*Merismopoedia*							
	Oscillatoria				+			
	Spirulina							
UNKNOWNS		+			+	+	+	+

4.4 High spatial and temporal resolution mapping of Galveston Bay water quality parameters from March 2010 to December 2012

4.4.1 Dataflow maps

The physio-chemical parameters mapped in Galveston Bay include water temperature, salinity, water clarity, dissolved organic matter, chl *a*, phycocyanin and phycoerythrin. After sensor calibration and blank correction, data were imported into Surfer (Version 8.0), a 3D contouring and surface plotting program (used default kriging method). Given the project generated >200 maps, below we only show a selection to highlight the major changes in the Bay.

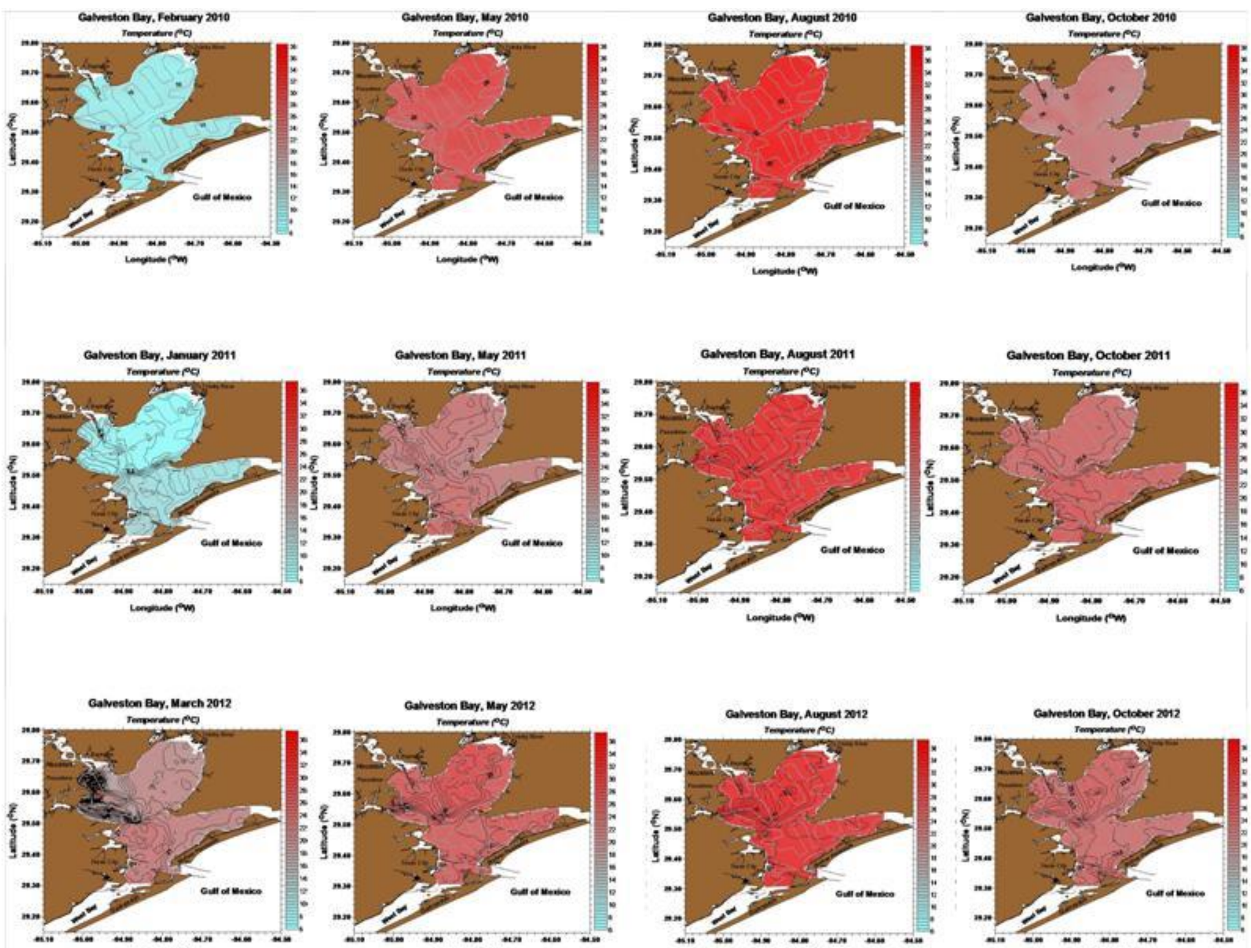


Figure 21 High spatial and temporal resolution maps of temperature (°C) measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales are the same for all maps and the temperature range is 6-36°C (teal to red respectively).

The surface water temperature ranges seen in these maps (Fig. 21) are typical for this Bay (Davis et al. 2007; Quigg et al. 2007; 2009a, b). Winter lows are generally 6.09 to 8.48 °C while summer highs are 32.95 to 34.54 °C. These salinity maps (Fig. 22) show that drought conditions reflected in the high salinity waters across the Bay began in the fall of 2010 and continued through to the end of 2011 with salinities ranging from 10.05 to 37.86 (min-max) throughout the Bay from January 2011-December 2011. In the spring of 2012, there were once again freshets that are typically associated with this time of year (Fig. 17) which introduced significant quantities of freshwater into the Bay. The range of salinities in 2010 and 2012 was 0 to 35.32 and 0 to 34.6 (min-max) respectively.

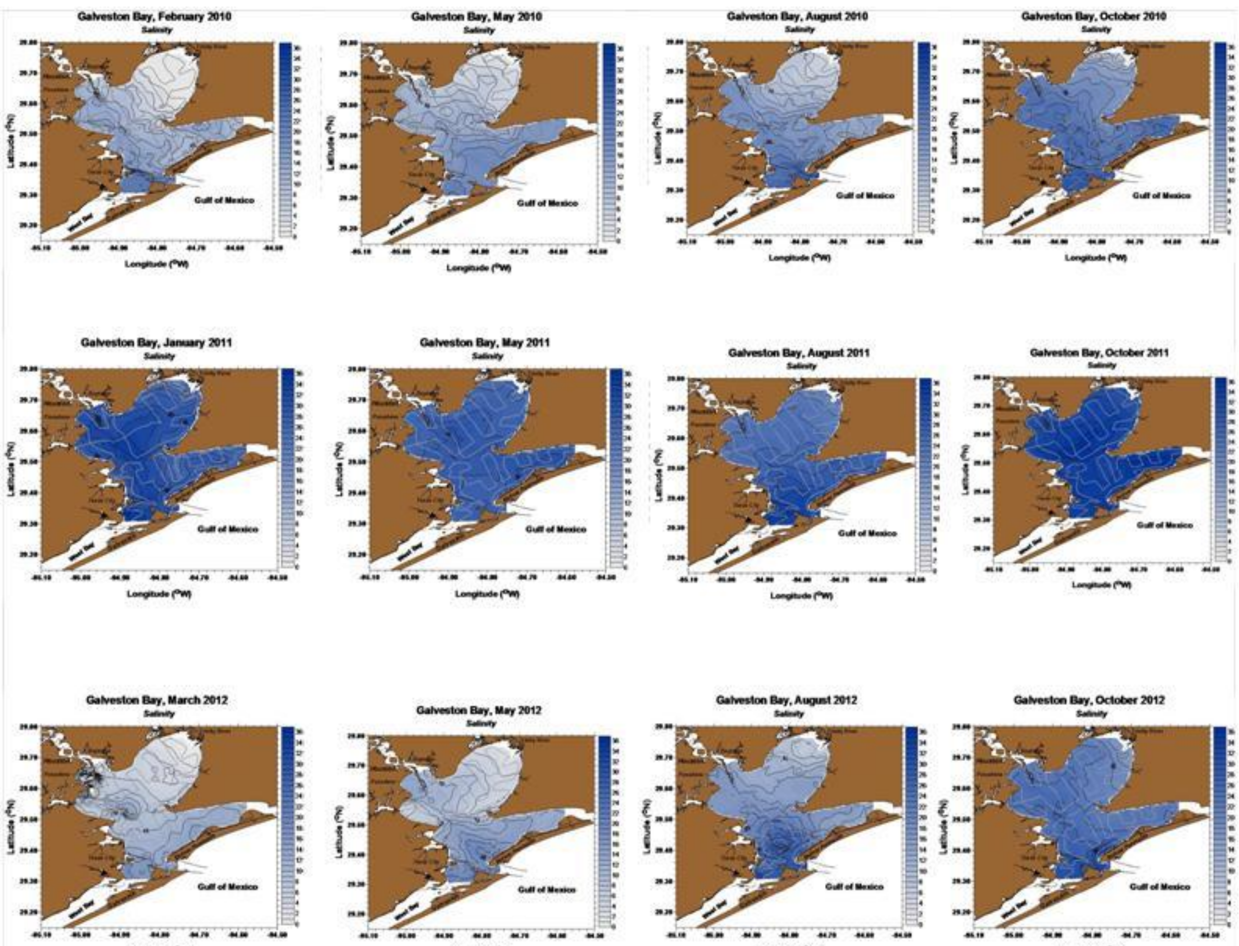


Figure 22 High spatial and temporal resolution maps of salinity measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the salinity range is 0-36 (white to blue respectively).

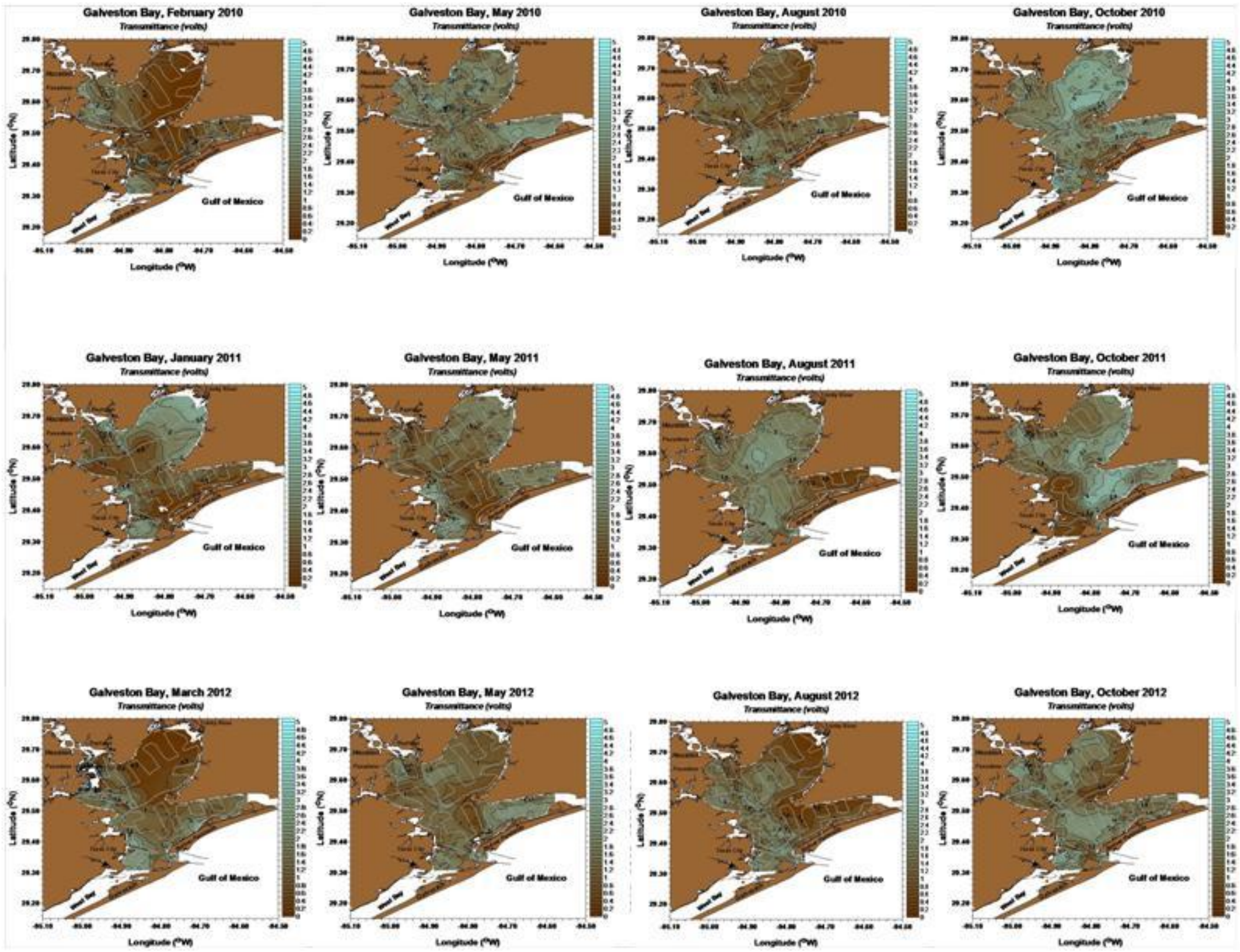


Figure 23 High spatial and temporal resolution maps of water clarity (beam transmittance), measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the water clarity range is 0-5 volts (brown to teal respectively).

Please note: the units herein are volts; we will not be able convert to concentrations for final report as the standard is still not available.

Galveston Bay is a shallow system and is therefore prone to wind mixing and turbid conditions. These water clarity maps (Fig. 23) are often an inverted view of the salinity maps, especially in the Trinity River estuary where freshets from the river can dramatically increase the turbidity. A good example of this can be seen in the February 2010 and March 2012 salinity and water clarity maps respectively. Higher freshwater inflows into the Bay lower salinities but increase turbidity.

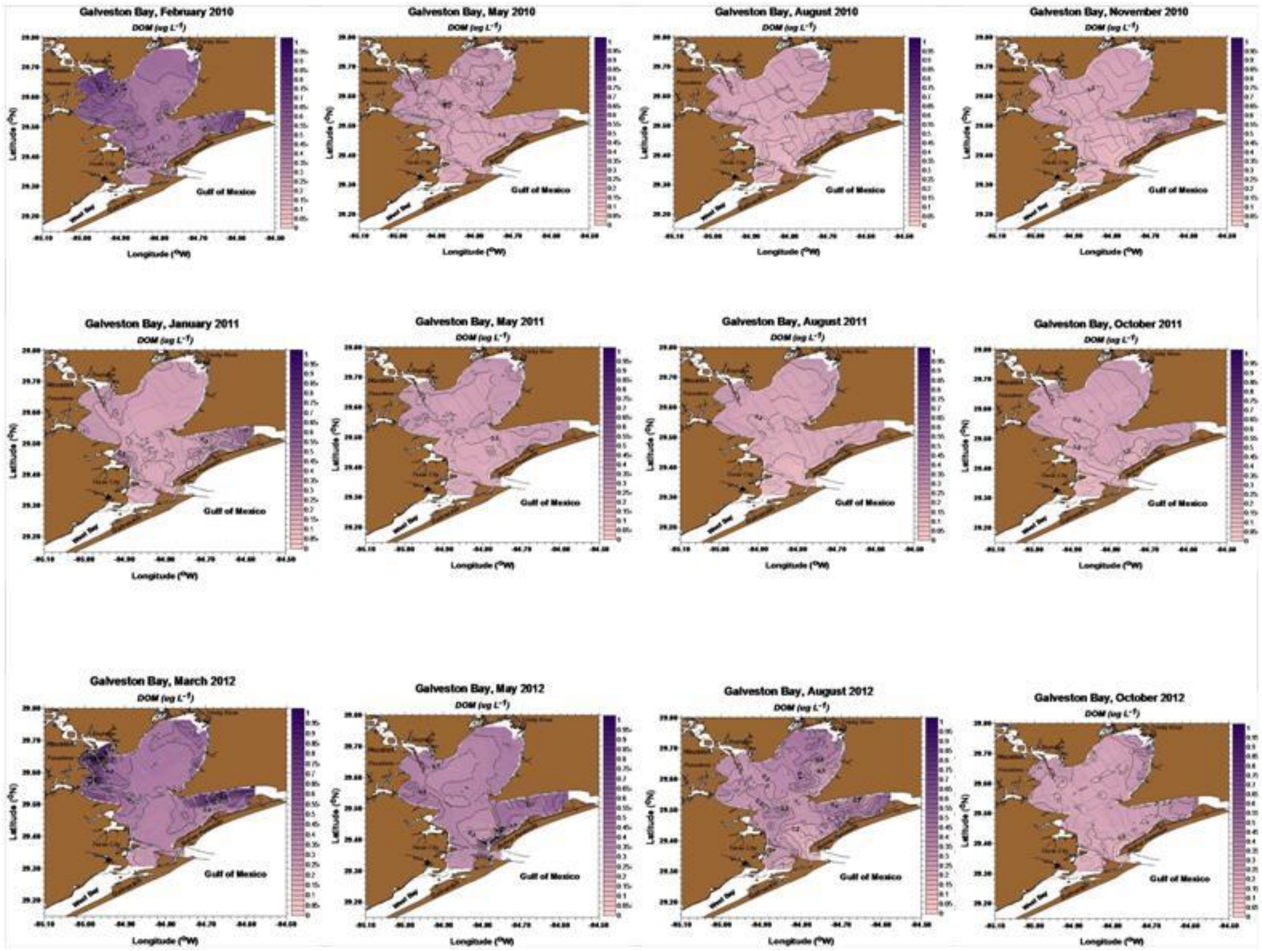


Figure 24 High spatial and temporal resolution maps of dissolved organic matter (DOM) measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the DOM range is 0-5 volts (pink to purple respectively).

Please note: the units herein are volts; we will not be able convert to concentrations for final report as the standard is still not available.

There appears to also be an inverse relationship between dissolved organic matter (DOM) measured in Galveston Bay (Fig. 24) and freshwater inflows (Fig. 17). DOM concentrations were typically low in 2011 during the drought. However, with the large spring freshets, DOM concentrations in the Bay generally increased. DOM ranged from 0.04 to 0.71 for most of 2011. However, in February 2010 and March 2012, DOM increased to 0.7 to 0.81 and 0.24 to 0.9 respectively (Fig. 24).

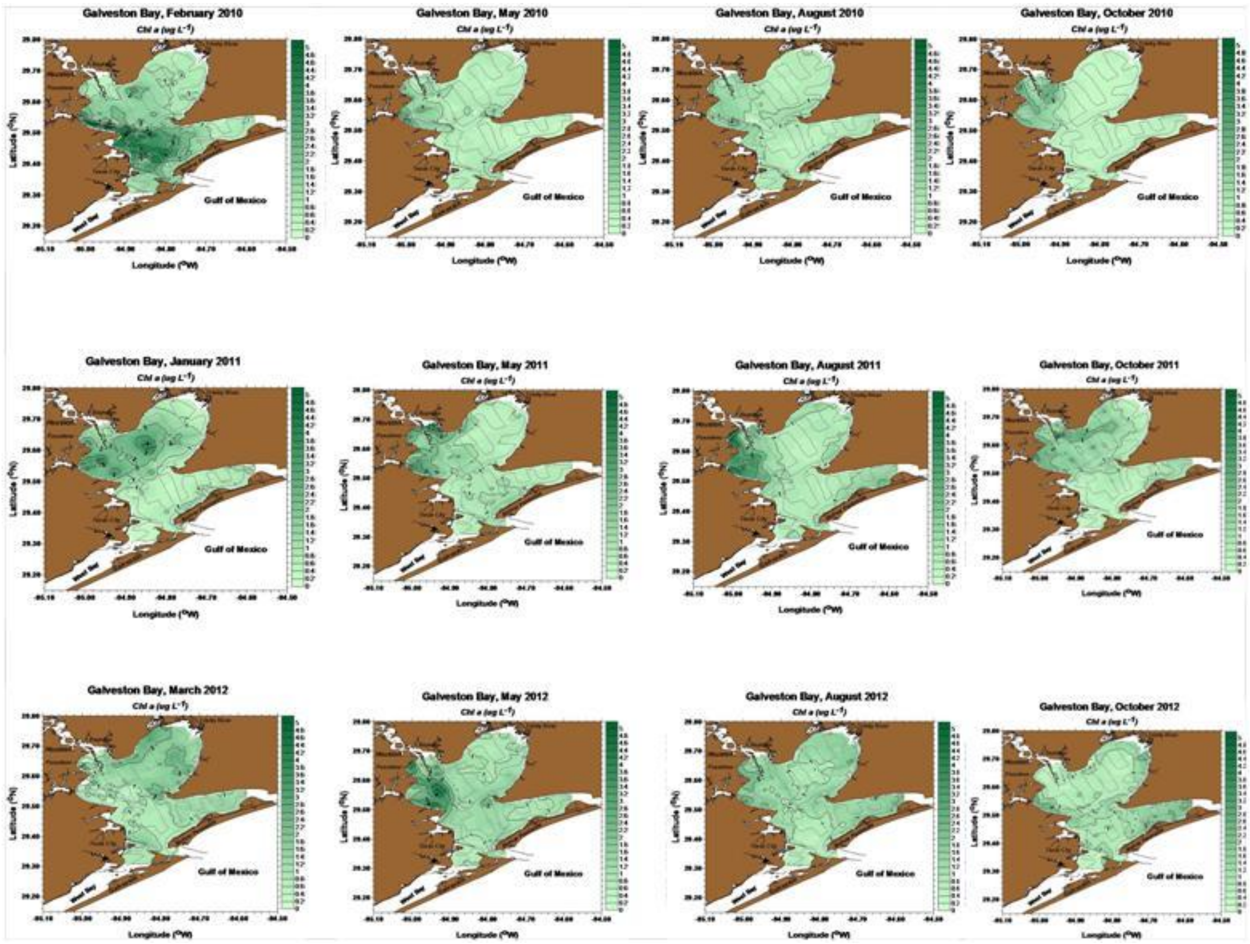


Figure 25 High spatial and temporal resolution maps of chlorophyll *a* measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the DOM range is 0-5 volts (light green to dark green respectively).

Please note: the units herein are volts; we will not be able convert to concentrations for final report as the standard is still not available.

Chlorophyll *a* concentration is measured as it is a proxy for phytoplankton biomass. Chlorophyll concentrations were highly variable during the project period (Fig. 25). There were typically higher concentrations on the west side of the Bay than on the east side of the Bay. Increases in chl *a* were also observed after several of the large freshets, but the magnitude was dependent on the timing of the freshet. Hot spots (high concentrations relative to area around them) of chlorophyll were observed in 2011 (Fig. 25). It is not clear if this reflects a general population increase or a localized bloom. There are a number of possible explanations which we will explore in the discussion below.

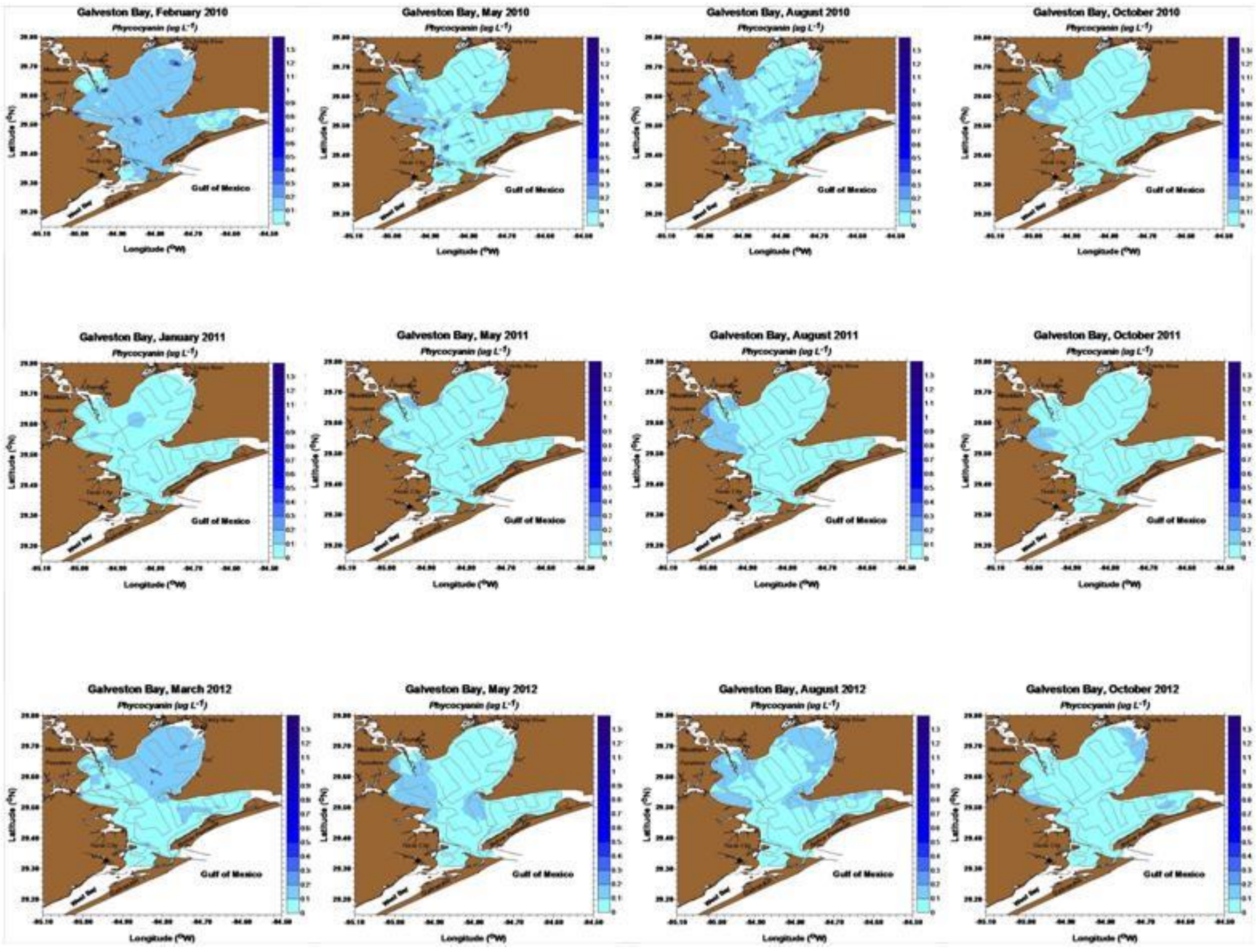


Figure 26 High spatial and temporal resolution maps of phycocyanin measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the 0-1.3 volts (teal to dark blue respectively).

Please note: the units herein are volts; we will not be able convert to concentrations for final report as the standard is still not available.

Phycocyanin is an accessory pigment that is commonly associated with cyanobacteria. From the maps above, it appears that certain conditions favor phytoplankton which utilize this pigment (Fig. 26). In particular, hotspots appear after major freshets – see February 2010 and March 2012 as well as during the summer months in the upper western side of Galveston Bay (Fig. 26).

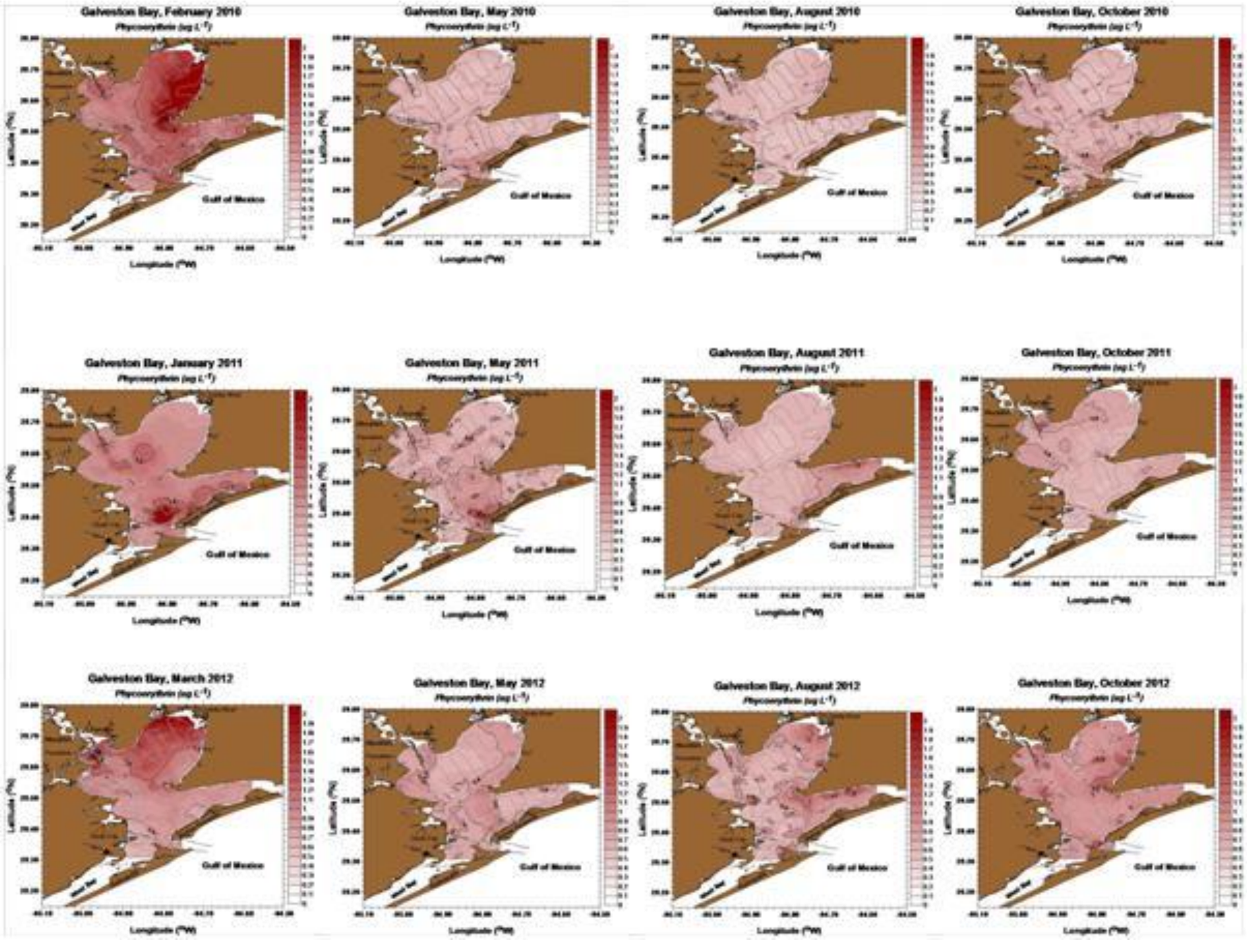


Figure 27 High spatial and temporal resolution maps of phycoerythrin measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. A selection of maps is included to show the major seasonal variations in this parameter. Scales were the same for all maps and the 0-2 volts (white to maroon respectively).

Please note: the units herein are volts; we will not be able convert to concentrations for final report as the standard is still not available.

Phycoerythrin is an accessory pigment that is commonly associated with cryptophytes in particular although it can also be found in some cyanobacteria and xanthophyta. From the maps above, it appears that certain conditions favor phytoplankton which utilize this pigment (Fig. 27). In particular, hotspots appear after major freshets – see February 2010, January 2011 and March 2012 but less so in a consistent manner during the summer months (Fig. 27).

4.4.2 Water quality measured at fixed stations

At the fixed stations (Fig. 1; Table 4), discrete water samples were collected to measure dissolved nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_3) and total particulate nitrogen (TN) and total particulate phosphorus (TP) from 2010 to 2012. The Trinity and San Jacinto Rivers are important sources of nutrients to Galveston Bay, with freshwater inflows and returned flows being the two major sources. On the other hand, the Gulf of Mexico is generally a poor nutrient source to the Bay. While dissolved nutrient concentrations are those most bioavailable to phytoplankton, total particulate nutrient concentrations are nonetheless an important component of the water quality characteristics of any system and may be available to some fraction of the community. Herein we present findings from four of these fixed stations to reveal the gradients in the water quality parameters in the Bay.

Station 12 is located (see coordinates in Table 4) most adjacent to the mouth of the Trinity River, located in the upper Trinity River Basin (Fig. 28). Dissolved and total nutrient concentrations were highly variable and did not appear to correlate with river flow, that is, high nutrients with high flows and vice versa. During 2011, the year of the drought, dissolved inorganic nitrogen (DIN) calculated as the sum of nitrate, nitrite plus ammonium were generally lower than in either 2010 or 2012 but not significantly. Only during 8 of the 36 months did DIN concentrations exceed 1 μmol/L suggesting that most available nitrogen (N) was being consumed by phytoplankton (Fig. 28). Phosphorus (P) concentrations ranged from 0.45 to 7.5 μmol/L at this station. By contrast, TN concentrations did appear to follow patterns in river flow during the study period, reaching ~115 μmol/L at times of peak discharge (Fig. 28). TP was variable at this station but did not follow patterns in river discharge (Fig. 28).

Station 6 located (see coordinates in Table 4) in the middle of Galveston Bay generally had lower nutrient concentrations than those measured at station 1, particularly during 2010 (Fig. 29). DIN and P concentrations were frequently <2 μmol/L and <5 μmol/L respectively suggesting that most available N and P were being consumed by phytoplankton (Fig. 29). TN and TP concentrations followed patterns observed at station 1 (Fig. 29).

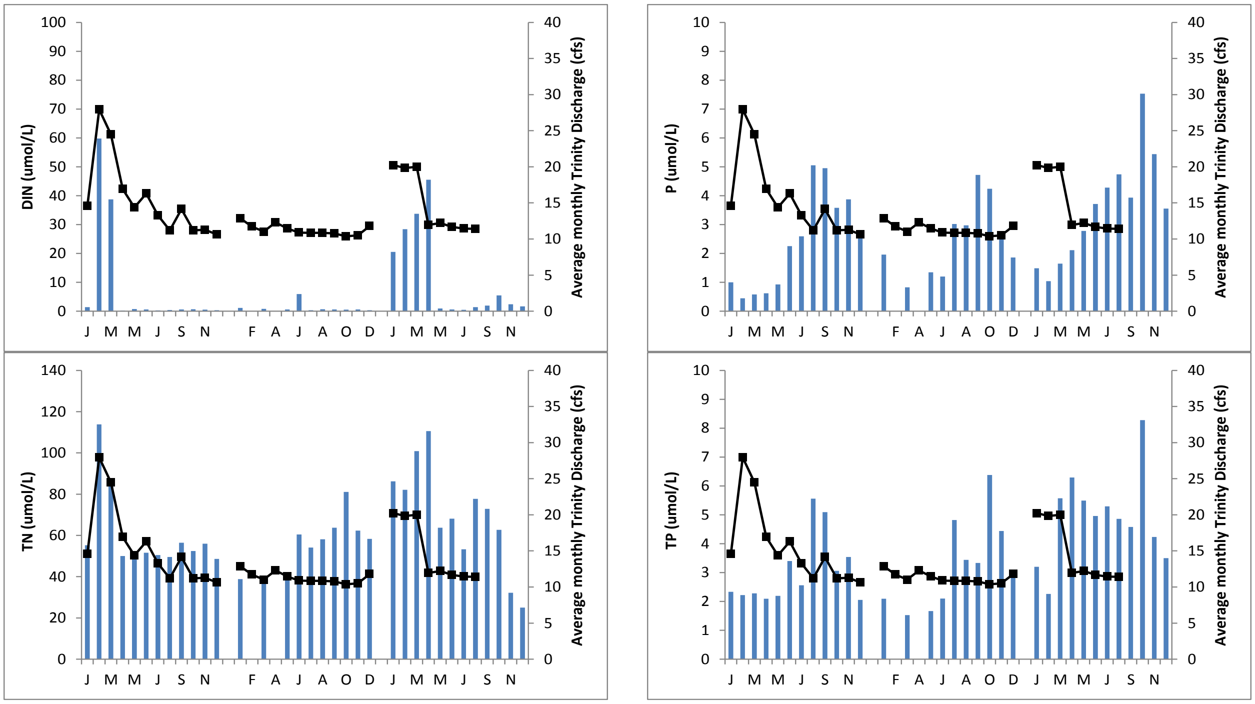


Figure 28 Dissolved inorganic nitrogen (DIN) and phosphorus (P; top) and total nitrogen (TN) and total phosphorus (TP; bottom) measured from 2010 to 2012 at station 12 (see Table 4) adjacent to the Trinity River discharge point. All nutrients were measured as μmol/L. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

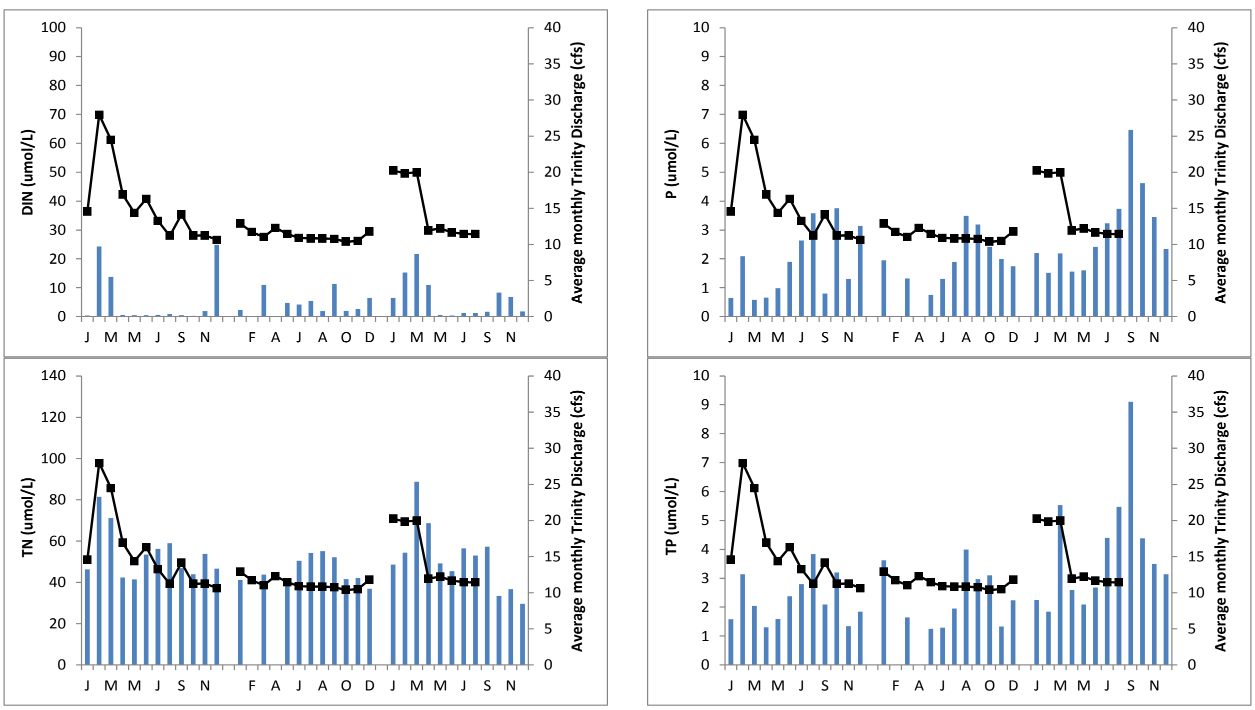


Figure 29 Dissolved inorganic nitrogen (DIN) and phosphorus (P; top) and total nitrogen (TN) and total phosphorus (TP; bottom) measured from 2010 to 2012 at station 6 (see Table 4) adjacent to the San Jacinto River discharge point. All nutrients were measured as μmol/L. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

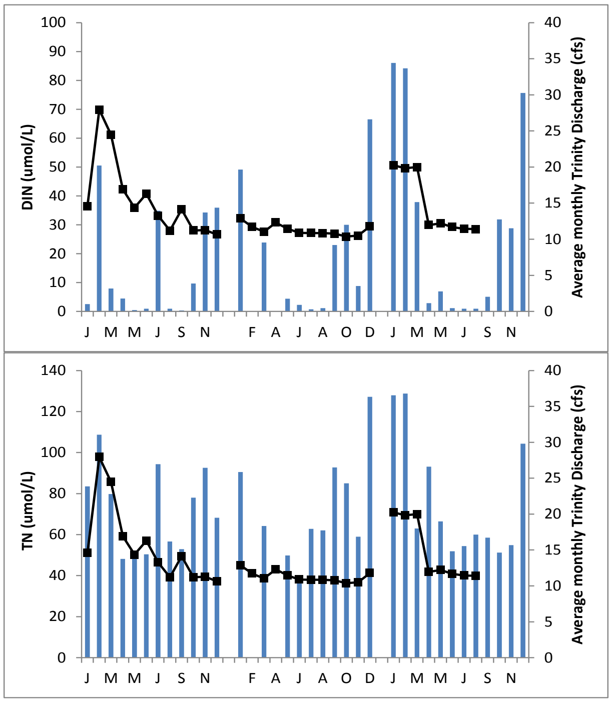


Figure 30 Dissolved inorganic nitrogen (DIN) and phosphorus (P; top) and total nitrogen (TN) and total phosphorus (TP; bottom) measured from 2010 to 2012 at station 4 (see Table 4) adjacent to the Trinity River discharge point. All nutrients were measured as μmol/L. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

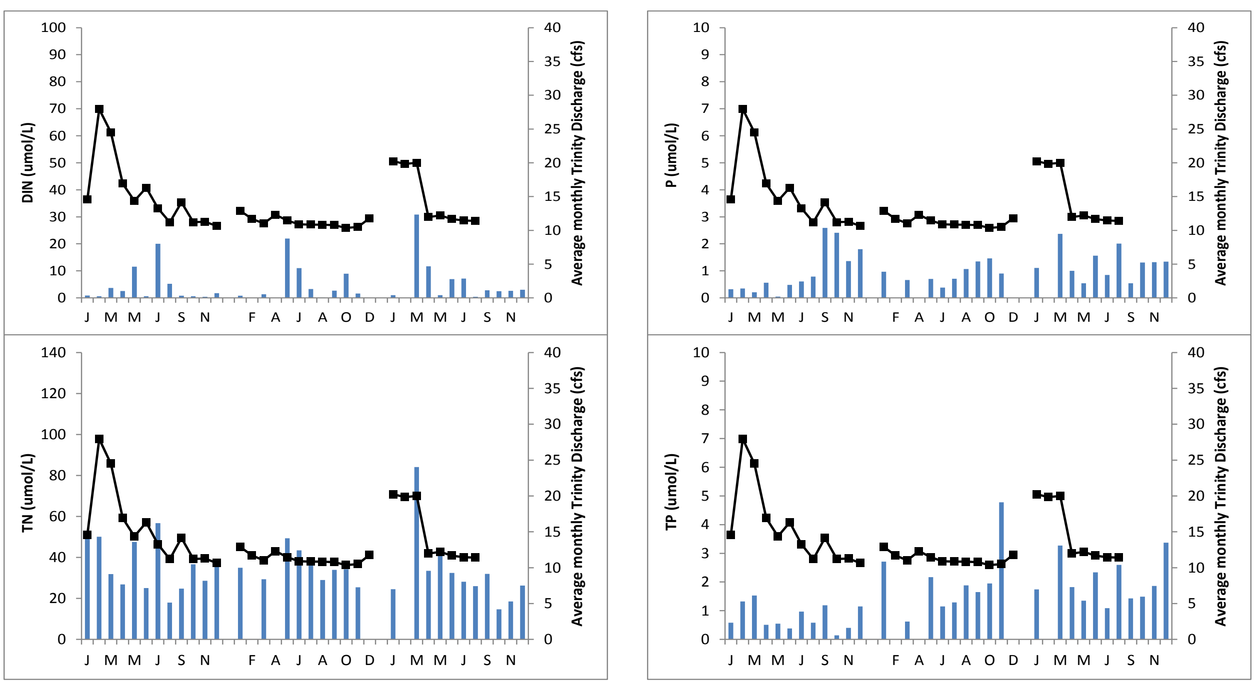


Figure 31 Dissolved inorganic nitrogen (DIN) and phosphorus (P; top) and total nitrogen (TN) and total phosphorus (TP; bottom) measured from 2010 to 2012 at station 29 (see Table 4) adjacent to the Trinity River discharge point. All nutrients were measured as μmol/L. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

Station 4 is located (see coordinates in Table 4) most adjacent to the mouth of the San Jacinto River, near the Houston Ship Channel, and frequently had much higher nutrient concentrations than those measured at any other station (Fig. 30). Dissolved inorganic nitrogen and phosphorus were between 10 and 70 µmol/L and 2 and 8 µmol/L respectively at least half the time samples were collected during the project period. By contrast, TN and TP concentrations were always greater than 37 µmol/L and ~1 µmol/L respectively.

Station 29 located (see coordinates in Table 4) closest to the Gulf of Mexico had generally very low DIN and P concentrations, much lower than those present at any other station (Fig. 31). DIN and P were <5 µmol/L and <2.5 µmol/L most of the times samples were collected. TN and TP concentrations were most often less than 37 µmol/L and ~1 µmol/L respectively by comparison to what was observed at Station 4 (Fig. 30).

In general, a DIN: P ratio in the range of 7:1 to 12:1 by mass is associated with plant growth being limited by neither phosphorus nor nitrogen. If the DIN:P ratio is greater than 12:1, phosphorus tends to be limiting, and if the DIN:P ratio is less than 7:1, nitrogen tends to be limiting (Howarth and Marino 2006). With just a couple of exceptions, DIN:P ratios were always < 1 at stations 12, 6 and 29 suggesting phytoplankton were N limited at these locations for most of the study period, especially during 2011 (Fig. 32). By contrast, at station 4, we found DIN:P ratios were frequently in the range of 7:1 to 12:1, especially from April-May to December. In the winter time, from January to March, DIN:P ratios were greater than 14 indicative of P limitation at this station.

Phytoplankton biomass estimated from chl *a* concentrations was highly variable both spatially and temporally when measured at the discrete stations (Fig. 33) as well as when measured using the Dataflow (Fig. 25). At stations 4, 6 and 12, a lower concentration of chl *a* was measured during 2011 relative to 2010 and 2012, ~ 15 µg/L compared with ~30 - 50 µg/L (Fig. 33). At station 29, chlorophyll concentrations were 7.4 µg/L ± 2.9 (standard deviation) with one exception. In February 2010, chlorophyll at this station was 34 µg/L (Fig. 33). As with the Dataflow maps, patterns in chlorophyll concentrations did not vary in relation to freshwater inflows from the Trinity River (Fig. 33).

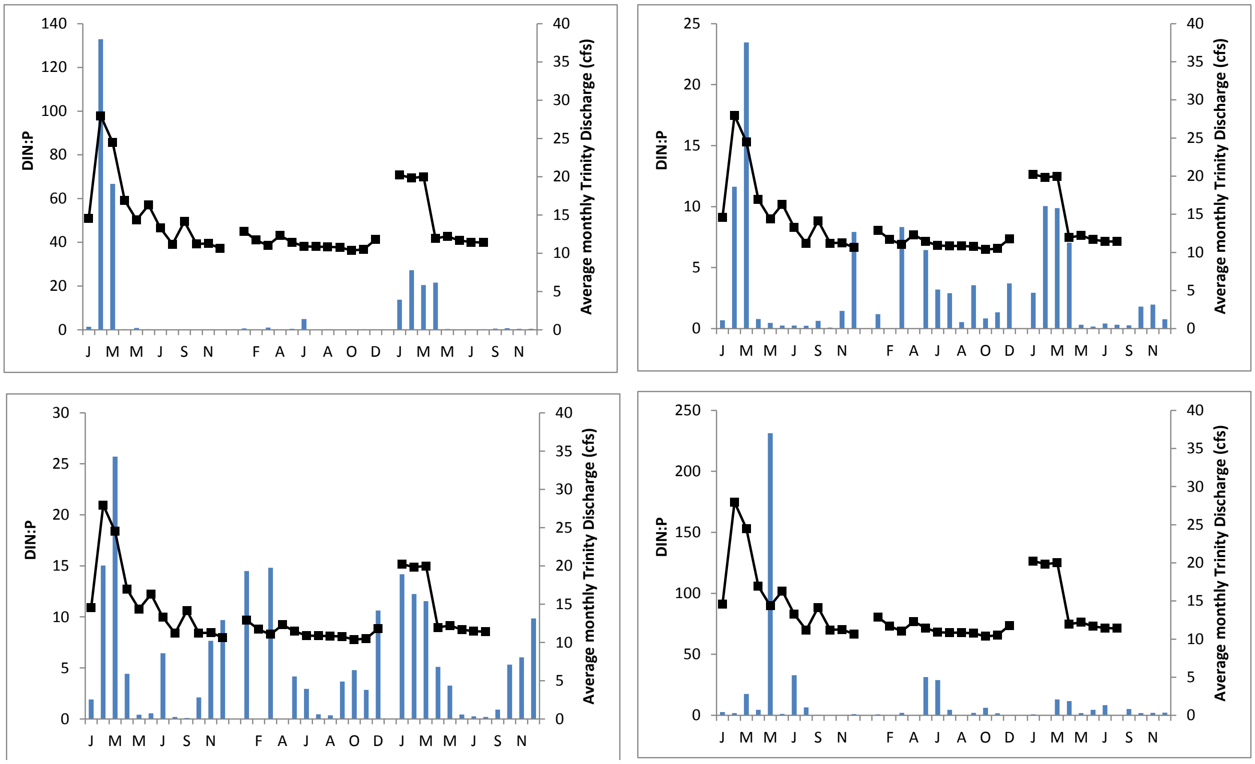


Figure 32 Dissolved inorganic nitrogen (DIN):phosphorus (P) ratios calculated for station 12 (top left), station 6 (top right), station 4 (bottom left) and station 29 (bottom right) during 2010 to 2012. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

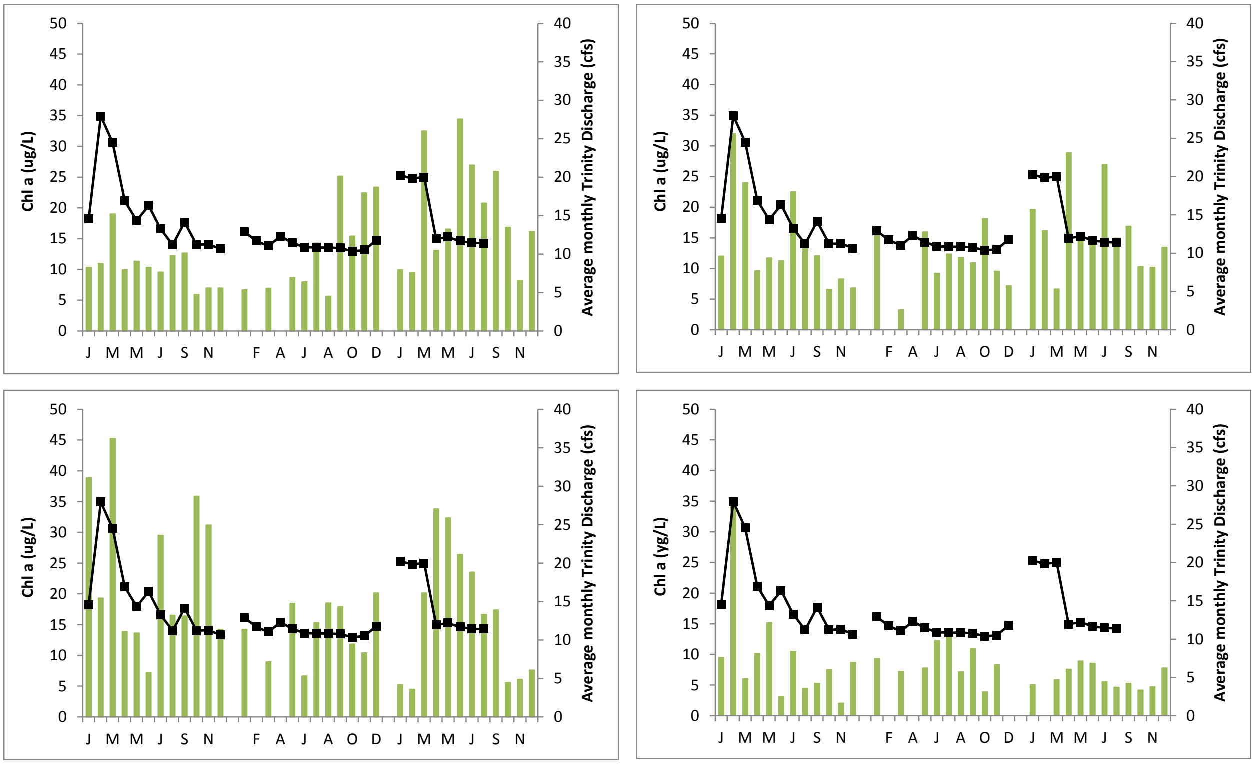


Figure 33 Chlorophyll *a* (ug/L) measured at station 12 (top left), station 6 (top right), station 4 (bottom left) and station 29 (bottom right) during 2010 to 2012. The secondary y-axis is the average monthly Trinity River discharge (cfs) data collected from the USGS.

4.5 Influence of nutrient and sediment load on the phytoplankton in Galveston Bay from March 2010 to December 2012

Figures 34 to 39 represent the findings of all 10 treatments performed during the RLAs: Control, +N, +NP, +P, +NA, +A, +Si, +ALL, G (grazing control), S (shaded light treatment) at the six fixed stations (see Table 4). In the initial experimental design, RLAs were planned for typical high flow (March; spring) and low flow (July; summer) periods. Whilst we hit these in 2010 and 2012, the findings for 2011 reflect a year-long low flow.

Figures 34 to 37 show that the +NP and +ALL treatments combined accounted for the greatest increase in chl *a* concentrations (phytoplankton biomass) in both the spring and summer months of 2010 and 2011. This change suggests co-limitation of phytoplankton by both nitrate and phosphate at all of the six fixed stations. A finding which is consistent with the prediction of many of the DIN:P ratios measured at the same stations (Fig. 32). The least change in biomass occurred at station 12 in March 2010 (Fig. 34) and March 2011 (Fig. 36) relative to the other stations during the same sampling periods. Station 12 is located in the upper Trinity River basin. During 2012, we found that phytoplankton appeared to be co-limited by nitrate plus ammonium (+NA) as often as in the +NP and +ALL treatments (Fig. 38 and 39).

Relative to the control treatments, we did not see a significant change in the +P or +Si treatments in any season or year suggesting the addition of these nutrients typically were not limiting (Fig. 34 to 39). We found chl *a* biomass doubled or tripled in the +A (ammonium) treatments relative to the control except at some stations in July 2011 (Fig. 37) in which no change was observed. Grazers were excluded from the +G (or grazing treatment) to determine if they limited increases in phytoplankton biomass in Galveston Bay. Given the chl *a* biomass in the grazing treatments was similar to that in the controls, we find that grazers do not limit primary productivity in Galveston Bay (Fig. 34 to 39).

Further, we were interested in measuring the role of sediment loading on regulating phytoplankton biomass. Whilst there is no direct way to measure this, we used shade cloth to reduce light penetration to the cubitainers in an effort to mimic the reduction in water clarity associated with sediment loading. We hypothesized this would be more important during high flow periods (March) than during low flow periods (July). We found that in all cases except August 2010, phytoplankton at station 12 were light limited, that is, their biomass increased relative to the control treatments in the +S (shade/sediment) treatments (Fig. 34 to 39). We also found this to be the case for phytoplankton at station 4 in March 2011 and March 2012 (Figs. 36 and 38 respectively). Stations 12 and 4 are adjacent to the Trinity and San Jacinto River mouths respectively suggesting these phytoplankton frequently deal with light stress induced by sediment loading.

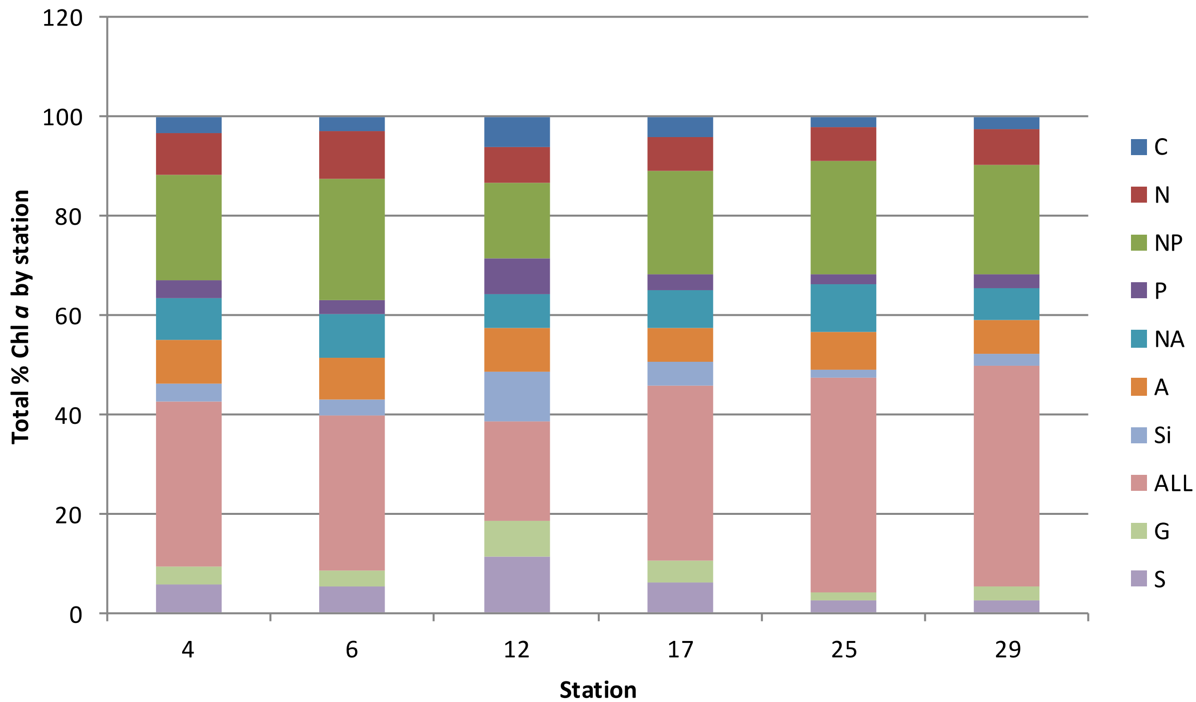


Figure 34 March 2010 chlorophyll *a* concentrations (µg/L) of all 10 treatments by fixed station.

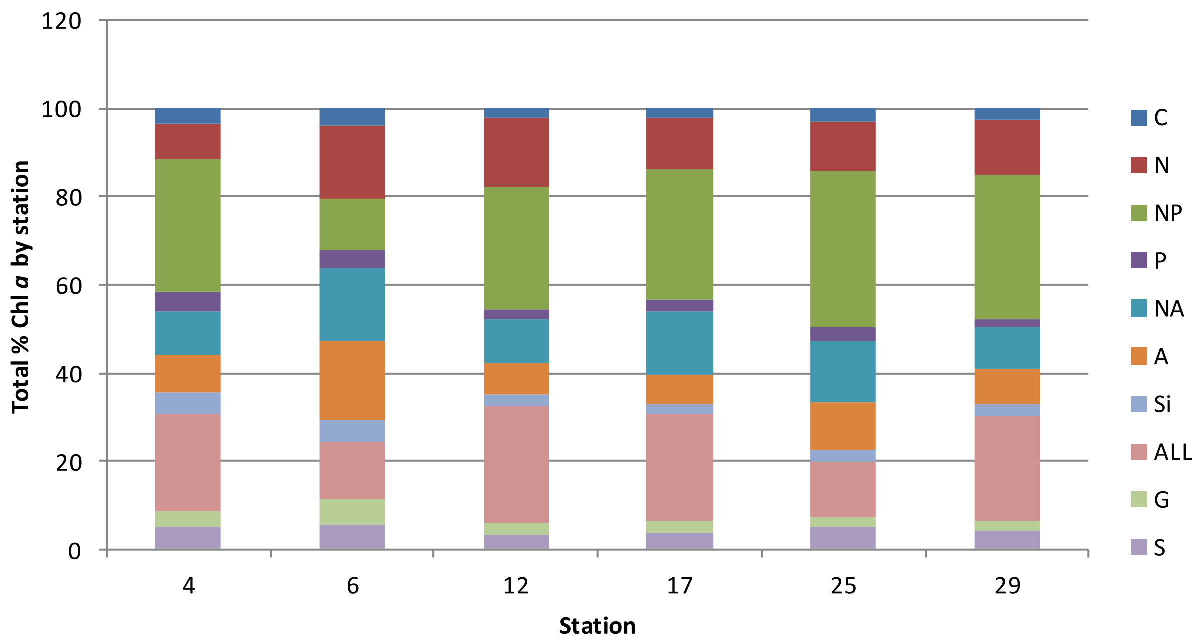


Figure 35 August 2010 chlorophyll *a* concentrations (µg/L) of all 10 treatments by fixed station.

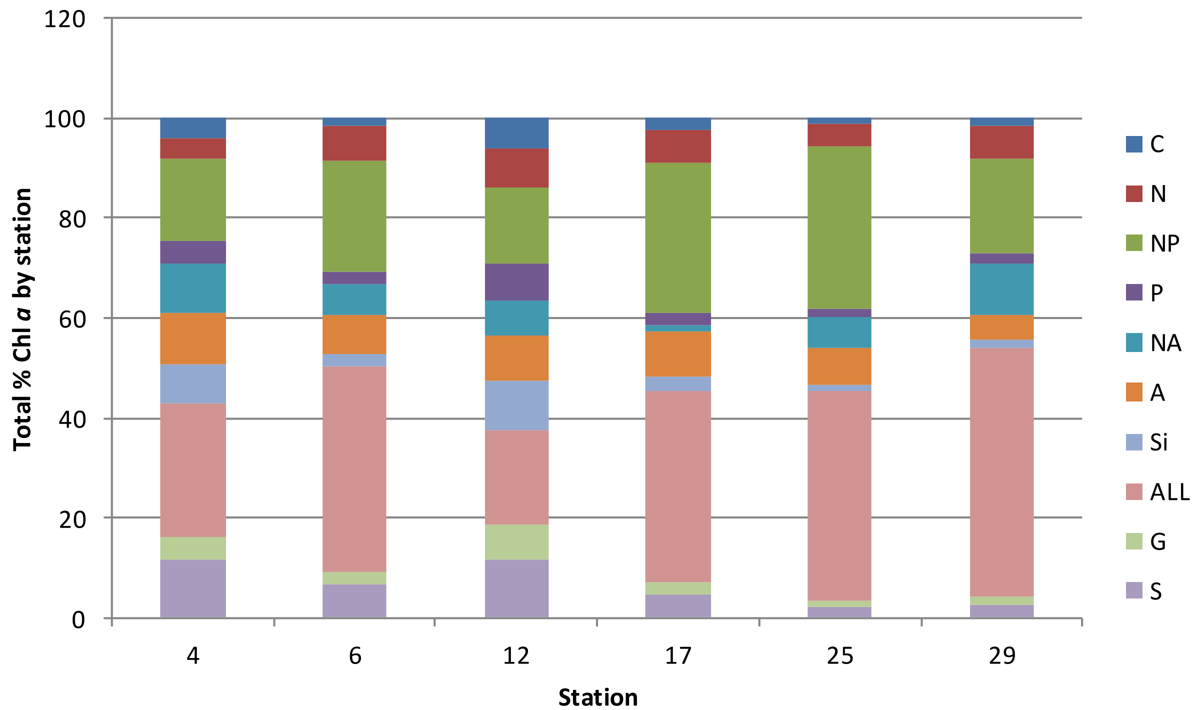


Figure 36 March 2011 chlorophyll *a* concentrations (μg/L) of all 10 treatments by fixed station.

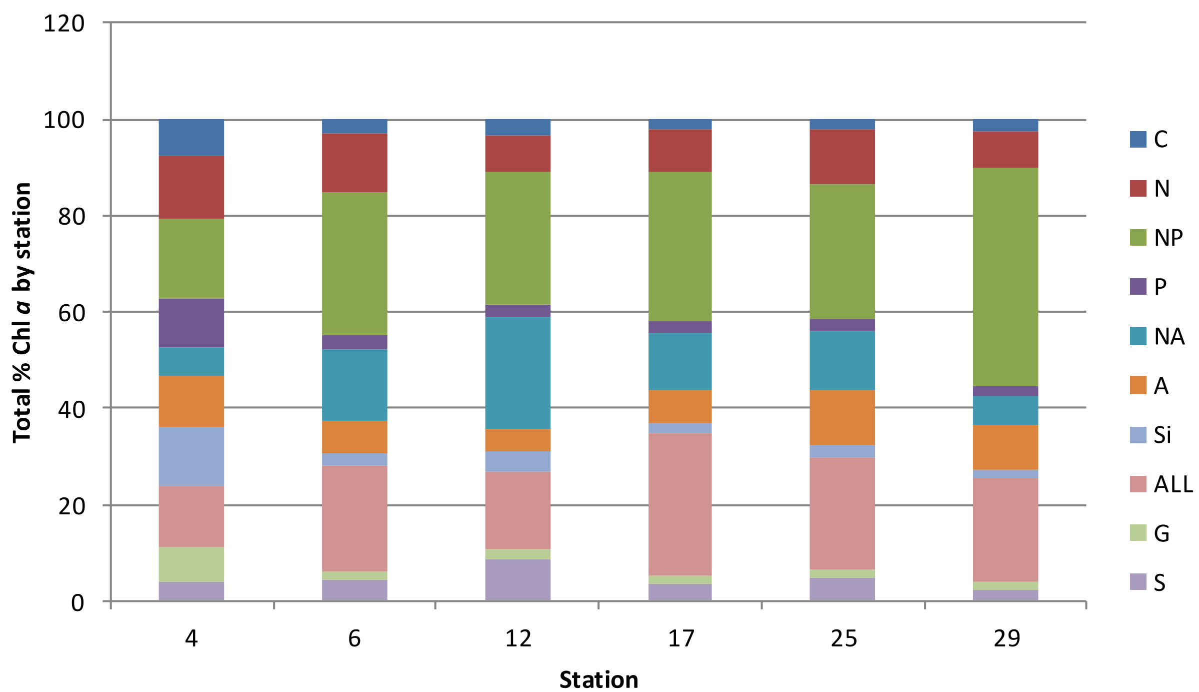


Figure 37 July 2011 chlorophyll *a* concentrations (μg/L) of all 10 treatments by fixed station.

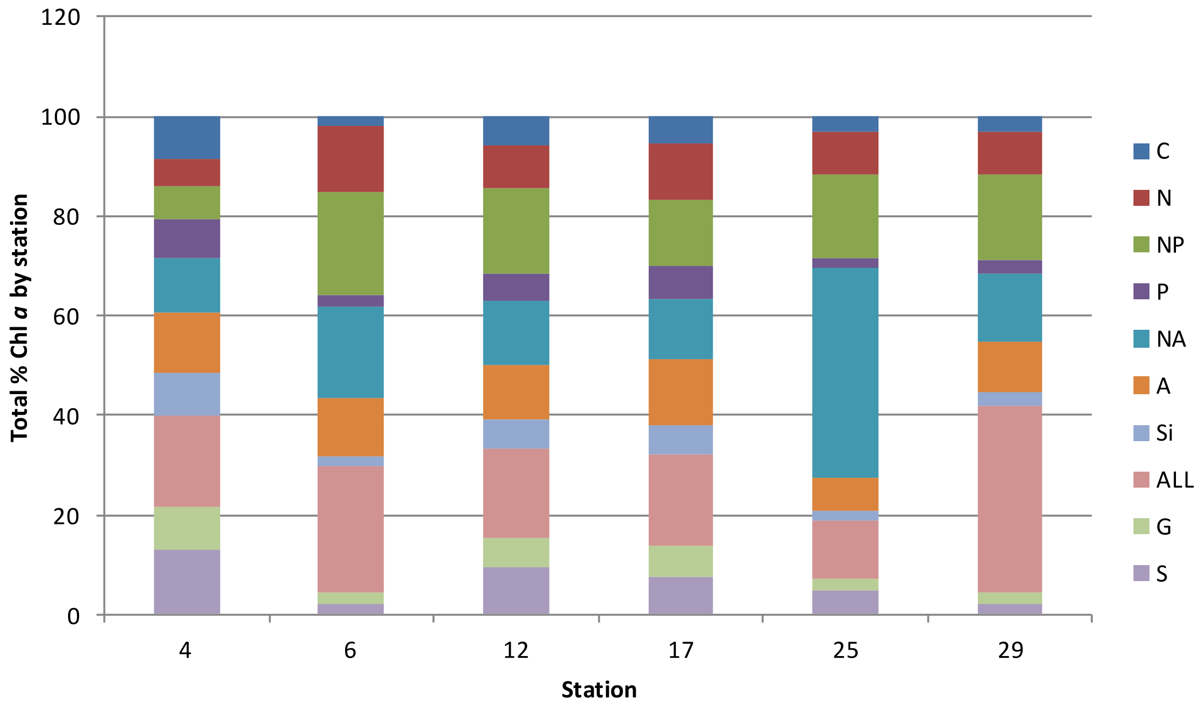


Figure 38 March 2012 chlorophyll *a* concentrations (µg/L) of all 10 treatments by fixed station.

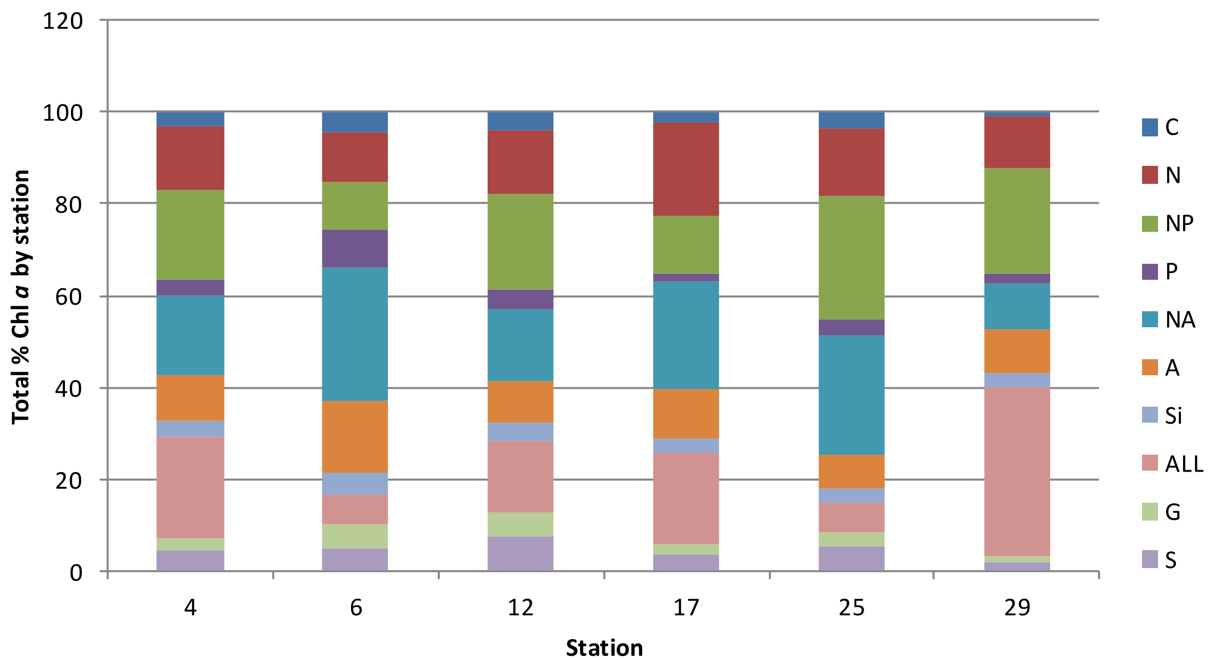


Figure 39 July 2012 chlorophyll *a* concentrations (µg/L) of all 10 treatments by fixed station.

In order to better see the significant responses in phytoplankton biomass changes associated with the addition of nutrients, we show our findings below in Figs. 40 to 45 from March 2010 until December 2012 for the +C (control), +N (nitrate), +NP (nitrate plus phosphorus), +NA (nitrate plus ammonium) and +ALL (nitrate, ammonium, phosphorus and silicate) treatments. While chl *a* concentrations in the control treatments were all less than 20 μg/L after a week long incubation except in March 2012 when it was closer to 50 μg/L, chl *a* concentrations rose to > 140 ug/L in the majority of +ALL treatment, and to > 200 μg/L in March 2012 (Figs. 40-45). During each March RLA, the greatest response was measured in the +ALL treatment whereas in July 2010 and 2011, the greatest response was measured in the +NP treatment.

Addition of only nitrogen also elicited a response greater than that in the control but this was not as significant (Figs. 40-45). Given the addition of nitrate (+N) or nitrate plus ammonium (+NA) elicited similar responses, we conclude that nitrate is more important to this population of phytoplankton than ammonium.

Fig. 45 shows that in July 2012 there were increases of all treatments relative to the control, particularly in station 4 (adjacent to the San Jacinto River) and in station 17 (mid-Trinity Bay). Lesser responses were observed at the remaining stations. Most notable in July 2012 was the significant response in treatments with nitrate (+N) and nitrate plus ammonium (+NP), suggesting these were the most important limiting nutrients driving phytoplankton production in all stations except for station 29 during this period.

The findings from summer 2012 (Fig. 45) which are different from those of other RLAs performed, may be explained by the large and prolonged freshwater inflow period earlier in the spring of 2012 (Fig. 17) followed by a month of very low flow. The long flow period may have flushed phytoplankton out of the Bay, that is, their growth rate could not outcompete their dilution rate. The subsequent period of low period may have left remaining populations without sufficient nutrients to continue growing.

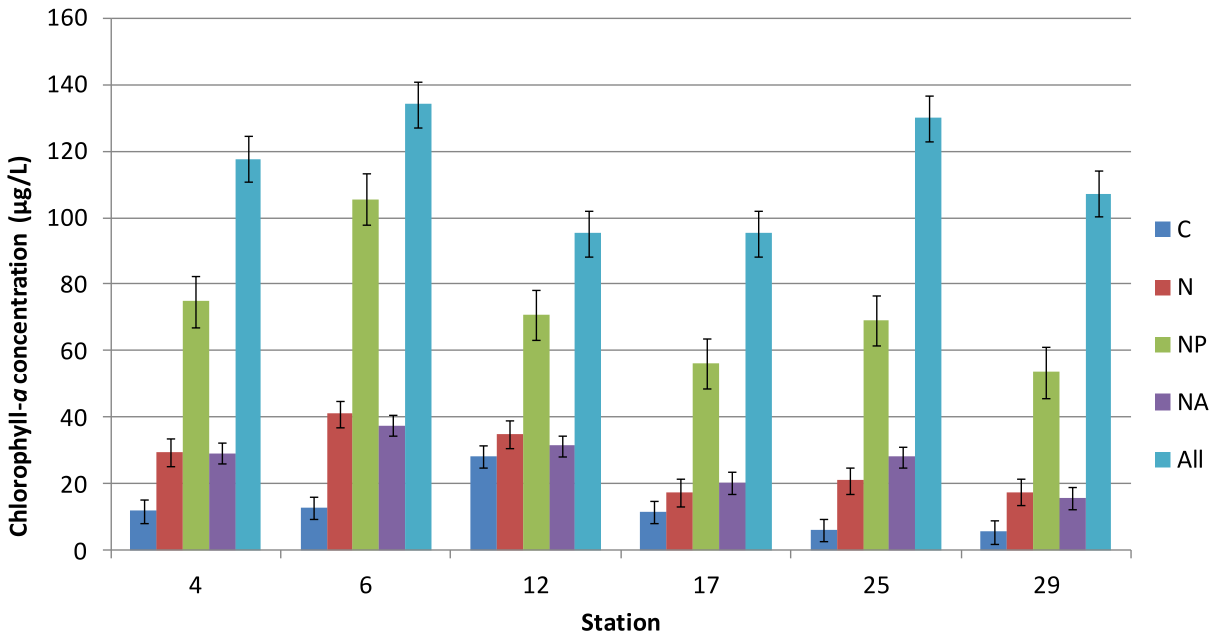


Figure 40 March 2010 chlorophyll *a* concentrations (µg/L) by station and treatment.

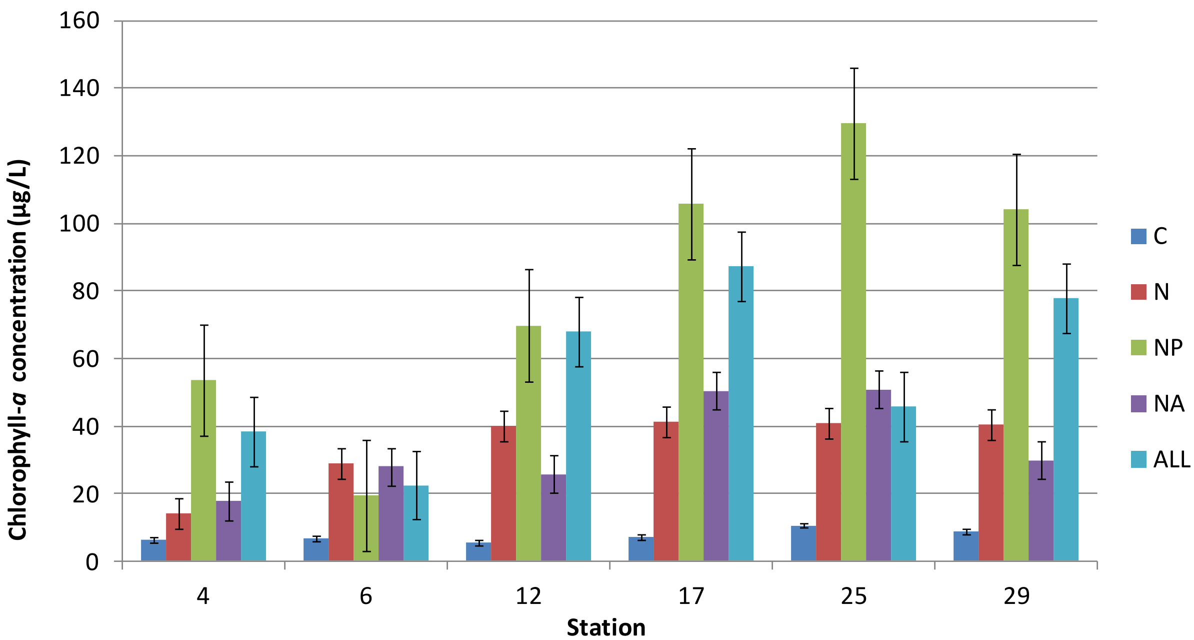


Figure 41 August 2010 chlorophyll *a* concentrations (µg/L) by station and treatment.

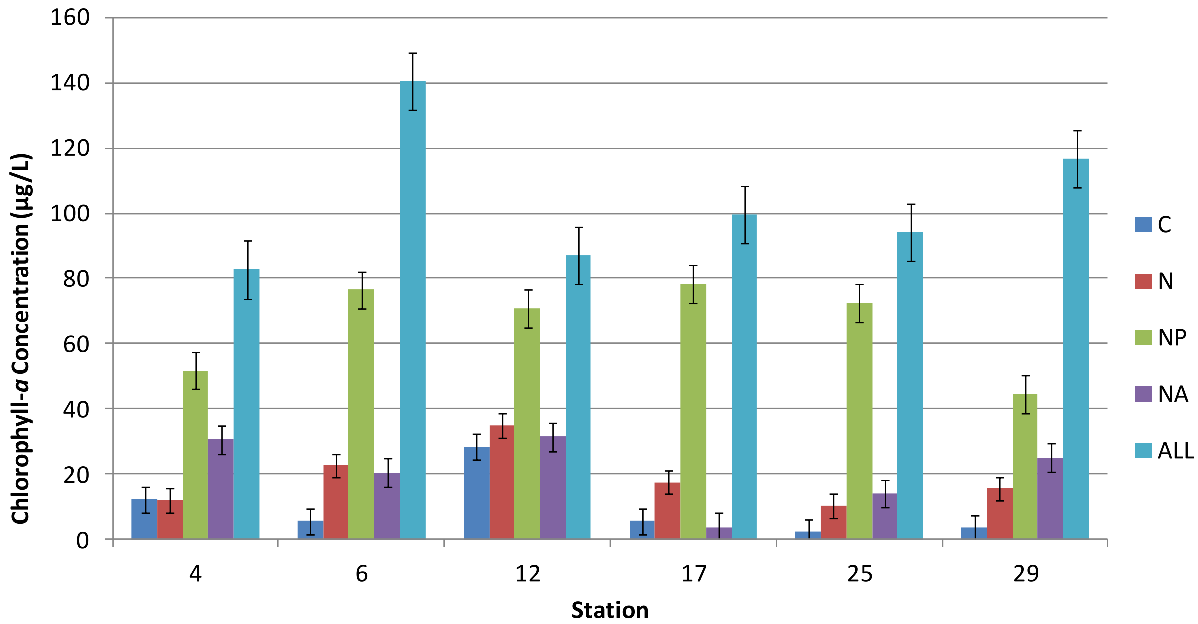


Figure 42 March 2011 chlorophyll *a* concentrations (μg/L) by station and treatment.

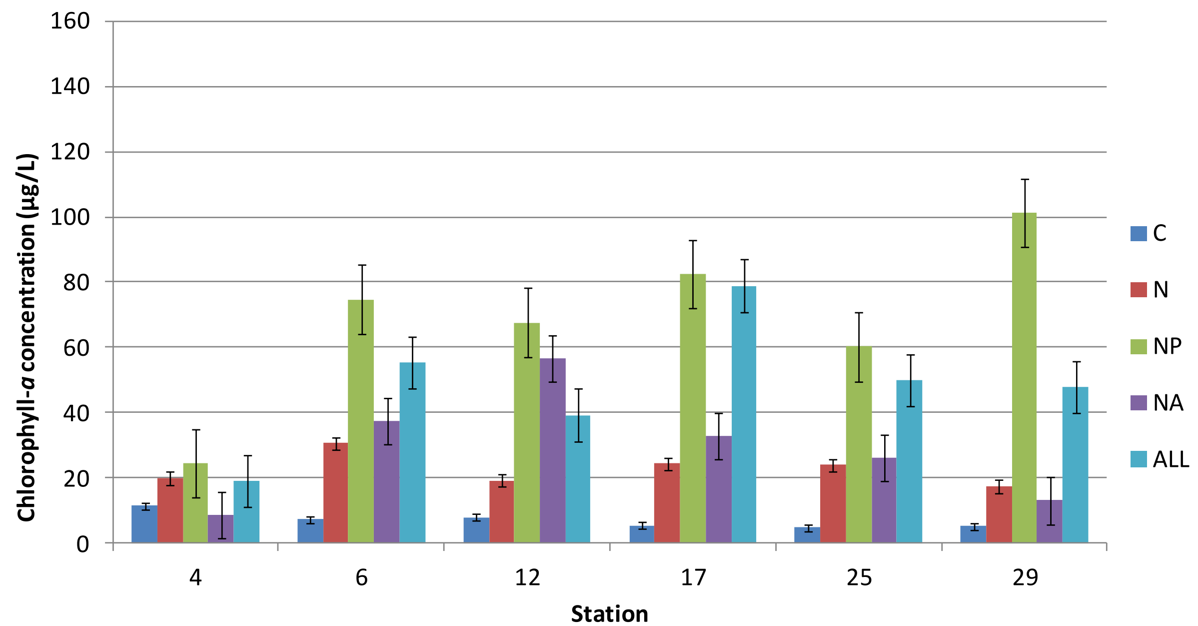


Figure 43 July 2011 chlorophyll *a* concentrations (μg/L) by station and treatment.

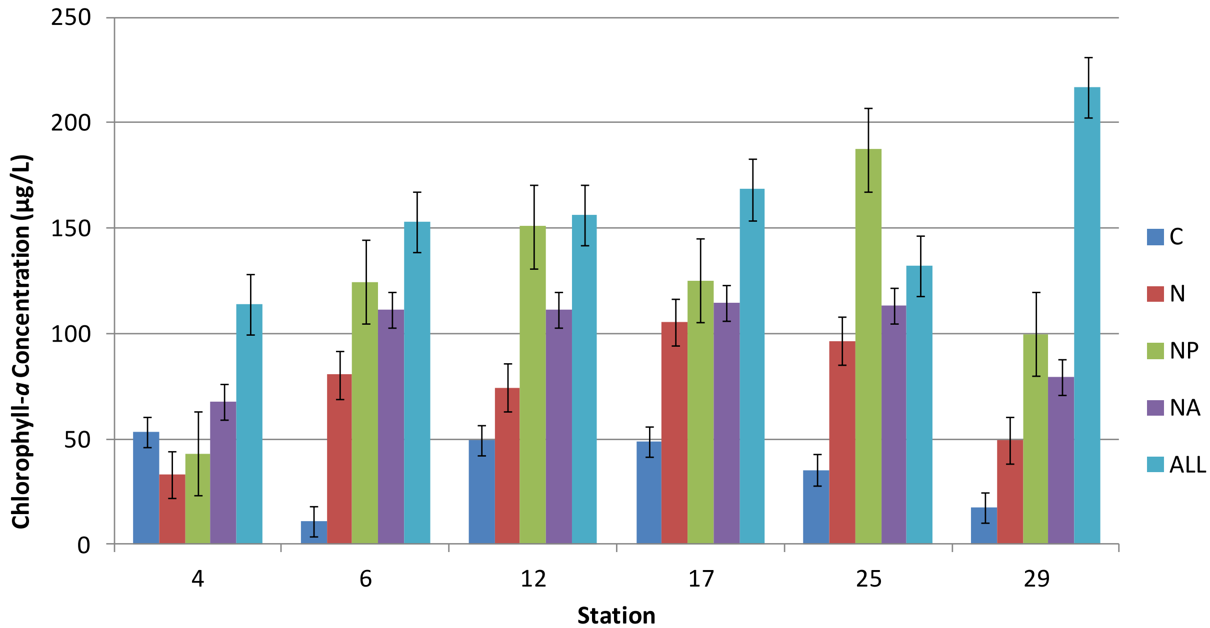


Figure 44 March 2012 chlorophyll *a* concentrations (µg/L) by station and treatment.

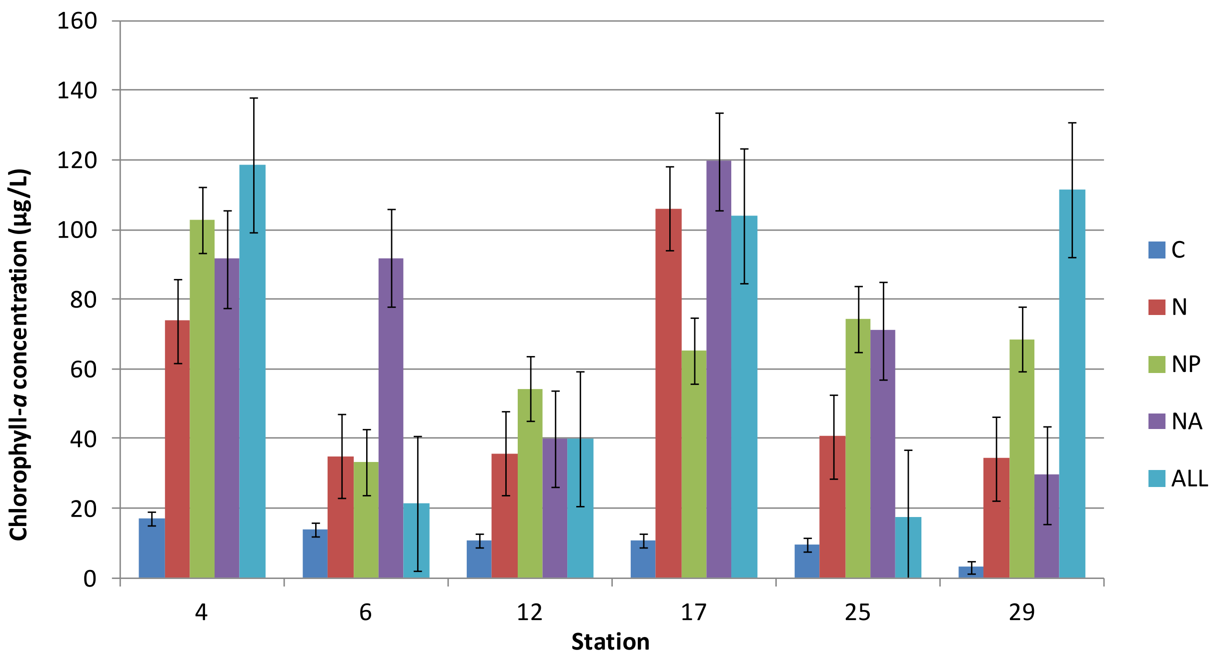


Figure 45 July 2012 chlorophyll *a* concentrations (µg/L) by station and treatment.

4.6 Distribution of *Rangia* clams in relation to salinity gradients in Galveston Bay

4.6.1 Determine the distribution of *Rangia* clams using historical datasets

To determine the suitability of *Rangia* spp. as a bio-indicator of freshwater inflows, historical datasets from TPWD were analyzed in conjunction with historic water quality data from TCEQ. The information collected from TPWD was plotted in GIS to examine both spatial and temporal patterns. *Rangia* clams, when present, are found in the Trinity River basin and East Bay (Fig. 46). Unexpectedly, we also found a decline in population density from the 1980s to the present. While in the 1980s, up to 2000 clams could be found in one sampling location, by the 2000s this decreased to at most, finding up to 50 clams at a single site.

Although different sampling strategies have been in place since collections started in the 1980s, it was found not to be the cause of the decline (Bill Balboa, pers. comm.). The cause of the decline will required further investigation, some of which was conducted as part of this project. A potential explanation for the decline would be a decrease in chlorophyll concentrations (proxy for food source) as a result of declines in nutrient levels in Galveston Bay.

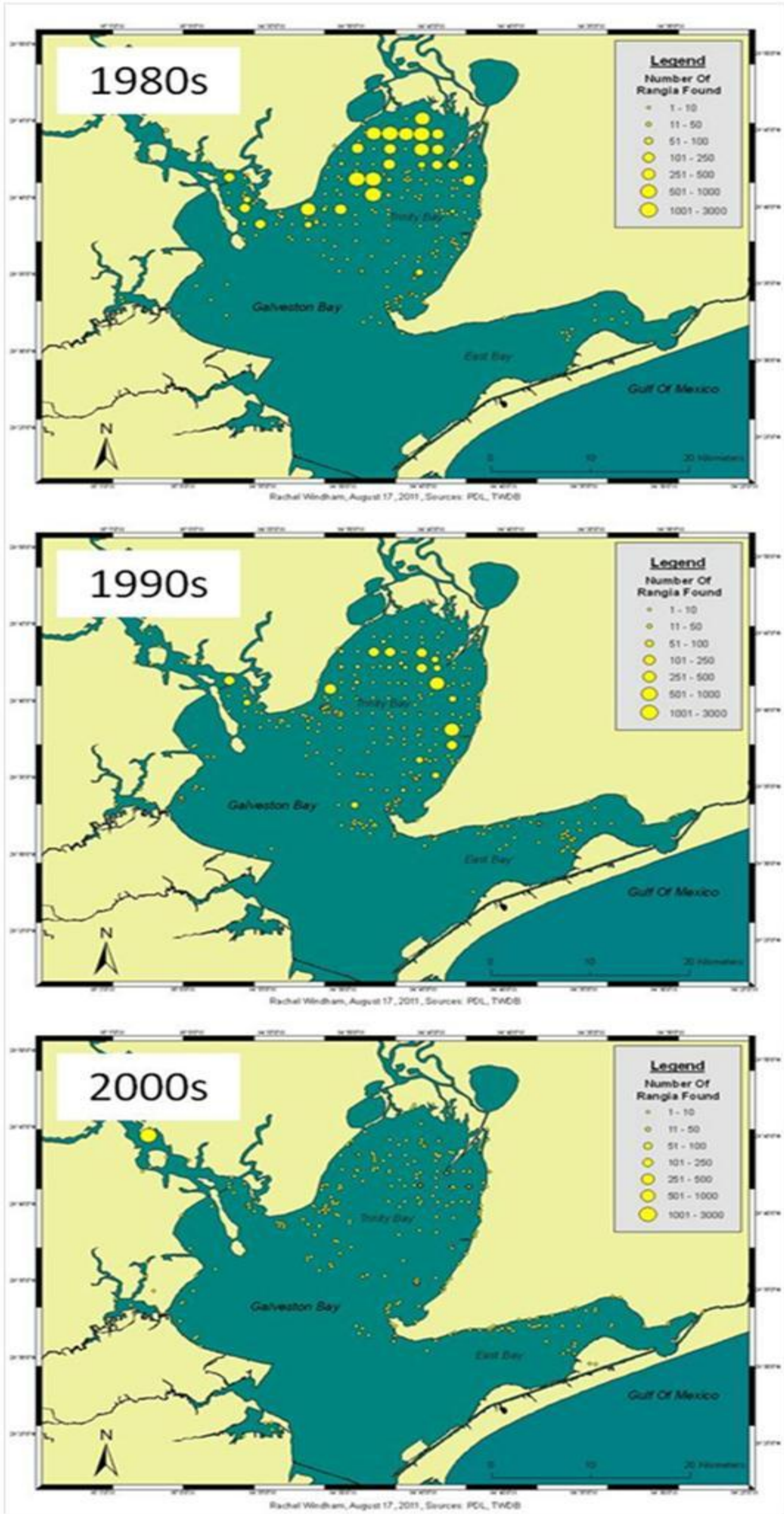


Figure 46 Decreasing numbers and shifts in the location of *Rangia* clams in Galveston Bay in the 1980s, 1990s and 2000s.

Using the TCEQ database provided by Lisa Gonzalez (Houston Advanced Research Center, Texas), nutrient and chlorophyll concentrations were plotted against time and a gradient from the Trinity River mouth (0 km) to the Gulf of Mexico (70 km) using SigmaPlot software to generate heat maps of each parameter.

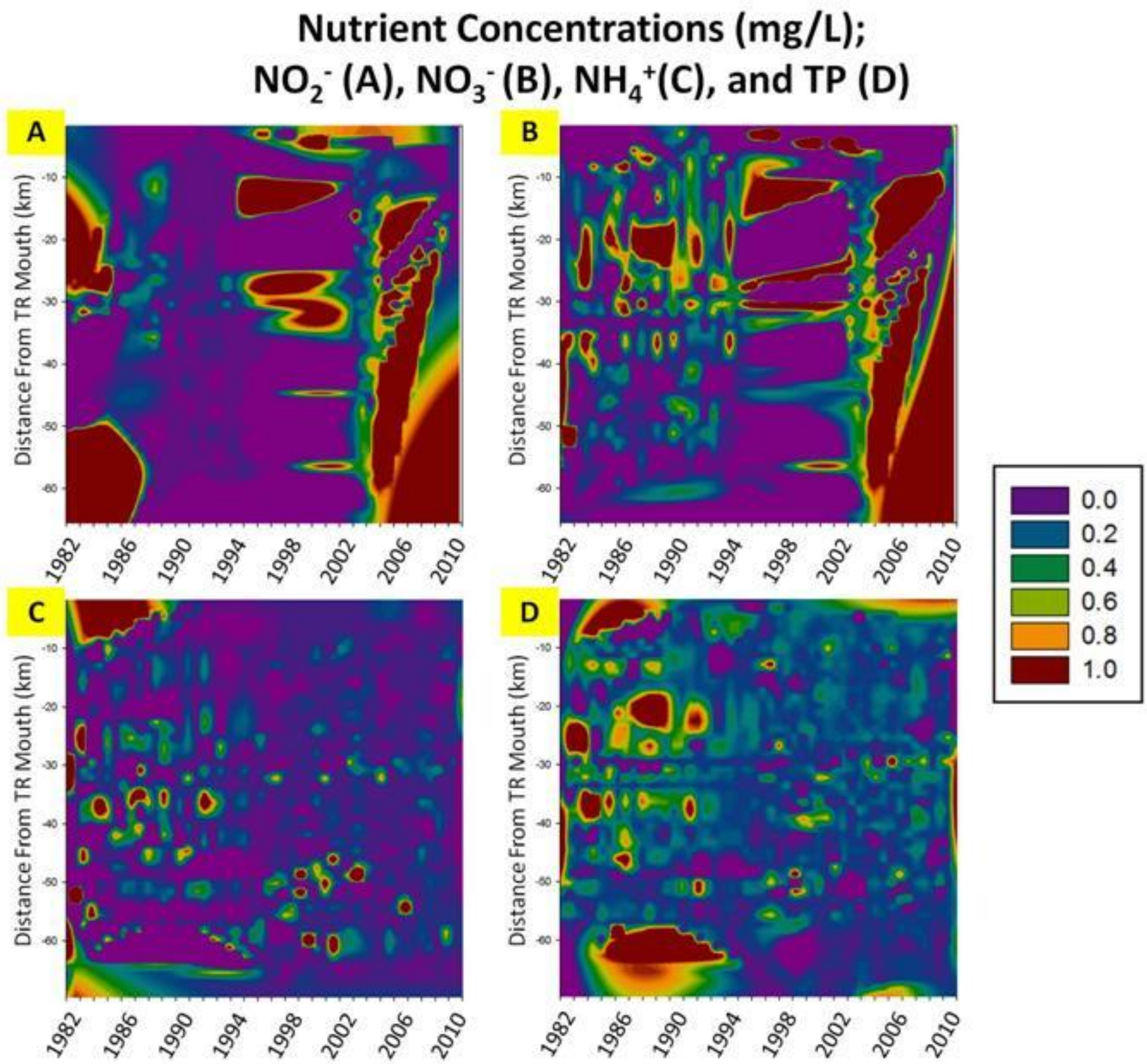


Figure 47 Shifts in nutrient concentrations in Galveston Bay from 1982 to 2010.

In Fig. 47, panels A, B and C display nitrate, nitrite and ammonium concentrations respectively. For these three nutrients, a lag in concentrations occurs at the transition between the 1980s and 90s with higher concentrations present before and after this time frame. While nitrate and nitrite resume former (higher) concentrations in the early 2000s, ammonium never recovers. Panel D displays total phosphorus concentrations (*Note: The TCEQ dataset for phosphate was not as extensive as that for N: nitrite, nitrate or ammonium, hence TP was used*). As with nitrate, nitrite and ammonium, a decrease in phosphorus was observed in the early 1990s. Like ammonium, phosphorus does not regain pre-1990s concentration levels as quickly as nitrate and nitrite. Also of note is that concentrations of nitrite and nitrate closest to the river mouth are lower in the 2000s compared to those measured near the mouth of the Bay opening to the Gulf of Mexico.

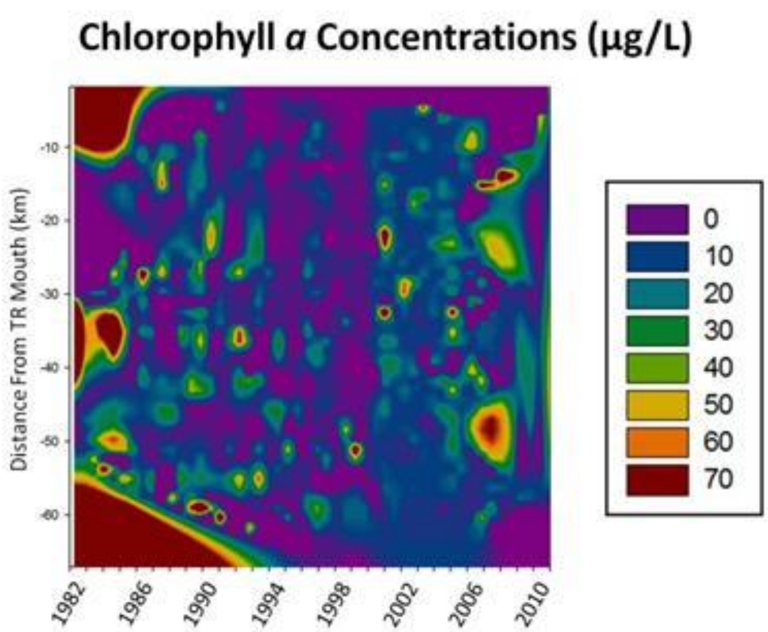


Figure 48 Shifts in chlorophyll *a* concentrations in Galveston Bay from 1982 to 2010.

Chlorophyll *a* concentrations from 1982-2010 are presented in Figure 48. Chlorophyll concentrations also decrease from the early 1990s. Chlorophyll *a*, a proxy for phytoplankton biomass in Galveston Bay, appears to have decreased indicating it was affected by nutrient levels, especially nitrogen. Though nitrogen sources, such as nitrate and nitrite, increase again in the early 2000s, chlorophyll levels do not recover in the same way. It is not clear what may be driving this change.

4.6.2 New surveys performed during 2010-2012 with concurrent salinity measurements

From March 2010 to December 2012 more than 800 clams were collected and analyzed using the methods outlined above.

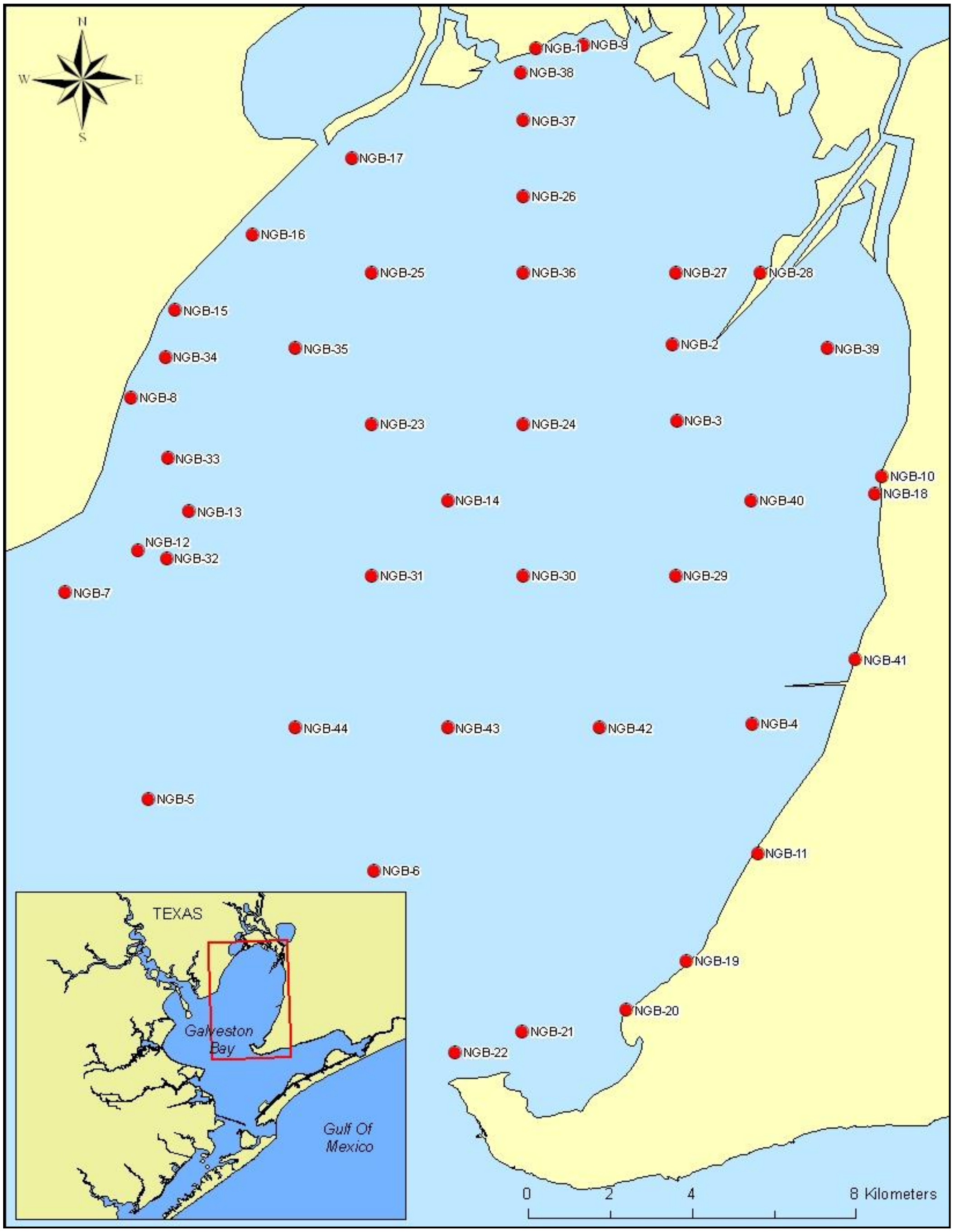


Figure 49 *Rangia* stations surveyed by TPWD in Trinity Bay (NGB), Texas, from October 2010 to May 2011.

Table 15 Presence (=1) or absence (=0) of clams and water quality parameters in Trinity Bay (NGB), Texas. (—) indicates parameter not measured.

TRINITY BAY (NGB)									
Month	Station	Coordinates (DD)		Presence/Absence	Temperature (°C)	Salinity	DO (mg/L)	POM (mg/L)	Depth (m)
October	NGB-1	N 29.774	W 94.772	1	26.4	9.1	6.6	—	0.4
	NGB-2	N 29.709	W 94.742	0	23.9	12.4	4.8	—	2.3
	NGB-3	N 29.692	W 94.741	0	24.0	13.6	5.0	—	2.7
	NGB-4	N 29.625	W 94.724	1	24.1	14.4	4.9	—	2.2
	NGB-5	N 29.609	W 94.857	0	23.9	18.7	4.9	—	3.1
	NGB-6	N 29.593	W 94.807	0	24.3	17.8	5.3	—	2.8
	NGB-7	N 29.654	W 94.875	1	25.1	16.7	5.4	—	2.9
	NGB-8	N 29.697	W 94.861	1	26.3	15.3	5.3	—	1.5
November	NGB-9	N 29.775	W 94.761	1	16.6	12.1	10.9	—	N/A
	NGB-10	N 29.680	W 94.696	1	17.1	16.4	10.0	—	N/A
	NGB-11	N 29.597	W 94.723	1	18.0	17.6	9.6	—	N/A
February	NGB-12	N 29.663	W 94.859	0	18.2	18.2	4.0	—	2.4
	NGB-13	N 29.672	W 94.848	1	18.9	17.5	4.3	—	2.5
	NGB-14	N 29.675	W 94.791	0	19.0	18.8	5.4	—	2.6
March	NGB-15	N 29.716	W 94.851	0	18.5	17.1	7.8	—	0.7
	NGB-16	N 29.733	W 94.834	0	19.4	16.9	8.3	—	0.6
	NGB-17	N 29.750	W 94.812	0	19.9	16.5	7.5	—	1.4
	NGB-18	N 29.676	W 94.697	1	20.1	18.1	7.5	—	0.7
	NGB-19	N 29.573	W 94.739	1	20.6	19.2	8.4	—	0.6
	NGB-20	N 29.563	W 94.752	0	20.9	19.5	8.6	—	0.7
	NGB-21	N 29.558	W 94.775	0	20.9	19.1	8.1	—	1.0
	NGB-22	N 29.553	W 94.790	0	20.5	19.5	7.9	—	1.3
April	NGB-23	N 29.691	W 94.808	0	23.3	22.6	6.7	—	2.5
	NGB-24	N 29.691	W 94.775	0	23.3	22.8	6.9	—	2.7
	NGB-25	N 29.725	W 94.808	1	23.2	20.8	7.3	—	2.2
	NGB-26	N 29.741	W 94.775	1	23.3	22.1	7.6	—	2.0
	NGB-27	N 29.725	W 94.741	0	23.3	22.7	7.4	—	2.2
	NGB-28	N 29.725	W 94.723	1	21.0	21.0	7.1	—	0.8
	NGB-29	N 29.658	W 94.741	1	23.5	22.4	7.3	—	2.5
	NGB-30	N 29.658	W 94.775	0	23.6	22.9	7.5	—	2.8
	NGB-31	N 29.658	W 94.808	0	23.9	23.7	7.1	—	2.9
May	NGB-32	N 29.662	W 94.853	0	27.1	23.1	5.0	85.3	3.1
	NGB-33	N 29.684	W 94.853	1	27.5	22.5	5.1	81.3	2.3
	NGB-34	N 29.706	W 94.853	1	27.9	21.8	5.9	88.3	1.8
	NGB-35	N 29.708	W 94.825	0	27.5	23.1	4.9	72.7	2.6
	NGB-36	N 29.725	W 94.775	0	27.5	22.6	5.2	84.3	2.7
	NGB-37	N 29.758	W 94.775	1	27.9	21.7	4.0	57.0	1.8
	NGB-38	N 29.768	W 94.775	1	29.3	21.1	8.1	101.0	0.7
	NGB-39	N 29.708	W 94.708	1	27.5	23.5	5.3	60.0	2.0
	NGB-40	N 29.675	W 94.725	0	27.2	24.9	5.6	72.7	2.5
	NGB-41	N 29.640	W 94.702	1	28.7	23.3	6.1	86.7	0.9
	NGB-42	N 29.625	W 94.758	0	27.4	26.4	5.5	56.5	2.8
	NGB-43	N 29.625	W 94.791	0	27.5	26.3	5.2	57.8	3.1
	NGB-44	N 29.625	W 94.825	0	27.3	25.7	5.0	61.0	3.2

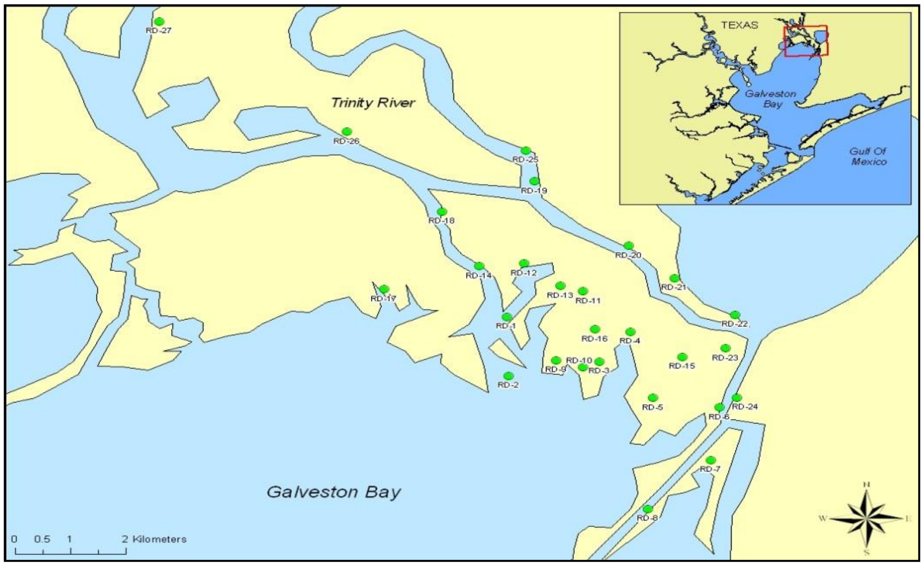


Figure 50 *Rangia* stations surveyed by TAMUG staff in the Trinity River and delta area (RD) from May 2011 to August 2011.

Table 16 *Presence (=1) or absence (=0) of clams and water quality parameters in the Trinity River and delta area (RD). — indicates parameter not measured.*

TRINITY RIVER & DELTA (RD)									
Month	Station	Coordinates (DD)		Presence/Absence	Temperature (°C)	Salinity	DO (mg/L)	POM (mg/L)	Depth (m)
May	RD-1	N 29.776	W 94.731	1	22.5	17.5	8.1	22.7	0.2
	RD-2	N 29.764	W 94.731	1	23.4	13.5	8.2	30.0	0.2
	RD-3	N 29.767	W 94.716	0	23.9	19.7	8.2	—	0.1
	RD-4	N 29.773	W 94.711	0	24.0	11.7	8.1	23.3	0.1
	RD-5	N 29.759	W 94.707	0	24.9	19.9	8.2	22.0	0.1
	RD-6	N 29.757	W 94.696	1	21.8	8.6	8.3	21.7	0.1
	RD-7	N 29.746	W 94.698	1	24.4	16.8	8.1	36.0	0.1
	RD-8	N 29.736	W 94.708	1	24.1	18.5	8.1	18.0	0.2
June	RD-9	N 29.767	W 94.723	1	29.1	13.2	7.1	228.7	0.1
	RD-10	N 29.765	W 94.719	1	29.1	12.8	7.5	212.7	0.1
	RD-11	N 29.781	W 94.719	0	29.6	12.5	7.6	112.7	0.1
	RD-12	N 29.787	W 94.728	1	30.7	14.3	8.6	163.3	0.1
	RD-13	N 29.782	W 94.722	0	31.1	13.7	9.3	142.0	0.1
	RD-14	N 29.786	W 94.735	0	28.4	14.9	7.2	126.0	0.1
	RD-15	N 29.768	W 94.702	1	31.0	12.4	8.9	134.0	0.1
	RD-16	N 29.773	W 94.717	1	29.9	14.2	8.0	129.3	0.1
July	RD-17	N 29.782	W 94.751	1	29.5	20.5	8.8	71.3	0.2
	RD-18	N 29.798	W 94.741	0	30.0	19.5	5.4	292.0	0.2
	RD-19	N 29.804	W 94.726	1	32.2	15.1	6.7	34.0	0.6
	RD-20	N 29.791	W 94.711	0	31.7	18.1	8.4	56.7	0.6
	RD-21	N 29.784	W 94.704	1	32.6	17.8	8.3	280.0	0.4
	RD-22	N 29.776	W 94.694	0	27.2	11.1	6.5	58.0	0.5
	RD-23	N 29.769	W 94.695	1	29.2	19.4	7.8	83.3	0.1
	RD-24	N 29.759	W 94.694	1	30.8	17.6	6.0	184.7	0.3
August	RD-25	N 29.810	W 94.728	0	32.1	6.2	5.5	54.0	0.9
	RD-26	N 29.814	W 94.757	1	33.7	15.0	10.0	62.7	0.3
	RD-27	N 29.837	W 94.787	1	31.8	13.4	8.5	98.7	0.4

Trinity River and delta area - Stations surveyed in the Trinity River and delta area (RD) (Fig. 49 and 50 above) showed a mean depth of 0.2 m. Water temperature increased from May to August as expected and stations surveyed within the same month were generally similar (Table 15). Salinity levels in RD were generally greater than 12 across all stations (Table 16) from May to August suggesting a decrease in freshwater inflows. DO levels were generally similar across all stations from May to August and did not fall below 5 mg/L (Table 16). POM levels varied across all stations from May to August with the lowest values in May and the highest values in July (Table 16).

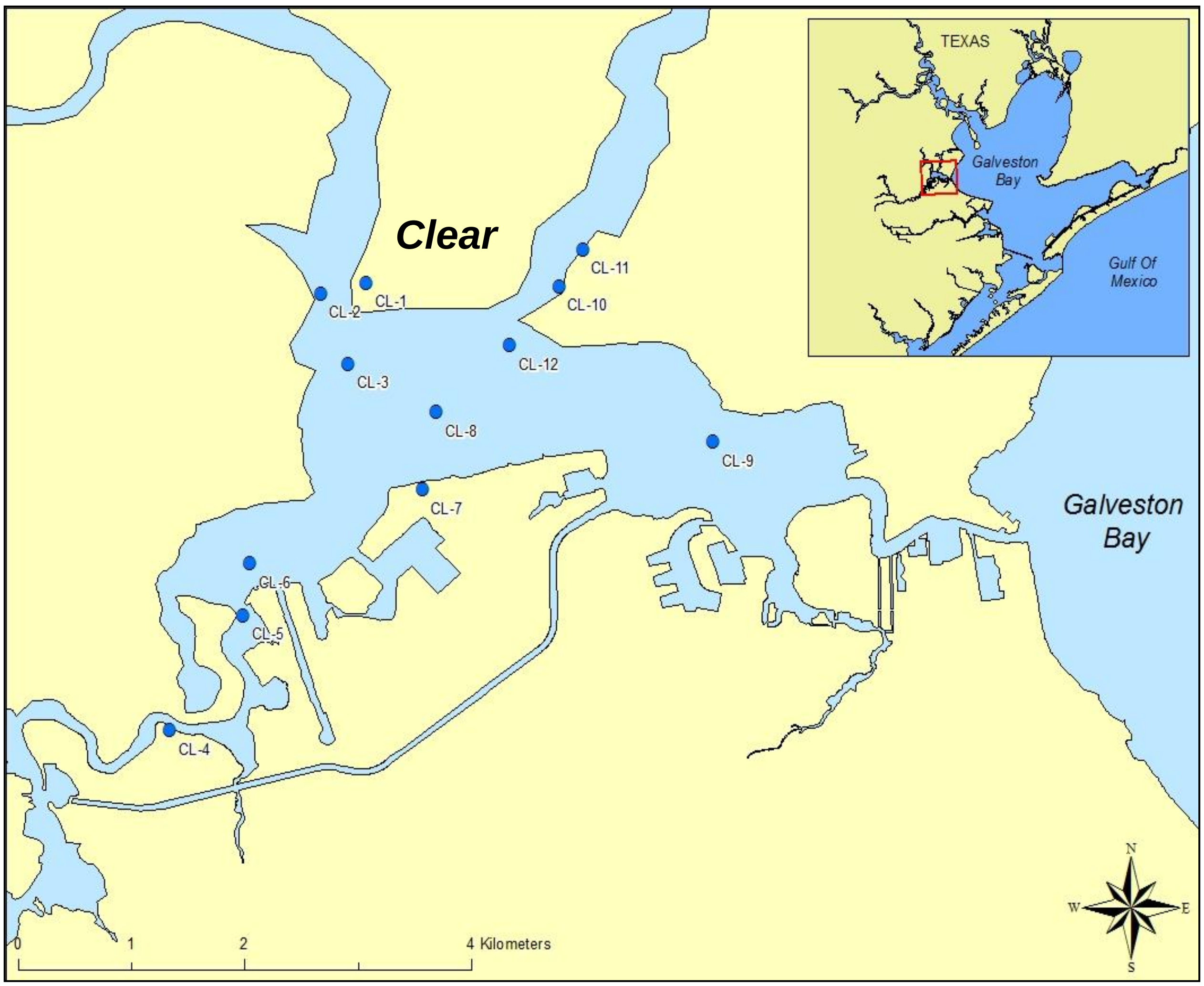


Figure 51 *Rangia* stations surveyed by TPWD in Clear Lake (CL), Texas, in June/ July 2011.

Table 17 Presence (=1) or absence (=0) of clams and water quality parameters in Clear Lake (CL), Texas. (—) indicates parameter not measured.

CLEAR LAKE (CL)									
Month	Station	Coordinates (DD)		Presence/Absence	Temperature (°C)	Salinity	DO (mg/L)	POM (mg/L)	Depth (m)
June	CL-1	N 29.567	W 95.069	1	30.2	19.9	5.6	—	0.9
July	CL-2	N 29.566	W 95.073	0	30.7	18.7	3.4	294.1	0.5
	CL-3	N 29.561	W 95.071	0	30.8	21.2	4.0	116.0	2.9
	CL-4	N 29.533	W 95.085	1	30.6	16.1	3.8	117.0	0.4
	CL-5	N 29.542	W 95.079	1	30.6	18.1	4.8	97.0	0.4
	CL-6	N 29.546	W 95.078	1	30.6	19.2	2.0	98.5	1.1
	CL-7	N 29.551	W 95.065	1	31.2	20.4	3.0	108.5	0.5
	CL-8	N 29.557	W 95.064	0	30.6	22.8	3.9	106.5	1.6
	CL-9	N 29.555	W 95.042	1	30.8	24.6	4.2	128.5	2.0
	CL-10	N 29.567	W 95.054	1	31.4	21.6	4.6	123.7	0.4
	CL-11	N 29.570	W 95.052	1	31.0	22.4	3.6	126.8	1.7
	CL-12	N 29.562	W 95.058	0	30.7	23.9	3.7	100.5	2.1

Clear Lake - Stations surveyed in Clear Lake (CL; Fig. 51) showed a mean depth of 1.2 m. Water temperature was generally similar across all the stations from June to July (Table 17). Salinity levels in CL were generally greater than 30 across all stations (Table 17). This suggests freshwater inflows were lowest in June and July in CL. DO levels were generally less than 5 mg/L across all stations (Table 17). POM levels were variable and generally over 100 mg/L across all stations in July (Table 17).

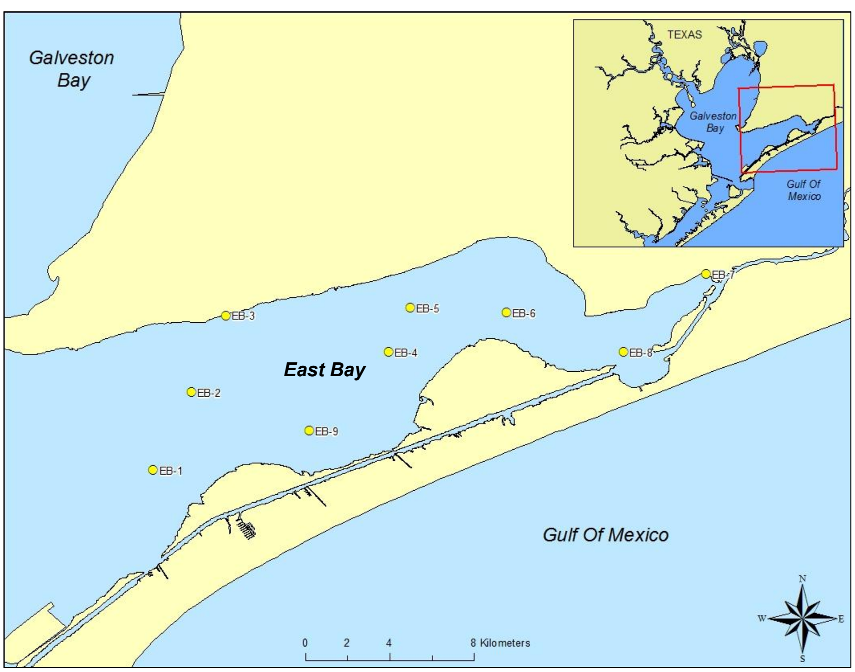


Figure 52 *Rangia* stations surveyed by TPWD in East Bay (EB) in August 2011.

Table 18 Presence (=1) or absence (=0) of clams and water quality parameters in East Bay (EB), Texas.

EAST BAY (EB)									
Month	Station	Coordinates (DD)		Presence/Absence	Temperature (°C)	Salinity	DO (mg/L)	POM (mg/L)	Depth (m)
August	EB-1	N 29.474	W 94.708	0	30.4	31.1	5.2	68.0	1.5
	EB-2	N 29.508	W 94.692	0	30.7	32.5	5.7	88.3	2.2
	EB-3	N 29.540	W 94.677	1	30.0	32.5	5.6	179.5	0.3
	EB-4	N 29.525	W 94.608	0	30.6	30.2	5.7	146.0	1.8
	EB-5	N 29.543	W 94.599	0	30.9	32.1	5.1	103.3	0.4
	EB-6	N 29.541	W 94.558	0	30.8	31.1	6.3	67.5	1.5
	EB-7	N 29.558	W 94.473	0	31.1	29.6	6.1	158.5	0.4
	EB-8	N 29.525	W 94.508	0	31.2	30.5	6.4	103.0	1.3
	EB-9	N 29.491	W 94.642	0	30.8	30.8	5.3	78.0	1.8

East Bay - Stations surveyed in East Bay (EB; Fig. 52) showed a mean depth similar to Clear Lake (1.2 m). Water temperature was generally similar across all the stations in August (Table 18). Salinity levels in EB were similar to salinity levels in CL (>30 across all stations) suggesting freshwater inflows were lowest in August for EB. DO levels were generally similar across all stations (Table 18). POM levels were variable across all stations and generally over 100 mg/L in August (Table 18).

Water quality parameters

A Kruskall-Wallis test revealed statistically significant differences in salinity, temperature and DO across the four different survey areas (NGB, RD, CL and EB). Follow up Mann-Whitney U tests showed that mean salinity, temperature and DO were significantly different (Table 19) between NGB stations and RD, CL and EB stations. Salinity was significantly lower and DO was higher at RD stations compared to CL and EB stations (Tables 16-18). Temperature was significantly lower at RD stations compared to EB stations and higher at RD stations compared to CL stations (Tables 16, 17, 18 and 19). Salinity, temperature and DO were significantly higher at EB stations compared to CL stations (Tables 18, 17 and 19). POM was not significantly different between NGB stations and RD stations or RD stations and CL stations. POM was significantly greater at CL and EB stations compared to NGB stations (Tables 17, 18 and 19). EB stations had significantly lower POM levels compared to RD and CL stations (Tables 16, 17 and 19).

Table 19 P-values from Mann-Whitney U tests of mean salinity, temperature (°C), DO (mg/L) and POM (mg/L) across all four survey areas (NGB, RD, CL and EB).

Survey Stations	Salinity	Temperature (°C)	DO (mg/L)	POM (mg/L)
NGB & RD	< 0.000	< 0.000	< 0.000	0.342
NGB & CL	0.035	< 0.000	< 0.000	< 0.000
NGB & EB	< 0.000	0.004	< 0.000	< 0.000
RD & CL	< 0.000	< 0.000	< 0.000	0.264
RD & EB	< 0.000	< 0.000	0.025	0.027
CL & EB	< 0.000	< 0.000	0.005	0.013

Rangia clam data

Rangia mean shell length was 48.9 ± 0.4 mm at NGB stations (mean ± SE), 55.7 ± 2 mm at RD stations, 34.6 ± 0.8 mm at CL stations and 39.7 ± 0.5 mm at EB stations. *Rangia* mean meat index was 10.6 ± 0.5 at NGB stations, 12.5 ± 0.5 at RD stations, 12.9 ± 0.2 at CL stations and 7.6 ± 0.3 at EB stations. A Kruskall-Wallis test revealed a statistically significant difference in *Rangia* shell length (mm), and meat index across the four survey areas (NGB, RD, CL and EB). Follow up Mann-Whitney U tests showed that shell length at NGB stations was significantly lower than RD stations but higher than CL and EB stations (Table 20 and Fig. 53). *Rangia* shell length at RD stations was significantly higher than CL and EB stations (Table 20 and Fig. 53). *Rangia* shell length at EB stations was significantly higher than CL stations (Table 20 and Fig 53). Meat index at NGB stations was significantly lower than RD and CL stations but higher than EB stations (Table 20 and Fig. 53). Meat index at RD and CL stations was significantly higher than EB stations (Table 20 and Fig. 53). *Rangia* median gonad to foot ratio was larger than foot (LTF) at NGB stations and RD stations, same as foot (SAF) at CL stations and smaller than foot (STF) at EB stations (Table 20). *Rangia* mean caloric content was 4670 ± 20 cal/g at NGB stations and 4760 ± 30 cal/g at RD stations. Caloric content of *Rangia* clams from stations CL and EB were not processed. *Rangia* shell length decreased with increasing salinity across all survey stations from RD to EB (Fig. 54). This suggests that *Rangia* physiology is affected by increased salinities. *Rangia* shell length did not vary with increasing temperature ($p > 0.05$) across all survey stations.

Table 20 P-values from Mann-Whitney U tests of mean gonad to foot ratios, shell length (mm) and meat index across the four survey areas (NGB, RD, CL and EB).

Survey Stations	Shell length (mm)	Meat Index	Gonad to foot ratio
NGB & RD	< 0.000	< 0.000	0.729
NGB & CL	< 0.000	< 0.000	< 0.000
NGB & EB	0.003	< 0.000	< 0.000
RD & CL	< 0.000	0.234	< 0.000
RD & EB	< 0.000	< 0.000	< 0.000
CL & EB	< 0.000	< 0.000	< 0.000

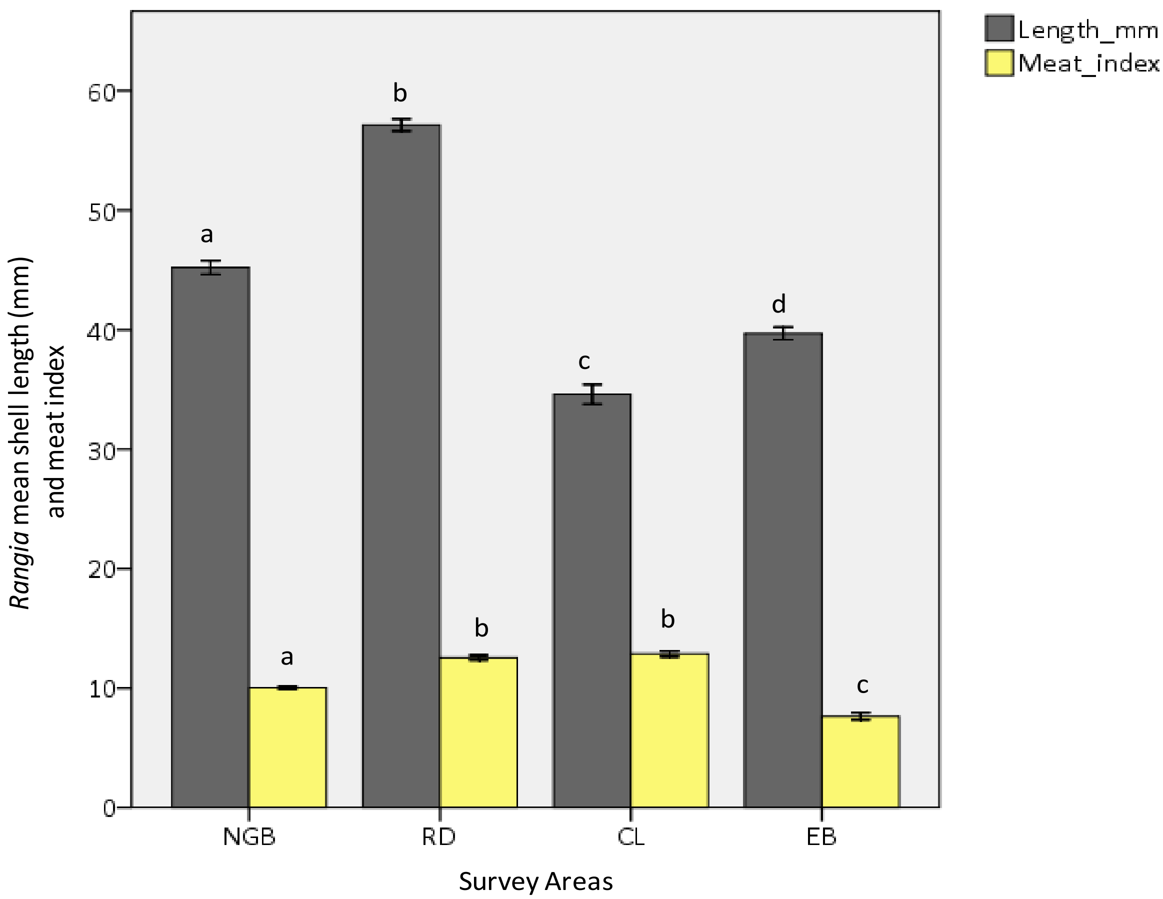


Figure 53 *Rangia* mean shell length (mm) and meat index surveyed at Trinity Bay (NGB), Trinity River and delta area (RD), Clear Lake (CL) and East Bay (EB) stations from October 2010 to August 2011. a, b, c and d denotes significantly different groups. Error bars represent +/- 1 SE.

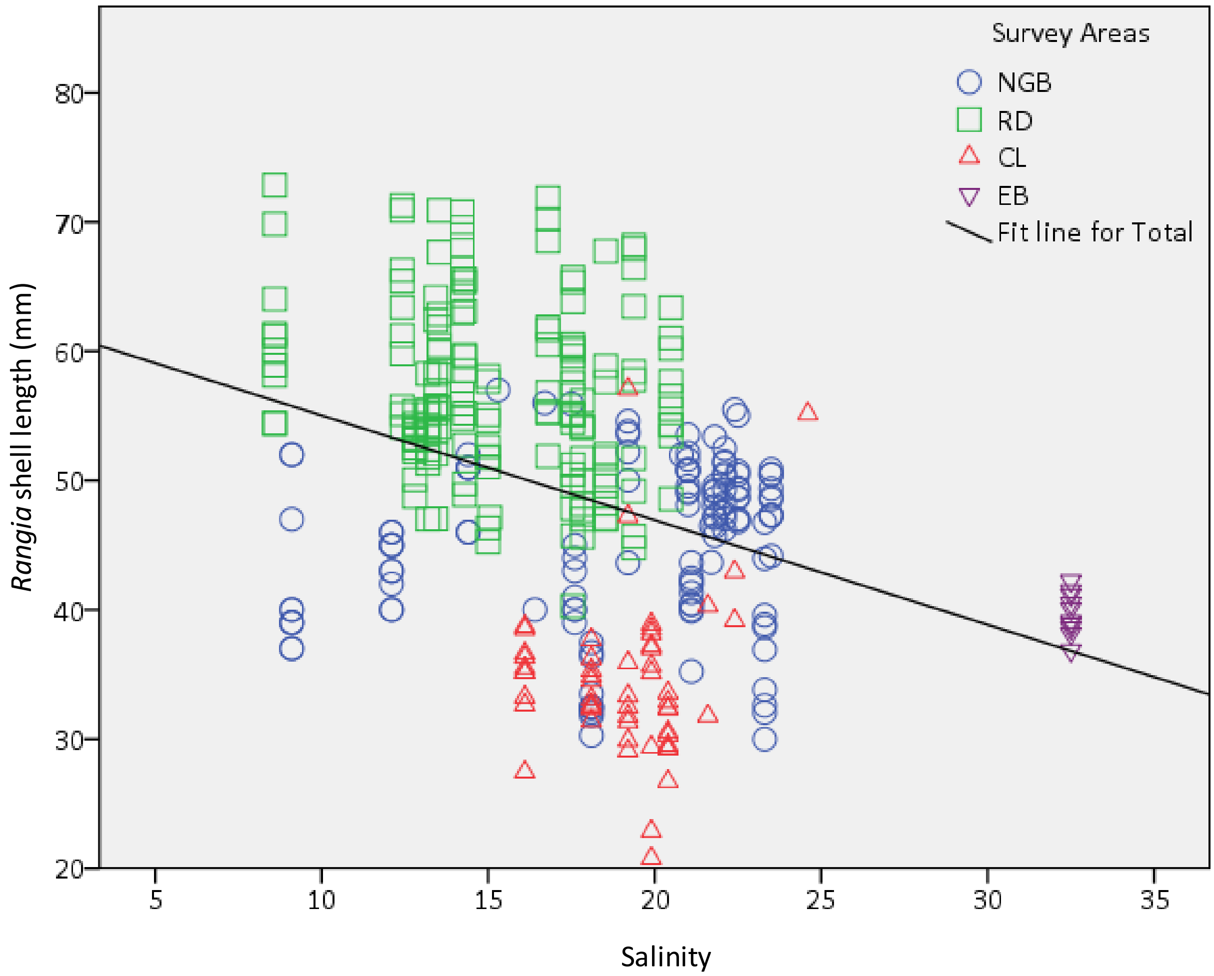


Figure 54 *Rangia* shell length (mm) in response to salinity surveyed at Trinity Bay (NGB), Trinity River and delta area (RD), Clear Lake (CL) and East Bay (EB) stations from October 2010 to August 2011.

4.6.3 **Conduct new surveys during 2010-2012 with concurrent salinity measurements. Focus will be Spring and Fall periods and assessment of adult gonadal condition as indicator of reproductive potential and spat settlement as indicator of larval survival.**

Two sites, NGB-1 (an exposed station in north Galveston Bay) and RD-8 (a river influenced station at the mouth of the Trinity River) have reliably produced clam samples since the beginning of the study period (Table 21; Fig. 55).

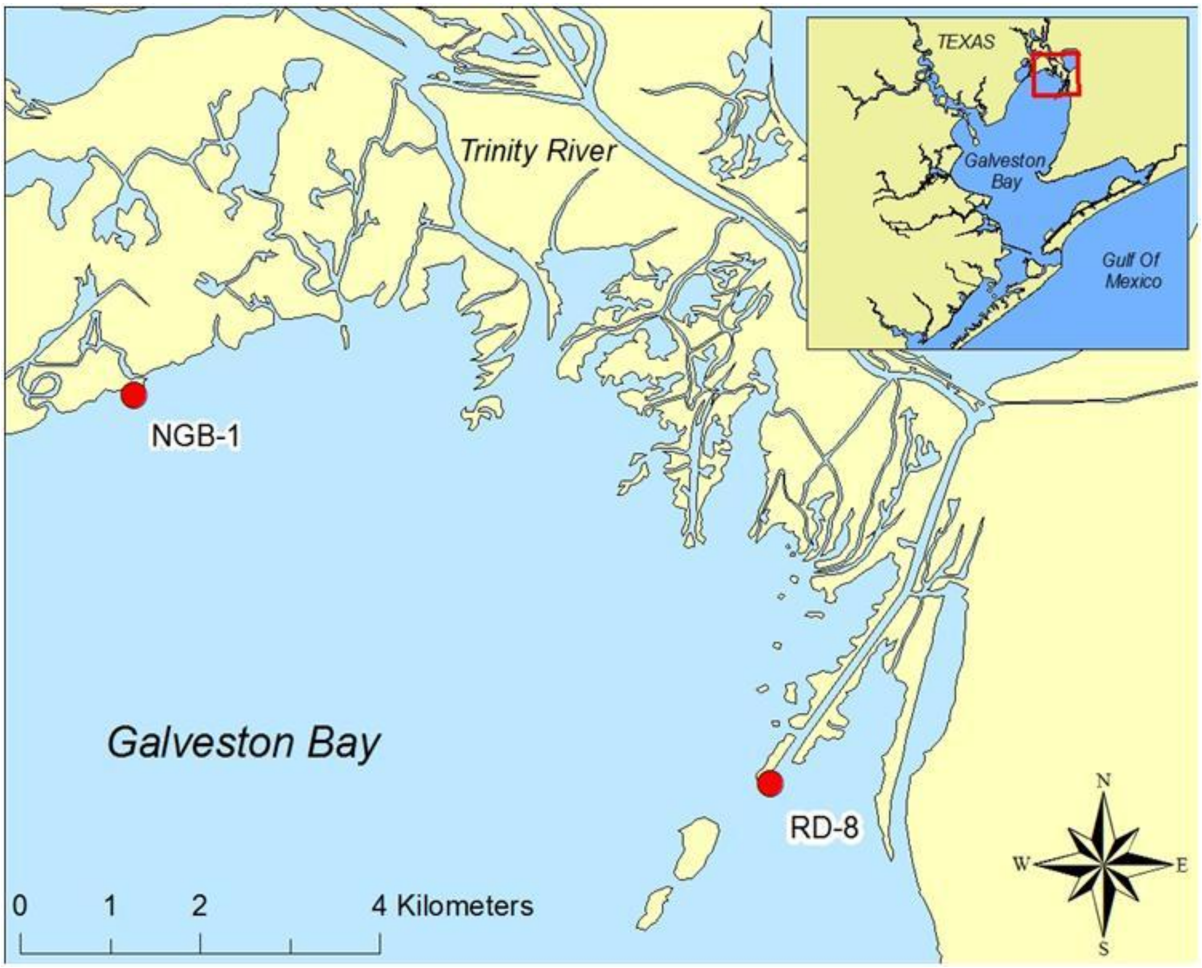


Figure 55 *Rangia* project sampling map. These sites were accessed with a small fiberglass boat and sampled with a metal quadrat to determine *Rangia* abundance.

Table 21 Latitude and longitude of sampling stations around the Trinity River Delta from which samples were collected.

Station	**Map number**	**Latitude**	**Longitude**
1	RD-8	29˚44.21'	-94˚42.51'
2	NGB-1	29˚46.45'	-94˚46.33'

The density (number of clams per square meter) of the clam populations at these sites are shown in Figure 56. The average clam density at each location is highly variable, from 1 to 6 clams/m^2. The biovolume (clam mass in grams per square meter) of the clam populations at these sites are detailed in Figure 57. The biovolume of clams at NGB-1 was typically lower (~70 g/m^2) compared with those at RD-8 which were more variable but closer to ~150 g/m^2 up until 2012. Average clam shell lengths were between 40 and 50 mm (Figure 58) at both NGB-1 and RD-8. There was no change during the sampling period. Average meat indexes (the percentage of wet meat that comprises the clam's total biomass) is shown in Figure 59. Though the values differ slightly from site to site, there seems to be a fluctuation in the meat indexes which increases in the spring-summer and decreases in the fall-winter. This may be due to a slight increase in gonadal mass between early spring and late fall, times when *Rangia* are likely to spawn and therefore purge some of their biomass.

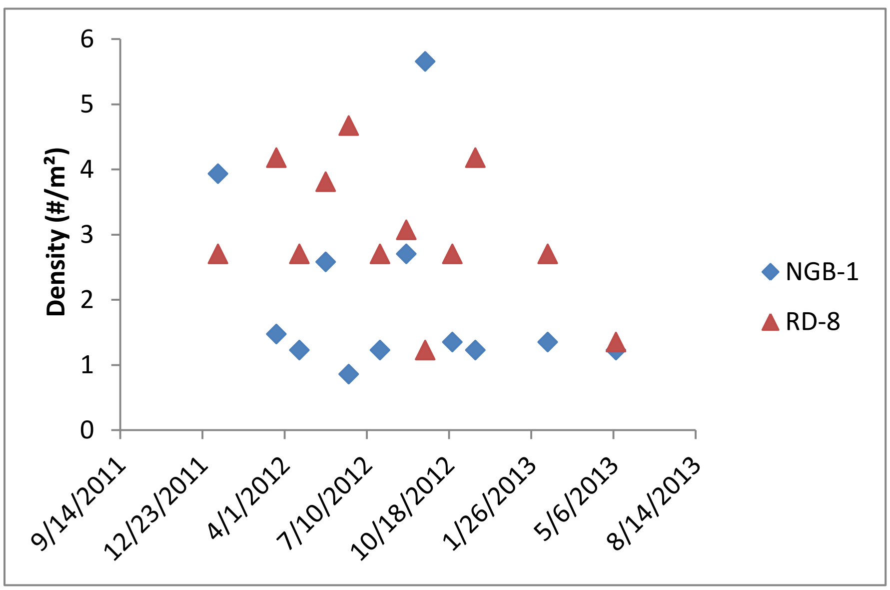


Figure 56 Clam density (#/m^2) at Stations NGB-1 and RD-8, 2012 to present.

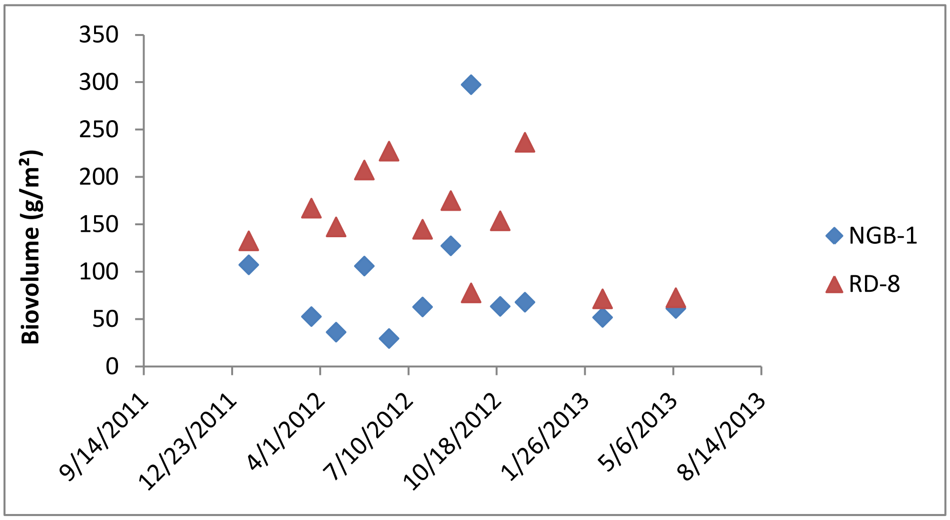


Figure 57 Clam biovolume at Stations NGB-1 and RD-8.

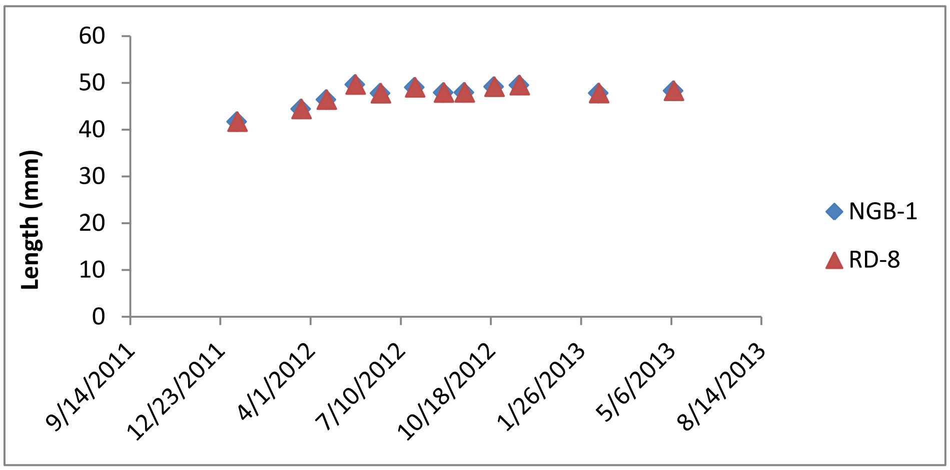


Figure 58 Average clam shell length at Stations NGB-1 and RD-8.

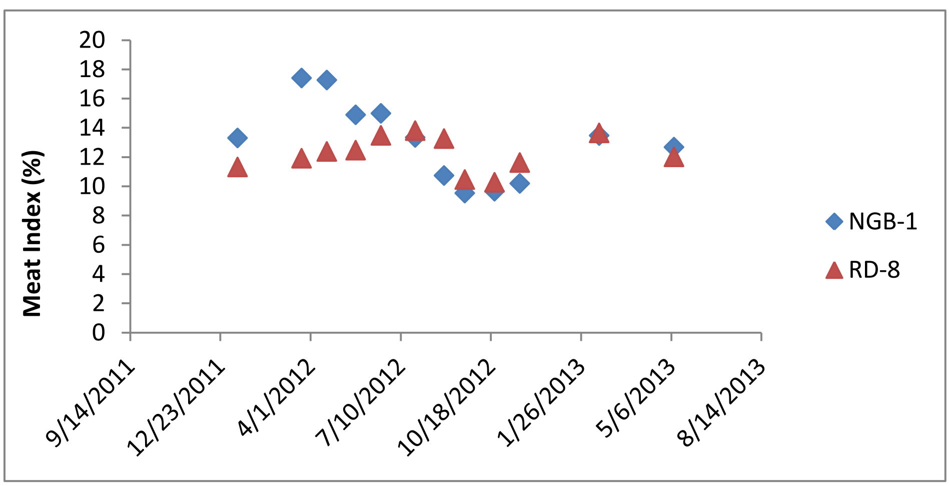


Figure 59 Average clam meat index at Stations NGB-1 and RD-8.

Sex ratios of the *Rangia* clams at NGB-1 and RD-8 and the incidence of parasitic infection are illustrated in Figures 60 and 61 respectively. Patterns between the two stations differ. Clams at RD-8 tend to remain more consistent with the sex ratio hovering around 1:1 from March to November but it then spikes to ~10 during the winter as a result of disproportionately more males than females present. At NGB-1, *Rangia* clams sex ratios are variable throughout the year.

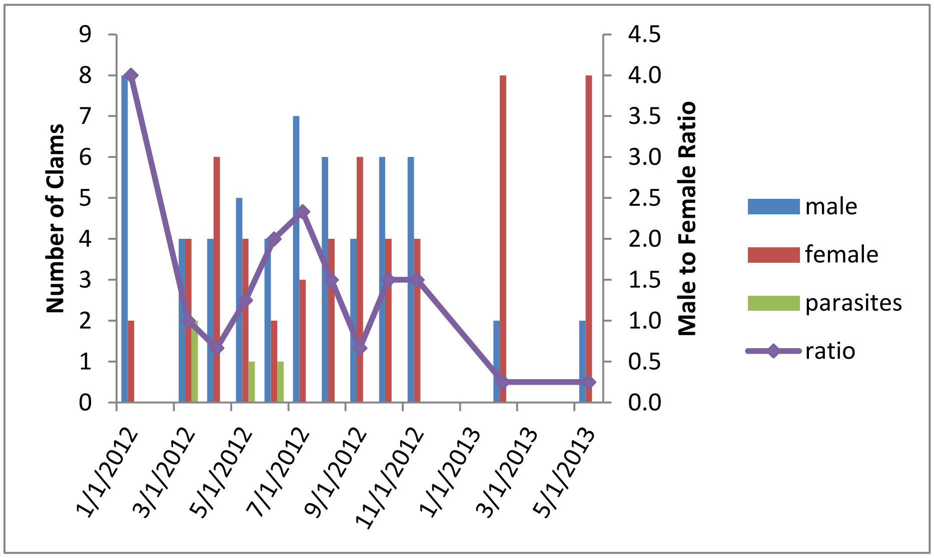


Figure 60 Numbers of male and female clams and incidences of parasitic infection along with M:F ratios at NGB-1.

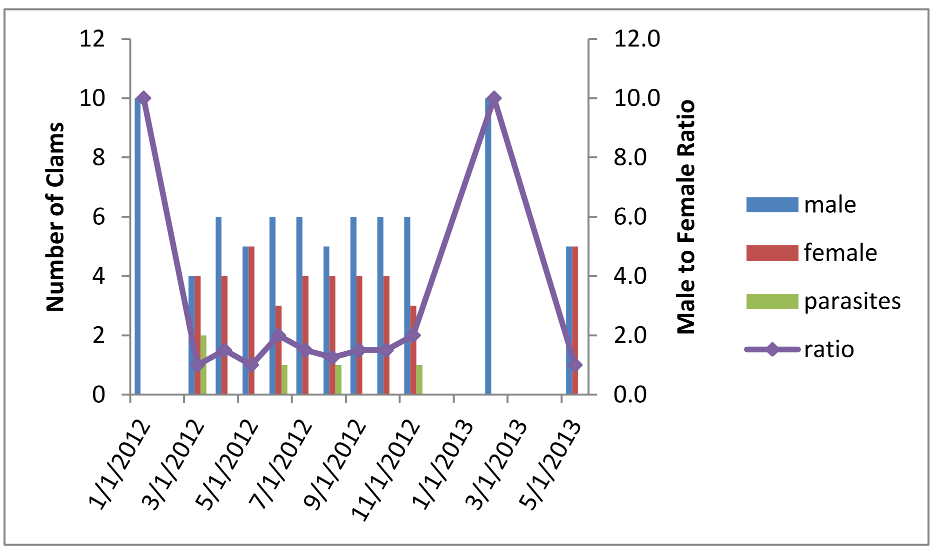


Figure 61 Numbers of male and female clams and incidences of parasitic infection along with M:F ratios at RD-8.

4.7 Isohaline maps

Isohaline maps for 360 months from January 1983 to December 2012 were produced based on TxBLEND simulations. These maps are available electronically by request to Dr. Quigg (quigga@tamug.edu).

4.7.1 Time series of percent of Bay area vs. inflow hydrographs

Fig. 12 shows a clear correlation between high inflows and greater percentage of Bay area with lower (greener) salinities is visually evident.

4.7.2 Maps and hydrographs of dataflow data vs. modeled daily average salinity

Maps of dataflow data vs. modeled daily average salinity were used to support visual evaluation model accuracy. Fig. 13 shows the pattern of the salinity gradient to be fairly consistent with the observed data, however in many locations TxBLEND overestimates salinities across the Bay on this day. For example, at station 21 TxBLEND predicted salinity at 23 PSU on August 16, 2010 whereas the observed salinity based from the dataflow measurement was 18 PSU. Fig. 14 shows a time series of predicted (TxBLEND) vs. observed (Dataflow) salinities at station 21. Both the model results and observed data show that salinities were rising at this location in the late summer and early fall of 2010, though TxBLEND predicts that salinities rose more quickly than they were observed. The pattern and comparisons shown in Fig. 13 and 14 are consistent across the Bay both temporally and spatially and are consistent with TWDB finds regarding the accuracy of the TXBLEND model namely results for salinity calibration demonstrated that the TxBLEND model for Galveston Bay was generally representative of observed salinities and trends, though long-term trends were simulated more accurately than short-term, high frequency variability, particularly in the upper estuary.

4.7.3 Statistical analysis presented spatially

The statistical results presented in Table 8 are displayed on maps in Figures 62 – 67. Following Moriasi et. al. (2007) guidelines, the colors in the figures indicate:

- green – very good,
- light green – good,
- orange – satisfactory and

- red – unsatisfactory.

The following observations are based primarily on visual observation of patterns in the dataflow sets. The maps include representation of the statistics for the TWDB datasonde sites which tend to have much longer and denser periods of record and do not in all cases show the identical patterns.

Figs. 62 and 63 provide estimates of model performance. These figures tell a similar story, namely that the model performance is very good to good in the middle areas of Galveston Bay and satisfactory to unsatisfactory nearer the river mouths and the gulf inlets. The coefficient of variation (Fig. 62) which is often criticized for being over sensitive to extreme values, depicts slightly better performance than the Nash – Sutcliff (Fig. 63).

Figs. 64 and 65 provide estimates of model accuracy both in absolute terms (Fig. 64) and normalized based on observed variance (Fig. 65). Again the model performs best in the middle area of the Bay. When normalized for observed variance (Fig. 65) increased accuracy is observed slightly further up in the Bay relative to its depiction as the absolute RMSE (Fig. 64).

Finally Figs. 66 and 67 provide insight into potential model bias. Negative values indicate that the model over predicts salinity relative to observed data while positive values indicate that the model under predicts salinity. Both of the following figures indicate that the model tends to over predict salinity (report higher salinities than observed) on the eastern (Trinity) side and under predict on western (San Jacinto) side. It should be stressed that bias on either side does not necessarily indicate a significant problem (see Table 8); however the largest discrepancies appear to be in the upper Trinity Bay, an area that has been recognized as being problematic in previous reports.

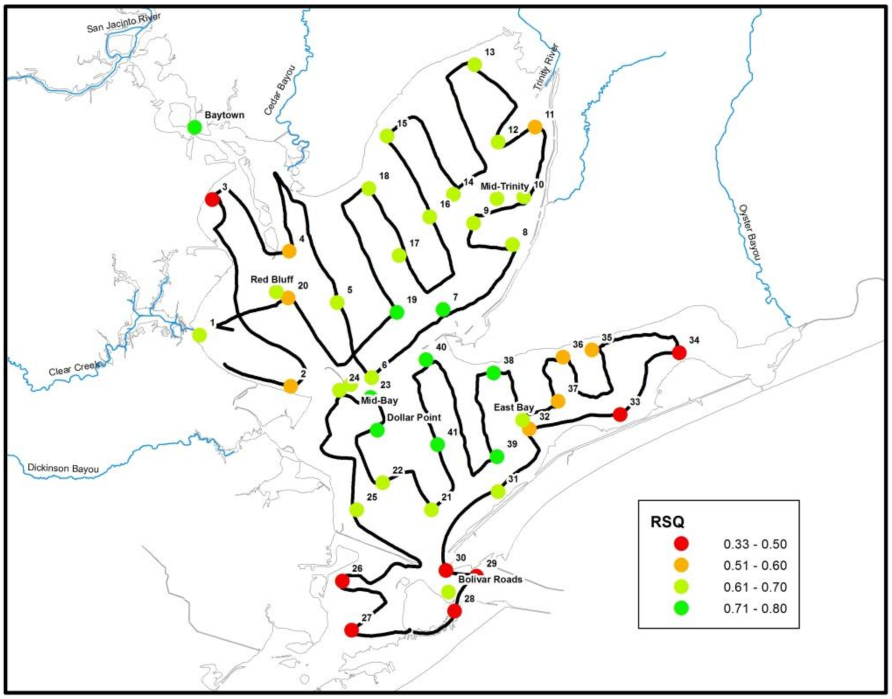


Figure 62 Coefficient of determination (RSQ) at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay.

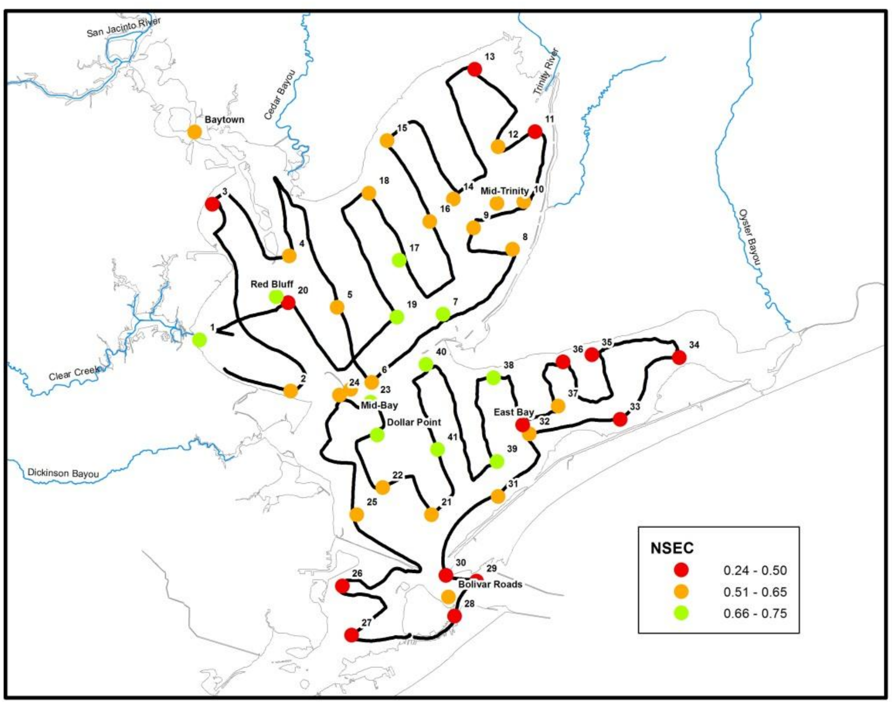


Figure 63 Nash-Sutcliffe Efficiency Criterion (NSEC) at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay**.**

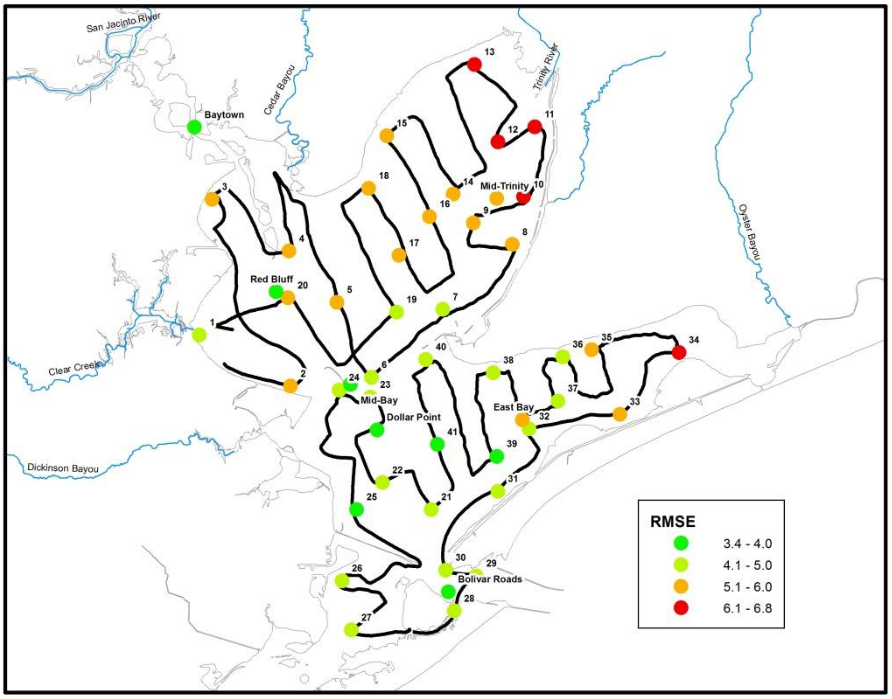


Figure 64 Root Mean Squared Error (RMSE) at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay.

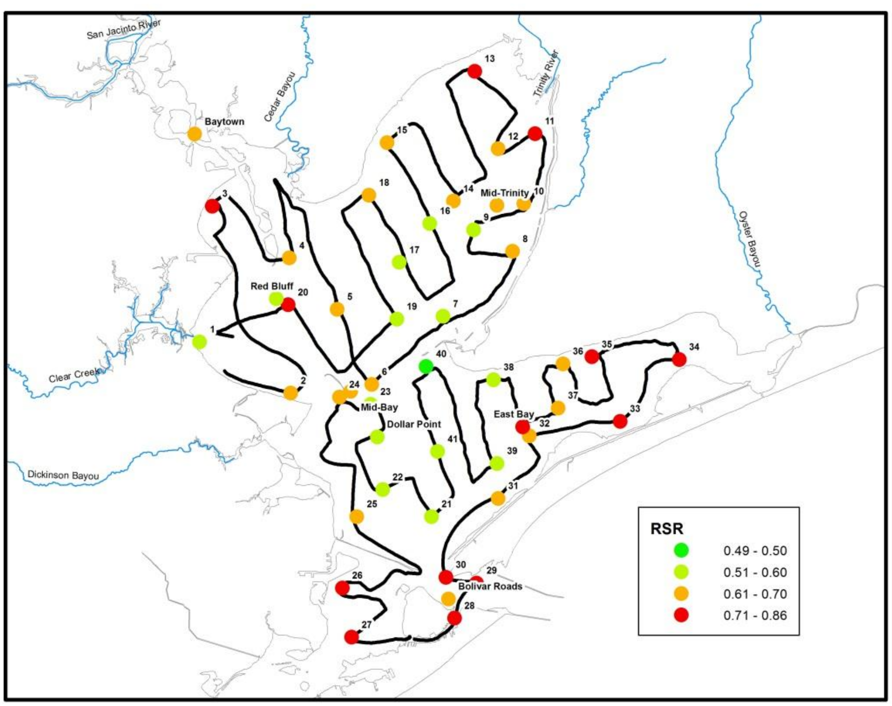


Figure 65 RMSE-observations standard deviation ratio (RSR) at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay**.**

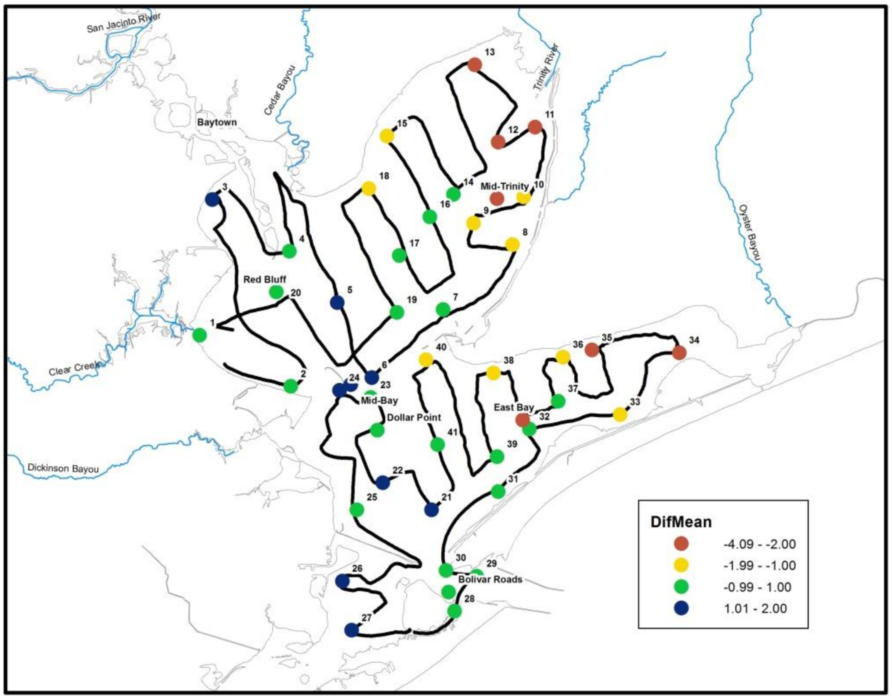


Figure 66 Differences in observed versus simulated means at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay.

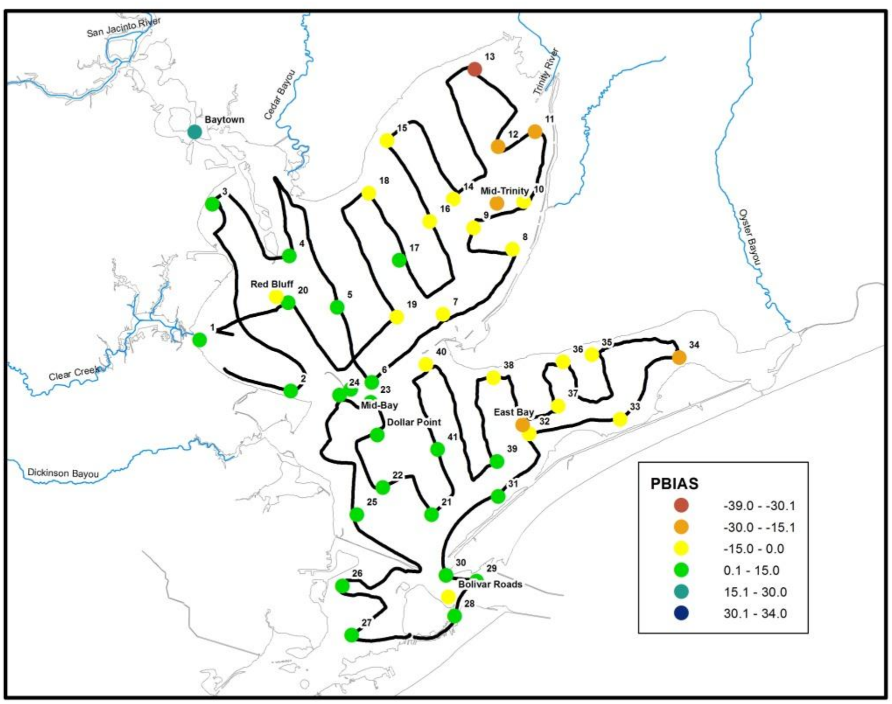


Figure 67 Percent bias (PBIAS) at 41 TAMUG dataflow stations and 8 TWDB datasondes in Galveston Bay.

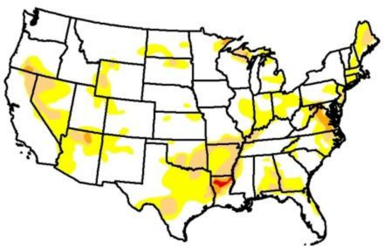
5. Discussion

This project focused on accessing both flora and fauna responses to freshwater inflows in Galveston Bay. Given the exceptional drought in 2011, the interpretation of our findings were complicated by this significant event. Specifically, in some cases we observed different patterns in 2012 relative to 2010 and 2011 but it is too early to know if these differences were associated with the drought event or if they were a part of the natural variability in this ecosystem.

5.1 2011 drought

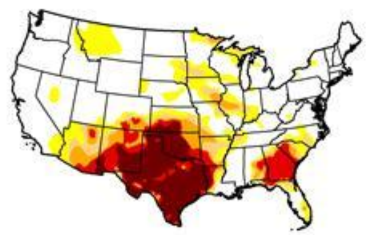
The project period covered the beginning of 2010 through to the end of 2012, thereby bracketing the drought which started in October 2010 and arguably ended mid 2012 (Fig. 68). Most of Texas in fact experienced a D4 or "exceptional drought", the most severe classification by the U.S. drought monitor (http://droughtmonitor.unl.edu/). From December 31, 2010 to March 31, 2011, the drought status around Galveston Bay changed from abnormally dry (D0) to exceptional (D4). Conditions were only alleviated in January 21, 2012 when drought conditions were changed to from exceptional to severe. However, it was not until July 31, 2012 that drought conditions were no longer measured in this ecoregion.

In terms of rainfall, 2011 was one of the top five driest years on record for the Galveston Bay watershed on record since records started in 1871 in Texas (Table 9). The cities of Houston and Galveston received ~ 30 to 50 percent of the expected normal rainfall during 2011. Concurrently, the City of Houston experienced the warmest year on record in 2011, matching the previous record set in 1962 while the City of Galveston recorded its second warmest year on record, with 2006 established as the warmest year since record keeping started (Table 10). The 30 year average (1981-2010) rainfall is 54.65 inches at Hobby Airport, but less than half this amount fell in 2011 (25.41 inches; Fig. 15).



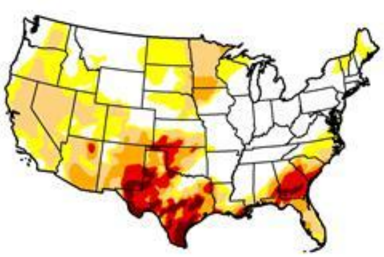
August 31, 2010 – Pre drought

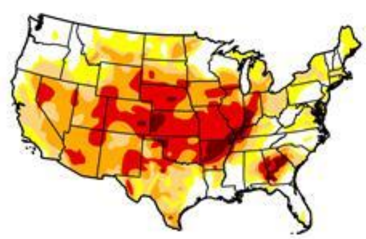
December 31, 2010



May 31, 2011

September 27, 2011





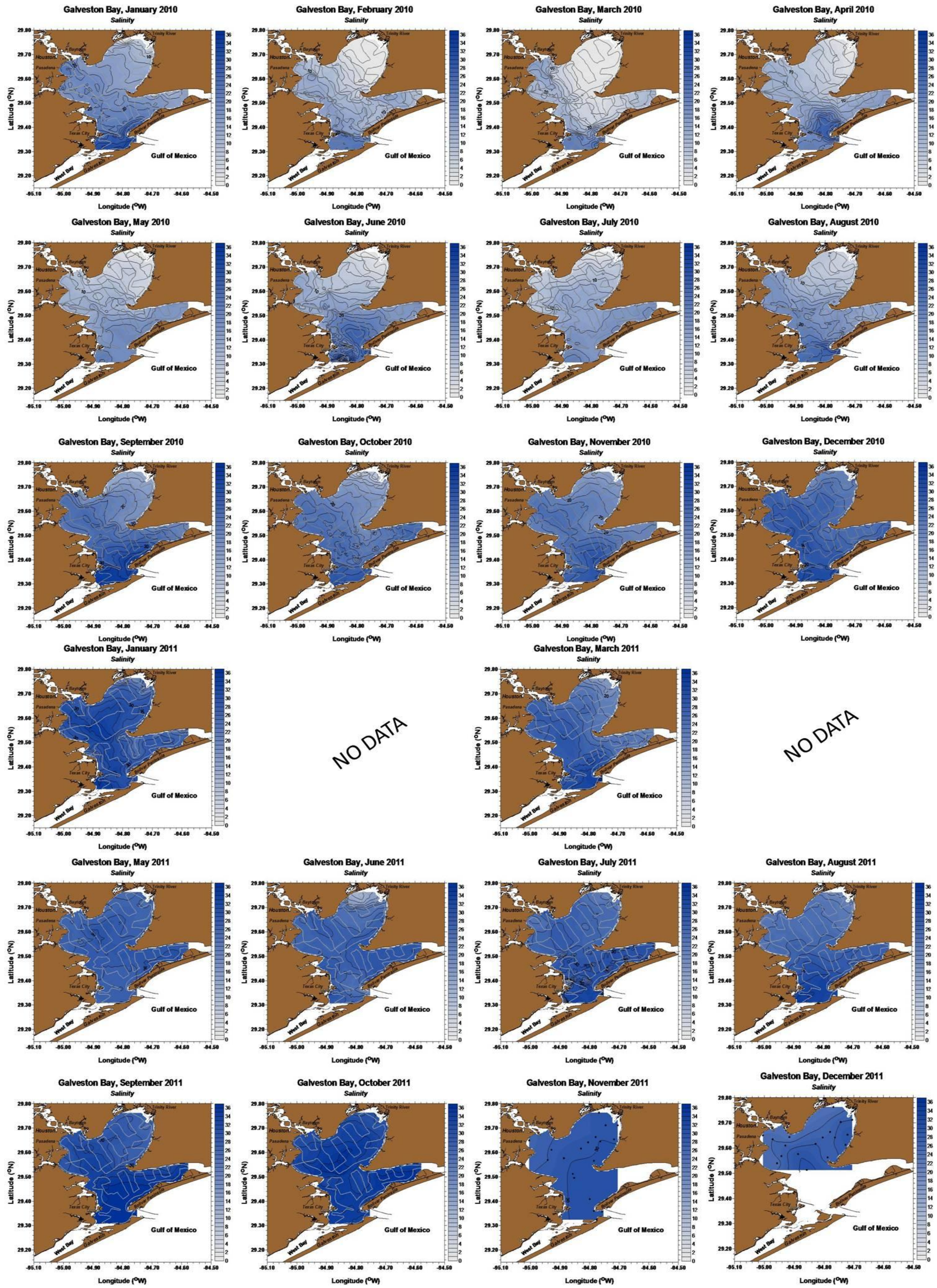
January 31, 2012

July 31, 2012 – Post drought

Drought Severity

D0 - Abnormally Dry
D1 Drought - Moderate
D2 Drought - Severe
D3 Drought - Extreme
D4 Drought - Exceptional

Figure 68 Maps showing the drought status in the US during the project period (http://droughtmonitor.unl.edu/archive.html).


Galveston Bay, January 2010
Salinity
Galveston Bay, February 2010
Salinity
Galveston Bay, March 2010
Salinity
Galveston Bay, April 2010
Salinity
Galveston Bay, May 2010
Salinity
Galveston Bay, June 2010
Salinity
Galveston Bay, July 2010
Salinity
Galveston Bay, August 2010
Salinity
Galveston Bay, September 2010
Salinity
Galveston Bay, October 2010
Salinity
Galveston Bay, November 2010
Salinity
Galveston Bay, December 2010
Salinity
Galveston Bay, January 2011
Salinity
NO DATA
Galveston Bay, March 2011
Salinity
NO DATA
Galveston Bay, May 2011
Salinity
Galveston Bay, June 2011
Salinity
Galveston Bay, July 2011
Salinity
Galveston Bay, August 2011
Salinity
Galveston Bay, September 2011
Salinity
Galveston Bay, October 2011
Salinity
Galveston Bay, November 2011
Salinity
Galveston Bay, December 2011
Salinity
Gulf of Mexico
Latitude (°N)
Longitude (°W)

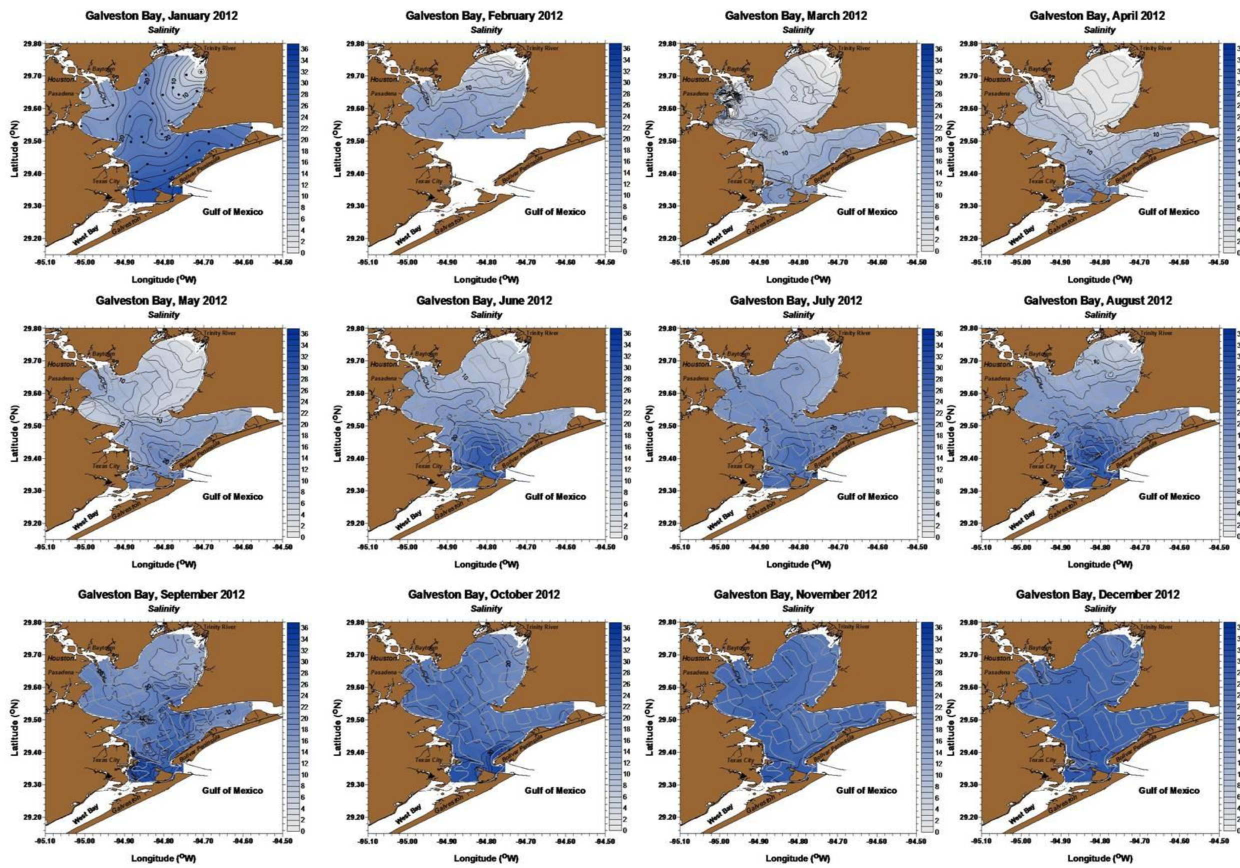


Figure 69 High spatial and temporal resolution maps of salinity measured monthly in surface waters of Galveston Bay from January 2010 to December 2012 following grid presented in Fig. 1. Scales were the same for all maps and the salinity range is 0-36 (white to blue respectively).

These changing conditions were reflected in the surface waters of Galveston Bay, particular in terms of salinity (Fig. 69). In Fig. 17, we see that significant freshwater inflows (freshets > 10,000 cfs) occurred during the spring of 2010 and 2012. When these occur, large areas of Galveston Bay surface water salinities drop to below 10. These inputs can be seen in 2010 and 2012 (white areas on maps below). The combination of a lack of freshwater inflow and lack of rainfall resulted in elevated salinities across the Bay starting in late 2010 and persisting until early 2012 (Fig. 69). Starting late summer of 2012 through to the end of the year, freshets were minimal and again salinities rose across Galveston Bay. Hence, during the project period, salinities in Galveston Bay were frequently higher than typically experienced (Quigg et al. 2007, 2009a,b; Quigg 2009, 2010).

5.2 Effect of freshwater inflows on phytoplankton in Galveston Bay – response to nutrient stress

There were many consequences for both the flora and fauna in Galveston Bay. With just a couple of exceptions, DIN:P ratios were always < 1 at stations 12, 6 and 29 suggesting phytoplankton were N limited at these locations for most of the study period, especially during 2011 (Fig. 32). This is consistent with findings in other coastal ecosystems which are reported to be frequently N limited (Howarth and Marino 2006) and previous studies in Galveston Bay (Quigg et al. 2007, 2009a,b; Quigg 2009, 2010). In the winter time, from January to March, DIN:P ratios were greater than 14 indicative of P limitation at this station. The switch from potential N to P limitation in the Bay has been previously observed by our group (Quigg et al. 2007, 2009a,b; Quigg 2009, 2010). By contrast, at Station 4, we found DIN:P ratios were frequently in the range of 7:1 to 12:1, especially from April-May to December which would indicate the phytoplankton in the upper San Jacinto River basin were neither N or P limited. The contentions on the regulation of phytoplankton biomass responses based on nutrient ratios were supported by the RLAs (Figs. 40 to 45) which showed that the addition of all nutrients (nitrate, ammonium, phosphorus and silicate) most frequently stimulated phytoplankton growth during March each year whereas in July 2010 and 2011, the greatest response was measured in the treatments with nitrate and phosphorus additions. There are two possibilities for these differences: (i) phytoplankton community compositions – see section 4.3 and (ii) temperature effects driving seasonal variations. Our findings suggest both these factors may have been important. Further supporting that freshwater inflows are an important driver in this Bay when it comes to phytoplankton responses are the findings in the RLAs performed during the summer 2012 (Fig. 45). Unlike previous RLAs performed, there was a large and prolonged freshwater inflow period followed by a month of very low flow. The long freshwater inflow period may have flushed phytoplankton out of the Bay, that is, their growth rate could not outcompete their dilution rate. The subsequent period of low flow period may have left remaining populations without sufficient nutrients to continue growing. Such responses have been observed in Chesapeake Bay and other systems (Malone et al. 1988; Fisher et al. 1999; Chan and Hamilton 2001).

5.2.1 Phytoplankton communities in Galveston Bay

Given we now have phytoplankton community data from 2005 to 2012 (minus 2007; identifications to genera level, enumeration and biovolumes), we used multivariate statistics to observe the biodiversity of phytoplankton genera identified throughout the study period. The presence-absence data for each station represented in Tables 12, 13 and 14 were analyzed with PRIMER-E V6 software using a taxonomic distinctness test. This method was developed as an extension to the Simpson diversity index which determines the probability of two random individuals from a sample belonging to the same type (i.e. species; Simpson 1949). Average taxonomic diversity (Δ) expands on this idea by determining the average taxonomic distance apart of two individuals from the same sample on a classification tree (Clarke and Warwick 2001). To remove the weighted bias of uneven count numbers, this test was applied to presence-absence data for comprehensive genus lists spanning all years of the study for each station which resulted in the determination of the average taxonomic distinctness (Δ^+) for each station (Clarke and Warwick 2001).

The results of the taxonomic distinctness tests (Δ^+) for each station were displayed using a funnel plot format (Figure 70). Each of these figures display a mean expected Δ^+ value (dotted line) and the upper and lower limits of the expected range of Δ^+ (solid lines) along with the actual Δ^+ values of the data from each year superimposed on the image. As it is more difficult to determine the change in distinctness of smaller sample sizes, the limits are wider for small numbers of species and narrower for large numbers (Clarke and Warwick 2001). Actual Δ^+ values that fall within the boundaries of the funnel represent that the sample data matched expected taxonomic distinctness trends while those that fall outside the range are reduced in some way.

Of the three stations, Station 1 has the highest values for average taxonomic distinctness and the best fits within the funnel of expected taxonomic distinctness (Fig. 70A). While most of the Δ^+ values for Station 2 also fall inside the expected range, these values are lower and suggest slightly less taxonomic distinctness (Fig. 70B). Station 6 has poor taxonomic distinctness compared to Stations 1 and 2 with low Δ^+ values within the funnel and several values dropping out of the expected range (Fig. 70C). These data reflect observations that phytoplankton samples were more diverse at Station 1 due to its proximity to regular freshwater influence.

A

B

C

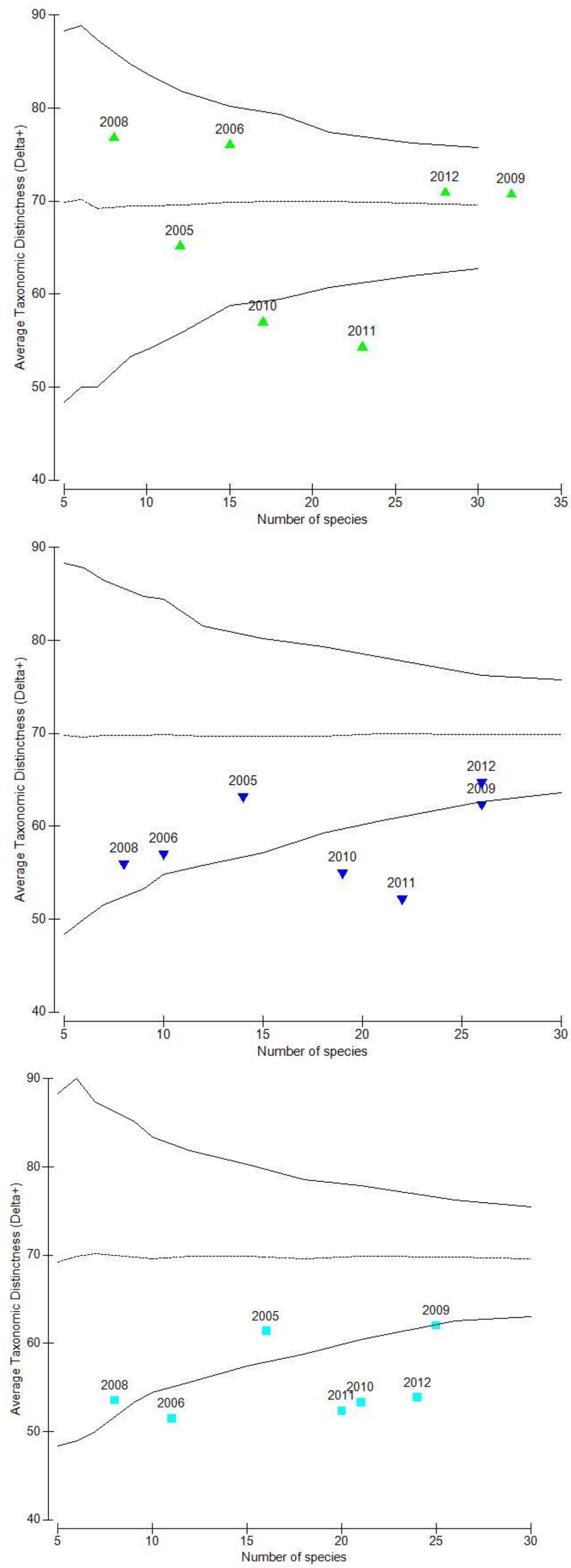


Figure 70 Taxonomic distinctness tests performed for the phytoplankton communities found at the three main stations (see summary Tables 12, 13 and 14) from 2005 to 2012 are displayed using a funnel plot format.

A, B and C represent phytoplankton communities present at Stations 1, 2 and 6 (corresponding to stations 12, 6 and 29 respectively in Figure 1; see also Tables 3 and 4 for latitudes and longitudes and other station details).

Freshwater inflows from the Trinity River help this station maintain a low salinity and are likely to usher nutrient pulses to the area which would stimulate a rich phytoplankton community. Station 2, which is more centrally located in Galveston Bay, may be subject to some of the Trinity River's far reaching effects but is equally likely to be influenced by the influx of saline waters from the Gulf of Mexico. Station 6 is heavily influenced by marine water from Gulf of Mexico and is more taxonomically distinct from Station 1 and 2.

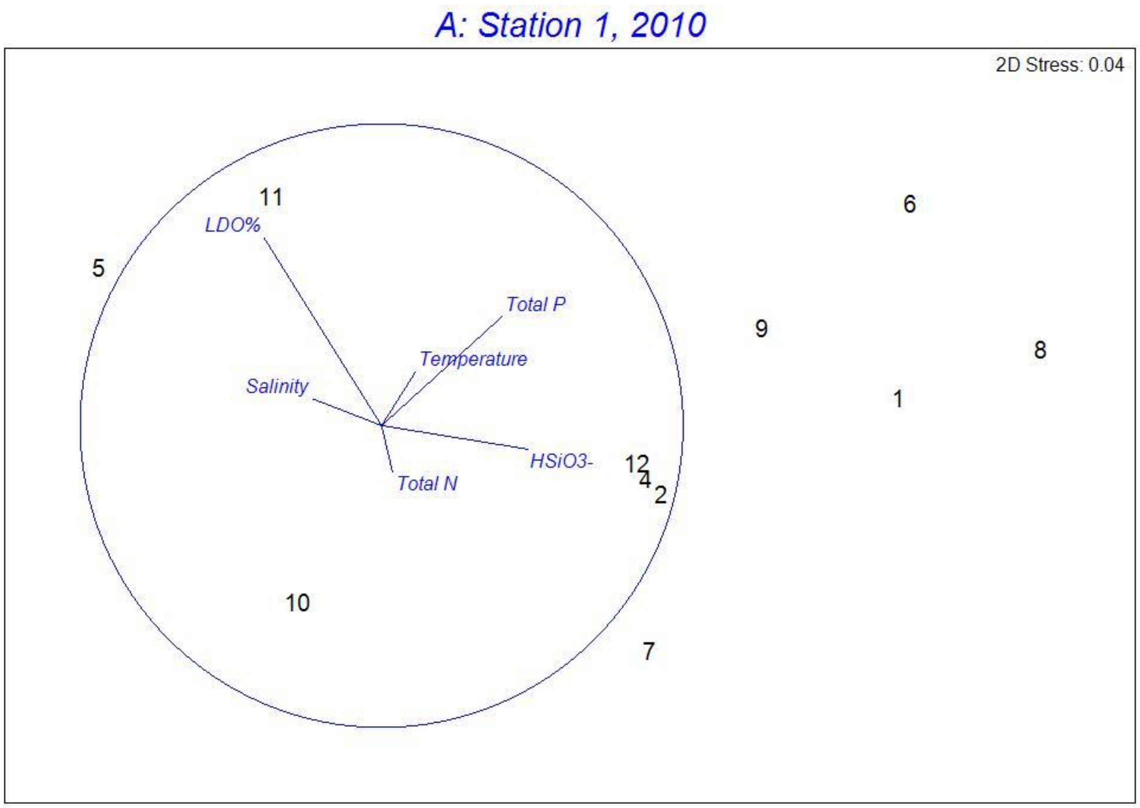
An important point of interest illustrated by Figures 70A, B and C is that all three stations experience a drop in taxonomic distinctness in 2010 and 2011. This implies a disturbance occurred in that time frame that negatively impacted phytoplankton communities especially at Stations 1 and 2. This reflects the consequences of the drought on phytoplankton populations, with limited freshwater inflows and nutrient pulses to phytoplankton communities in those sensitive areas. This may also help explain why our findings in the RLAs for 2012 are so different from those performed in 2010 and 2011 (see above).

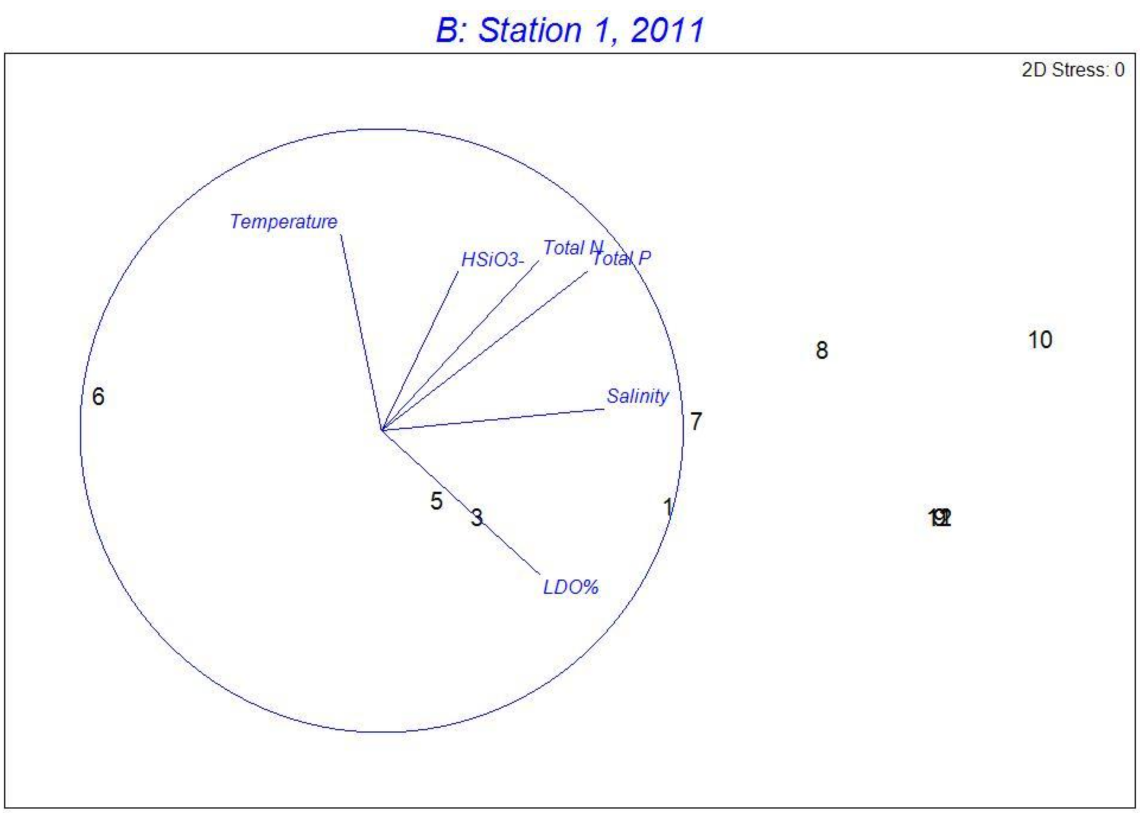
5.2.2 Interactions between biotic (phytoplankton) and abiotic factors

To determine which environmental variable or combination of variables contributed most strongly to the drop in average taxonomic distinctness in phytoplankton at all stations in 2010 and 2011 shown in Figures 70A, B and C the algal group data represented in Figures 18, 19 and 20 were analyzed using multivariate statistics. First, algal group count data were organized into six separate tables for each sample site in 2010 and 2011 respectively. Using PRIMER-E V6 software, these data were individually transformed and processed into similarity matrices using the Bray-Curtis coefficient (Bray and Curtis 1957). These matrices were then used to plot six separate multi-dimensional scaling (MDS) plots representing each station in 2010 and 2011 respectively. MDS plots reflect the similarity of samples by using their proximity in space to represent their relatedness (Clarke and Warwick 2001). The goodness of fit or stress of each plot is represented in the upper right corner of each figure. Stress values below 0.1 represent ideal goodness of fit (Clarke and Warwick 2001). The numbers in each plot represent the month in which each sample was collected.

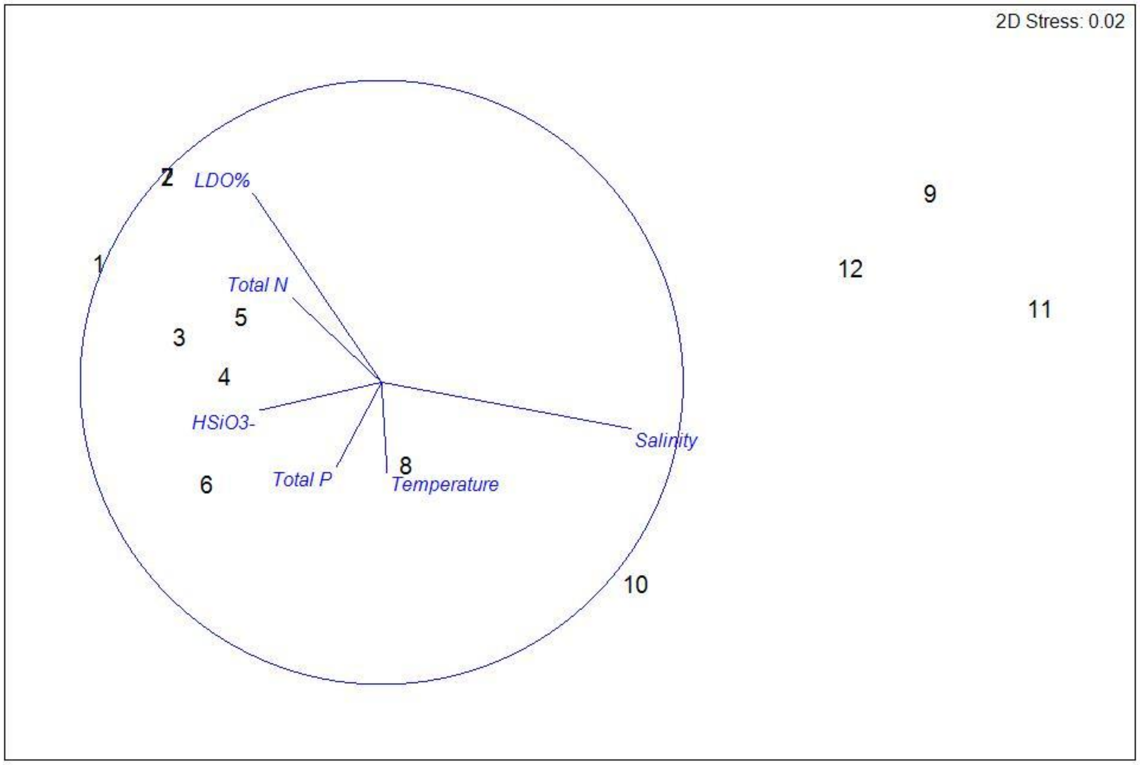
In addition, environmental data parameters from the Dataflow used during sample collection and results of nutrient analysis conducted on surface water from Stations 1, 2 and 6 were organized into six separate tables depending on station and year. The values in these environmental data tables were normalized using PRIMER-E and compared with the biological algal group data of the same station and year by plotting them as vectors and superimposing them on the biological MDS plots. Vectors were determined by Pearson correlation with vectors of longer lengths representing greater influence and directions indicating which of the biological variables they influenced most strongly (Clarke and Warwick 2001). The outcomes of the MDS are shown in Figure 71 below.

Figures 71A and B represent Station 1 in the year 2010 and 2011 respectively with the numbers on each plot representing the month of the corresponding sample data. In Fig. 71A, no distinct groups are formed between the months and none of the environmental variables display a particularly strong influence on the arrangement of the biological variables. However, in Fig. 71B, data from July to December 2011 appear to separate out toward the right of the plot along the path of the salinity vector. This suggests that the algal group compositions at Station 1 in 2011 were affected by a change in salinity. Similar to Figures 71A and B, Figures 71C and D represent Station 2 in the year 2010 and 2011 respectively. In Fig. 71C, data from September to December 2010 appear to separate out toward the right of the plot along the path of the salinity vector. This pattern is reinforced in Fig. 71D in which data from August to December 2011 are grouped tightly under the strong salinity vector. These plots suggest that the algal group compositions at Station 2 were affected by a change in salinity in both the year 2010 and 2011. In the style of the previous figures, Figures 71E and F represent Station 6 in the year 2010 and 2011 respectively. Much like the MDS plot for Station 1 in 2010, Fig. 71E displays no distinct groups forming between the months and no dominant influence of any environmental variables. However, in Fig. 71F, data from July to December 2011 form the most distinct grouping of all previous plots and separate out toward the right of the plot in the direction of the salinity vector. These findings suggest that the algal group compositions at Station 6 in 2011 were greatly affected by salinity fluctuations.

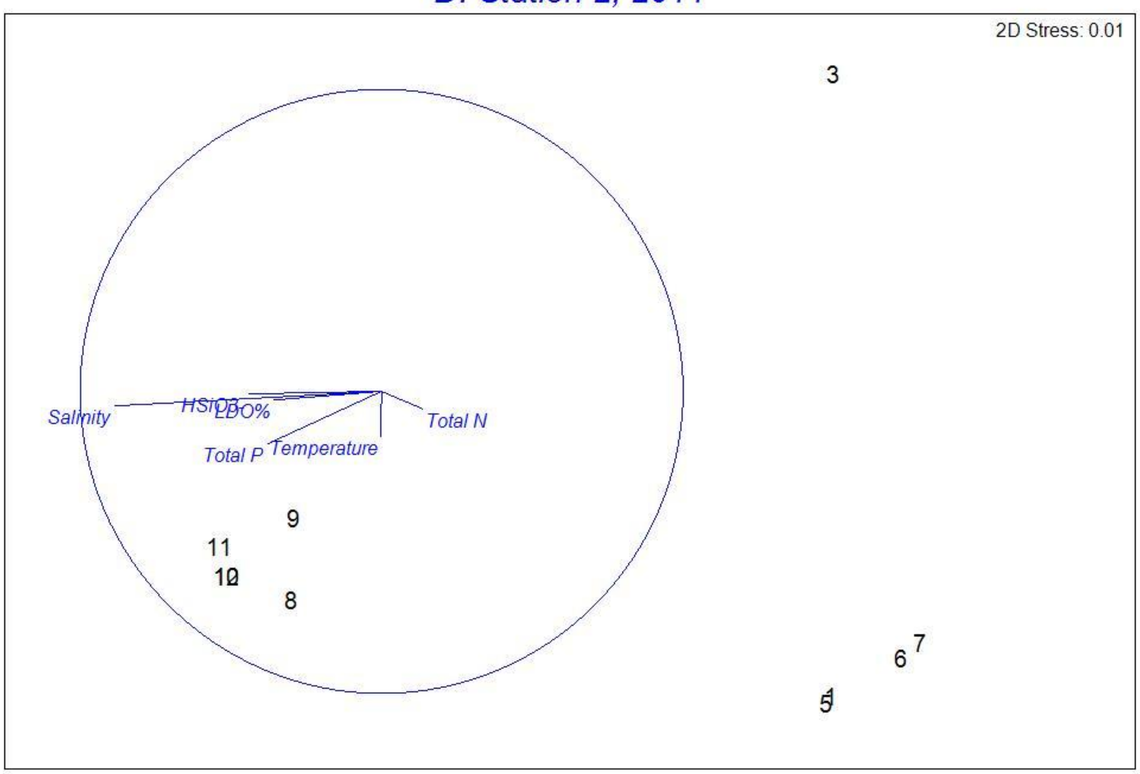

A: Station 1, 2010
2D Stress: 0.04
11
LDO%
6
5
Total P
9
Temperature
8
Salinity
1
HSiO3-
12
4
2
Total N
10
7

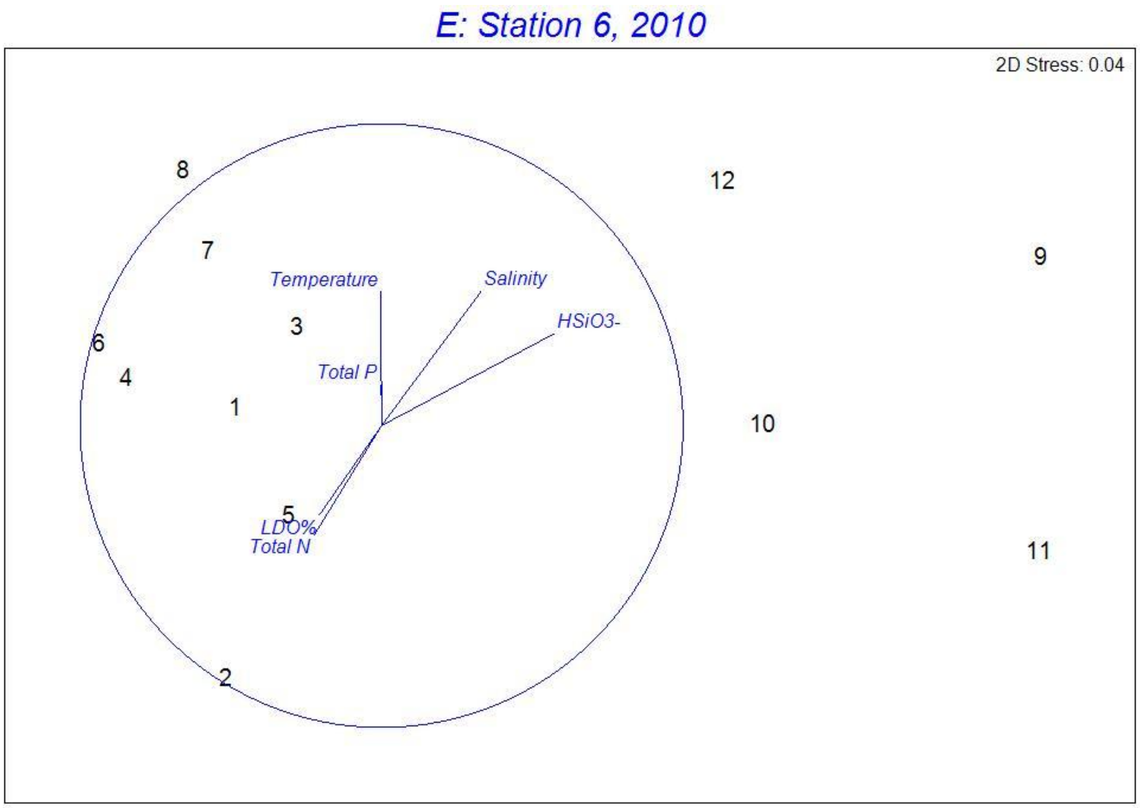

B: Station 1, 2011
2D Stress: 0
Temperature
HSiO3-
Total N
Total P
8
10
6
Salinity
7
5
3
1
LDO%

C: Station 2, 2010



D: Station 2, 2011





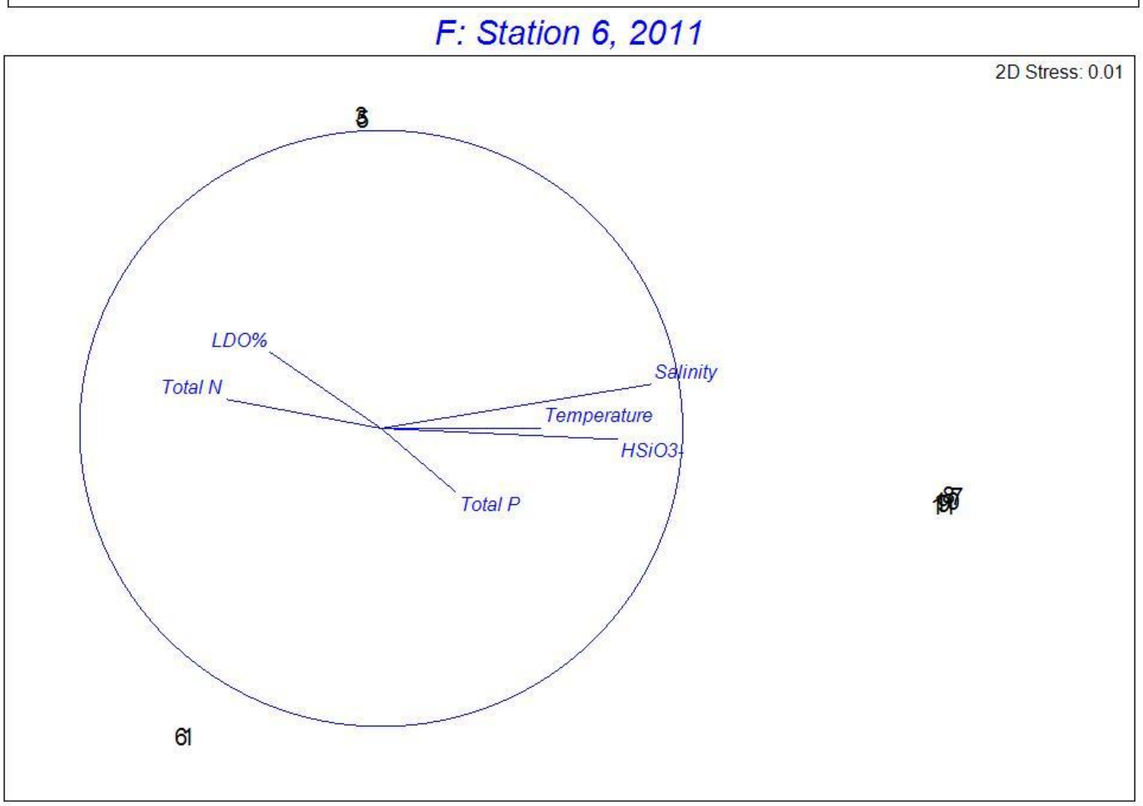


Figure 71 MDS plots of similarity of biological variables for Stations 1, 2 and 6 in 2010 and 2011 (A, B, C, D, E and F respectively) with vector overlays of environmental variable influences.

Though the previous MDS figures are useful visual tools, they only suggest trends and do not show which of the vectors is most statistically important to the biological parameters. Keeping this in mind, further multivariate statistical analysis was conducted. Using a biological and environmental stepwise test (BEST), the most statistically influential environmental factors for each station and year were determined by finding the variable or combination of variables that induced a grouping of biological variables similar to those groups formed in their natural MDS plots. The variables or combinations of variables that yielded the highest weighted Spearman rank coefficient (ρ) for each station and year are represented in Table 22 below. The value of ρ is a reflection of the similarity ranks of each variable (Clarke and Warwick 2001). If ρ approaches 0, it indicates an absence of any match between biological and environmental patterns; however, if ρ is higher than 0.5 and approaches 1, it denotes a strong correlation between biological and environmental variables (Clarke and Warwick 2001).

Table 22 Environmental variables most influential in Galveston Bay algal group count similarities in 2010 and 2011 and their respective ρ coefficients.

Year	Station	ρ	Important Environmental Variable(s)
2010	Station 1	0.405	% Dissolved Oxygen
	Station 2	0.555	Salinity
	Station 6	0.308	$HSiO_3^-$ Concentrations
2011	Station 1	0.519	Salinity, Total Nitrogen
	Station 2	0.550	Salinity
	Station 6	0.772	Salinity

Observing the results of the BEST test for each station and year, it is clear that in 2010, only the phytoplankton community at Station 2 was significantly impacted by any environmental variable, in this case salinity. While algal group counts at Station 1 and 6 were influenced by % dissolved oxygen and silicate concentrations respectively, the values of their ρ coefficients indicate that the correlations are weak. However, in the record drought year of 2011, all stations were strongly impacted by changes in salinity. Furthermore, Station 1 was strongly affected by a combination of salinity and total nitrogen fluxes. Because Station 1 is closest to the mouth of the Trinity River, the phytoplankton community at this location likely suffered a decrease in average taxonomic distinctness (Fig. 70) due to a lack of freshwater inflow and nitrogen influx typical of normal conditions.

5.3 *Vallisneria americana*- use as a bio-indicator in Galveston Bay?

As part of the BBEST process, *Vallisneria* was identified as a potential bio-indicator for freshwater inflows to Galveston Bay (Espey et al. 2009). Changes in submerged aquatic vegetation (SAV) including that for *Vallisneria americana* distribution can be linked to direct effects in hydrologic changes such as physical disturbances in the water (e.g. flow velocities, stage and residence times) (Dobberfuhl et al. 2009). Indirect effects of changes in water quality influenced by hydrologic alterations include saltwater intrusion, increased algal production, nutrient concentrations, and light availability (Dobberfuhl et al. 2009). Climatic disturbances such as severe droughts and tropical storms and hurricanes can impact SAV distribution and abundance by increasing salinities and light attenuation and completely removing the plant from the site (Sagan 2007). Ambient light conditions can impact salinity effects on SAV (Dobberfuhl et al. 2009). Submerged aquatic vegetation metabolism can be influenced by increased salinity can which can have negative effects on the plant if energy is already restricted by light availability and photosynthetic capacity (Dobberfuhl et al. 2009). French and Moore (2003) found increased light conditions allowed SAV (i.e. *Vallisneria americana*) to tolerate increased salinity (up to 5); consistent with the findings of Dobberfuhl et al. (2009) which reported that limited light conditions will decrease SAV salinity tolerance.

A study by Sagan (2007) in Florida summarized the changes in SAV distribution within the lower St. Johns River Basin from 1996-2007. *Vallisneria* beds were found in oligohaline/mesohaline environments and extended from the lower St. Johns River into the upper St. Johns estuary. The presence of well-established beds of *Vallisneria* in these areas coincide with literature that report adult plants are more tolerant of salinities of 10 (Espey et al. 2009) up to as high as 12 (Twilley and Barko 1990; French and Moore 2003). From 1998-2007, *Vallisneria* was the dominant SAV in terms of latitudinal distribution, within bed distribution and coverage despite extreme droughts brought on from 1999-2000 and 2006-2008 in the St. Johns River Basin. This is likely due to the fact that this species was found starting in the oligohaline/mesohaline reach and up to 100 miles upstream where salinities would typically decline.

Growth of *V. americana* can potentially occur over a wide range of temperatures. Barko et al. (1982) reported a decline in plant dry biomass, shoot density and length in temperatures of 16 °C or less. They did see an increase in growth with increased temperatures from 16-28 °C with optimal growth conditions observed at 28-32 °C. Temperatures for seed germination are most favorable at >22 °C (Jarvis and Moore 2008). Other water quality parameters such as pH, dissolved oxygen, total suspended solids and chl *a* concentration can create a threshold for the distribution of *V. americana*. *Vallisneria* is less common in areas with a pH of less than 6 (Crisé et al. 1985; Hunt 1963) indicating a sensibility to acidification. Hunt (1963) found *V. americana* to be one of the most abundant plants of the lower Detroit River where pH levels ranged from 6.5 to 9.0. Campbell (1939) found this species far enough downstream from sewage effluent where dissolved oxygen levels began to increase. Brinley (1942) measured dissolved oxygen levels from 3.0 to 5.0 mg/L in an area of a polluted stream comparable to Campbell (1939). In the Detroit River where *Vallisneria* was found dissolved oxygen levels were generally above 8.0 mg/L (Hunt 1963). Kemp et al. (2004) calculated water quality thresholds for SAV and found in salinities of 0.5-18 to be most appropriate. They reported total suspended solids and chl *a* concentrations greater than 15 (mg/L and μg/L, respectively) exceeded the limits for the presence of primarily fresh and brackish water SAV.

Sediment composition can also play an important role in SAV distribution. Smart and Barko (1985) found that *V. americana* grows on fine textured sediment comprised of < 20% organic content. Hunt (1963) found this species on substrates ranging from gravel to hard clay but optimal growth on silty sand. In laboratory experiments, Rybicki and Carter (1986) found that the number of viable *Vallisneria* plants grown from tubers (overwinter buds) significantly increased in both silty clay and sand substrates at a depth of 10cm. Jarvis and Moore (2008) found seed germination of *V. americana* was enhanced in sediments composed of ≤ 3% organic content and > 40% sand.

5.3.1 Historical distributions of *Vallisneria* in Galveston Bay

Vallisneria plants have been documented in the Trinity River delta of Galveston Bay and surrounding wetland areas (Fig. 72). Adair et al. (1994) found *Vallisneria* was the dominant SAV in shallow and oligohaline (< 10) waters of Trinity Bay, Texas. These authors also reported *Vallisneria* beds were extensive with a high biomass along the northeast shorelines of the river delta. The presence of sand bars along the Trinity River delta attenuate wave action and turbidity northeast of the bars and make this primarily muddy substrate conducive for SAV growth (Adair et al. 1994).

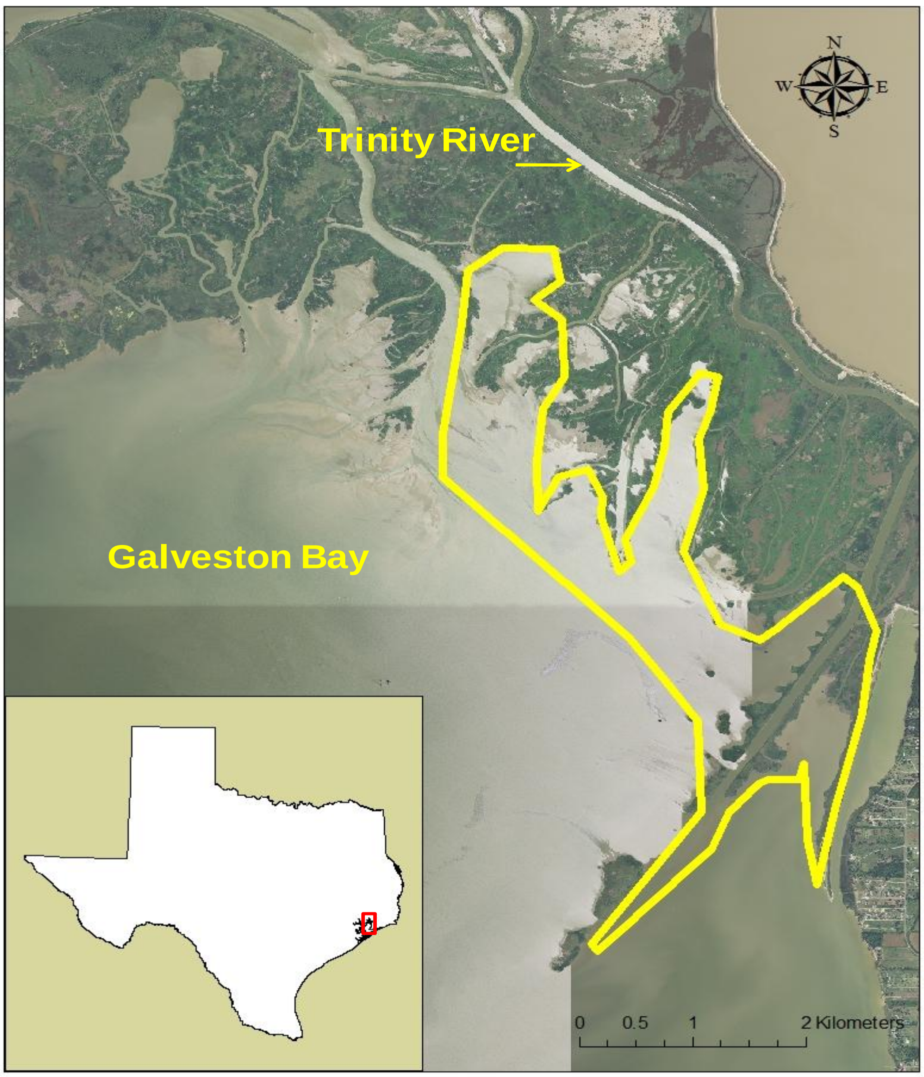


Figure 72 Distribution boundary of *V. americana* compiled by Texas Parks and Wildlife Department based on described surveys conducted by Adair et al. (1994). Map source Texas Natural Resources Information System (TNRIS) 2010 NAIP aerial images at http://www.tnris.org/.

5.3.2 Effect of drought on *Vallisneria* in Galveston Bay

Despite extensive search during the period of this study at > 20 stations visited between March 2010 and December 2012 (Fig. 73), we did not find *Vallisneria* plants in the Trinity River basin of Galveston Bay in location predicted based on the studies of Pulich (2006) and Adair et al. (1994) or based on the map provided by TPWD (Fig. 72). Stations were added further away from the boundary map (see Fig. 73) but this did not resolve the issue.

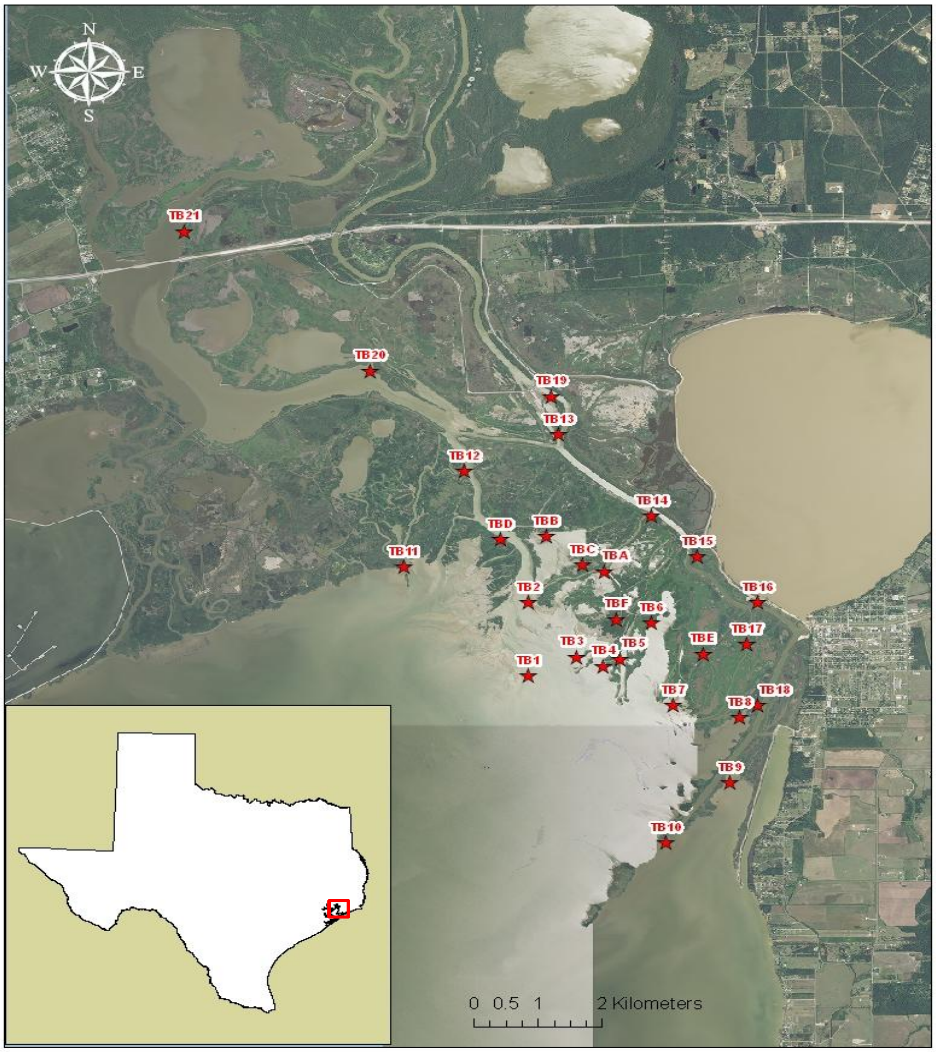


Figure 73 2011 survey stations for *V. americana* and water quality and sediment characteristics along the Trinity River and delta area in Texas. Map source Map source Texas Natural Resources Information System (TNRIS) 2010 NAIP aerial images at http://www.tnris.org/.

We hypothesize that the elevated salinities throughout the study period (see Figs. 22 and 69) would not have been conducive to the plants which require salinities of < 5 germinate (Campbell 2005; Jarvis and Moore 2007) and up to 10 for growth and survival of adult plants (Twilley and Barko 1990; French and Moore 2003; Espey et al. 2009). Dobberfuhl et al. (2009) hypothesized that *V. americana* would experience salinity stress within thirty days of salinities > 5 and eventual mortality > 15. Hence, the complete absence of *Vallisneria* plants can be considered an indication of the consequences of prolonged long flow periods, particularly in 2011, when salinities at all stations remained > 12 for the year (Fig. 69).

That we did not see the plants return in 2012, despite the large freshets in the spring (Fig. 17) may be in part because salinities were still relatively high (Fig. 69) or it may be due to some other combination of factors that we do not yet understand. For example, the combination of a drought associated with prolonged low flows, reduced nutrient concentrations and sediment flux to the Trinity River Basin. Hence, it may take more than one season for the plants to recover.

A further possibility is that although Adair et al. (1994) did actually visit the sites and examine the *V. americana*, the distribution boundary of *V. americana* compiled by TPWD (Fig. 72) using TNRIS 2010 NAIP aerial images (http://www.tnris.org/) was not ground-truthed. Hence, these maps may reflect *Ruppia* beds, observed in this location during the study (see Fig. 8) or some other SAV or combination of SAVs. Therefore, the absence of *V. americana* during the study may reflect a more long term absence than originally believed. Adair et al. (1994) found *Vallisneria* meadows accounted ~ 15% of the SAV community in Galveston Bay. This was reported as a decline relative to previously published studies from the 1970s. The decline in SAV in the Galveston Bay complex appears to coincide with major shorefront development which negatively impacts the water quality in this important ecosystem.

While the present studies' findings may be an anomaly associated with sampling during a drought period, it certainly highlights the need for long term monitoring of *V. americana* distributions if they are to be used as a bio-indicators for Galveston Bay health. Further, it highlights the fundamental outcome of reduced freshwater inflows to the Bay for a prolonged period and the need to carefully consider any strategies proposed for the use of freshwater upstream of the Bay.

5.4 *Rangia* sp. - use as a bio-indicator in Galveston Bay?

Estuarine organisms can be considered as meaningful bio-indicators of environmental conditions, especially benthic macroinvertebrates because of their sedentary lifestyle (Reish 1986; Bilyard 1987). *Rangia* clams maybe important bio-indicators as they provide a link between primary producers and consumers; these nonselective filter feeders convert plant detritus and phytoplankton into clam biomass (Darnell 1958) and in turn are preyed upon by fish, crustaceans, mollusks and ducks (references cited in Hopkins et al. 1973). *Rangia* clams can also help improve water quality due to their filter feeding abilities and subsequently enhance the presence of submerged aquatic vegetation (Officer et al. 1982). In Galveston Bay, both *Rangia cuneata* and *Rangia flexuosa* can be found.

5.4.1 *Rangia cuneata* versus *Rangia flexuosa*

Rangia cuneata is a species of mollusk that inhabits brackish waters along the Atlantic coast from Chesapeake Bay to the Gulf of Mexico (Fairbanks 1963; Tenore et al. 1968; Wakida-Kusunoki and MacKenzie 2004; Wolfe and Petteway 1968). *Rangia cuneata*, known as the Atlantic *Rangia*, common *Rangia*, *Rangia* clam, brackish water clam, or estuarine clam, is a dominant bivalve of the Gulf of Mexico estuaries (Wong et al. 2010). This species is found in low salinity estuarine environments (Cain 1973; Hopkins 1970; Parker 1966) ranging from 5-20 (Swingle and Bland 1974). *Rangia cuneata* has the ability to osmoregulate which allows for this species to respond to sudden salinity changes in low salinity environments (Bedford and Anderson 1972). *Rangia cuneata* also tolerates salinities that are too low for other estuarine species (0-13) such as oysters (Hopkins and Andrews 1970).

Although *Rangia flexuosa* is closely related to *Rangia cuneata* (LaSalle and De la Cruz 1985), it is not reported in the literature as often. *Rangia flexuosa*, known as brown *Rangia* (LaSalle and De la Cruz 1985) is not found along the Atlantic coast but inhabits coastal waters from Louisiana down to Mexico (Wakida-Kusunoki and MacKenzie 2004). *Rangia flexuosa* is also a brackish water clam found primarily in river-influenced bays where salinities vary from fresh to brackish over extended periods of time (Sheridan et al. 1989). The two species can occur together and are often found in sub-tidal zones (Wakida-Kusunoki and MacKenzie 2004). *Rangia flexuosa* is a lot less common than *Rangia cuneata* and easily distinguished from its close relative by the short posterior lateral tooth and the non-distinct pallial sinus (LaSalle and De la Cruz 1985). *Rangia* species vary in size among different populations which is often linked to differences in environmental salinities (Hopkins et al. 1973). This is why *Rangia* morphology such as shell length is commonly used as a growth indicator of mollusks in response to environmental conditions (Tenore et al. 1968). For the purpose of this study however, we did not distinguish between these two species in terms of understanding their response to freshwater inflows. In future studies however, that may be appropriate given the two species have different environmental niches.

5.4.2 Current distributions of *Rangia* sp. in Galveston Bay

We sampled *Rangia* populations in Galveston Bay from October 2010 to December 2012. Preliminary sampling trips conducted with TPWD personnel focused on determining the presence or absence of clams throughout the Bay. The initial sampling matrix (see summary Figure 74 below) was designed to visit areas in which *Rangia* had been found historically (see Figs. 2 and 46). Using the Trinity River and delta area as a detailed example here (Fig. 75), locations sampled for *Rangia* clams are shown with red and green symbols to indicate the absence and presence of clams respectively. Unexpectedly, not only did we find fewer clams but also in fewer locations than expected both in this area and other parts of Galveston Bay (Figs. 49-52).

Characterization of the current extent of *Rangia* clams indicates that *Rangia* clam shell length was largest in the areas with lowest salinities (Fig. 54). This is consistent with earlier studies in which *Rangia cuneata* were found only in parts of Galveston Bay where salinity was typically less than 15 (O'Heeron 1966). Further, Parker (1966) found *Rangia cuneata* in the upper Trinity Bay where salinities were 4-16 but found them to be "scarce" in the lower Trinity Bay and East Bay where salinities were 9-25 and 16-24, respectively. In addition *Rangia cuneata* was the only species found suggesting that *Rangia flexuosa* is less tolerant to such elevated salinity levels in the current Galveston Bay.

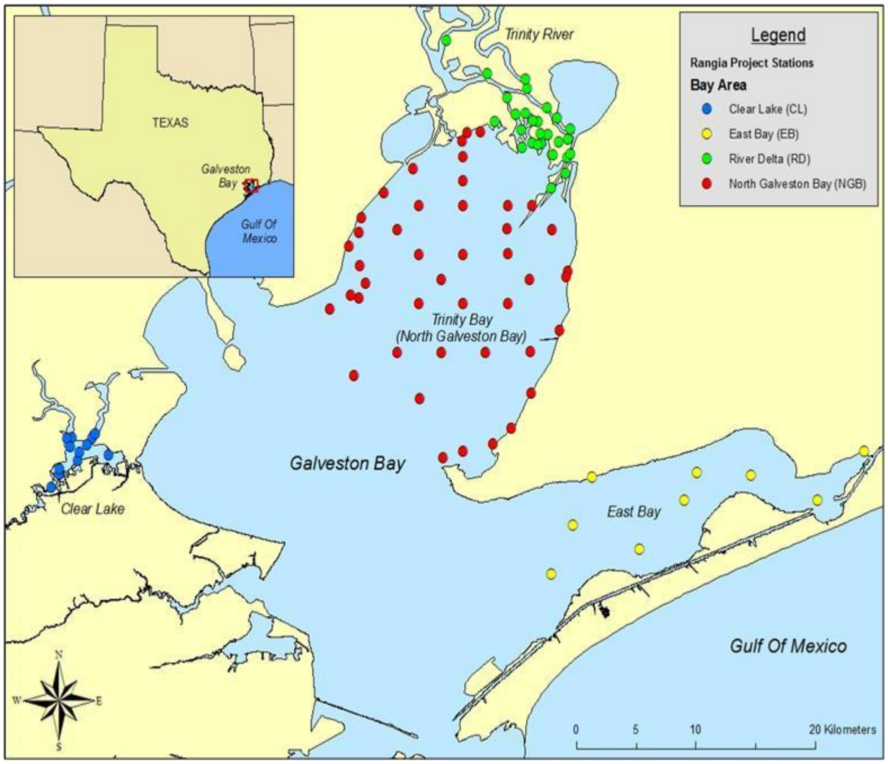


Figure 74 Survey stations for *Rangia* clams and water quality and sediment characteristics in Galveston Bay, Texas, from October 2010 to December 2012. More details can be found in Figs. 49-52 above.

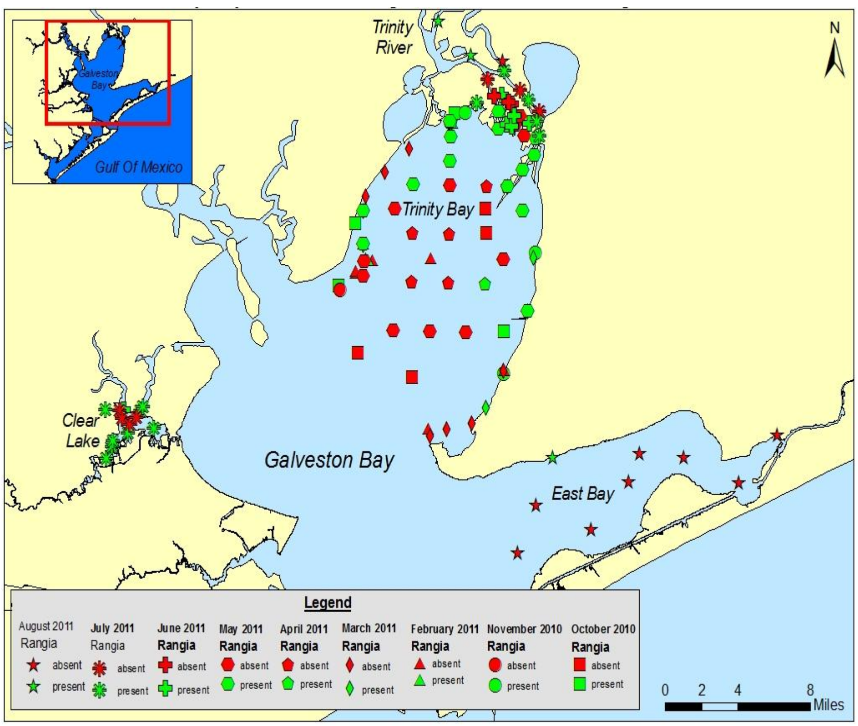


Figure 75 Presence vs. Absence of *Rangia* clams throughout Trinity Bay and surrounding areas, October 2010 to August 2011.

Rangia clams in the present study were generally found within the salinity range for clams found in Maryland, Louisiana and southern Texas (references cited in Hopkins et al. 1973). In conjunction with depleted freshwater inflow into Galveston Bay during the current project's study period, DO levels across the Bay were above the hypoxic threshold providing favorable conditions for the presence of *Rangia* clams. This is in contrast to the study in Lake Pontchartrain, Louisiana, where increased saline intrusion resulted in low DO levels due to salinity stratification and subsequent decreases in macroinvertebrates such as *Rangia cuneata* (Poirrier et al. 2009).

We measured a suite of variables in an effort to understand the overall health of the *Rangia* clams in Galveston Bay. We found that in East Bay, where salinities were highest, all clam samples were males suggesting males are more tolerant to stressed environmental conditions than females (data not shown) while those in the Trinity River and delta area had both males and females (Figs. 60 and 61). Hence, salinity plays an important role in reproductive potential for these clams.

Rangia meat index (the ratio of wet meat weight to shell weight) can also be a useful relationship in monitoring environmental parameters on bivalves (Tenore et al. 1968). Favorable environmental conditions are indicated by a high meat index, whereas a low meat index suggests more adverse conditions (Allen 1963). In the present study, *Rangia* clams with the highest meat index were found in the river delta stations where the substrate consisted primarily of sand suggesting this particular area provides favorable conditions for *Rangia* clams.

Rangia caloric content values (not shown) were similar to those found by Bagatini et al. (2007) and in a previous study we performed on a subset of samples (Parnell et al. 2011). Bagatini et al. (2007) measured the caloric content of a riverine bivalve, *Corbicula fluminea*, which ranged from 5000 cal/g to 4800 cal/g. Parnell et al. (2011) found the highest caloric values in *Rangia* from May to July at the river delta stations. This coincides with *Rangia's* reproductive period beginning in early through midsummer as found by Cain (1975).

Because of the drought of 2011 and the associated decreased freshwater inflow into Galveston Bay with concurrent widespread and prolonged increased salinity, we were not able to accurately address concerns related to the health of therefore current *Rangia* distributions in response to freshwater inflow. That is, our sampling effort bracketed the drought such that it is difficult to distinguish drought affects from natural effects on these clams. Therefore, it is necessary to continue the *Rangia* monitoring program in Galveston Bay.

Further, the lack of significant relationships between water quality parameters, particularly salinity, and *Rangia* characteristics was most likely a result of the non-repetitive sampling of survey stations. Therefore, future efforts will focus on monitoring water quality parameters and collecting *Rangia* clam samples at a few stations within each survey area on a monthly basis. In addition, we will quantify *Rangia* clams by using a coring device to explain their distribution based on abundance rather than simply presence/absence. Based on repetitive monthly surveys we expect to find significant relationships between environmental conditions and *Rangia* characteristics and distribution over time. We would like to provide resource managers more insight on using *Rangia* clams as bio-indicators of freshwater inflows that are critical to Galveston Bay.

5.4.3 Towards understanding current distributions of *Rangia* sp. in Galveston Bay relative to historical distributions and abundances

We are currently investigating the possible reasons for both the overall decline in *Rangia* populations since the early 1980s (Fig. 46), a phenomenon observed in this and all other bays along the Texas coast (Bill Balboa, pers. comm.) and whether the present changes are associated with the 2011 drought or reflect the long term overall phenomena associated with reduced flows and thus increased salinities.

One hypothesis is a long term decline in food supply associated with concurrent declines in nutrient supply to the Bay as a result of the Clean Water Act of 1972. Lester and Gonzalez (2002) previously reported this observation (decrease in chl *a*, nitrate and phosphate) but not its consequences – hence further investigation will be required.

An alternative hypothesis was proposed by Dr. William Wardle (Texas A&M University at Galveston; pers. comm.), who suggested that an increase in parasites may be responsible for the overall decline. During 2012 when we examined parasitic loads, we did not find a correlation between clam health, salinity or other parameters which could be used to link this to overall changes on decadal scales in the Galveston Bay *Rangia* populations. Hence, further studies are required.

5.5 Develop better models of salinity for Galveston Bay

Whilst we initially set out to develop a better understanding of the use of these flora and fauna as biological indicators of the effects of freshwater inflows in Galveston Bay, we found that the first task at hand was to develop better models of salinity on both spatial and temporal scales. As part of this task, TxBLEND was updated and the outputs compared to salinities measured by mapping with a Dataflow. Visual and statistical methods indicate that model performance is at least satisfactory and in many cases good or very good during most times and at most locations (Fig. 12 and Table 8 versus Figs. 62 to 67). Consistent with previous findings of issues by the TWDB, the TxBLEND model for Galveston Bay was generally representative of observed salinities and trends, though long-term trends were simulated more accurately than short-term, high frequency variability, particularly in the upper estuary.

Further work will involve discussions (already initiated) with the TWDB to identify possible refinements to the model calibration to improve model performance. In addition, the next step to try to couple TxBLEND with other water quality constituents (e.g., chl *a*, nutrients) is under consideration. In addition to the missing and filled in data (see methods section above), two other issues related to freshwater inflow data are outstanding which could not be resolve within the time constraints of the current study. First, as can be seen in Fig. 76 below, there appears to have been a fairly significant decrease in diversions from coastal watersheds after 2000. This decrease is entirely attributable to a single watershed (8010), the more recent estimates are consistent with TCEQ records and older TCEQ records would suggest that the estimates included in TxBLEND prior to 2000 may be overestimated.

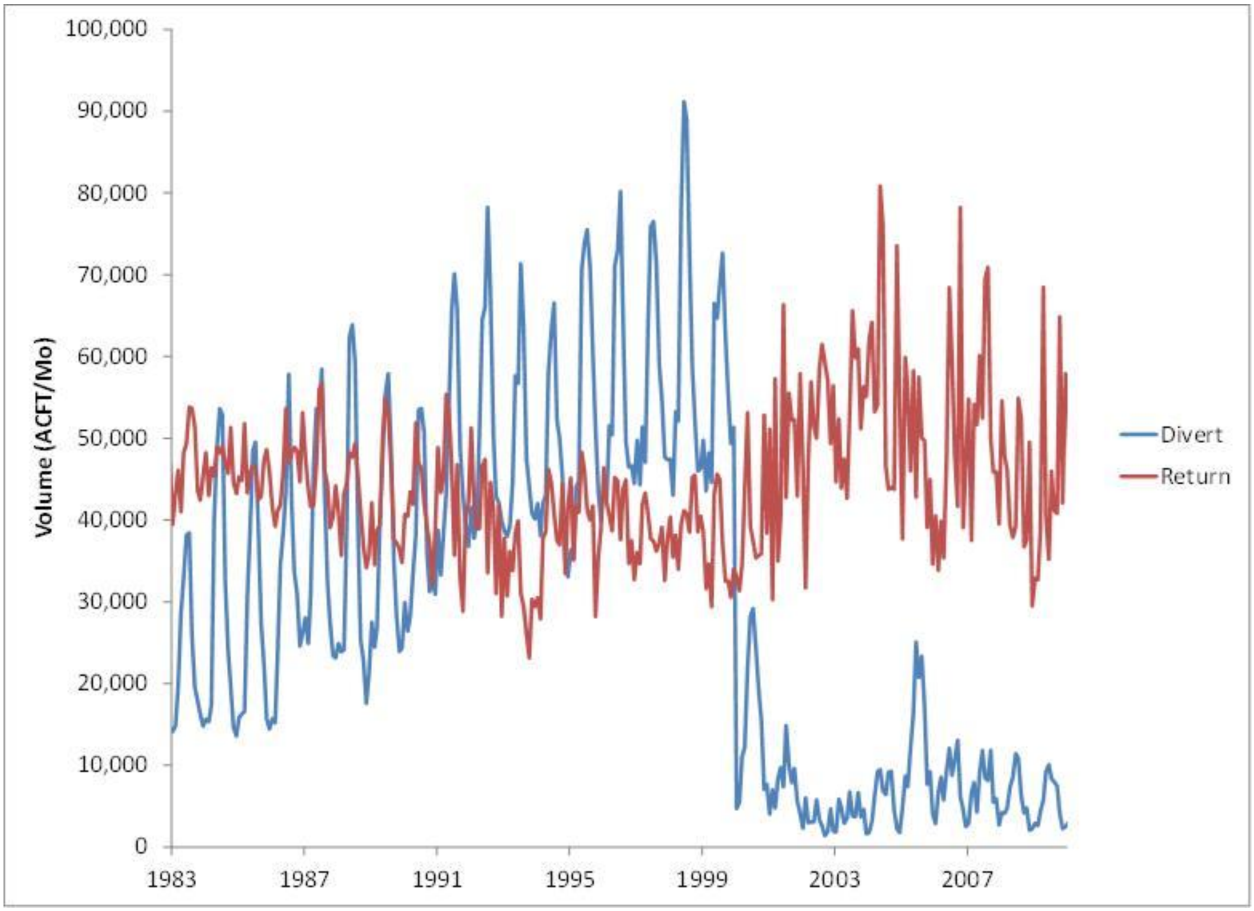


Figure 76 Diversions (blue) and returns (red) from coastal watersheds in Galveston Bay.

Second the TxBLEND model does not include estimates of runoff, diversion or returns from subwatershed 8020. This is a historical issue with the model that is under review by the TWDB. This subwatershed includes Lake Anahuac, and records and analysis describing how it affects inflows are currently lacking.

Additional future effort will be to determine whether the model can be used in place of measured data. The appropriateness of using a model in any context is dependent upon, among other things, the level of precision and accuracy that is necessary to address a scientific or policy question of interest. Analysis of model performance conducted in this study is generally supportive of the continued use of the TxBLEND model to inform policy decisions related to the management of freshwater inflows.

At the culmination of the first round of the SB3 process the TCEQ made a rule (30 Tex. Admin. Code §298.225) stating that "(a) A water right application in the Trinity or San Jacinto river basins, which increases the amount of water authorized to be stored, taken or diverted as described in §298.10 of this title (relating to Applicability), shall not reduce the long-term frequency on either a seasonal or annual basis at which the volumes of freshwater inflows, to Galveston Bay occur", as described in Figure 76.

Table 23 Bay and Estuary Freshwater Inflow Standards for the Galveston Bay System (adapted from Lee et al. 2001).

Bay and Estuary Freshwater Inflow Standards for the Galveston Bay System

Basin	Annual Inflow Quantity (af)	Annual Target Frequency	Winter Inflow Quantity (af)	Winter Target Frequency	Spring Inflow Quantity (af)	Spring Target Frequency	Summer Inflow Quantity (af)	Summer Target Frequency	Fall Inflow Quantity (af)	Fall Target Frequency
Trinity	2,816,532	50%	500,000	40%	1,300,000	40%	245,000	40%	N/A	N/A
	2,245,644	60%	250,000	50%	750,000	50%	180,000	50%	N/A	N/A
	1,357,133	75%	160,000	60%	500,000	60%	75,000	60%	N/A	N/A
San Jacinto	1,460,424	50%	450,000	40%	500,000	40%	220,000	40%	200,000	40%
	1,164,408	60%	278,000	50%	290,000	50%	100,000	50%	150,000	50%
	703,699	75%	123,000	60%	155,000	60%	75,000	60%	90,000	60%

af = acre-feet

Although the process, through which the values in the Table 23 were derived, involved inputs and refinements from many levels, the technical origins of these values can be found in the freshwater inflow needs study conducted by TPWD (Lee et al. 2001). In that study, while TxBLEND was used to conduct what was described as a verification analysis, neither the regression approach used to develop these inflow values nor the TxBLEND verification analysis considered *Rangia* or *Vallisneria*. The rules developed by TCEQ beg a number of questions that might now be investigated with the TxBLEND model, the most obvious being whether these inflows would be expected to produce salinity conditions in Galveston Bay supportive of these two species as well as other recreationally and commercially important species including oysters and a variety of fish. Furthermore, it would seem that a modeling exercise might be the best way to evaluate whether the granting of a water rights application would be expected to violate the long term frequency targets defined in the rule and how predicted long term frequencies might be expected to affect habitat conditions for these species. More generally, the TxBLEND model would also be an appropriate tool for accessing the impacts of regional and state water plans.

This would likely include linking the state's water availability models (WAMs), used to predict future inflows, to the TxBLEND model to predict resulting salinity conditions. Finally, TxBLEND might also be employed to evaluate various strategies, developed as part of either SB1 or SB3, to protect and maintain the ecological health of Galveston Bay.

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