FINAL REPORT

Phytoplankton Responses to Freshwater Inflows in the Trinity-San Jacinto Estuary

By:
Antonietta S. Quigg (Ph.D.)
Principal Investigator

To:
Texas Water Development Board
P.O. Box 13231, Capital Station,
1700 N. Congress Ave., Rm. 462
Austin, TX 78711-3231

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Texas A&M University at Galveston
Department of Marine Biology
5007 Avenue U, Galveston, Texas, 77551

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List of Abbreviations and Acronyms

CCC	Coastal Coordination Council
CDOM	Colored dissolved organic matter
CMP	Coastal Management Program
GBEP	Galveston Bay Estuary Program
GPP	Gross Primary Production (mg O_2 L^{-1} hr^{-1})
NOAA	National Oceanic and Atmospheric Administration
NPP	Net Primary Production (NPP = GPP – R; mg O_2 L^{-1} hr^{-1})
PAR	Photosynthetic active radiation (μmol m^{-2} s^{-1})
PHYTO-PAM	Phytoplankton Pulse-Amplitude Modulated Fluorometer
R	Respiration (mg O_2 L^{-1} hr^{-1})
*rel*ETR	relative electron transport rate (μmol electrons m^{-2} s^{-1})
RLA	Resource Limitation Assay
TAMUG	Texas A&M University at Galveston
TGLO	Texas General Land Office
TSS	Total Suspended Solids
TWDB	Texas Water Development Board
USGS	United States Geological Service

1. Abstract

The Galveston Bay Estuary Program identified an "examination of the impacts of freshwater inflow and bay circulation" as priority areas in its comprehensive conservation management action plan for 2001-2005. Specifically to ensure *beneficial* freshwater inflow necessary for a salinity, nutrient and sediment loading regime adequate to maintain the 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 freshwater inflows and modes of nutrient loading have on estuaries. Hence, water quality, primary productivity and phytoplankton community structure was monitored in response to freshwater inflows in Galveston Bay. The project spanned a range of inflow conditions into the Galveston Bay estuary between January and December 2008. Spatial maps generated from monthly sampling campaigns with a Dataflow unit provided a clear depiction of inflow effects on water quality in the system. In the fall/spring, repeated, large freshwater inflow events freshened much of the bay, introduced nutrients and lowered water clarity. In the summer/fall, freshets were infrequent. Noticeable differences in the northern section (upper bay) versus the southern section (lower bay) of Galveston Bay in terms of water quality, primary productivity and community composition, much of which was related to aforementioned river inflow effects on salinity, nutrients and to a lesser degree sediment loading. The findings of this study indicate that phytoplankton communities were co-limited by N (as nitrate) and P (as orthophosphate) for much of the year. Understanding of the linkages between the magnitude of freshwater inflows, nutrient and sediment loading on phytoplankton community structure and productivity for the Galveston Bay ecosystem remains a challenge. This requires further data collection in the Galveston Bay system, enumeration of existing phytoplankton samples from 2006, and additional analysis will enable us to further clarify the importance of pulsed inflow events in this estuarine system.

Freshwater inflows and the health of Galveston bay: influence of nutrient and sediment load on the base of the food web.

2. Introduction

With a rapidly expanding urban population in Texas coastal municipalities (TWDB 2001; 2007), water regulators and managers are faced with the challenge of meeting rising human needs for water supply and water quality, while maintaining critical freshwater inflows to estuaries to preserve ecosystem health. The Galveston Bay Estuary Program identified an "examination of the impacts of freshwater inflow and bay circulation" as a priority area in its comprehensive conservation management action plan for 2001-2005 (GBEP, 2001; Longley 1994). Specifically to address Section 11.147 (a) of the Texas Water Code which defines "*beneficial inflows*" as those that provide a "*salinity, nutrient, and sediment loading regime adequate to maintain an ecologically sound environment in the receiving bay and estuary system that is necessary for the maintenance of productivity of economically important and ecologically characteristic sport or commercial fish and shellfish species and estuarine life upon which such fish and shellfish are dependent*." A clear understanding of the downstream ecological impacts of changes in freshwater inflows on estuaries remains a priority for resource managers and scientists alike.

This program builds on an earlier Texas General Land Office – Coastal Management Program (TGLO-CMP) project - Cycle 10: The impact of changing freshwater inflows on the health of Galveston Bay (Quigg et al. 2007). The Cycle 10 program focused on monitoring water quality, primary productivity and phytoplankton community composition in response to freshwater inflows to the Galveston Bay estuary. The present program (TGLO-CMP project - Cycle 12 and TWDB Contract # 0804830792) will focus specifically on the effect of nutrient and sediment loads as a component of freshwater inflows to Galveston Bay. These loads introduce nutrients (N, P, Si, organic matter, various pollutants) and reduce water transparency (sediment loading). At the base of the food web, primary producers are the most sensitive to such changes in water quality. By monitoring the response of primary producers in Galveston Bay to nutrient and sediment loading, the present program aims to provide valuable information for TGLO-CMP,

TWDB and other state agencies endeavoring to determine best management practices for water supply and water quality.

2.1 Nutrient loading

The degree of nutrient and sediment loading are important factors contributing to water quality and ecosystem health in estuaries (Longley, 1994; Nixon 1995). In Texas, studies have shown that changes in freshwater inputs 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). The changes observed in these studies are ultimately linked to nutrient and to a lesser degree, sediment loading. Coastal wetland loss in Louisiana has however, have been attributed mostly to the reduction in sediment loading as a result of freshwater diversion (Boesch et al. 1984). Factors equally important, but not as often addressed, include the magnitude of flushing and nutrient loading, the mode of nutrient loading, and the ratios of potentially limiting nutrients within the load (Malone et al, 1988; Chan and Hamilton, 2001)

In 1999, Guillen published a report indicating that primary production in Galveston Bay was phosphorus (P) limited while more recently Örnólfsdóttir et al. (2004) reported that it was nitrogen (N) limited. These two studies provide conflicting findings, potential, as a result of the different approaches used. Nonetheless, they both raise similar questions:

(i) What nutrient(s) limit phytoplankton growth in Galveston Bay?

(ii) Does the limiting nutrient change on spatial and/or temporal scales ? and if so, what are they?, and

(iii) What is the interaction between nutrient and sediment load on phytoplankton production?

Örnólfsdóttir et al. (2004) examined nutrient limitation on spatial (transect from Trinity River into Galveston Bay) and temporal (year long study) scales. Consistently, N was the nutrient limiting growth of phytoplankton. Diatoms were the taxa that most often responded to the

addition of N sources. The resulting shift in phytoplankton community composition towards diatoms may not initially raise concerns because this taxa is not typically associated with harmful algal blooms. Nonetheless, there are a number of noxious species which reside in Texas estuaries, particularly species of *Nitzschia* and *Pseudonitzschia* (Fryxell et al. 1991), which have been associated with shellfish poisoning from eating mussels and oysters contaminated with domoic acid (Dickey et al. 1992; Villac et al. 1993). Further, Buyukates and Roelke (2005) found that plankton assemblages receiving nutrient loads in a pulsed mode had less accumulated phytoplankton biomass and supported greater secondary productivity, while assemblages receiving a continuous inflow resulted in a phytoplankton bloom and demise of the zooplankton community. Shifts in phytoplankton composition and physiology are likely to change the nutritional value of phytoplankton to consumers, ranging from zooplankton to higher trophic levels.

2.2 Sediment loading

Given that primary productivity is light driven, and that sediment loads decrease water clarity, the interaction between these this components of freshwater inflows clearly needs to be addressed. Sediment loading into Galveston Bay would be predominately from the two main river sources: San Jacinto River (northwest) and Trinity River (northeast). Given that Galveston Bay is relatively shallow, wind driven mixing would also play an important role in maintaining particulates in the water column and/or benthic resuspension. Less is known about the role of sediment loading in regulating primary producers in estuarine systems.

2.3 Galveston Bay

The Trinity-San Jacinto Estuary, also referred to as Galveston Bay (Fig. 1), is in the largest watershed on the Texas coast (see Thronson and Quigg, 2008 for details). It is one of 22 systems that are part of the National Estuary Program (see details at www.gbep.state.tx.us). It faces some of the greatest conservation challenges of any system in Texas. This complex is adjacent to the most populated and industrialized area of the state. Suburban and industrial development are reducing critical wetland habitat at a faster rate than anywhere else along the coast (www.gbep.state.tx.us). The majority of Texas' hazardous chemical spills and the largest oil spills occur in this system; domestic and industrial wastewater also flow into this bay

(www.tpwd.state.tx.us). Periodic dredging of the Houston Ship channel as well as several smaller ports is a significant conservation threat to this bay (GBEP 2001). Exotic species like Chinese tallow, giant salvinia, water hyacinth and grass carp also threaten native habitats throughout the bay. In an investigation of fish kills occurring along the Texas coast from 1951 to 2006, Thronson and Quigg (2008) found that Galveston and Matagorda Bays had the highest number of fish kill events and total number of fish killed.

Fig. 1. Texas General Land Office map of the Trinity-San Jacinto estuary.

Given Galveston Bay is predicted to experience the largest population growth of any of the Texas coastal municipalities in decades to come (TWDB 2001, 2007), it's imperative that we understand how it responds to freshwater inflows – total discharge, pulses of differing magnitude, circulation patterns and/or returned flows – resulting from alterations in its watershed. We need to understand how the present Galveston Bay ecosystem complex responds

to nutrient and sediment loading from freshwater inflows in order to develop a conceptual understanding of the downstream ecological impacts of future mitigation strategies for freshwater inflows and modes of nutrient loading into this system. Specifically, how do changes in nutrient and sediment loading affect primary productivity and phytoplankton community composition? If the basis of the food web is altered, the impact will be transmitted to all higher trophic levels.

2.4 Objectives

The main objective of this program was to support continued research aimed at determining the effect of nutrient and sediment loads, both components of freshwater inflow, on primary productivity and the phytoplankton community in Galveston Bay. Building on data collection efforts underway with the support of several agencies - Texas Water Development Board (TWDB), Texas General Land Office – Coastal Management Program (TGLO-CMP), Texas Sea Grant and the Galveston Bay Estuary Program (GBEP) - this study obtained monthly high resolution spatial and temporal mapping of water quality parameters along with phytoplankton productivity and composition during 2008. In order to better understand phytoplankton response to freshwater inflow, resource limitation assays (RLA) were used to determine if nutrients or sediment loading have the greatest effect on phytoplankton productivity and community composition. The resulting data and conclusions will be essential for developing the next generation of predictive models relating freshwater inflow to bay health.

Specific objectives:

(i) High spatial and temporal resolution mapping of Galveston Bay,

(ii) Define influence of nutrient and sediment load on the phytoplankton in Galveston Bay, and

(iii) Measurement of primary production & phytoplankton community composition in Galveston Bay.

3. Methods

High spatial and temporal resolution mapping was performed monthly whenever possible. From January to August 2008, poor weather conditions (high winds) were typically the most critical

factor preventing field work. The TAMUG campus evacuated three times in the summer of 2008 because of Tropical Storm Eduardo (August 5th), Hurricane Gustav (September 2nd) and then Hurricane Ike (September 13th). On September 13, 2008, Hurricane Ike made its final landfall over Galveston, Texas as a Category 2 hurricane on at 2:10 a.m. CDT. Ike was the third most destructive hurricane to ever make landfall in the United States. It was the ninth named storm of the 2008 Atlantic hurricane season. Hence sampling in September through to December 2008 was conducted with a great deal of care as there was a great deal of debris in Galveston Bay. This resulted in a reduced sampling effort for the remainder of 2008 for safety reasons.

3.1 Water Quality

Real-time flow data from a USGS monitoring station (Trinity River at Romayor) near the river's mouth was used determine the freshwater inflow into Galveston Bay from January to December 2008.

The Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physio-chemical parameters in shallow aquatic systems (Madden and Day 1992), was used to map along a tightly gridded transect, Galveston Bay (Fig. 2). This integrated instrument system concurrently measured water temperature, conductivity, salinity, water clarity (beam transmittance), chlorophyll *a* (*in situ* fluorescence), dissolved organic matter (DOM; *in situ* fluorescence), and photosynthetic active radiation (PAR). 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.

In most cases water quality surveys took two successive days, however, in some cases poor weather conditions did not permit us to do so (see section 3). GPS and Dataflow information was used to create highly detailed contour maps of water quality parameters in relation to physiographic features using Surfer.

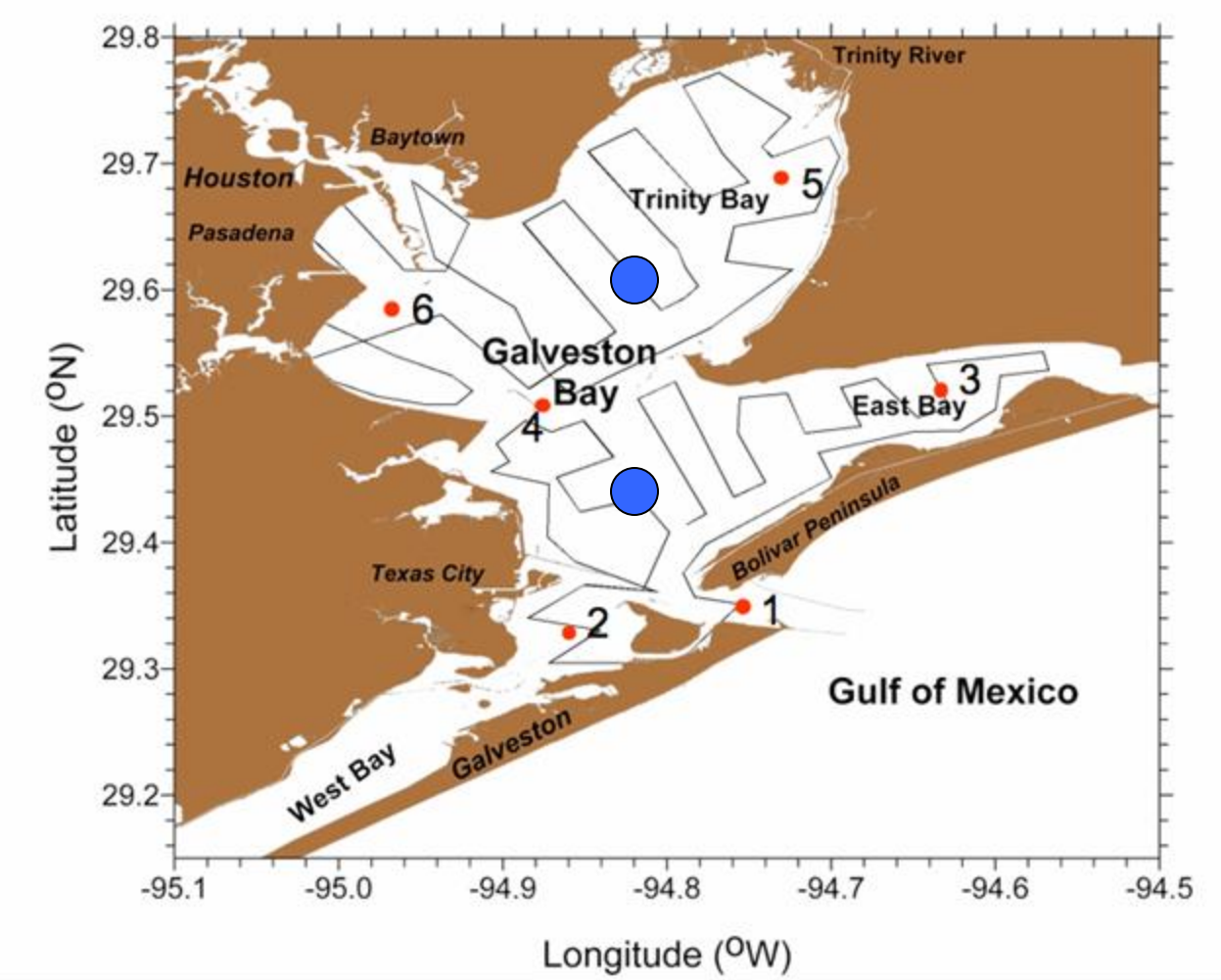


Fig. 2 Galveston Bay water quality parameters were examined along a tightly gridded transect shown by the grey line. The northern part of the bay would typically take a day to complete, and the southern part a second day. Six fixed stations were sampled for additional information such as nutrients, primary productivity and phytoplankton community composition. Blue circles show locations of RLA stations.

Discrete water samples were collected from six fixed stations (Fig. 2, Table 1) for laboratory analysis of total suspended solids (TSS), nutrients, and for the calibration of Dataflow unit (see section 3.3), primary productivity (see section 3.2), phytoplankton community structure analysis (see section 3.3 and 3.4) and for RLA's (see section 3.5).

Water from each station was filtered (GF/F; Whatman) onto two separate filters under low vacuum (< 130 kPa) pressure for *chlorophyll* analysis. Filters were folded and frozen at -20°C for chlorophyll analysis and at -80°C for pigment analysis. Chlorophyll *a* (chl *a*) and phaeophytin *a* (phae *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.

Station	Latitude	Longitude
1	29°21.51'	94°46.12'
2	29°18.55'	94°52.88'
3	29°32.90'	94°34.72'
4	29°33.13'	94°48.27'
5	29°36.56'	94°55.88'
6	29°41.77'	94°51.16'
North RLA	29°37.01'	94°49.66'
South RLA	29°25.75'	94°50.68'

Table 1: Latitude and longitude of fixed sampling stations in Galveston Bay.

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 using analytical auto-analyzer according to Hansen and Koroleff (1999).

3.2 Primary productivity

In-field phytoplankton responses to inflow events were investigated by measuring water column productivity at 6 fixed locations distributed across the Galveston Bay (Fig. 2) using widely-accepted *in situ* light and dark bottle techniques. Productivity measurements were made during each of the Dataflow samplings trips.

Bottles were filled with surface water taken from a particular location. The bottles were kept in flow-through containers on the boats bow; water was continuously circulated across the bottles

to keep them at ambient temperatures. 'Light' bottles were covered with 50% shade cloth and 'dark' bottles were purchased in black. The amount of oxygen dissolved in the original water sample (initial) was recorded (mg O_2 L^{-1}); the light/dark bottles were incubated for at least a few hours before a final O_2 concentration was measured.

In the light bottle, Gross Primary Production (GPP) and Respiration (R) occurred while only respiration took place in the dark bottle. The difference between these two processes is Net Primary Production (NPP). GPP, R and NPP were calculated according to the following equations:

(1) NPP (mg O_2 /L/hr) = (GPP - R) = (Light - Initial)

(2) R (mg O_2 /L/hr) = (Initial - Dark)

(3) GPP (mg O_2 /L/hr) = (NPP + R) = (Light - Dark)

Values were expressed, per square meter, as rates were totaled to the base of the euphotic zone by multiplying productivity by Secchi depth (Wetzel & Likens, 2000).

3.3 Phytoplankton community structure

The relative abundance of microalgal groups in mixed species assemblages can be assessed microscopically (samples collected, but not yet examined) and/or using the diversity and phylogenetic association of specific photosynthetic accessory pigments (chlorophylls and carotenoids). Microalgal photopigments provide reliable measures of the relative abundance of characteristic algal groups (Millie et al. 1993, Jeffrey et al. 1997). Photopigment composition is also significantly (linearly) correlated with species cell counts (Jeffrey et al. 1997). Mackey et al. (1996) have developed a factor analysis algorithm (CHEMTAX) for calculating algal class abundances (both in terms of relative and absolute numbers) based on biomarker photopigments. CHEMTAX is a useful and accurate statistical method for converting pigment concentrations into estimates of cell numbers (Wright et al. 1996).

High performance liquid chromatography (HPLC), which provides rapid and accurate quantification of chlorophylls and carotenoids, was used for photopigment-based chemosystematic characterization of microalgae (Millie et al. 1993, Jeffrey et al. 1997, Pinckney

et al. 1998). Aliquots (0.3 to 1.0 L) of water collected from the 6 fixed stations (Fig. 2) were filtered under a gentle vacuum (<50 kPa) onto 4.7 cm diameter glass fiber filters (Whatman GF/F), immediately frozen, and stored at -80 C. Frozen filters were then placed in 100% acetone (3 mL), sonicated, and extracted at -20 C for 12 - 20 h. Filtered extracts (200 µL) were injected into a Spectra-Physics HPLC equipped with a single monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and two polymeric (Vydac 201TP, 0.46 x 25 cm, 5 µm) reverse-phase C_{18} columns in series. This column configuration was devised to enhance the separation of structurally similar photopigments and degradation products. Monomeric columns provide strong retention and high efficiency, while polymeric columns select for similar compounds with minor differences in molecular structure and shape (Van Heukelem et al. 1994, Jeffrey et al. 1997). A nonlinear binary gradient, adapted from Van Heukelem et al. (1994), was used for pigment separations (Pinckney et al. 1998). Solvent A consists of 80% methanol:20% ammonium acetate (0.5 M adjusted to pH 7.2) and solvent B is 80% methanol: 20% acetone. Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards, including chlorophylls *a*, *b*, β-carotene (Sigma Chemical Company), fucoxanthin, and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison to extracts from phytoplankton cultures and quantified using the appropriate extinction coefficients (Jeffrey et al. 1997).

3.4 Phytoplankton Pulse - Amplitude Modulated Fluorometer (PHYTO-PAM)

The pulse-amplitude-modulation (PAM) measuring principle is based on selective amplification of a fluorescence signal which is measured in the presence of intense, but very short (µsec) pulses of actinic light (Papageorgiou and Govindjee, 2004). In the PHYTO-PAM, light pulses are generated by an array of light-emitting diodes featuring 4 different wavelengths: blue (470 nm), green (520 nm), light red (645 nm) and dark red (665 nm). This feature is very useful for distinguishing algae with different types of photosynthetic accessory pigments of freshwater and marine algae (Jakob et al. 2005). Green algae (Chlorophytes and Prasinophytes) can be distinguished from Diatoms plus Dinoflagellates and Cyanophyta.

Further, valuable information on the photosynthetic performance and light saturation characteristics of a phytoplankton community can be obtained by measuring the relative electron transport rate (*rel*ETR). Light response curves were generated by measuring the change in quantum yield (Y) with increasing PAR. These resemble the photosynthesis-irradiance curves known from gas exchange and C14-fixation measurements (Falkowski and Raven 1997). The advantage of the PHYTO-PAM technique was that it can be done in minutes, is non-invasive and requires no isotopes. Gas-exchange techniques and C14-fixation require hours to a day, isotopes for the latter technique and so restrict the total number of samples which can be examined. The PHYTO-PAM approach promises to be particularly suited to monitoring programs designed to assess inter-annual variability in phytoplankton community composition, productivity and biomass. It is sensitive to 0.1 µg chlorophyll L^{-1} (Nicklisch and Köhler 2001) and allows for statistically robust experimental design given many samples can be examined within a short period of time.

The PHYTO-PAM was used to determine the content of *active* chlorophyll in water samples (1 L) collected from 40 to 42 stations across Galveston Bay, including the 6 fixed stations shown in Fig. 2. Water samples were collected in acid-washed dark bottles and stored in a cooler at ambient temperatures. After dark acclimation, they were processed using the PHYTO-PAM. The minimal fluorescence of dark-adapted samples (F) was recorded as it provided an estimate of the chlorophyll content of the water samples and the proportions of the different types of algal groups given that all 4 wavelengths were used. Light response curves were generated for each sample so that photosynthetic performance and light saturation characteristics of the phytoplankton community could be deconvoluted.

3.4 Resource Limitation Assays

Resource limitation assays (RLA) were undertaken to identify which resource (nutrient(s) and/or light) limited phytoplankton growth at sampling sites in Galveston Bay during the study period. These bioassays were carried out essentially as described by Fisher et al. (1999) on water samples collected from two sites (North RLA and South RLA; Table 1). Surface (0 - 0.5 m) water was collected in 20 L acid washed carboys (total thirty-two carboys). An additional water

sample (20 L) was taken from each site and returned to the laboratory – this water was used to measure the initial water quality (nutrients, TSS, etc..) and phytoplankton characteristics of the sample (see below). Each bottle was triple rinsed prior to filling.

Triplicate carboys were then randomly selected for one of six treatments: a control (no addition), +N (30 μmol L^{-1} NO_3^-), +P (2 μmol L^{-1} PO_4^{3-}), +NP (30 μmol L^{-1} NO_3^-, 2 μmol L^{-1} PO_4^{3-}) (final concentrations in each treatment), a "grazing control; GC" and a "light" treatment. The +GC refers to treatments in which no nutrients were added (as done for the control) but for which water was pre-filtered with a 380 μm filter before filling each carboy. In order to determine the effect of an increased sediment load and concurrent reduced light penetration on phytoplankton productivity and community structure, one carboy from each location was covered with shade cloth resulting in a 50% reduction in light penetration; this treatment is referred to as "light".

Treatments were incubated outdoors at ambient water temperature and turbulence and under 50% ambient sunlight in an outdoor facility shown below (Fig. 3). These free floating corrals were designed to fit 8 carboys in each of four quadrants. Carboys were randomly loaded into this unit within hours of sample collection. Treatments were then left for a week before being sub-sampled as described below. The sampling protocol was designed to assess the natural variability in phytoplankton community assemblages.

Initial (Day 0) and final (Day 7) samples were handled in the same manner. Carboys were collected and processed as quickly as possible either in the laboratory or outdoors in a low light (shaded) environment. Each carboy was shaken vigorously to mix contents. The following samples were collected in accordance to the methods described above: chlorophyll, nutrients (dissolved and total), TSS, and PHYTO-PAM.

The response potential of phytoplankton in each treatment was quantified according to the phytoplankton response index (PRI) of (Fisher *et al.* 1999). The PRI was calculated by determining the phytoplankton growth response as the ratio of the maximum biomass relative to the initial biomass. Also included was a response classification (as recommended by Fisher *et al.*

1999) to accommodate for errors and temperature differences between assays; the threshold for a significant response was set to 140 fold > than the control.

Figure 3: Resource Limitation Assays were performed using (A) acid washed 20L carboys, (B) and (C) once nutrients were added to the appropriate treatments, carboys were loaded into the corrals, and (D) the treatments were left outside in corrals under ambient conditions for a week before being retrieved.

4. Results

4.1 Freshwater Inflow into Galveston Bay during 2008

Real-time freshwater inflow measured as daily discharge to Galveston Bay from January 01 to December 31 2008 was downloaded from the USGS monitoring gage located on the Trinity River at Romayor (08066500). Monthly sampling campaigns (total of 10) are shown (red spots) on Figure 4. A total of 2,100 thousand cubic feet per sec of water was discharged in 2008. As

can be seen in Fig. 4, three significant freshwater inflow events (>10,000 cubic feet per sec) occurred in 2008: first from February 17 to 23 (total of 192,400 cubic feet per sec), a second prolonged flow from March 10 to May 12 (total of 1,250,600 cubic feet per sec) and a third event from September 14 to 18 (total of 61,100 cubic feet per sec). It could be argued that the last event was not a "true" freshwater inflow in the sense of that the data given this was recorded immediately after Hurricane Ike which made landfall on September 13th 2008. This last peak more likely reflects that drainage of surge waters back into Galveston Bay. Hence, in 2008, there were two significantly freshwater inflow events during the winter and spring months.

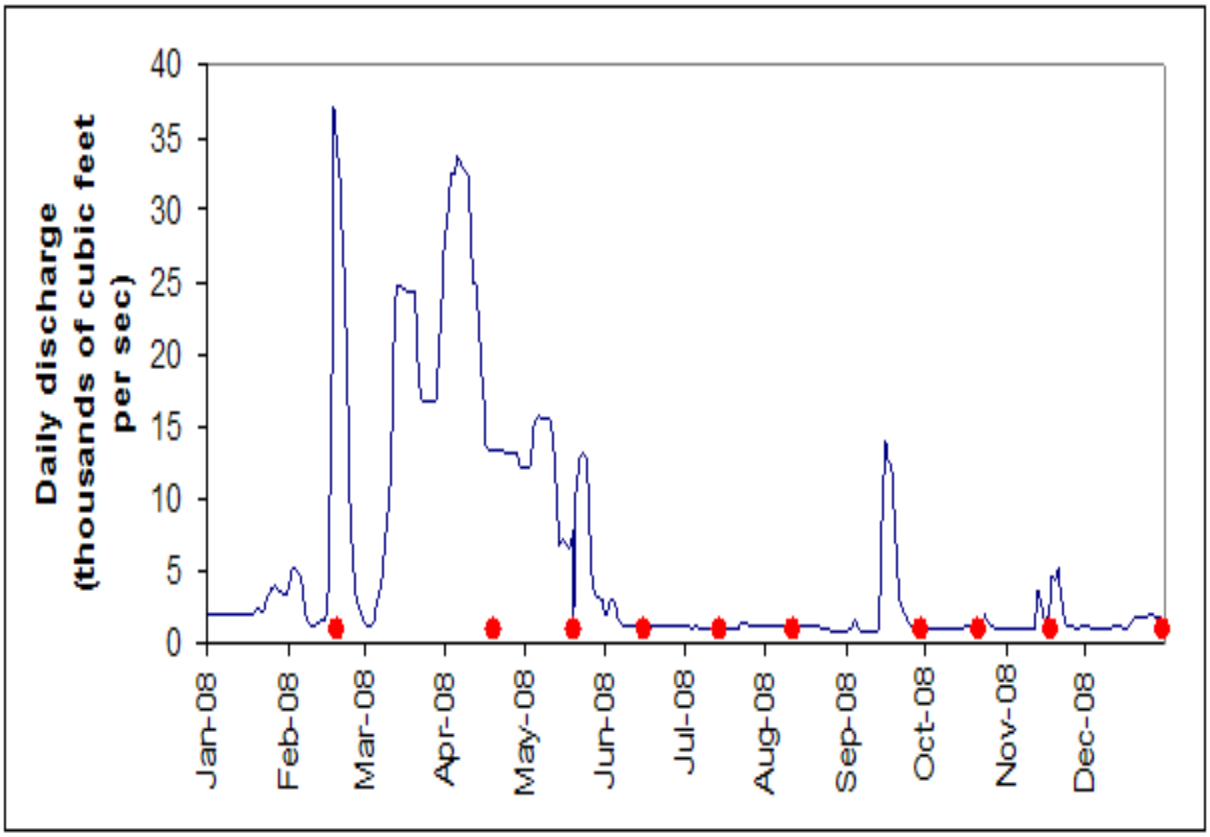


Fig. 4 Daily discharge of freshwater into Galveston Bay from January 01 to December 31 2008. Real-time flow data was downloaded from the USGS monitoring station located in the Trinity River at Romayor (08066500) located near the river's mouth. Red spots indicate timing of monthly field trips.

Period of record	Average Discharge (cfs)	% relative to highest
1925-1934	6737.36	64
1935-1944	8449.1	80
1945-1954	7330.5	69
1955-1964	6007.2	57
1965-1974	7198.4	68
1975-1984	7212.6	68
1985-1994	10562*	100*
1995-2004	9758.5	92
2005	8858	84
2006	1828	17
2007	14480	137
2008	**6214**	**59**

Table 2 Decadal average discharge (cfs × 1000) measured at the USGS monitoring gage located on the Trinity River at Romayor (08066500) from 1925 to 2004, then annual averages.

Relative to previous decadal average discharges measured at the USGS monitoring gage located on the Trinity River at Romayor (08066500), 2008 had lower than average flows (Table 2). In our case, the comparison was made with flows during 1985 – 1994, which was the period of greatest flows, almost two times greater than the average flow during 2008. The flow in 2007 was also twice that measured in 2008 (Table 2).

4.2 Temporal and spatial distributions of water quality parameters in Galveston Bay

The physio-chemical parameters mapped in Galveston Bay include water temperature, conductivity, salinity, water clarity, chlorophyll *a*, and dissolved organic matter. After sensor calibration and blank correction, data was imported into Surfer, a 3D contouring and surface plotting program. Spatial characteristics of the various water quality parameters in Galveston

Bay for April and July 2008 are shown in Fig. 5A and B below. These months were chosen as they represent "wet" and "dry" periods in Galveston Bay respectively (all others are included in Appendix A).

During April, water temperatures varied from 19°C to 22°C (Fig. 5A). By July 2008, temperatures had risen significantly to between 29°C and 31°C. In the cooler months such as April, elevated temperatures were often observed adjacent to and in East Bay, reflecting the shallower nature of this part of the bay. These temperature ranges are typical for estuaries and bayous in Texas (Longley 1994; Davis et al. 2007; Quigg et al. 2007).

Salinities (and conductivities) were significantly lower across Galveston Bay in April relative to July 2008, particularly in the Trinty River basin were values were much lower (23 PSU) relative to the rest of the Bay (average 40 PSU) (Fig. 5A) reflecting the influx of freshwater inflow from the Trinity River as shown in Fig. 4. Salinities were generally higher in August 2008 (Fig. 5A); however, the bay was not completely marine as was observed in previous years (Davis et al. 2007; Quigg et al. 2007). Rather a gradient of salinities (brackish to marine) was still observed across the estuary.

The magnitude of freshwater entering Galveston Bay early in the year had a long and significant influence of the system's salinity gradient. Highest salinities were recorded near the Bolivar and West Bay reflecting the interactions with the Gulf of Mexico and reduced circulation in this area due to the Texas City Dike respectively (Fig. 5A).

Water clarity was measured as transmittance (water clarity) which typically decreases in the water column due to the input of silty runoff, domestic and industrial wastewater discharge, flooding and chronically high flow rates and/or algal growth from nutrient enrichment (eutrophication). In Galveston Bay, transmittance was generally lower (darker color) after freshwater inflow events (see April; Fig. 5B). The extent and distribution of the reduce water clarity mirrors salinities, suggesting that freshwater inflow is the most important factor controlling transmittance after large freshwater inflow events. On the other hand, during periods of low freshwater inflow, transmittance increases, as seen in July 2008 (Fig. 5B) but not

uniformly across the bay nor in relation to salinity. During periods of low freshwater inflow, factors other than freshwater inflow appear to be regulating water clarity in Galveston Bay.

Colored dissolved organic matter (CDOM) also affects the transparency of water, the fate and bioavailability of nutrients and contaminants, and the supply of energy to pelagic microbial food webs (Curtis and Adams 1995; and references therein). Aquatic ecosystems vary in the relative contribution of CDOM from the catchment (allochthonous) and CDOM produced within the system (autochthonous). The distribution of CDOM in a water body provides details on the efficiency of carbon cycling in that system, by both the phototrophic community (that produce it) and the heterotrophic community (that consume it). There was significantly more CDOM in Galveston Bay in April (average at each of 42 stations = 0.46 ug/l) than in July 2008 (average 0.24 ug/l) (Fig. 5B). This finding indicates that allochthonous sources of CDOM were the primary source after large freshwater inflow, while autochthonous maybe more important during low freshwater inflow.

Chlorophyll concentration was measured as a proxy for the biomass of phytoplankton. In April 2008, despite higher freshwater inflow, average chlorophyll concentrations were 0.87 ug/L (Fig. 5B), generally greater (average 0.56 ug/l) than those observed during the summer (July 2008; Fig. 5B). freshwater inflow are an important source of nutrients to the phytoplankton community in Galveston Bay. This will be examined further below.

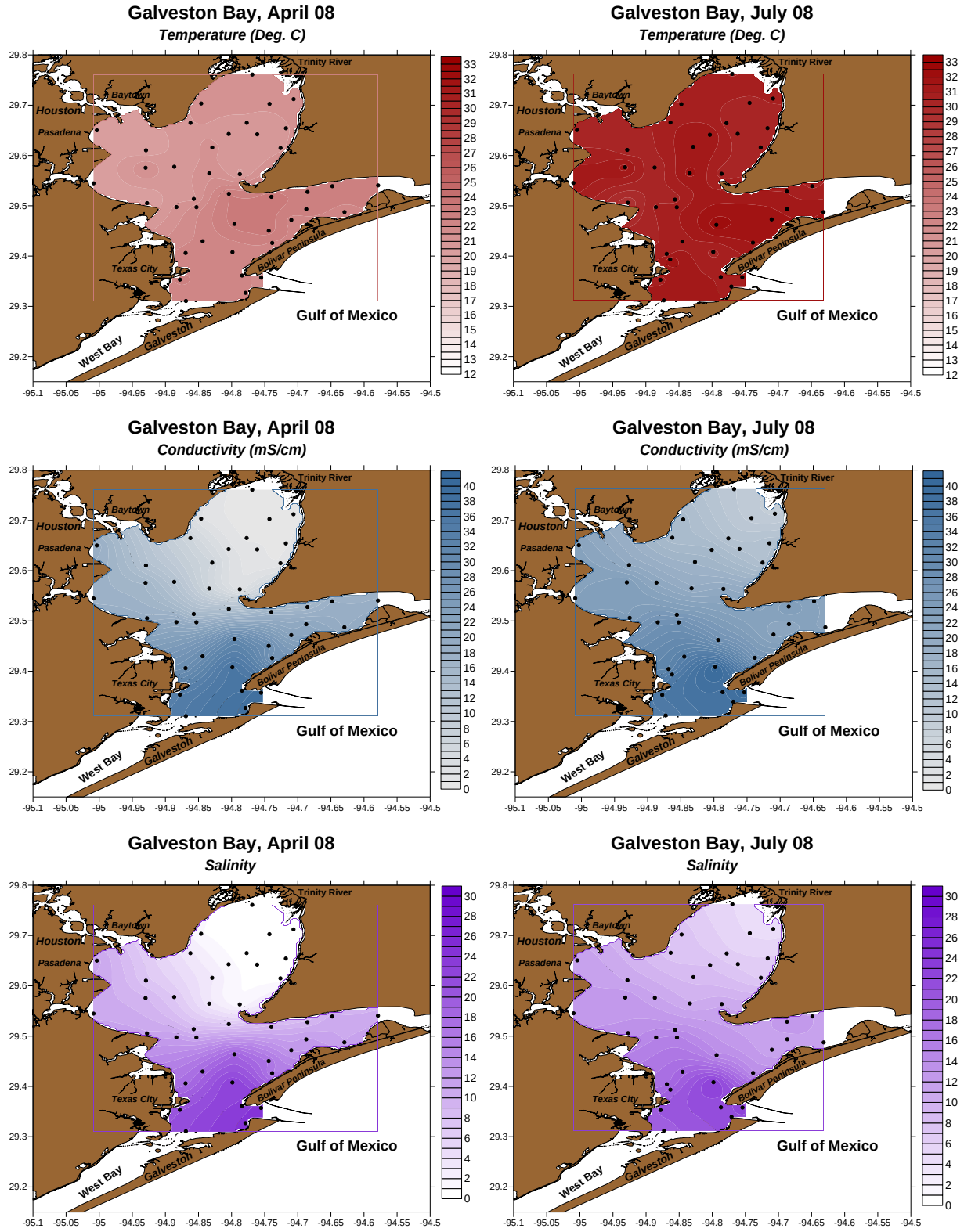


Fig. 5A Temporal (April and July 2008) and spatial patterns of temperature (°C), conductivity (mS cm^{-1}) and salinity as measured with the Dataflow in Galveston Bay.

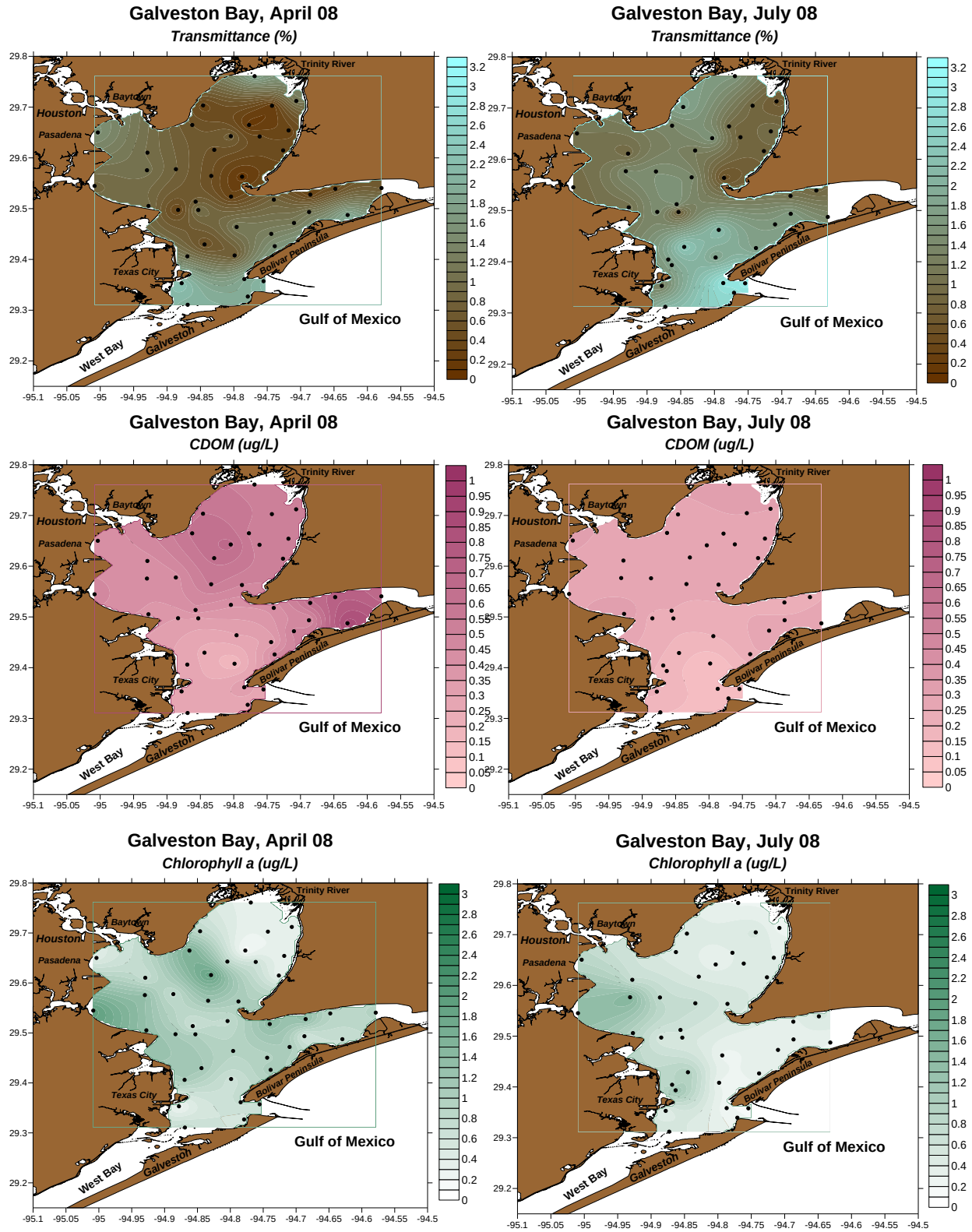


Fig. 5B Temporal (April and July 2008) and spatial patterns of transmittance (%), dissolved organic matter (ug L^{-1}) and in vivo chlorophyll a (ug L^{-1}) as measured with the Dataflow in Galveston Bay.

4.3 Temporal and spatial distributions of total suspended solids in Galveston Bay

In order to summarize our findings, and make it simpler to examine trends, data collected from the six fixed stations was averaged into seasonal bins of winter (December - February), spring (March – May), summer (June – July) and fall (August – November). Normally August would fall into the summer category but because the sampling took place in the last days of that month, in this instance, it made more sense to include it with the fall data set.

Total sediment loading into Galveston Bay can be estimated from measurements of total suspended sediment (TSS) concentrations (Fig. 6). TSS was typically greatest at all stations during the winter and summer (63% of all TSS in 2008) and lowest in the spring (17% of all TSS in 2008). Further, TSS was typically greater near station 1 and progressively decreased closer to station 5 and 6, which were those closer to the river basins. Station 1 is located at Bolivar pass (see Figure 2) which is a rather shallow area which experiences a huge amount of ship traffic. The greater TSS at this station is thus more likely reflecting the unique hydrography of this location and the frequent resuspension events rather than the true TSS loading at this location. Unfortunately, there is no simple way for us to distinguish what might be the true TSS value.

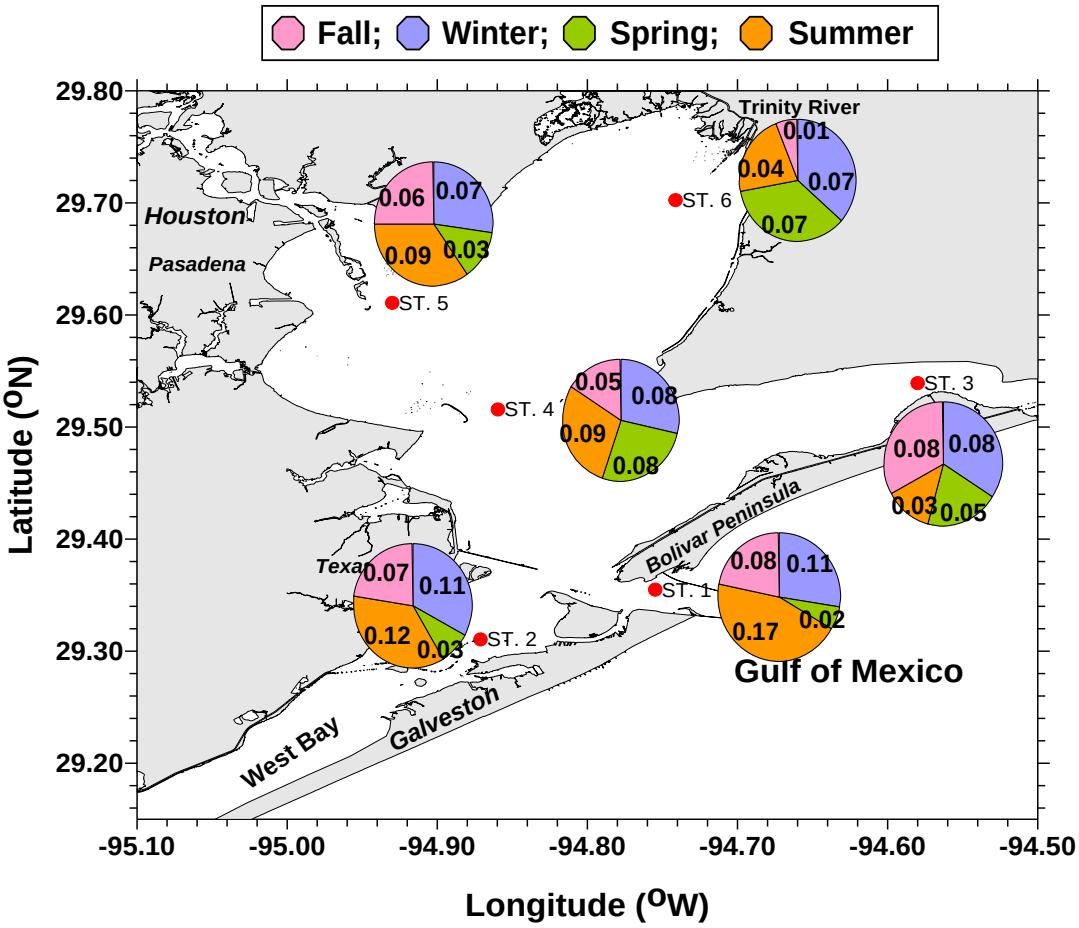


Fig. 6 Seasonal changes in total suspended sediment (TSS; mg/l) loads in Galveston Bay.

4.4 Temporal and spatial distributions of nutrient concentrations in Galveston Bay

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. Other rivers and tributaries in the complex may contribute, but to a lesser extent. The Gulf of Mexico on the other hand is a poor nutrient source to the bay.

Dissolved nitrite plus nitrate concentrations ranged between 0.09 (below detection limit) and 43 μM while dissolved phosphate concentrations ranged from 0.12 and 7.2 uM (Fig. 7). It appears that in 2008, the San Jacinto River was frequently a greater source of dissolved nutrients to Galveston Bay than the Trinity River (Station 5 and 6 respectively, Fig. 7).

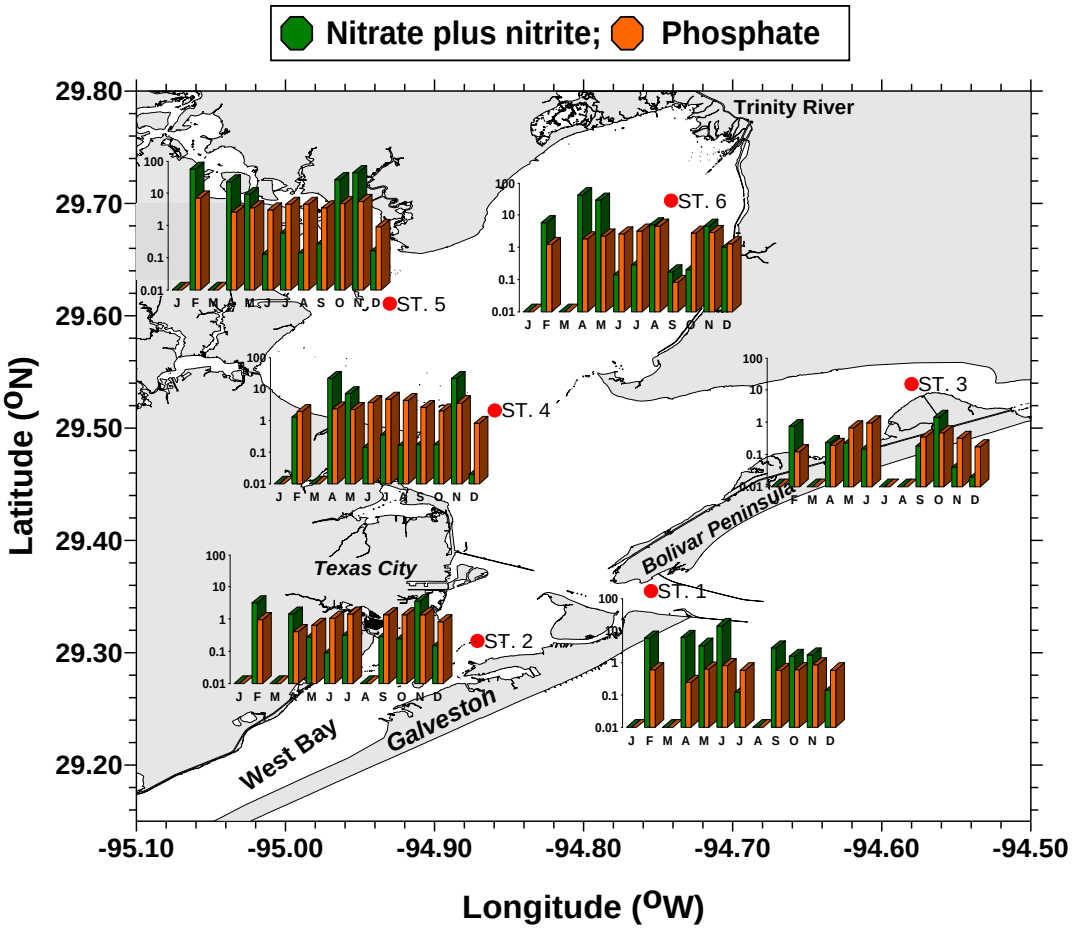


Fig. 7 Monthly dissolved nitrite plus nitrate (μM; green) & orthophosphate (μM; orange) measured in Galveston Bay during 2008 at 6 fixed stations. The y-axis was log transformed (0.01 to 100) so that the four-fold range in the data could be included on one axis.

The higher annual dissolved nutrient concentrations in the San Jacinto River basin may also reflect inputs from the Houston Ship Channel and urbanization and industrialization along the lower San Jacinto River complex which includes the Houston metropolitan area. Typically, lowest nitrate and phosphate concentrations were measured closest to the Gulf of Mexico (Station 1), in West Bay (Station 2) and East Bay near Rollover Pass (Station 3) as is illustrated in Fig. 7 below. Similar such nitrogen and phosphate concentrations and distribution patterns were reported by Pinckney (2006) and Quigg et al. (2007) for Galveston Bay.

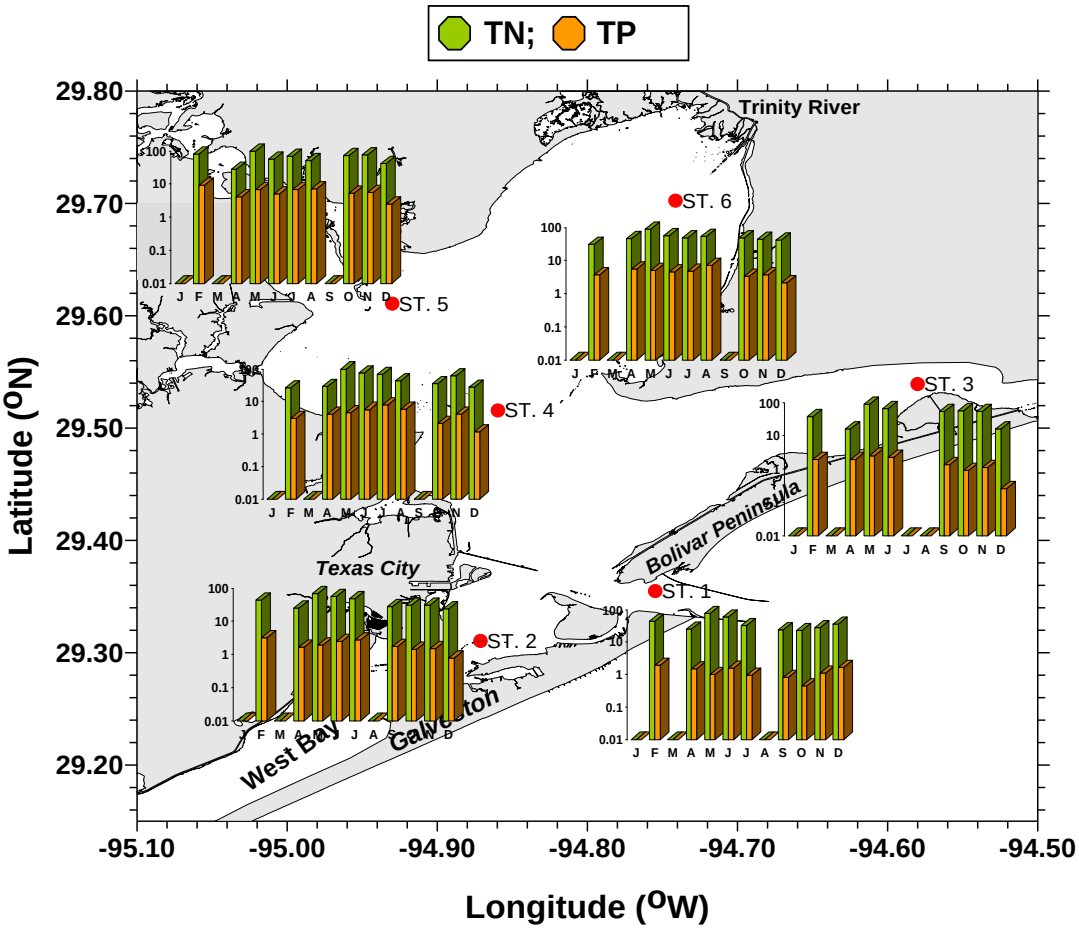


Fig. 8 Monthly total particulate nitrogen (TN, μM; green) & phosphate (TP, μM; orange) measured in Galveston Bay during 2008 at 6 fixed stations. The y-axis was log transformed (0.01 to 100) so that the four-fold range in the data could be included on one axis.

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. Total particulate nitrogen (TN) and phosphate (TP) concentrations were greater at all stations in Galveston Bay (Fig. 8) than dissolved concentrations. Similar qualitative patterns in TN and TP distributions were observed to those reported for dissolved nutrient concentrations.

Patterns in nitrate plus nitrite concentrations did not parallel those of phosphate (Fig. 7) nor did TN and TP concentrations (Fig. 8), consistent with our understanding that different processes

regulate these nutrient concentrations. Given the San Jacinto and Trinity Rivers are thought to be the major sources of nutrient inputs into this system (in agreement with measured data as seen in Fig. 7 and 8), and that river flow has seasonal patterns (Fig. 4), we examined the seasonal changes (as defined in section 4.3) in nutrient distributions.

By comparing N as nitrate plus nitrite to phosphate (Fig. 9A) and TN to TP (Fig. 9B) respectively, seasonal patterns did appear. Dissolved nutrient concentrations were greatest in fall and spring, the latter by at least an order of magnitude (Fig. 9A), and significantly lowest consistently in the summer and winter. On the other hand, total particulate nutrient concentrations were greatest in the summer but did not vary significantly during the other seasons (Fig. 9B).

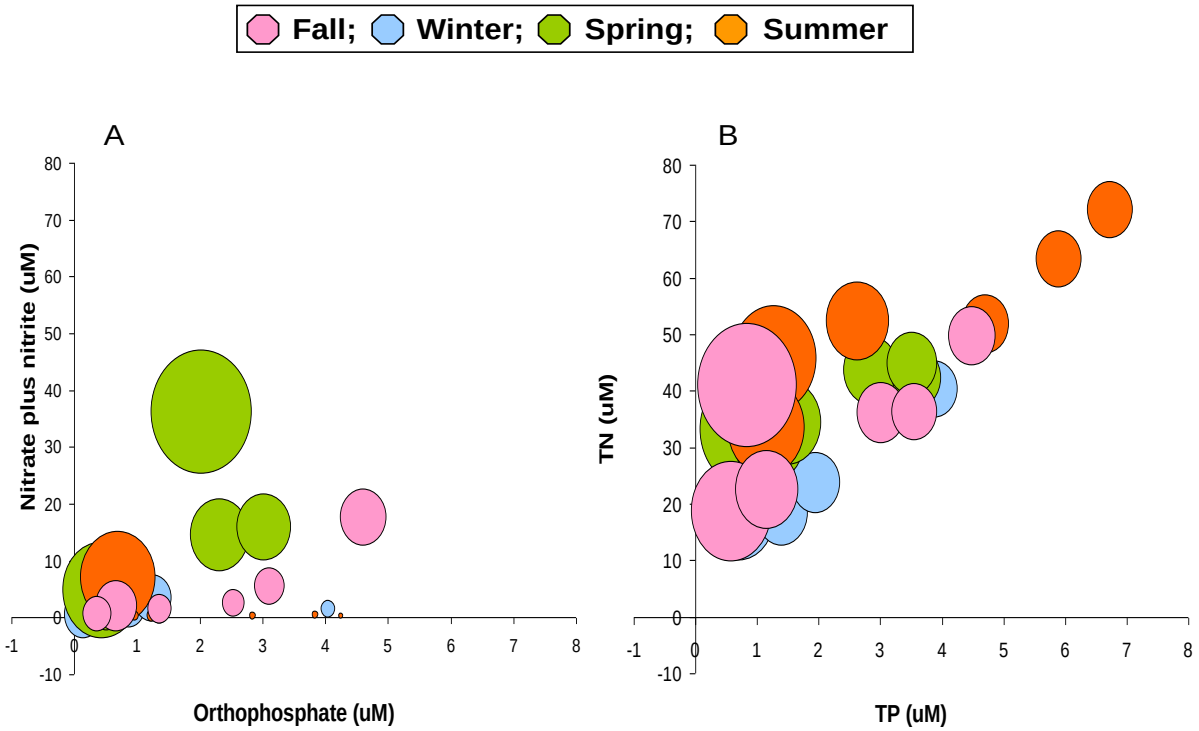


Fig. 9 Seasonal changes in dissolved (A, left) and total (B, right) nutrient concentrations (uM) in Galveston Bay. 9A: N as nitrate plus nitrite versus orthophosphate. 9B: TN versus TP. The x- and y-axis are concentrations; the bubbles where calculated as nutrient ratios and provide a visual of the magnitude of nutrients in each season.

4.5 Temporal and spatial distributions of primary production in Galveston Bay

Primary production was measured during each monthly sampling trip at the 6 fixed stations throughout Galveston Bay. Net primary production (gC m^{-2} d^{-1}) was highest in the northern and western sectors of Galveston Bay year round, with lowest rates measured in East Bay (Station 3) and at Bolivar Road (Station 1) at the opening of this Bay with the Gulf of Mexico, as shown in Figure 10. Primary productivity was greater in the San Jacinto River basin than in the Trinity River basin (Stations 5 and 6 respectively; Fig. 10).

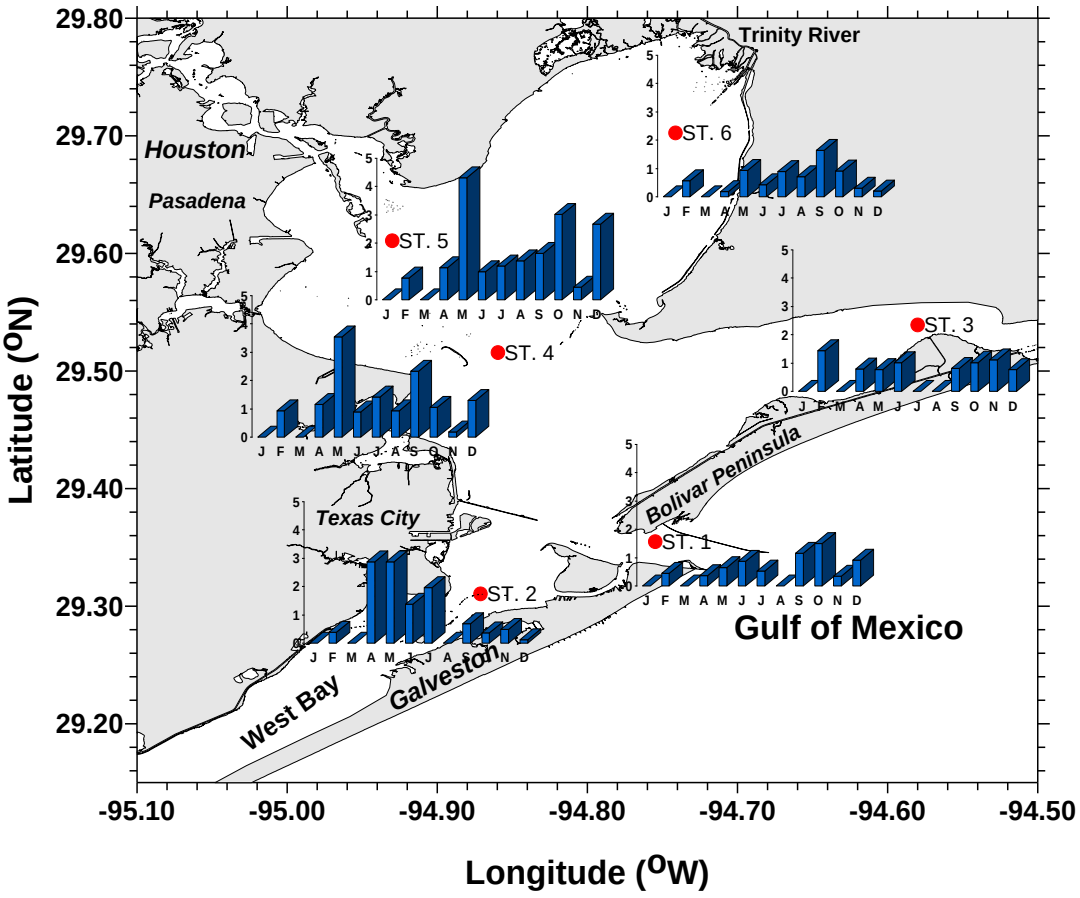


Fig. 10 Monthly gross primary production rates (gC m^{-2} d^{-1}) measured in Galveston Bay during 2008 at 6 fixed stations.

When looking at monthly data, strong seasonal patterns in primary production in Galveston Bay did not appear (Fig. 10), particularly in the upper part of the Bay as has been previously reported (Quigg et al. 2007). By examining annual primary productivity rates measured across the Bay (Fig. 11), most of the primary production did indeed occur during the spring months (36%) and the least in the winter (19%) (seasons defined in section 4.3) which is certainly consistent with previous reports.

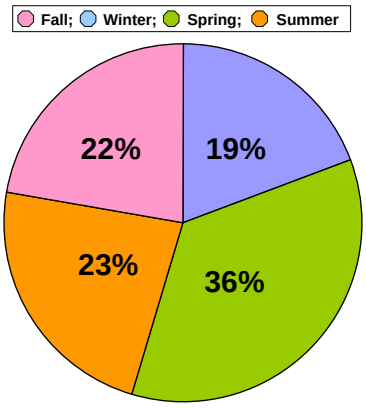


Fig. 11 Seasonal primary production (as a % of total).

4.6 Phytoplankton community structure

Phytoplankton are a diverse group of microscopic organisms ranging in size from 1 to 2000 μm. While some species are readily identifiable using microscopic techniques, the vast majority of species remain difficult to distinguish. Using High Performance Liquid Chromatography, the major groups of phytoplankton in a water sample can be defined on the basis of their pigmentation patterns (Pinckney et al. 1998; Örnólfsdóttir et al. 2004; Pinckney 2006). In this study, we followed changes in the following major groups and groupings of phytoplankton:

(i) Cyanobacteria / Prochlorophytes (blue),

(ii) Chlorophytes / Prasinophytes (green),

(iii) Diatoms / Dinoflagellates (orange),

(iv) Haptophytes /Prymnesiophytes (purple), and

(iv) Cryptophytes (pink).

Changes in phytoplankton community composition were site dependent in Galveston Bay (Fig. 12). In general terms, there were more Cyanobacteria and Prochlorophytes (blue) in the San Jacinto and Trinity River basins for most of the year while Diatoms and Dinoflagellates (orange) generally made up a larger fraction of the community in the southern sector of the bay,

particularly at Bolivar and in East Bay. Haptophytes and Prymnesiophytes (purple) and Cryptophytes (pink) accounted for less than 10% of the total community.

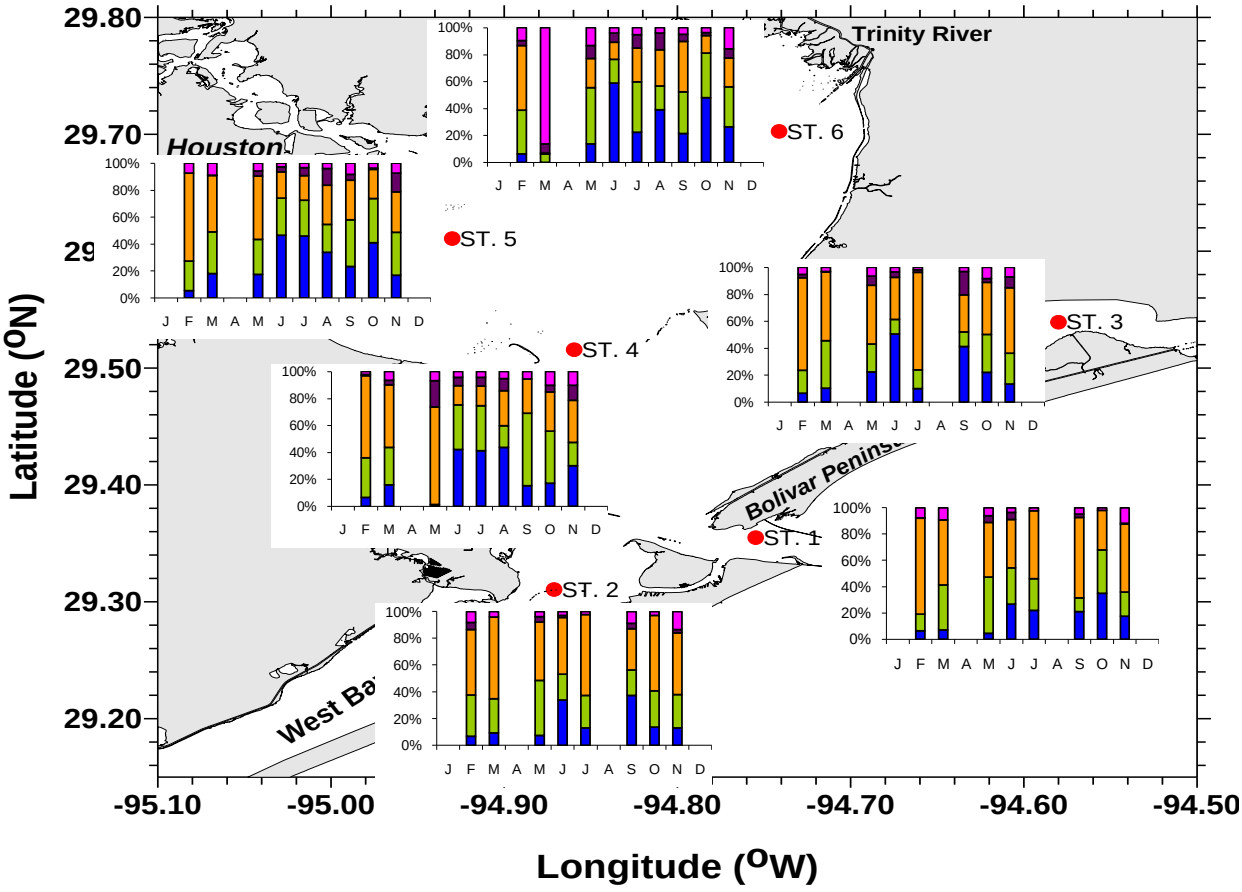


Fig. 12 Monthly changes in phytoplankton community composition throughout Galveston Bay during 2008 as measured at 6 fixed stations. Cyanobacteria / Prochlorophytes (blue), Chlorophytes / Prasinophytes (green), Diatoms / Dinoflagellates (orange), Haptophytes /Prymnesiophytes (purple), and Cryptophytes (pink).

In March, we detected a Cryptophyte (pink) bloom at station 6 (Trinity River basin) (Fig. 12); we were not able to identify the culprit. In May, we detected a large bloom at station 4 (mid-bay) which accounted for 70% of the community (orange: Diatoms / Dinoflagellates) (Fig. 12). A dinoflagellate (yet to be identified) was responsible and caused large areas of the bays water to be discolored a darkish red. No reports of fish kills or other deleterious effects associated with the bloom were recorded indicating it may not have been a harmful form or in a harmful state.

Large scale blooms may have been present at other times of the year but may not have been detected since they vary in duration from days to weeks and our sampling scheme was monthly. Further, plankton tows were taken and will be used to identify the components of the phytoplankton community in the future.

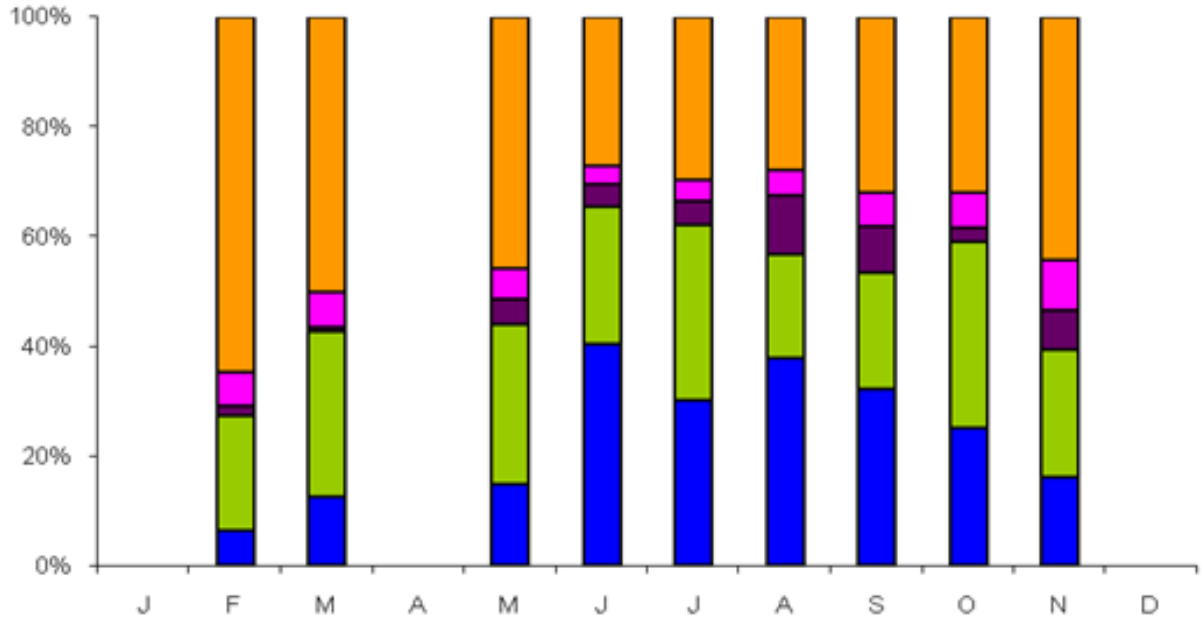


Fig. 13 Monthly changes in phytoplankton community composition in Galveston Bay during 2008 at all fixed stations. Cyanobacteria / Prochlorophytes (blue), Chlorophytes / Prasinophytes (green), Diatoms / Dinoflagellates (orange), Haptophytes /Prymnesiophytes (purple), and Cryptophytes (pink).

By compiling the yearly data, seasonal changes in the major players were noticeable. Cyanobacteria and Prochlorophytes (blue) were more pronounced in the warmer and summer months and less abundant in the cooler and winter months (Fig. 13). Diatoms and Dinoflagellates (orange) showed the opposite trend with lower biomass in warmer months and greater biomass in the cooler months. Similar such trends have been observed for these groups previously in Galveston Bay (Örnólfsdóttir et al. 2004; Pinckney 2006). Interestingly, the Chlorophytes and Prasinophytes (green) had the same relative abundance year round (Fig. 13). It will be interesting to examine the community composition in plankton tows to determine which species are present at different times of the year; this will be conducted in the future. Cryptophytes (pink) like Cyanobacteria and Prochlorophytes (blue) showed a seasonal oscillation; this group is poorly

studied and so it is not yet known what their normal patterns may be in estuarine systems such as Galveston Bay.

4.7 PHYTO-PAM

The PHYTO-PAM uses different fluorescence wavelengths to distinguish between Cyanophyta (blue; 470 nm), green algae which includes both Chlorophytes and Prasinophytes (green; 520 nm) and Dinoflagellates plus Diatoms (light red; 645 nm) on the basis of their photosynthetic accessory pigments (Jakob et al. 2005). As Chlorophytes plus Prasinophytes (chlorophylls a and b) and Dinoflagellates plus Diatoms (chlorophyll a and c) respectively use the same fundamental pigments, these taxonomic grouping cannot be distinguished from each other; only from other phytoplankton taxa.

We collected 42 water samples from across Galveston Bay at locations also mapped with the Dataflow instrument including our 6 fixed sampling stations (Fig. 2). This is the second time this technology has been used to map on a large scale (see Quigg et al. 2007). The PHYTO-PAM did not detect Chlorophytes and Prasinophytes (green algae) during 2008 in Galveston Bay. This is a similar finding to that in our earlier investigation (Quigg et al. 2007). This indicates that the instrument requires further software 'training'; this is an issue we are currently addressing since the HPLC data clearly indicates that Chlorophytes and Prasinophytes (green algae) are important in Galveston Bay (Figs. 12 and 13). Consistent with the HPLC data and with previous studies in this and other ecosystems (Pinckney *et al.* 1998; Pinckney 2006; Davis et al. 2007; Quigg et al. 2007), the phytoplankton community in Galveston Bay at different times of the year was either dominated by Cyanophyta (blue) or Dinoflagellates plus Diatoms (orange/brown).

The advantage of the PHYTO-PAM is that rather than simply investigating the amount (biomass of photosynthetic material) of each group present, we are able to examine the potential of that photosynthetic material for photosynthesis. The yield (Y) as measured using the PHYTO-PAM provides such information; it is the ratio of variable to maximum fluorescence in a given water sample. A yield of 0.83 reflects a physiologically healthy phytoplankton community while lower yields indicate the community is under some environmental stress (Falkowski and Raven 1997).

The yield value however, does not provide details on the nature of that stress which may be from light (too high or too low), nutrients – typically lack thereof, a combination of these or some other kind of stressor.

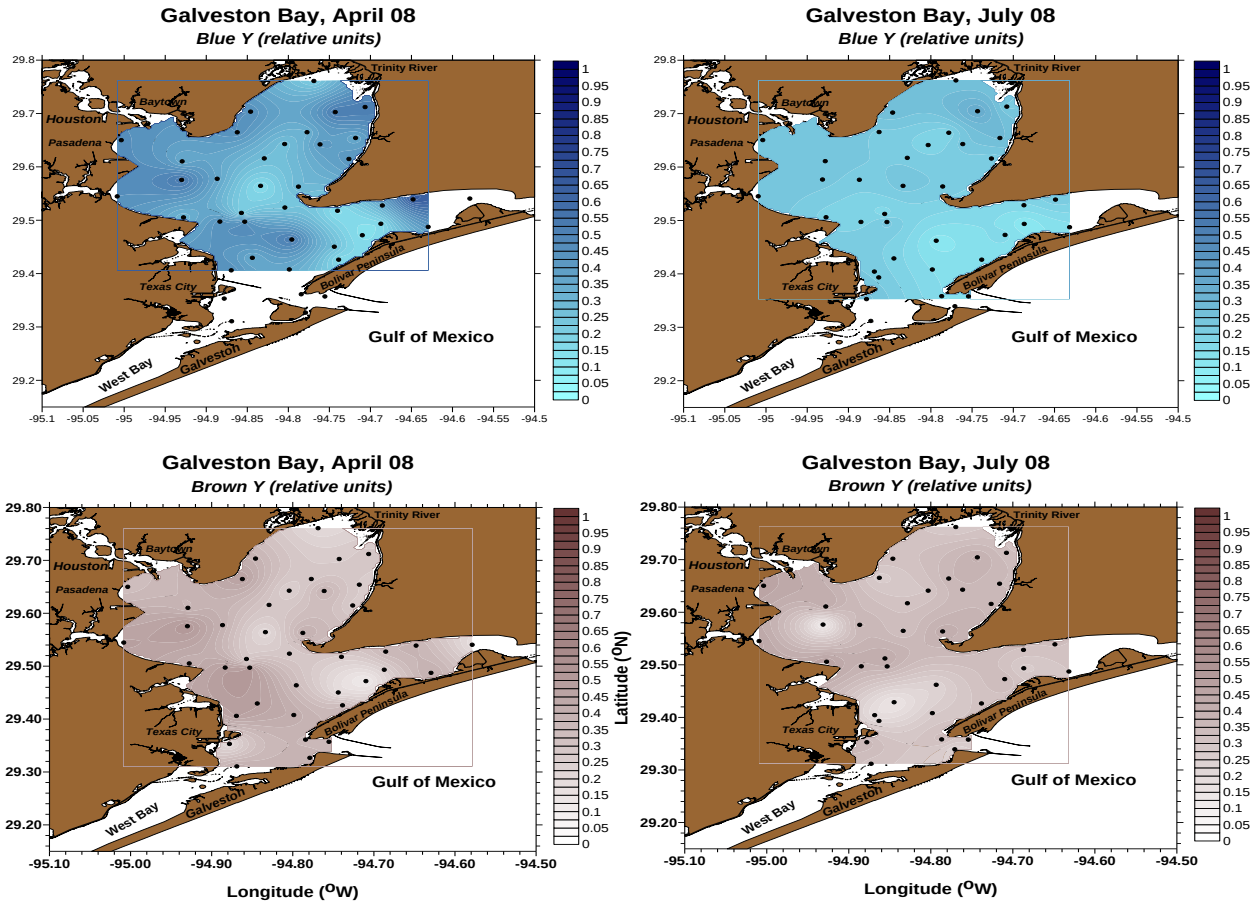


Fig. 14 Spatial maps of revealing the efficiency of photosynthesis (measured as Y or yield) for Cyanophyta(blue) and Dinoflagellates plus Diatoms (brown) as measured with a PHYTO-PAM.

Cyanophyta had a higher photosynthetic efficiency (Y) during periods of high flow (April 2008) relative to periods of low flow (July 2009); clearly benefiting from the introduction of nutrients in the flow (Fig. 14; upper panels). A similar pattern, but more complex in terms of the spatial responses, was also observed for the Dinoflagellates plus Diatoms (Fig. 14; lower panels). Yields in the northern sector of Galveston Bay were 0.6 and greater for both groups, particularly in April (Fig. 14). The overall lower yields observed in July were partially explained by the lower flows, but given that high yield values were still record at a significant number of stations,

indicates a more intricate set of interactions was controlling the photosynthetic efficiency of the phytoplankton present.

4.8 Resource Limitation Assays

The influence of nutrient and sediment load was determined by performing a series of "resource limitations assays" (RLA's). In the scope of work, we had planned to work at the 6 standard stations (Table 1) during low flow periods and immediately after high flow periods. Given this was the first time we performed the RLA's, we did not appreciate the logistics of conducting such experiments on the scale of Galveston Bay. Also, because it was a difficult year in the field (see section 3 above), we radically modified our sampling protocol. We performed the RLAs:

(i) in order to capture both high and low freshwater inflow,

(ii) by sampling in only two locations: North RLA and South RLA, we focused on understanding the influences in the northern and southern sectors of the Bay respectively, and

(iii) treatments which provided information on the response of the community to sediment loading, nutrient additions and grazers.

We feel the data collected in 2008 is nonetheless valuable and provides much information on the influence of freshwater inflow on the phytoplankton community, in keeping with our original objectives.

RLA's were conducted in April, May, July, August, October and November (Fig. 15) in order to capture variations in freshwater inflow as well as seasonal changes in phytoplankton responses to nutrient and sediment loading. As RLA's were conducted according to Fisher et al. (1999), the analysis approach described by the authors was also followed (see methods above). The phytoplankton response index (PRI) was presented on the same scale in all cases (Fig. 15) so that findings could be comparable between months as well as treatments. In order to accommodate for errors and temperature differences between assays, the threshold for a significant response was set to 140 fold > the control. Based on the threshold value, none of the treatments used to examine the effect of grazing and sediment loading had a significant response above the control level (Fig. 15).

In all but one case, the addition of both nitrate and phosphate (+NP) together resulted in a significant PRI. During July, the PRI was < 140 at the North station only when both N and P were added. In majority of RLA's, PRI values were greatest in the +NP treatments relative to all other treatments (Fig. 15) reflecting a co-limitation of phytoplankton populations. Further, phytoplankton from the southern station (purple bars in Fig. 15) responded more intensely to treatments than those from the northern station (blue bars in Fig. 15); this will be discussed below. Only in August 2008 did the addition of phosphate (+P) elicit a small but significant phytoplankton response (PRI = 270) in the population collected at the southern station of Galveston Bay (Fig. 15). The addition of nitrate (+N) typically elicited an observable response in both sectors of the bay with only a few exceptions: April (PRI < 140 in the North and South stations) and July (PRI < 140 at the North station only).

In all RLA's, there was no significant response of the phytoplankton in either the grazing or the sediment loading treatments (Fig. 15). This indicates that the community was not limited by both these factors year round. This will be discussed further below. While the phytoplankton response index (PRI) provides information on how the community as a whole responded to the various RLA treatments, we measured phytoplankton community compositional changes in each set of assays to examine the response of the major phytoplankton groups. There were similar responses when comparing RLA's conducted in the north versus south portions of Galveston Bay; there were also responses which varied with freshwater inflows. In Fig. 16 below, we summarized the findings from RLA's conducted in May and November 2008.

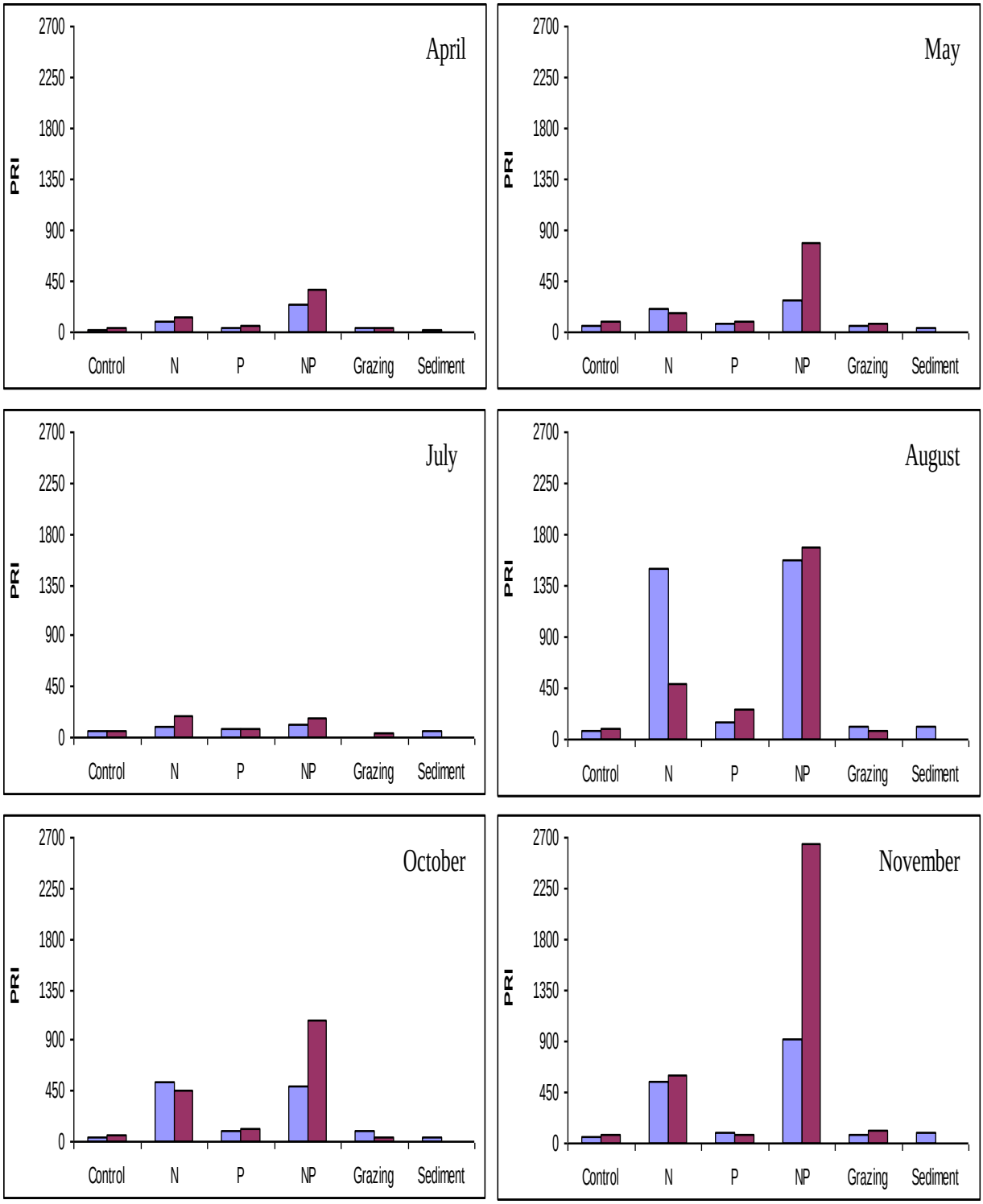


Fig. 15 Outcomes of the resource limitation assays scaled between 0 and 2700. Assays were performed in the northern (blue) and southern (purple) sections of Galveston Bay during 2008. Water was collected from locations indicated on Figure 2. Changes in phytoplankton biomass were calculated as the phytoplankton response index (PRI) which normalizes the changes in biomass to the initial concentration.

In general, in RLA's conducted in the northern sector of the bay, we measured increases in Cryptophyte (pink) populations in the nutrient addition treatments (+N, +P, +NP) relative to control treatments, but no significant increases in other groups. A concurrent decrease in Cyanobacteria and Prochlorophytes (blue) was observed, particularly in those treatments to which nutrients had been added (Fig. 16). Whilst the proportion of Chlorophytes and Prasinophytes (green) did not change significantly in May; we observed an increase in their populations in November in RLA-N, particularly in the +N and +sediment loading treatments.

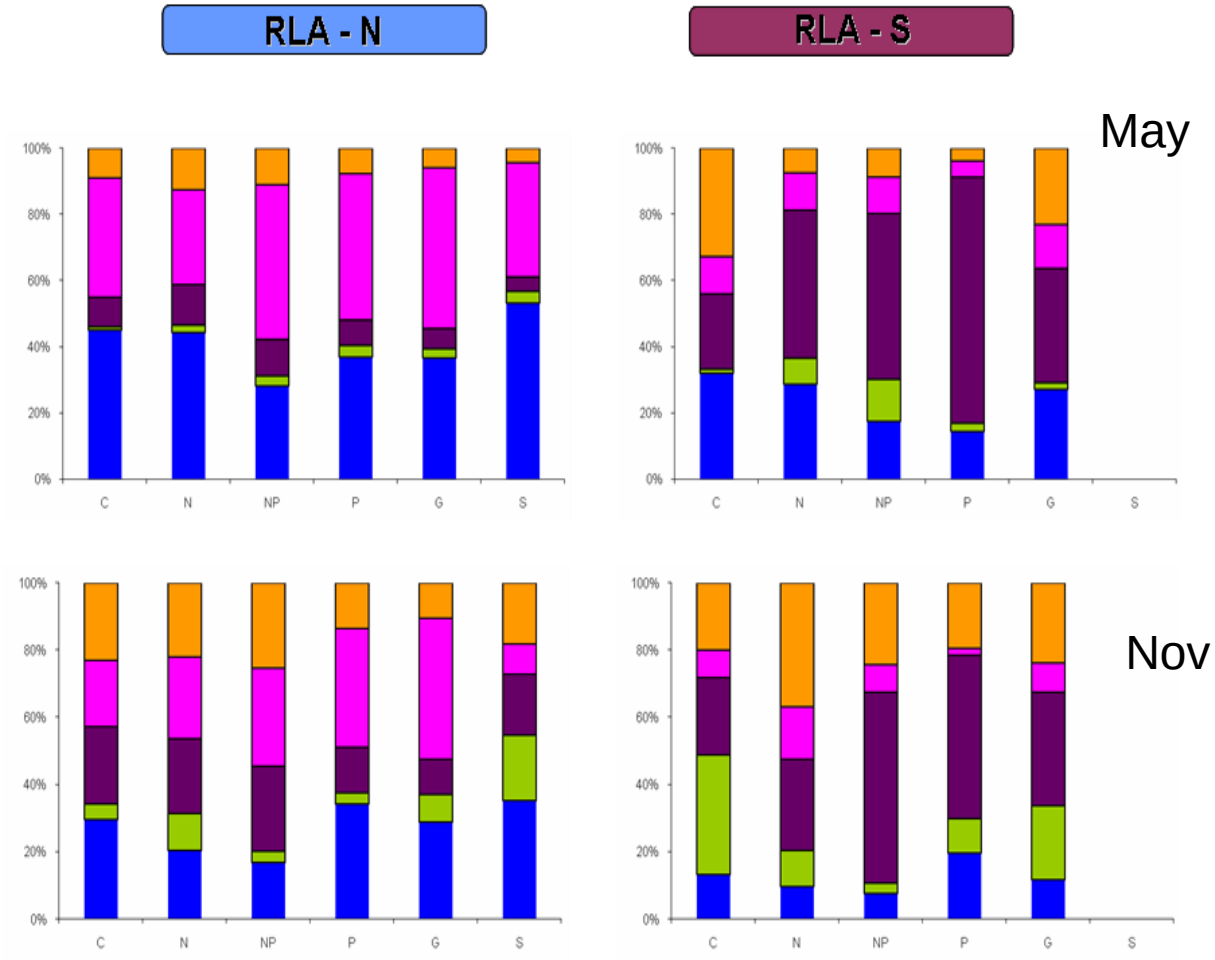


Fig. 16 Outcomes of the resource limitation assays. Assays were performed in the northern (left) and southern (right) sections of Galveston Bay during 2008. Changes in phytoplankton biomass were examined as changes in the distributions of the major phytoplankton taxa: Cyanobacteria / Prochlorophytes (blue), Chlorophytes / Prasinophytes (green), Diatoms / Dinoflagellates (orange), Haptophytes /Prymnesiophytes (purple), and Cryptophytes (pink). Sediment samples were lost in RLA-S.

In contrast, in RLA's conducted in the southern sector of the bay, we measured increases in Haptophytes and Prymnesiophytes (purple) populations in *all* treatments relative to control treatments and significant decreases in other groups. This finding suggests that Haptophytes and Prymnesiophytes could outcompete the other groups if conditions changed in the southern section of Galveston Bay so that there were either increased nutrient concentrations and/or sediment loading or reduced grazing (Fig. 16). Relative to the control, the biomass of Cyanobacteria and Prochlorophytes (blue) did not change significantly regardless of the time when the assays were conducted (Fig. 16). The same can be said of Cryptophyte (pink) populations with one exception, the +P nutrient treatment – both in May and November (Fig. 16). Whilst the proportion of Chlorophytes and Prasinophytes (green) did not change significantly in May (except in the +N and +NP treatments) as was observed in the northern sector of the Bay; we observed an decrease in their populations in November in RLA-S, the opposite response of our observation in RLA-N (Fig. 16).

5. Discussion

Understanding of the downstream ecological impacts of changes to freshwater inflows on estuaries is complicated by the large spatial and temporal scales which need to be examined in order to define relationships between water quality, nutrient and sediment loading and different levels of the trophic cascade which are either directly (e.g., phytoplankton) or indirectly impacted (e.g., fish, birds). Any understanding is further complicated by human induced changes such as industrialization, urbanization, redirection of flows, the introduction of returned flows from waste water treatment and a myriad of other factors, not all of which are currently known. Section 11.147 (a) of the Texas Water Code specifically defines "beneficial inflows" as those that provide a "salinity, nutrient, and sediment loading regime adequate to maintain an ecologically sound environment in the receiving bay and estuary system that is necessary for the maintenance of productivity of economically important and ecologically characteristic sport or commercial fish and shellfish species and estuarine life upon which such fish and shellfish are dependent." Yet to be defined, however, is a method for determining the amount of flow sufficient to meet these estuarine ecosystem requirements in light of all the other pressures being exerted in estuaries.

5.1 Freshwater inflows

In Texas, natural freshwater inflows are known to vary in magnitude and duration, with most significant flow events occurring from Fall to Spring, with little or no flow events occurring in the summer. Periodic hurricanes and tropical storms in the summer have been known to greatly increase freshwater inflow into Texas estuaries, although these and their impacts are not predictable. In 2008, frequent and large (>7000 cfs) freshwater inflow events were observed in Galveston Bay between February and May with some 1,689 thousand cfs entering the bay on the Trinity River side; five large pulses brought in 80% of the annual discharge (2,100 thousand cfs) for the year. Based on the USGS stream gage monitoring station located on the Trinity River at Romayor, a large freshet (61,100 cfs) entered the bay between September 14 and 18 (Fig. 4). Given Hurricane Ike which made landfall on September 13th 2008, this may reflect the drainage of surge waters back into Galveston Bay rather than a genuine freshwater inflow event. While the pattern of inflow events in 2008 are not unusual for this estuary, what does vary from year to year is that magnitude, duration and number of freshwater inflows. By comparison, during 2006, the annual discharge (1828 thousand cfs) was three times less than that observed in 2008. Similarly, freshets of >7000 cfs (seven total) occurred in the fall and spring, with little flow between May and October (Quigg et al. 2007). By contract, the annual discharge in 2007 was double that measured in 2008 (Table 2). The pulsed hydrology observed in the Trinity-San Jacinto estuary is common in many estuaries and can account for much of the annual loading of nutrients and sediment (Brock 2001; Paerl et al. 2001; Davis et al. 2004, 2007).

A unique aspect of the approach in this research program is the use of a Dataflow to map water quality parameters with high resolution on both spatial and temporal scales. The findings for Galveston Bay after a period of significant high flow (April 2008) and low flow (July 2008) are presented in Fig. 5 revealing the complex system level response. In general, salinity and water clarity decreased in response to a pulse of freshwater inflow whilst chlorophyll *a* and dissolved organic matter increased (Fig. 5). These patterns were most apparent in the northern section of Galveston Bay, in the area above San Leon (to the west) and Smith Point (to the east) relative to the southern section. The response was clearly dependent on the magnitude of the freshwater inflow event and to a lesser extent on the timing (see maps in Appendix A for further detail). For the latter, concurrent freshwater inflow events, such as those observed in the Spring of 2008, had

a bigger influence on the downstream water quality characteristics than smaller individual events (Figs. 4 and 5; Appendix A). In 2006, freshets were typically shorter in duration and of smaller magnitude. Concurrently, the area influenced by this freshwater inflow was restricted to the Trinity River (see Quigg et al. 2007). As observed with other studies in Galveston Bay (Pinckney 2006; Davis et al. 2007; Quigg et al. 2007), the Gulf of Mexico did not appear to have a significant effect on water quality in the upper part of Galveston bay, rather only in the southern most end in the area most adjacent to Bolivar Road and in West Bay. The level of complexity of the findings of this research program is reflected simply in examining the small subset of the data (Fig. 5). Multivariate multi-dimensional statistical approaches will be required to elucidate general patterns which may point the most important factors affecting spatial and temporal responses in water quality. Future efforts will also benefit from a careful comparison of data collected in 2006 with that in 2008.

5.2 Nutrient and sediment loading into Galveston Bay

The Trinity and San Jacinto Rivers are important sources of nutrient and sediments to Galveston Bay, with freshwater inflows and returned flows being the two major sources (Brock 2001). Other rivers and tributaries in the complex contribute but to a significantly lesser extent. We examined the effect of nutrient and sediment loading in several ways.

The majority of the sediment load carried by the Trinity River arrived in the Trinity River basin during the winter and spring (Fig. 6; Station 6). In the San Jacinto River basin, the sediment load was high year round except during the spring (Fig. 6; Station 5) which indicates that processes other than freshwater inflows may be important in regulation of the sediment load in this part of Galveston Bay. The Houston Ship Channel and its associated facilities line the upper western sector of this estuary and likely have a greater influence of sediment load than natural processes. Further, the higher sediment load almost year round on this side of the estuary may be associated with flows (including returned flows) which originate at the head of the watershed, in the Dallas / Fort Worth area. This dynamic certainly would influence not only the sediment load but also the nutrient loading.

Both dissolved and total particulate nutrient concentrations were measured as part of this study Figs. 7 – 9). It appears that the San Jacinto River was a greater source of nutrient loading than the Trinity River (Fig. 7 & 8). Lowest nutrient loading was measured in the stations most adjacent to the Gulf of Mexico, and in particular, in West bay (Fig. 7 & 8). Similar such patterns were observed in Galveston Bay in 2006 (Quigg et al. 2007). Seasonal patterns of nutrient distribution occurred across Galveston Bay (Fig. 9). Dissolved nutrient concentrations were greatest in fall and spring, the latter by at least an order of magnitude, and significantly lower in the summer and winter. On the other hand, total particulate nutrient concentrations were greatest in the summer but did not vary significantly during the other seasons (Fig. 9). It appears that dissolved nutrient loads are regulated by allochthonous processes (freshwater inflows) while particulate loads are regulated by autochthonous processes. For the latter, higher particulate loading appears to reflect nutrient loading associated with the Houston Ship Channel, urbanization and industrialization along the upper San Jacinto River complex and wind driven mixing towards the opening of Galveston bay with the Gulf of Mexico at the southern most end of the Bay.

The Redfield ratio (16:1 atoms of N: atoms of P) is the ratio of elements needed for 'balanced' or healthy growth of phytoplankton (Redfield 1934, 1958).While phosphorus is the proximal limiting nutrient element of concern in fresh waters, nitrogen is the proximal nutrient limiting productivity in marine systems (Nixon, 1995; Howarth and Marino, 2006). Estuarine phytoplankton are often nutrient-limited by N and/or P – the limiting factor(s) varies on a number of scales including proximity to nutrient source (natural or anthropogenic), season, and/or other as yet unknown factors. Water column N:P ratios (dissolved and particulate) were generally < than 16 (not shown), indicating phytoplankton population in Galveston Bay would be typically N limited year round. This finding is consistent with earlier similar studies for Galveston Bay (Örnólfsdóttir et al. 2004; Pinckney 2006; Quigg et al. 2007). This assertion is further supported by the findings in the resource limitation assays which also showed that for most months of the year, phytoplankton were N-limited (Fig. 15). What made 2008 a unique year was that there was also wide spread co-limitation of phytoplankton production, that is, that the addition of both N and P resulted in a higher phytoplankton responses than the addition of N sources alone (Fig. 15). One possibility may be the higher overall freshwater influx experienced

early in 2008 relative to other years when studies were performed (e.g., 2006 in Quigg et al. 2007). Consistent with this observation is that the greatest phytoplankton response indices were always measured in RLA South, that is, the resource limitations assays conducted in the Southern portion of Galveston Bay.

5.3 Phytoplankton productivity and community composition

Primary productivity was greater in the San Jacinto River basin than in the Trinity River basin (Stations 5 and 6 respectively; Fig. 10) which is consistent with the lower sediment loading (Fig. 6 and the higher nutrient loading (Figs. 7 and 8). Lowest production was measured in the areas adjacent to the Gulf of Mexico (Fig. 10). This corresponded with lower nutrient loads (Figs. 7 and 8) but not with the sediment loading observed (Fig. 6). Given that both nutrients and light are the most important drivers of primary productivity (Falkowski and Raven, 1997), this finding was not entirely unexpected. It does however reflect the complexity of the system.

When looking at monthly data, strong seasonal patterns in primary production in Galveston Bay did not appear (Fig. 10). In a 2006 study, we did find seasonal patterns, particularly in the upper part of the Bay (Quigg et al. 2007). It is not entirely clear why seasonal patterns were not observed in 2008; this will be a point for further investigation. Seasonal patterns are observed in many estuarine systems (e.g., Chan and Hamilton, 2001; Pinckney et al.1998).

The dominant phytoplankton groups in Galveston Bay in 2008 were the Cyanobacteria and Prochlorophytes in the warmer months and the Diatoms and Dinoflagellates in the cooler months (Fig. 13). Örnólfsdóttir et al. (2004) and Pinckney (2006) have previously reported this pattern in these particular major groups; this finding may have more to do with changing water temperatures than other autochthonous factors. Further, if the spatial and temporal patterns of these major groups are examined carefully at the six fixed stations throughout Galveston Bay, the results reflect both the internal complexity of the system and that different driving factors of the phytoplankton community composition are important in different parts of the bay (Fig. 12). This aspect has not been previously examined in great detail and will be a source of further studies. Using the PHYTO-PAM, we have gained an insight into the physiology of these major

groups and have observed that freshwater inflows have a significant impact in influencing phototroph responses (Fig. 14).

Unlike previous studies, we also found that Chlorophytes and Prasinophytes were big players in Galveston Bay during 2008 (Fig. 12 and 13). In addition, we also detected two major phytoplankton blooms events: a Cryptophyte (pink) bloom in March at station 6 (Trinity River basin) and a large dinoflagellate bloom at station 4 (mid-bay) in May (Fig. 12). The culprits have not yet been identified but this will be the effort of future studies. Further, the culprits appeared not to have been harmful in nature as there were no reports of fish kills or other signs of ecosystem disruption (Winston Denton, TPWD, pers. comm). It is not immediately clear why we observed this pattern in Galveston Bay in 2008. This finding lends support to the argument that long terms studies are needed in such systems to understand the natural and/or inherent variability.

The use of resource limitation assays not only enabled us to examine the response of the phytoplankton community as a whole to the addition of nutrients (+N, +P, +NP) but also to an increase in sediment loading (+S) and the removal of grazers (G) (Fig. 15). By taking these a step further and examining the phytoplankton community response we gained insights into potential driving factors for community shifts. Most notable of our observations was the finding that Cryptophyte (pink) populations increased in the nutrient addition treatments relative to control treatments in assays conducted in the northern sector of the bay (RLA-N) but not those in the southern sector (RLA-S) (Fig. 16). Instead, we observed increases in Haptophytes and Prymnesiophytes (purple) populations in *all* treatments relative to control treatments and significant decreases in other groups in RLA-S assays. Given that Cryptophyte (pink) and Haptophytes and Prymnesiophytes (purple) populations do not normally dominate in Galveston Bay (Fig. 13) but did so under these modified conditions, suggests that significant alternations in water quality (nutrient and sediment loading) in Galveston Bay could potentially change the baseline phytoplankton community. However, future studies will be valuable in repeating these kinds of experiments to determine if the changes observed are unique to 2008 or an intrinsic response of the Galveston Bay system. Changes in phytoplankton populations at this level would

have a significant effect on economically important and ecologically characteristic sport or commercial fish and shellfish species.

6. Conclusions and future directions

We need to understand how the present Galveston Bay ecosystem complex responds to freshwater inflows – pulses, high flow and low flow periods – in order to develop a conceptual understanding of the downstream ecological impacts of future changes to freshwater inflows and modes of nutrient loading into this system. The approach in this study – Dataflow and PHYTO-PAM mapping on fine spatial and temporal scales - promises to be particularly suited to monitoring programs designed to assess variability in water quality, primary productivity and phytoplankton community composition studies. They allow for statistically robust experimental design given the large number of samples that can be examined within a short period of time. The project spanned a range of inflow conditions into the Galveston Bay estuary between January and December 2008. Spatial maps generated from monthly sampling campaigns with a Dataflow unit provided a clear depiction of inflow effects on water quality in the system. In the fall/spring, repeated, large freshwater inflow events freshened much of the bay, introduced nutrients and lowered water clarity. In the summer/fall, freshets were infrequent. Noticeable differences in the northern section (upper bay) versus the southern section (lower bay) of Galveston Bay in terms of water quality, primary productivity and community composition, much of which was related to aforementioned river inflow effects on salinity, nutrients and to a lesser degree sediment loading. The findings of this study indicate that phytoplankton communities were co-limited by N (as nitrate) and P (as orthophosphate) for much of the year.

8. Bibliography

Arar. E.J. and Collins, G.B. 1997. Method 445.0, *In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence. *In* Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices, 2nd Edition. National Exposure Research Laboratory, Office of research and development, USEPA., Cincinnati, Ohio (EPA/600/R-97/072, Sept. 1997).

Boesch, D., *et al.* 1984. Deterioration of coastal environments in the Mississippi deltaic plain: options for management. pp 447-466, In: The Estuary as a Filter. Academic Press. Orlando.

Brock, D.A. 2001. Uncertainties in individual estuary N-loading assessments. p. 171-185. *In* R.A. Valigura et al. [eds.], Nitrogen loading in coastal water bodies: An atmospheric perspective. Coastal and Estuarine Studies 57. American Geophysical Union, Washington, DC.

Buyukates, Y., D. and Roelke. 2000. Influence of nutrient loading mode to accumulation of algal biomass and secondary productivity: An experimental study with management implications. *Eos* 80:235-236.

Chan, T., D. and Hamilton. 2001. Effect of freshwater flow on the succession and biomass of phytoplankton in a seasonal estuary. *Mar. Freshwater Res.* 52:869-884.

Curtis, P. J. and H. E. Adams. 1995. Dissolved Organic Matter Quantity and Quality from Freshwater and Saltwater Lakes in East-Central Alberta. *Biogeochemistry***,** 30: 59-76

Davis S., *et al.* 2004 Importance of episodic storm events in controlling ecosystem structure and function in a Gulf Coast estuary. *Journal of Coastal Research*. 20:1198-1208

Davis S., *et al.* 2007 Use of High-Resolution Spatial Mapping to Estimate Plankton Response to Freshwater Inflows Entering Galveston Bay: Importance to Watershed Development and Ecosystem Health. *Final Report for the Galveston Bay Estuary Program*, Texas Commission on Environmental Quality.

Dunton, K., *et al.* 1995. Annual variations in biomass and distribution of emergent marsh vegetation in the Nueces River Delta. Proc. of 24th Water for Texas Conference. Austin.

Falkowski, P. G. and Raven, J. A. 1997 Aquatic Photosynthesis. Blackwell.

Fisher, T.R., Gustafson, A.B., Sellner, K.R., Lacuture, R., Haas, L.W., Magnien, R., Karrh, R. & Michael, B.1999. Spatial and temporal variation in resource limitation in Chesapeake Bay. *Marine Biology* 133: 763-778.

GBEP 2001. The Galveston Bay Plan. The comprehensive conservation and management plan for the Galveston Bay Ecosystem. GBNEP#49. 457pp.

Hansen, H. P., and F. Koroleff. 1999. Determination of nutrients. p. 159-228. In K. Grasshoff, K. Kremling and M. Ehrhardt [eds.]. *Methods of Seawater Analysis.* Wiley.

Heilman, J., *et al.* 1999. Tower-based conditional sampling for measuring ecosystem-scale carbon dioxide exchange in coastal wetlands. *Estuaries* 22:584-591.

Howarth, R. W. and Marino, R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnology and Oceanography* 51, 364-382.

Jakob, T., *et al.* 2005. Estimation of chlorophyll content and daily primary production of the major algal groups by means of multiwavelength-excitation PAM chlorophyll fluorometry: performance and methodological limits. *Photosynthesis Research* 83: 343-361

Jeffrey, S. W., Mantoura, R. F. C. & Wright, S. W. [Eds.] 1997. *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods.* UNESCO, Paris, 450 pp.

Longley, W.L. 1994. Freshwater inflows to Texas bays and estuaries: ecological relationships and methods for determination of needs. Texas Water Development Board and Texas Parks and Wildlife Department. Austin. TX. 386pp.

Mackey, M., *et al.* (1996) CHEMTAX-a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. *Mar Ecol Prog Ser* 144:265-283

Malone, T., *et al.* 1988. Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Mar. Ecol. Prog. Ser.* 48:235-249.

Montagna, P.A., and R.D. Kalke. 1992. The effect of freshwater inflow on meiofaunal and macrofaunal populations in the Guadalupe and Nueces Estuaries, TX. *Estuaries* 15:307-326.

Millie, D. F., *et al.* 1993. Microalgal pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications. *Can. J. Fish. Aquat. Sci.* 50:2513-27.

Madden, C. and J. Day. 1992. An instrument for high speed mapping of chlorophyll–*a* and physico-chemical variables in surface waters. *Estuaries*. 15:421-427.

Nicklisch A and Köhler, J. 2001. Estimation of primary production with Phyto-PAM fluorometry. *Ann Report Inst Fresh Ecol Inland Fish Berlin* 13: 47-60

Nixon, S.W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41:199-219.

Örnólfsdóttir, E.B., *et al.* 2004. Nutrient pulsing as a regulator of phytoplankton abundance and community composition in Galveston Bay, Texas. *Journal of Experimental Marine Biology and Ecology*. 303: 197-220.

Paerl, H, *et al.* 2001. Ecosystem impacts of three sequential hurricanes (Dennis, Floyd, and Irene) on the United States' largest lagoonal estuary, Pamlico Sound, NC. *PANS*. 98(10):5655-5660.

Papageorgiou, G.C. and Govindjee. 2004. Chlorophyll a Fluorescence: A Signature of Photosynthesis. Springer. pp. 818.

Pinckney, J. L., *et al.* 1998. Annual cycles of phytoplankton community structure and bloom dynamics in the Neuse River Estuary, North Carolina. *Mar. Biol.* 131:371-82.

Pinckney, J. 2006. System-scale nutrient fluctuations in Galveston Bay, Texas (USA), p. 141-164. In J.C. Kromkamp, J.F.C. de Brouwer, G.F. Blanchard, R.M. Forster, and V. Creách (Eds.). Functioning of Microphytobenthos in Estuaries, Royal Netherlands Academy of Arts and Sciences, Amsterdam.

Quigg, A., Davis, S.E. & Roelke, D.F. 2007. Changes in freshwater inflows and how they effect Texas Bays. Final Report of the Coastal Coordination Council pursuant to National Oceanic and Atmospheric Administration Award No. NA05NOS4191064. pp. 47.

Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnson memorial volume. Liverpool, U.K., Liverpool University, 176-192.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. American Scientist, 46, 205-221.

Riera, P., *et al.* 2000. Utilization of estuarine organic matter during growth and migration by juvenile brown shrimp *Panaeus aztecus* in a south Texas estuary. *Mar. Ecol. Progr. Ser.* 199:205-216.

Roelke, D., *et al.* 1999. A model of phytoplankton competition for limiting and non-limiting nutrients: Implications for development of estuarine and near shore management schemes. *Estuaries* 22:92-104

Roelke, D.L. 2000. Copepod Food-Quality Threshold as a Mechanism Influencing Phytoplankton Succession and Accumulation of Biomass, and Secondary Productivity: A Modeling Study with Management Implications. *Ecol. Model.* 134:245-274.

Texas Water Development Board. 2001. Water for Texas: Summary of Regional Water Plans.

Texas Water Development Board. 2007. State Water Plan for Texas: Highlights of the 2007 State Water Plan. GP-8-1.

Thronson, A. and Quigg, A. 2008 Fifty five years of fish kills in Coastal Texas. *Estuaries and Coasts.* 31: 802–813

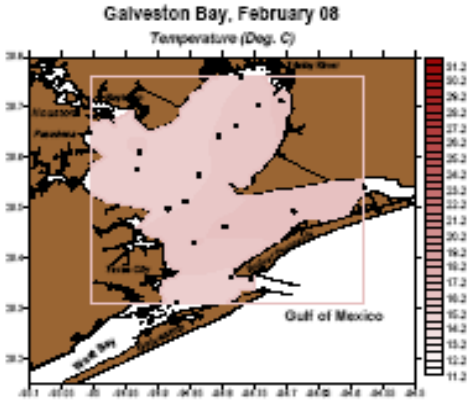
Ward, G., *et al.* 2002. Experimental river diversion for marsh enhancement. *Estuaries* 25:1416-1425.

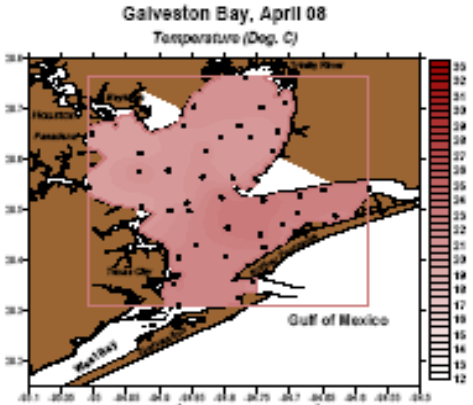
Wetzel, R. G. & Likens, G. E. 2000. *Limnological Analyses* Third Edition, Springer. 429 pages.

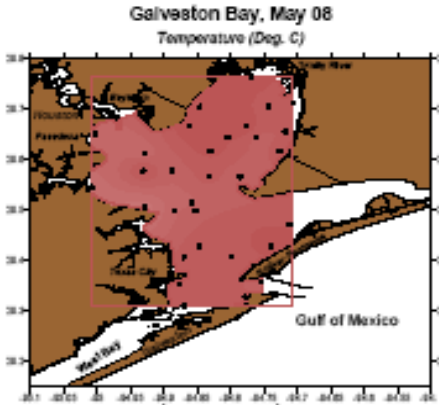
Appendix A

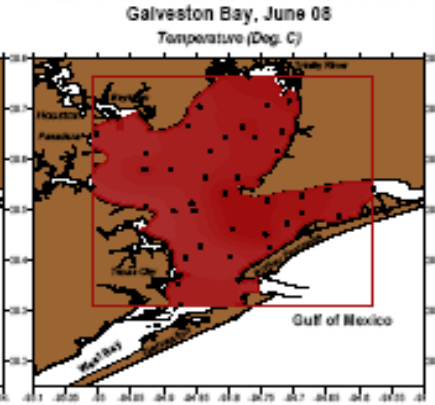
Temporal and spatial patterns of water quality parameters measured with the Dataflow in Galveston Bay.

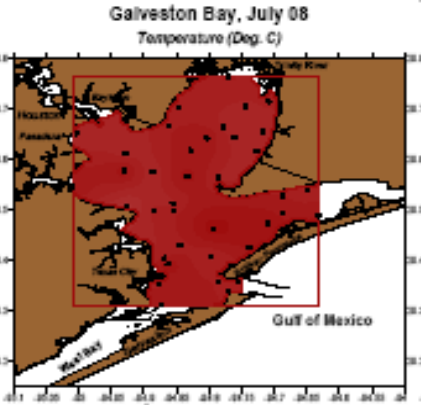
2008 Temperature Deg. C

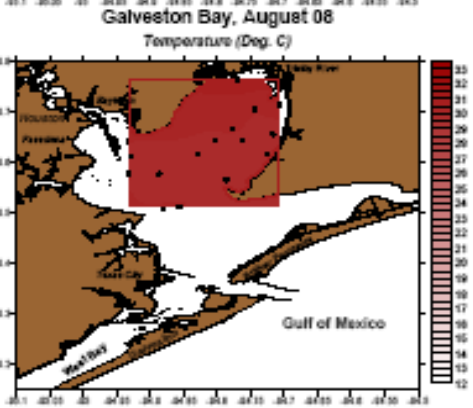


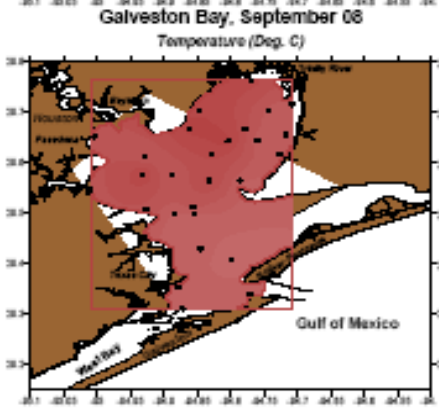


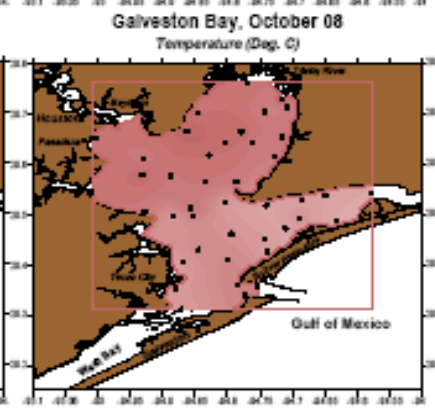


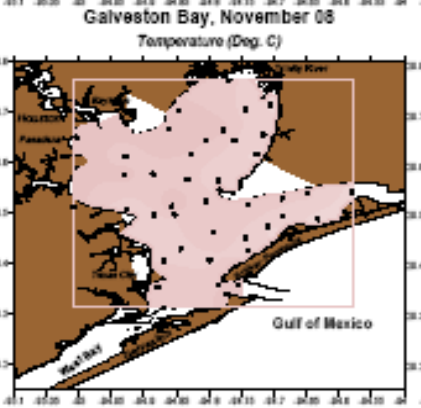


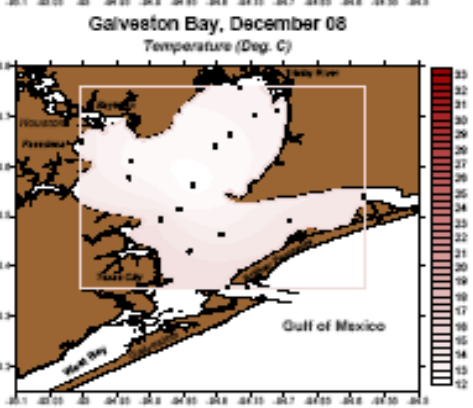




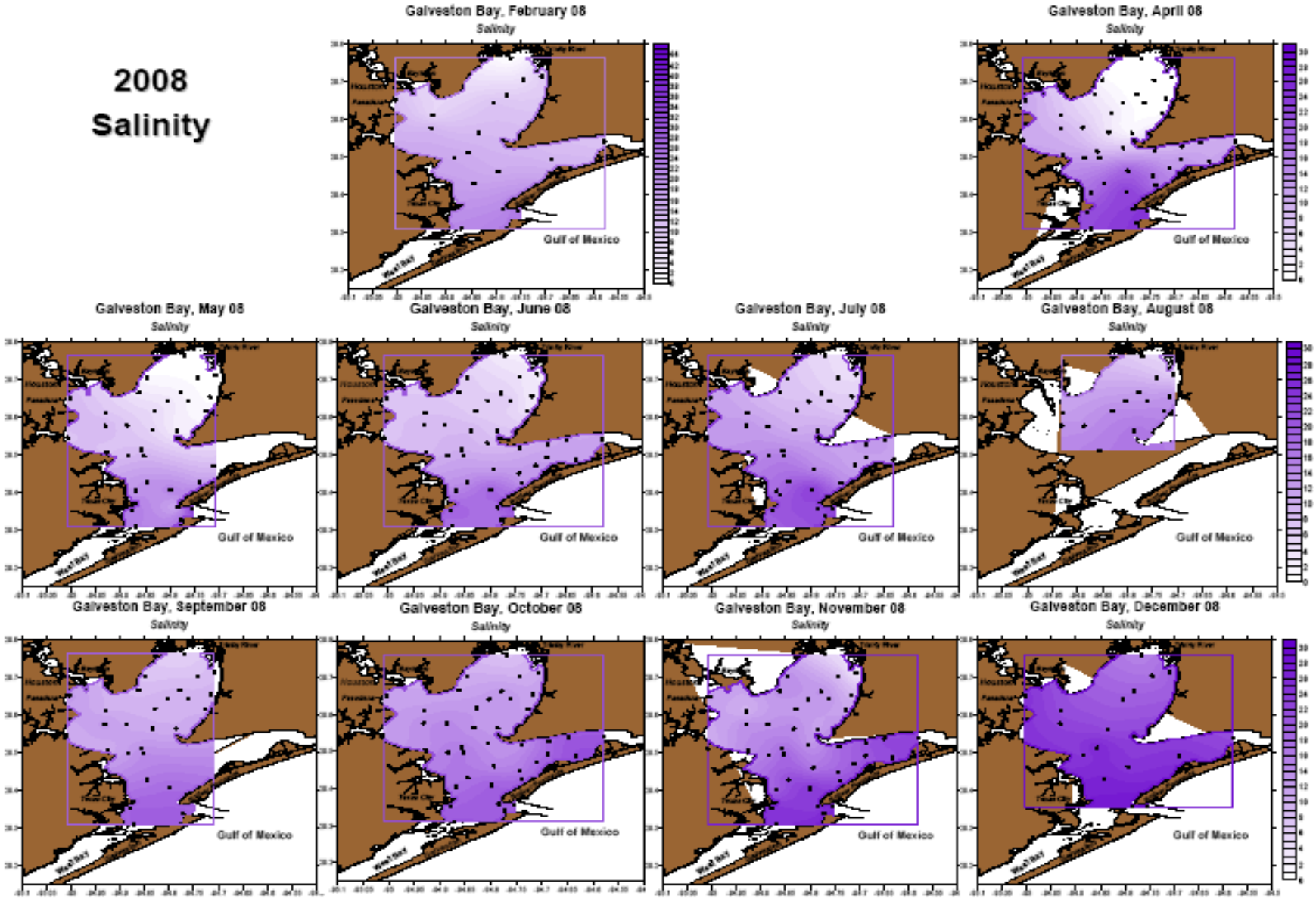




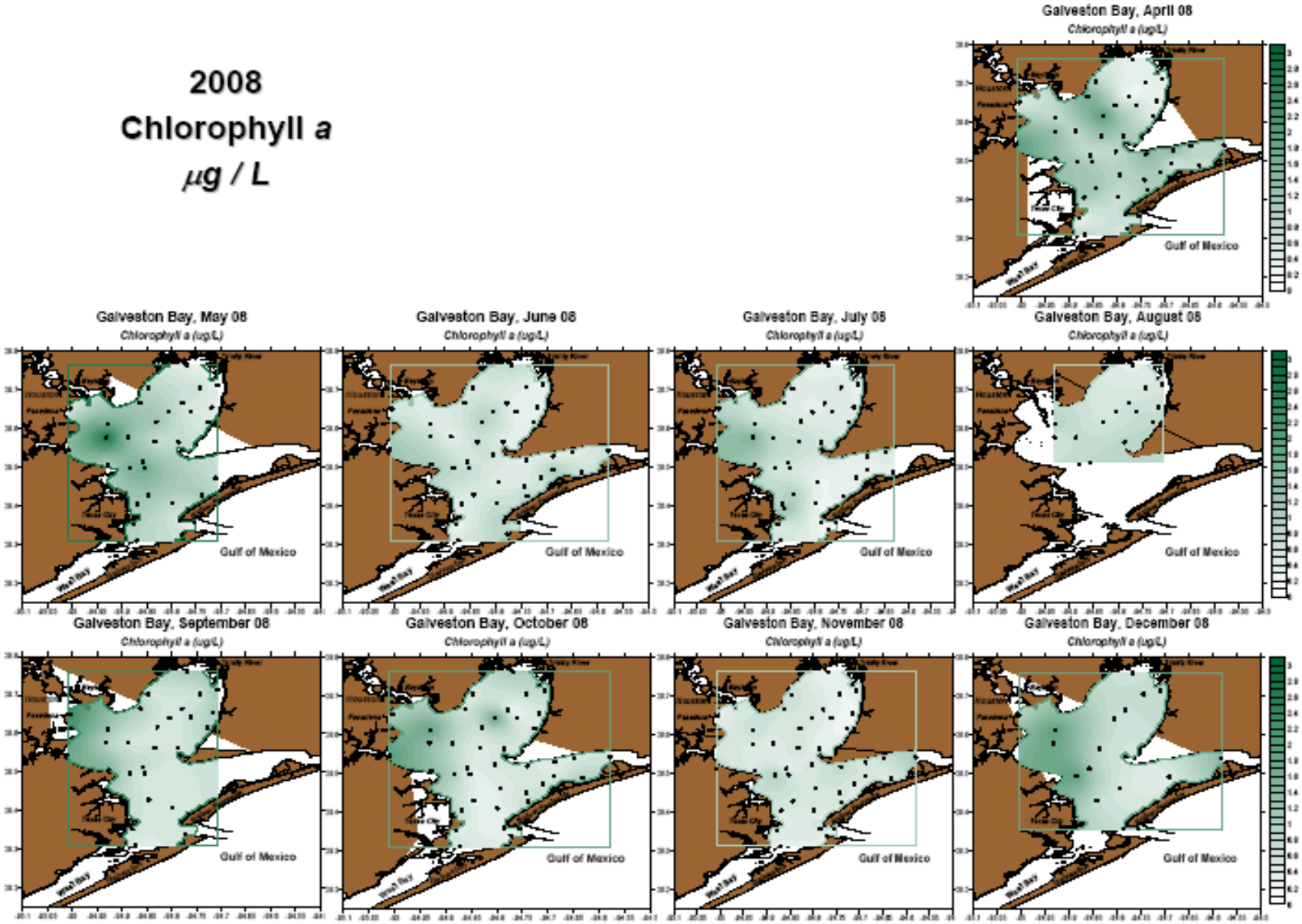




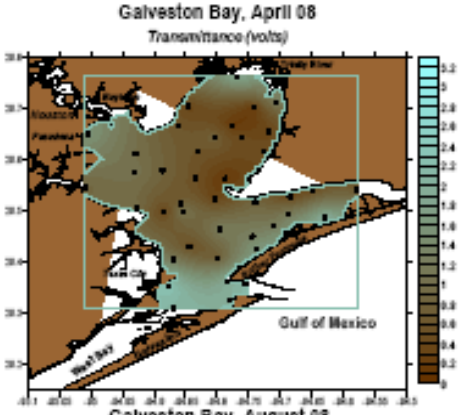
2008 Salinity

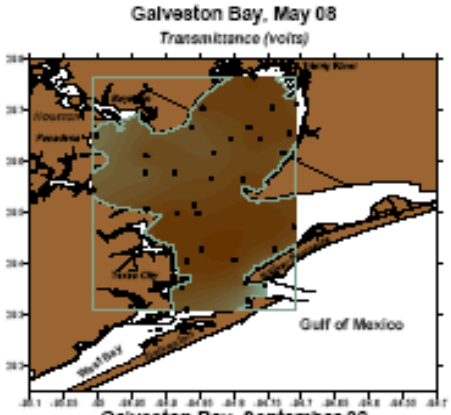


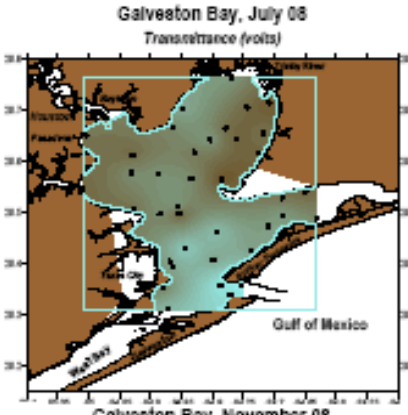
2008
Chlorophyll *a*
μg / L

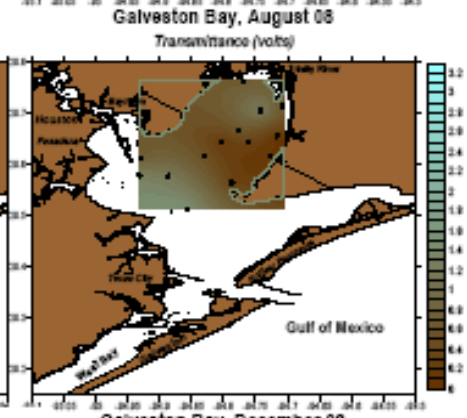


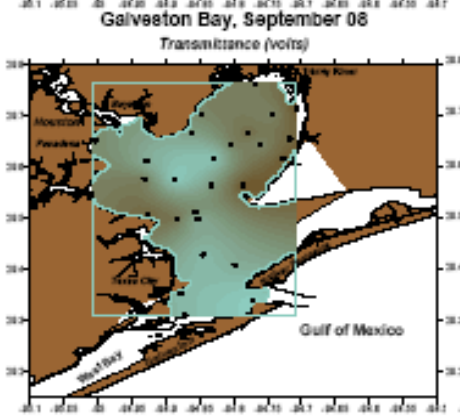
2008 Transmittance (volts)

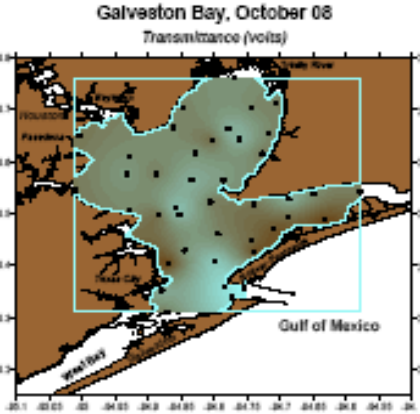


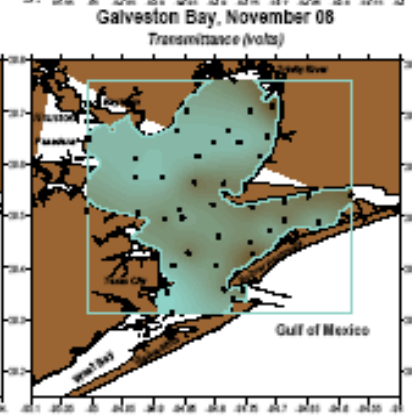


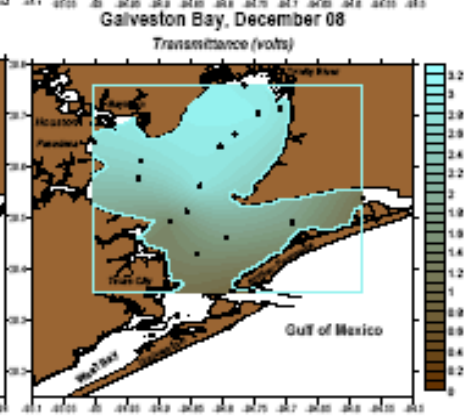




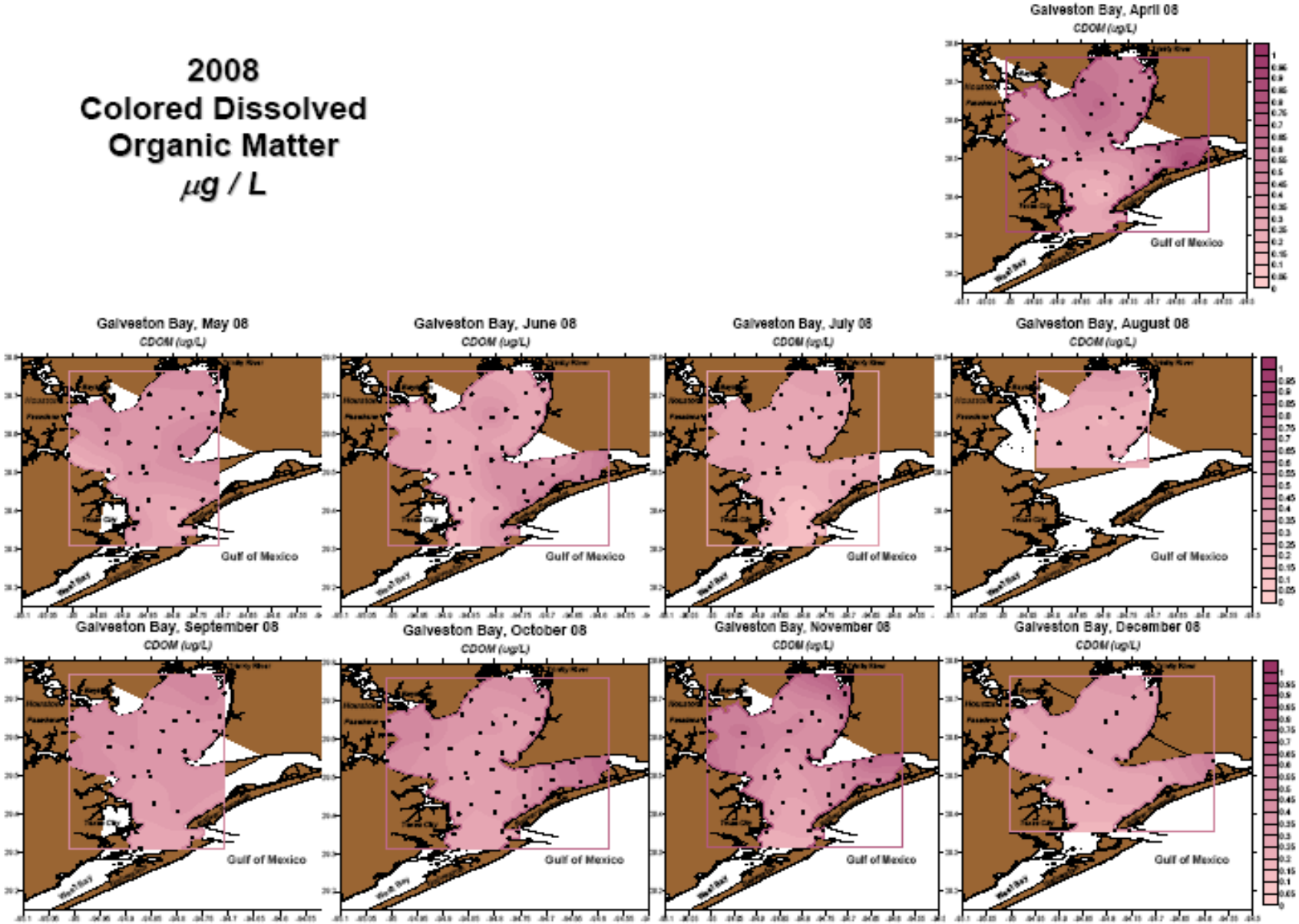








2008
Colored Dissolved Organic Matter
$\mu g / L$



Appendix B

Data collected from the six fixed stations (Table 1, Figure 2) was averaged into seasonal bins:

winter (December - February),

spring (March – May),

summer (June – July), and

fall (August – November).

Normally August would fall into the summer category but because the sampling trip took place at the end of the month, in this instance, it made more sense to include it with the fall data set.

Temporal and spatial distributions of total suspended solids in Galveston Bay

TSS (mg/mL)	Station	Winter	Spring	Summer	Fall
	1	0.11	0.02	0.17	0.08
	2	0.11	0.03	0.12	0.07
	3	0.08	0.05	0.03	0.08
	4	0.08	0.08	0.09	0.05
	5	0.07	0.03	0.09	0.06
	6	0.07	0.07	0.04	0.01

Temporal and spatial distributions of primary production in Galveston Bay

Gross primary production rates (gC m-2 d-1)	Station	Winter	Spring	Summer	Fall
	1	0.67	0.50	0.70	1.00
	2	0.25	2.87	1.66	0.51
	3	1.10	0.78	1.02	0.98
	4	1.12	2.35	1.16	1.12
	5	1.72	2.72	1.10	1.62
	6	0.38	0.56	0.66	0.89

Chlorophyll (ug l-1)	Station	Winter	Spring	Summer	Fall
	1	2.67	1.17	0.38	0.48
	2	2.38	2.69	3.04	0.61
	3	0.58	2.14	0.69	1.10
	4	3.43	3.99	4.83	1.38
	5	2.96	3.77	1.12	2.12
	6	3.60	1.78	0.57	1.36

Temporal and spatial distributions of nutrient concentrations in Galveston Bay

Dissolved Nitrate + nitrite (μM)	Station	Winter	Spring	Summer	Fall
	1	3.00	4.80	7.08	2.04
	2	1.66	0.85	0.21	1.36
	3	0.38	0.23	0.15	0.54
	4	0.66	14.46	0.24	5.49
	5	1.46	15.80	0.36	17.65
	6	3.43	36.05	0.22	2.46

Dissolved phosphate (μM)	Station	Winter	Spring	Summer	Fall
	1	0.60	0.44	0.71	0.68
	2	0.88	0.52	1.24	1.36
	3	0.15	0.42	0.97	0.37
	4	1.36	2.32	4.26	3.12
	5	4.06	3.03	3.84	4.61
	6	1.25	2.02	2.85	2.54

Total particulate nitrogen (μM)	Station	Winter	Spring	Summer	Fall
	1	26.67	33.02	45.63	18.64
	2	22.61	31.43	52.23	22.51
	3	17.65	34.38	33.38	40.88
	4	18.33	43.58	71.94	36.09
	5	40.15	42.07	63.26	49.73
	6	23.70	44.80	51.80	36.27

Total particulate phosphate (μM)	Station	Winter	Spring	Summer	Fall
	1	1.21	0.82	1.28	0.59
	2	1.33	1.20	2.63	1.16
	3	0.72	1.50	1.15	0.85
	4	1.41	2.85	6.73	3.02
	5	3.90	3.59	5.90	4.49
	6	1.96	3.52	4.72	3.56