FINAL REPORT

Galveston Bay Plankton Analysis

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List of Abbreviations and Acronyms

cfs	cubic feet per sec
FWI	fresh water inflows
GBEP	Galveston Bay Estuary Program
HPLC	High Performance Liquid Chromatography
PHYTO-PAM	Phytoplankton Pulse Amplitude Modulated fluorometer
QAPP	Quality Assurance Protection Plan
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 Program
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, the goal is to ensure *beneficial* freshwater inflows necessary for a 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 freshwater inflows on estuaries, specifically phytoplankton communities. Hence, 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 2005 and December 2006. Using a mutli-variate and mutli-dimensional statistical approach, we did find that specific genera of diatoms and dinoflagellates, the two major groups, occur in specific parts of Galveston Bay at species times of the year (Figures 7 to 9). For example, the diatoms *Navicula*, *Thalassionema, Thalassiosira* and to a lesser extent *Pleurosigma,* were most abundant at Stations 1 and 4 (Bolivar Pass near mouth and Morgan's Point in the middle of Galveston Bay respectively) during the spring and summer months (March to August 2005). *Cylindrotheca* and many of the other diatoms were present mostly at Station 6 (Trinity River basin), but to a lesser extent in other parts of Galveston Bay. These euryhaline phytoplankton are common to many bays and estuaries throughout the world and form the base of many important trophic food webs, supporting zooplankton and fish populations. Unfortunately, we also identified species which have been associated with harmful algal blooms. These include the diatoms *Pseudo-nitzschia* and *Nitzschia* and the dinoflagellates *Akashiwo, Dinophysis, Prorocentrum* and *Protoperidinium.* These phytoplankton species have been implicated in fish kills and shellfish hatchery closures around the world. We did not detect any *Karenia brevi* nor *Pfiesteria* sp. in our microscopic examination – blooms of these two species in Galveston Bay would severely disrupt the Galveston Bay ecosystem. Understanding the linkages between the magnitude of freshwater inflows and phytoplankton community structure in Galveston Bay remains a challenge; requiring further data collection in the Galveston Bay system, enumeration of existing newly collected phytoplankton samples from 2008-2009, and analysis of additional parameters in order to further clarify the importance of inflow events in this estuarine system.

Galveston Bay Plankton Analysis

2. Introduction

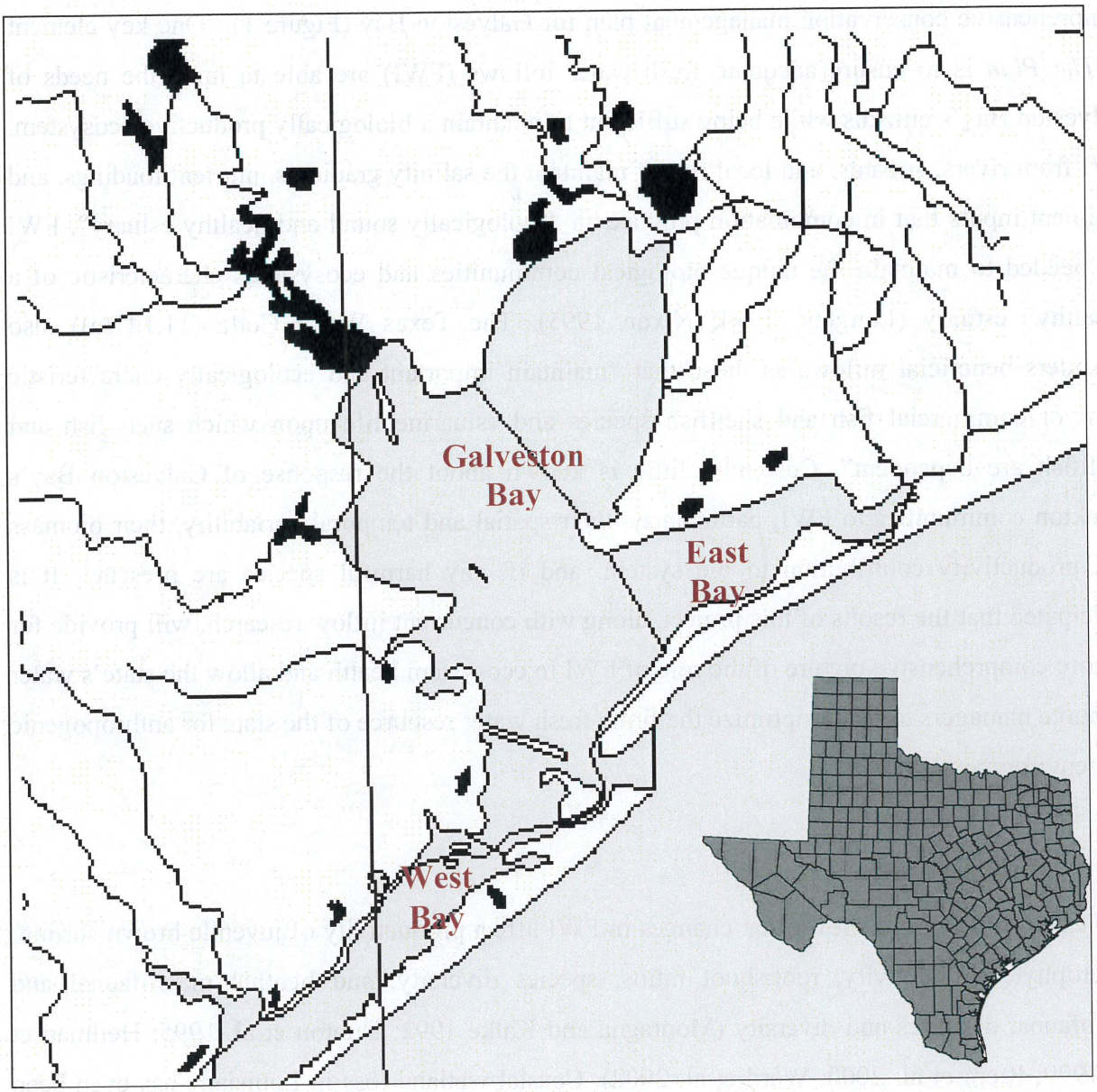
The Galveston Bay Estuary Program (GBEP) of the Texas Commission on Environmental Quality (TCEQ) is charged with implementing the Galveston Bay Plan *(The Plan),* a comprehensive conservation management plan for Galveston Bay (Figure 1). One key element of *The Plan* is to ensure adequate fresh water inflows (FWI) are able to meet the needs of Galveston Bay's citizens while being sufficient to maintain a biologically productive ecosystem. FWI from rivers, streams, and local runoff maintain the salinity gradients, nutrient loadings, and sediment inputs that in combination produce an "ecologically sound and healthy estuary". FWI are needed to maintain the unique biological communities and ecosystems characteristic of a "healthy" estuary (Longley, 1994; Nixon 1995). The Texas Water Code (11.147(a)) also considers beneficial inflows as those that "maintain important and ecologically characteristic sport or commercial fish and shellfish species and estuarine life upon which such fish and shellfish are dependent". Currently, little is known about the response of Galveston Bay's plankton communities to FWI, particularly, their spatial and temporal variability, their biomass and productivity contribution to the system, and if, any harmful species are present. It is anticipated that the results of this project, along with concurrent inflow research, will provide for a more comprehensive picture of the role of FWI in ecosystem health and allow the state's water resource managers to better optimize the finite fresh water resource of the state for anthropogenic and environmental uses.

2.1 Background

In Texas, studies have shown that changes in FWI affect productivity of juvenile brown shrimp, macrophyte productivity, root:shoot ratios, 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 even been attributed to a 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).

Figure 1.

Map showing location of Galveston Bay and the lesser bays of the Galveston Bay Estuary complex, also referred to as the Trinity-San Jacinto Esutary.



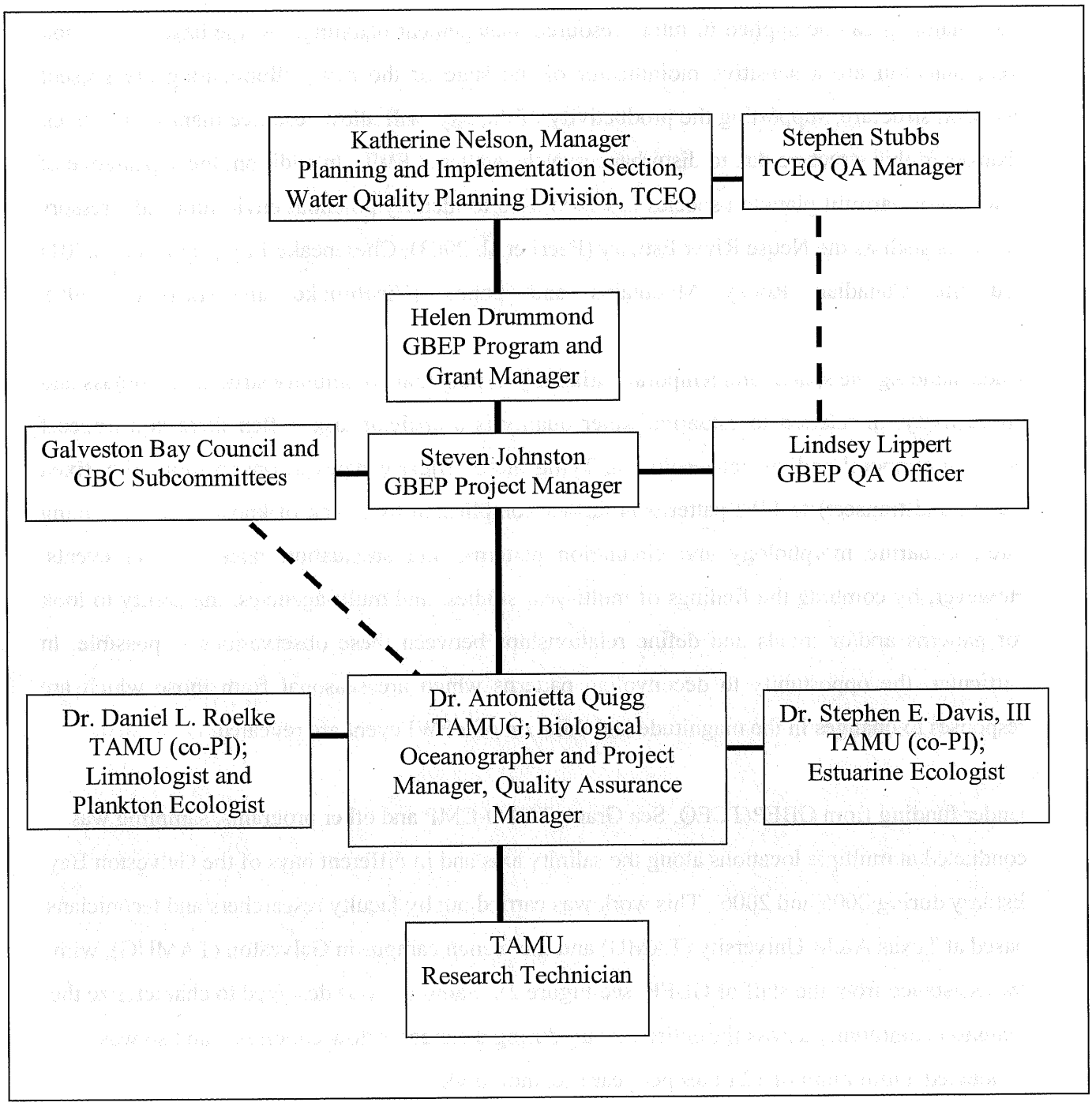
The Galveston Bay area is likely to see the largest population growth along the Texas coast in the next few decades (TPWD, 2007). There is pressing need to understand how present Galveston Bay responds to nutrient and sediment loading from FWI in order to develop a conceptual understanding of the effect of FWI on the plankton community so that this understanding can be applied in future resource management planning. At the base of the food web, plankton are a sensitive bioindicator of the state of the bay. Illuminating the present plankton structure, supporting the productivity of the bay, will allow resource managers to track changes in this structure due to disturbances such as altered FWI. In addition, the appearance of new and/or harmful plankton 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).

Understanding the spatial and temporal variability in plankton community structure, biomass and productivity, in relation to estuarine water quality is a difficult task. Often there is a tradeoff between temporal and spatial resolution. Tying these different types of observations (i.e. fixed station and transect) to FWI patterns is further complicated by a lack of knowledge on loading rates, estuarine morphology and circulation patterns, and attenuation rates of FWI events. However, by combing the findings of multi-year studies, and multi-agencies, the ability to look for patterns and/or trends and define relationships between these observations is possible. In particular, the opportunity to deconvolute patterns which are seasonal from those which are responses to changes in the magnitude and duration of a FWI event are revealed.

Under funding from GBEP/TCEQ, Sea Grant, TGLO-CMP and other programs, sampling was conducted at multiple locations along the salinity axis and in different bays of the Galveston Bay Estuary during 2005 and 2006. This work was carried out by faculty researchers and technicians based at Texas A&M University (TAMU) and the branch campus in Galveston (TAMUG), with the assistance from the staff of GBEP (see Figure 2). Sampling was designed to characterize the plankton community across the entire estuary during different inflow conditions and so was conducted a minimum of 12 times per year (i.e. monthly).

Figure 2.

Organizational chart of project personnel



We sampled a range of pulsed events and dry periods during the study period. Samples for High-Performance Liquid Chromatography (HPLC) pigment analyses were collected to infer plankton community structure at fixed stations. The results of these analyses and subsequent pigment ratios will be periodically checked against microscopic analysis. The microscope analysis is especially important validation of the HPLC work, but more importantly it allows us to identify the important plankton species in Galveston Bay and how their presence in the community is altered by changes in water quality, seasons and other perturbations in the Galveston Bay system. All collection procedures and equipment to be used are described below. Tabular and graphical products will be submitted for communication of these findings to GBEP, the scientific community, management agencies and public.

2.2 Project/Task Description

The purpose of this project is to examine, under a microscope, the plankton samples collected in 2005 and 2006 from a previous GBEP/TCEQ funded project, in order to examine the effect of salinity, nutrient and sediment loading on plankton community structure. The 2005-2006 samples were analyzed using High Performance Liquid Chromatography (HPLC) using protocols described by (Millie et al. 1993, Jeffrey et al. 1997) in combination with the program CHEMTAX (Mackey et al. (1996) to measure for chlorophyll a biomass (Jeffrey et al. 1997) and plankton community structure. A Phytoplankton Pulse Amplitude Modulated (PHYTO-PAM) fluorometer was used in 2006 to complement both the measurement of primary productivity using traditional techniques, and to determine what the major taxonomic groups were present (Jakob et al. 2005). The findings of the HPLC/CHEMTAX and PHYTO-PAM studies have been reported in Davis et al. (2007) and Quigg et al. (2007) respectively. While each of these methods provides a level of understanding to the plankton community present on a biomass, production or gross taxonomic scale (Division level) at any given time, none provides details of the actual species present, their numbers and/or biovolume. Funding at the time, however, did not afford a more rigorous and detailed analysis. The plankton samples will be identified to species level, enumerated and the biovolume calculated. A digital photo library will also be established and made available on the PI's website for other researchers and agency personnel.

2.3 Project objectives, tasks, and schedule of deliverables

Program Element 1: Sample Analysis

Objective: To examine samples collected from the Galveston Bay Estuary as part of a monthly sampling program in 2005 to 2006, specifically examining plankton community composition.

Task 1.1 Train a research technician in plankton identification and enumeration techniques.

Task 1.2 Examine samples collected in 2005 to 2006.

Task 1.3 Maintain updated QAPP information should an approved QAPP revision be made and maintain updated documentation on data acquisition methodologies on data tracking sheets.

Program Element 2: Data Analysis

Objective: The link between riverine inflows (and corresponding loading of materials) and spatial patterns in phytoplankton community structure will be accomplished through analysis of the data collected in 2005 and 2006. These data will be translated into tabular and graphical products for distribution to the GBEP Project Manager, or GBC Subcommittees upon request.

Task 2.1 Analyze spatial patterns in plankton community structure derived from microscopic analyses.

Task 2.2 Compare findings from microscopic analysis to those performed using HPLC in determining the major plankton divisions in Galveston Bay over the sampling period.

Task 2.3 Link temporal patterns in inflows and loadings to spatial patterns in estuary-wide plankton community structure through multivariate statistical analyses.

Program Element 3: Dissemination of Data

Objective**:** Data on plankton community structure will be provided as graphical and tabular analyses to GBEP Project Manager in quarterly progress reports and in a final report. This information will be made available to the GBEP, GBC and GBC subcommittees in written and electronic format (as requested).

Task 3.1 Work with GBEP Project Manager to develop a report on the importance of inflow events and nutrient loading to Galveston Bay system.

Task 3.2 Provide GIS-based maps, graphs, and interpretations of information to the GBEP Project Manager and GBC subcommittees.

3. Methods

High spatial and temporal resolution water quality mapping were performed monthly, whenever possible. The findings have been reported in Davis et al. (2007). On several occasions, field work was not possible due to poor weather conditions. Discrete water samples were also collected from six fixed stations (Table 1) for laboratory analysis of many parameters including phytoplankton community structure analysis (see section 3.2) and for phytoplankton tows (see section 3.3). All protocols and procedures were followed as outlined in the projects Quality Assurance and Protection Plan (QAPP).

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

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'

3.1 Freshwater inflows

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 2005 to December 2006. The data presented is that previously checked by the USGS's QAPP.

3.2 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).

3.3 Plankton collection

Community phytoplankton were collected by 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 was preserved in an acid cleaned HDPE rectangular bottle (125 mL; Nalgene) using Glutaraldehyde (final 5%). *Total Phytoplankton* were assayed by measuring 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.

3.4 Plankton Enumeration and Identification

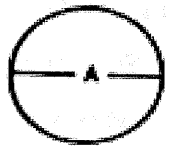
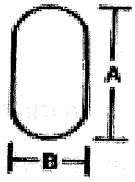
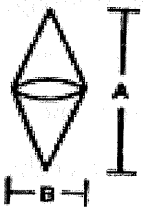
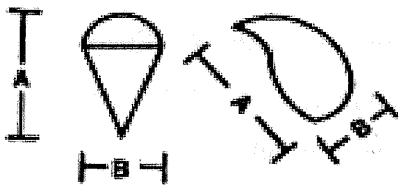
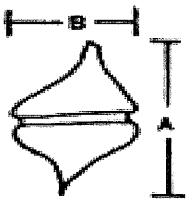
Samples were examined microscopically for general species identification with the assistance of Tomas (1997). Digital photographs of representatives of each species were recorded along with the magnification, sizes and any other distinguishing detail.

Utermohl chambers (Utembol, 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. We used descriptions in Table 2 (from Wetzel and Likens (1991) to determine cell dimensions and biovolumes. A detailed protocol is included in Appendix 1. This describes the fine specifics from the assembly of the chambers to the enumeration process.

3.5 Statistical Analysis

The program R© was used to examine the plankton data in order to perform a multi-variate and multi-dimensional analysis and present findings.

Table 2. *In order to calculate the volume for each of the phytoplankton cells, the following measurements and formula were used (from Wetzel and Likens 1991).*

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*

4. Results

4.1 Freshwater Inflow into Galveston Bay during 2005 and 2006

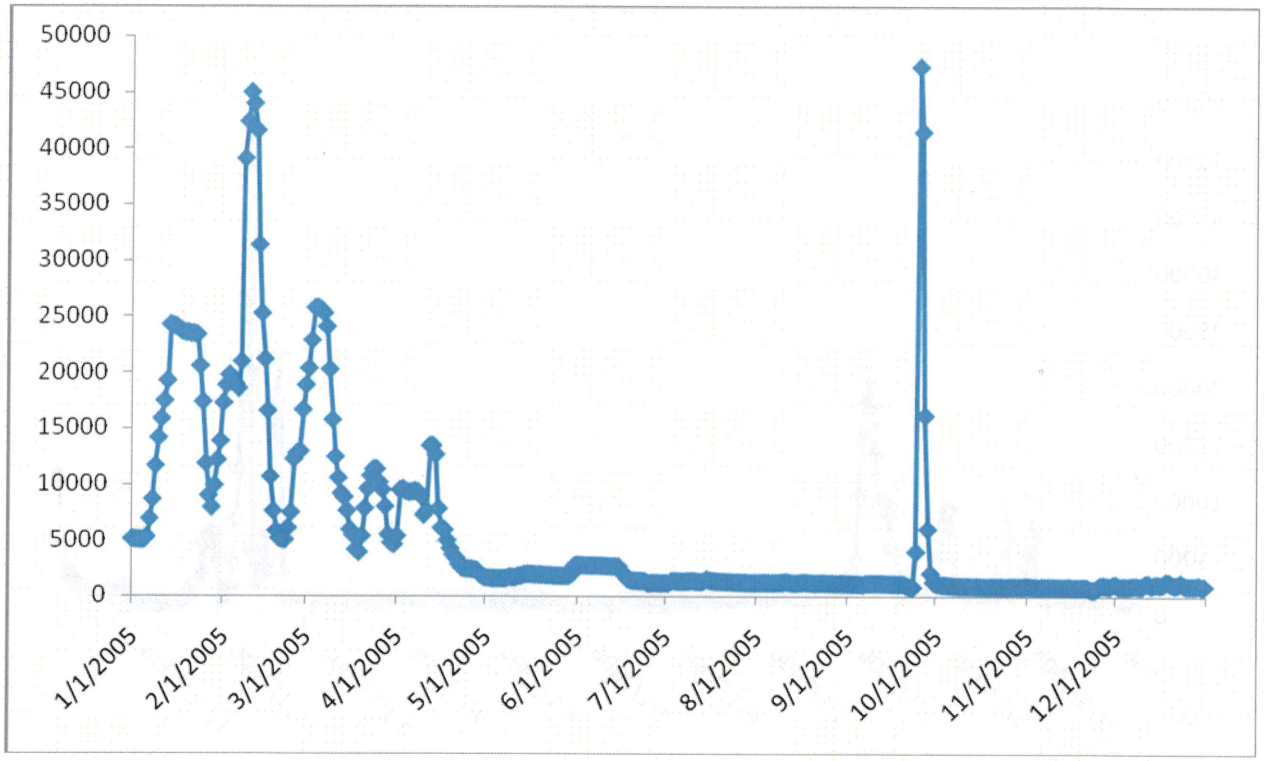
Real-time freshwater inflow measured as daily discharge to Galveston Bay from January 01 2005 to December 31 2006 was downloaded from the USGS monitoring station located on the Trinity River at Romayor (08066500). Given the two years were quite different in terms of discharge characteristics, we have presented them separately.

A total of 2,039,564 cubic feet per sec (cfs) of water was discharged into Galveston Bay in 2005 (Fig. 3). Approximately 5% of this however was not attributed to flow from the Trinity River per se, rather it was associated with the passage of Hurricane Rita in September 2005. In a three day period associated with this hurricane, 105,000 cfs was recorded at the Romayor USGS monitoring station. It could be argued that this event was not a "true" freshwater inflow in the sense of that the flow was recorded immediately after Hurricane Rita which made landfall on September 23rd 2005. This peak more likely reflects that drainage of surge waters back into Galveston Bay. Significant freshwater inflows pulses (>7000 cfs) or "freshets" did occur in 2005. If Hurricane Rita is excluded from the analysis, these freshets were all recorded during the winter and spring (Figure 3). Only one of the freshets was greater than 30,000 cfs.

In 2006, less than 50% of the 2005 freshwater inflow to Galveston bay was recorded, that is, 959,413 cfs for the year compared with 2,039,564 cfs in 2005 (Figure 4). Also, unlike 2005, significant freshwater inflow pulses (>7000 cfs) occurred in both the winter/spring and during the fall. In general, the magnitude of freshets was typically lower in 2006 compared to 2005, with freshets typically between 10,000 and 20,000 cfs (Figure 4). Again, only one of the freshets was greater than 30,000 cfs; this occurred in the fall of 2006. Hence, in 2006, there were significantly freshwater inflow events during the wet months.

Figure 3.

Daily discharge of freshwater into Galveston Bay from January 01 to December 31 2005. Real-time flow data was downloaded from the USGS monitoring station located in the Trinity River at Romayor (08066500). Freshwater daily discharge is presented on the y-axis in units of cubic feet per sec.



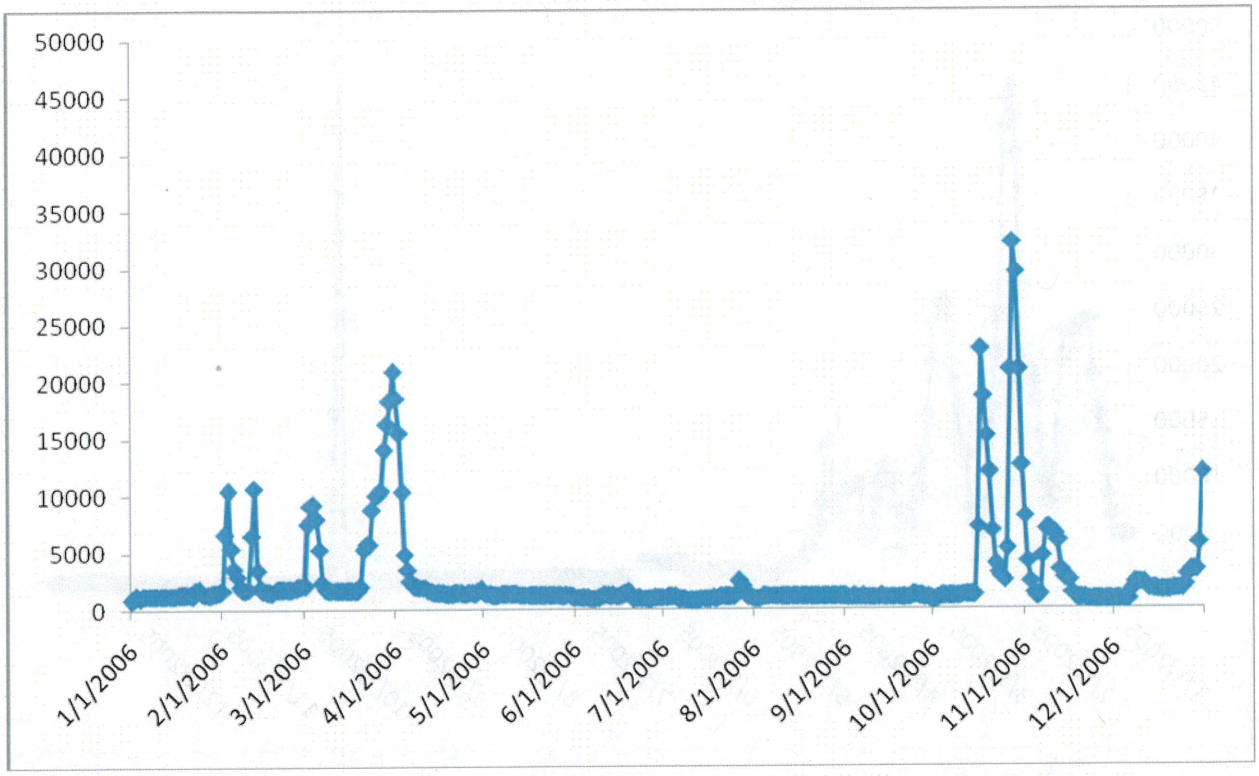
	2005
Total discharge for 12 months (cfs)*	2039564
Number of peaks greater than 7000 cfs	6
Number of peaks greater than 30000 cfs	2
Number of winter/spring peaks greater than 7000 cfs	5
Number of fall peaks greater than 7000 cfs	1
Number of continuous dry days (less than 7000 cfs)#	257

* Note: Short (3 days) period of elevated discharge in September 2005 a result of Hurricane Rita - not fresh water discharge per se; accounts for 5.15% of total discharge

\# Hurricane Rita days excluded from analysis

Figure 4.

Daily discharge of freshwater into Galveston Bay from January 01 to December 31 2006. Real-time flow data was downloaded from the USGS monitoring station located in the Trinity River at Romayor (08066500). Freshwater daily discharge is presented on the y-axis in units of cubic feet per sec.



	2006
Total discharge for 12 months (cfs)	959413
Number of peaks greater than 7000 cfs	7
Number of peaks greater than 30000 cfs	1
Number of winter/spring peaks greater than 7000 cfs	4
Number of fall peaks greater than 7000 cfs	3
Number of continuous dry days (less than 7000 cfs)	196

4.2 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 tecnhiques, the vast majority of species remain difficult to distinguish. Using HPLC, 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) Diatoms & Chrysophytes (light blue)
(ii) Cryptophytes (purple)
(iii) Dinoflagellates 1 +2 (yellow)
(iv) Euglenophytes + Chlorophytes (red)
(v) Cyanobacteria (relative to chlorophyll a concentration) (dark blue)

Six stations in Galveston Bay were sampled in 2005 and 2006 (Table 1). They were choosen to represent different kinds of habitats within the estuary, as well as the lesser bays of this ecosystem. Further, they were choosen to assess community composition along a salinity gradient from potentially freshwater in the Trinity River basin (station 6) to marine in Station closest to the Gulf of Mexico (station 1). The location of the six stations is shown below in Figure 5.

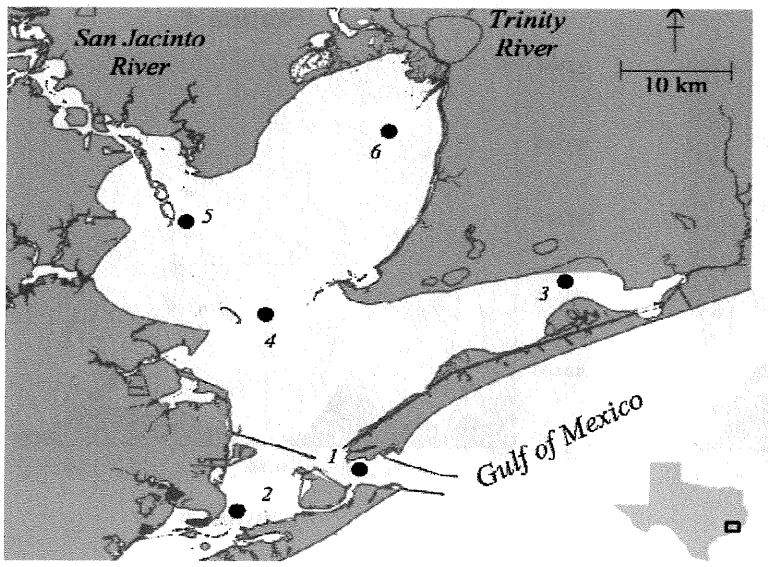
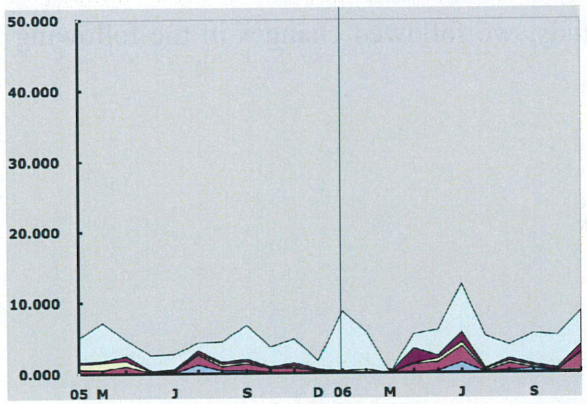


Figure 5.

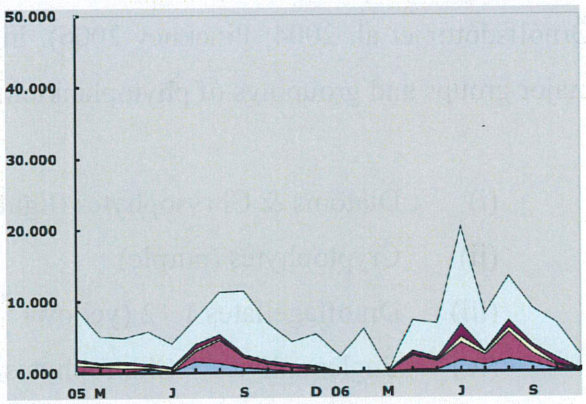
Locations of the six fixed sampling stations in Galveston Bay and its lesser bays: West Bay and East Bay.

Figure 6.

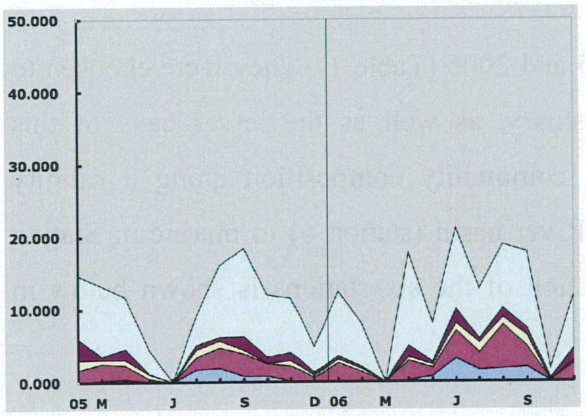
Phytoplankton community structure in 2005 and 2006 measured using HPLC and deconvoluted using CHEMTAX. Phytoplankton divisions were grouped in some cases to simplify the information presented. In these cases, individual groups represented less than 5% of the total community. In each case, the concentration is in units of ug L^{-1}.



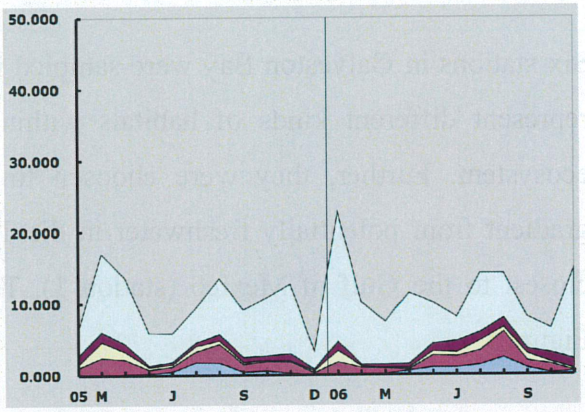
Station 1



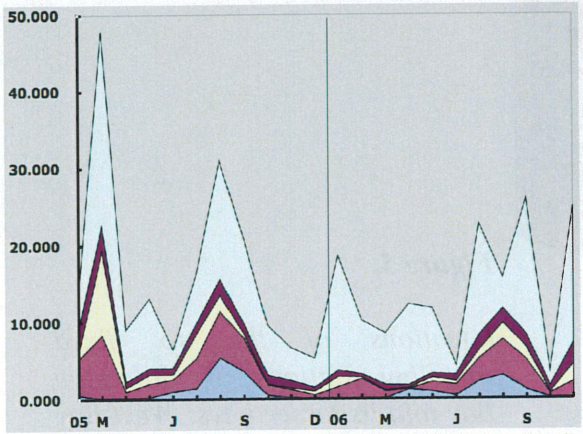
Station 2



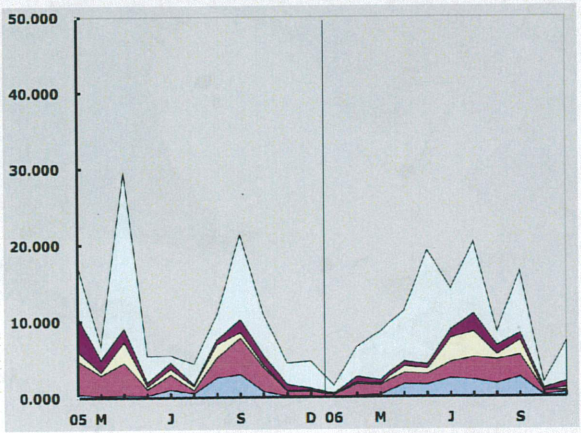
Station 3



Station 4



Station 5



Station 6

Phytoplankton community composition was station and time dependent throughout Galveston Bay as can be seen in Figure 6 above. In general terms, the Galveston Bay estuary in 2005 and 2006 was dominated by Diatoms & Chrysophytes (light blue) at all stations and all times of the year. Next in terms of contribution to the overall biomass was Euglenophytes + Chlorophytes (red). Cyanobacteria (dark blue) were more pronouced in the warmer and summer months and less abundant in the cooler and winter months, although they did appear for an extended period (into the fall) in 2006 (Figure 6). Interestingly, the Cryptophytes (purple) had the same relative abundance year round; this group is poorly studied and so it is not yet know what their normal patterns may be in estuarine systems such as Galveston Bay.

Our data indicates that the least amount of phytoplankton (in terms of biomass) are present in the southern section of Galveston Bay, adjacent to the Gulf of Mexico, with increasing amounts moving north towards the San Jacinto (Station 5) and Trinity (Station 6) rivers in both 2005 and 2006 (Figure 6). Previous studies have seen a similar trend (e.g., Davis et al. 2007; Quigg et al. 2007) and related the findings to nutrient gradients. Freshwater inflows not only intoduce freshwater but also carry an important nutrient and sediment load. While nutrients stimulate phytoplankton growth, the sediment load reduces light availability in the water column. Hence, phytoplankton in the river basins have ample nutrients but low light. At the mouth of the bay, the opposite pattern is observed, that is, phytoplankton have ample light but the water is nutrient poor.

4.3 Phytoplankton enumeration and identification

Given a series of events during the course of this project which reduced our overall effective work time, we chose to examine phytoplankton samples collected only from stations 1, 4 and 6 thereby examining a gradient from the mouth of Galveston Bay to the Trinity River basin. Our findings have been summarized in a series of tables. In these tables, the presence of individual phytoplankton genera has been rated according to the following system:

- = absent	where:	- = 0%
R = rare		R < 10%
C = common		C ≥ 10% but ≤ 50%
A = abundant		A > 50%

Table 3. Frequency of each Genus during each month at Station 1.

		Station 1								
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept
DIATOMS	*Asterionellopsis*	C	-	-	-	-	-	-	-	-
	Chaetoceros	-	-	-	-	-	-	R	-	-
	Coscinodiscus	-	C	-	-	-	C	R	R	-
	Cylindrotheca	C	-	-	-	-	R	-	C	A
	Ditylum	-	-	-	-	-	-	-	-	-
	Grammatophora	R	-	-	C	-	-	-	-	-
	Guinardia	-	-	-	-	-	-	C	-	-
	Navicula	C	R	R	C	-	C	C	C	C
	Nitzschia	-	-	-	-	-	-	-	C	C
	Odontella	-	-	-	-	-	R	-	-	-
	Pleurosigma	R	-	-	-	R	C	R	C	R
	Pseudo-nitzschia	-	R	-	-	-	-	-	-	-
	Skeletonema	R	-	-	-	-	-	-	-	-
	Thalassionema	R	-	-	C	-	R	-	-	-
	Thalassiosira	C	C	C	C	A	C	R	R	-
DINOFLAGELLATES	*Akashiwo*	-	-	-	-	-	-	R	-	-
	Ceratium	-	-	-	-	-	R	R	-	-
	Dinophysis	-	-	-	-	-	R	-	-	-
	Noctiluca	R	-	-	-	-	-	-	-	-
	Prorocentrum	-	C	C	-	-	C	R	-	R
	Protoperidinium	R	-	-	-	-	-	-	-	-
UNKNOWNS	UK A Jan	R	-	-	-	-	-	-	-	-
	UK B Jan	R	-	-	-	-	-	-	-	-
	UK C Jan	R	-	-	-	-	-	-	-	-
	UK D Jan	R	-	-	-	-	-	-	-	-
	UK E Jan	R	-	-	-	-	-	-	-	-
	UK F Jan	R	-	-	-	-	-	-	-	-
	UK G Jan	R	-	-	-	-	-	-	-	-
	UK H Jan	R	-	-	-	-	-	-	-	-
	UK A Feb	-	R	-	-	-	-	-	-	-
	UK A Mar	-	-	C	-	-	-	-	-	-
	UK B Mar	-	-	C	-	-	-	-	-	-
	UK C Mar	-	-	R	-	-	-	-	-	-
	UK D Mar	-	-	R	-	-	-	-	-	-
	UK A Apr	-	-	-	R	-	-	-	-	-
	UK B Apr	-	-	-	R	-	-	-	-	-
	UK C Apr	-	-	-	-	-	-	-	-	-
	UK A May	-	-	-	-	C	-	-	-	-
	UK B May	-	-	-	-	-	-	-	-	-
	UK C May	-	-	-	-	-	-	-	-	-
	UK A Jun	-	-	-	-	-	R	-	-	-

***Table 4**. Frequency of each Genus during each month at Station 4.*

		Station 4								
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**
DIATOMS	*Asterionellopsis*	C	-	-	-	-	-	-	-	-
	Chaetoceros	-	-	-	-	-	-	-	-	-
	Coscinodiscus	R	-	-	-	-	R	-	R	R
	Cylindrotheca	R	R	-	-	C	R	-	C	R
	Ditylum	-	-	-	-	-	-	-	-	R
	Grammatophora	-	-	-	R	-	-	-	-	-
	Guinardia	-	-	-	-	-	-	-	-	-
	Navicula	C	C	-	R	-	C	A	A	C
	Nitzschia	-	-	-	-	-	-	-	R	C
	Odontella	-	-	-	-	-	-	-	-	R
	Pleurosigma	-	C	-	-	C	C	C	R	-
	Pseudo-nitzschia	-	-	-	-	-	-	-	-	-
	Skeletonema	-	-	-	-	-	-	-	-	-
	Thalassionema	-	-	-	A	C	C	-	C	-
	Thalassiosira	-	A	A	-	C	-	C	-	C
DINOFLAGELLATES	*Akashiwo*	-	-	-	-	-	-	-	-	-
	Ceratium	-	-	-	-	-	R	-	-	R
	Dinophysis	-	-	-	-	-	R	-	-	-
	Noctiluca	-	-	-	-	-	-	-	-	-
	Prorocentrum	R	-	A	-	-	R	-	-	R
	Protoperidinium	-	-	-	-	-	-	-	-	-
UNKNOWNS	UK A Jan	-	-	-	-	-	-	-	-	-
	UK B Jan	-	-	-	-	-	-	-	-	-
	UK C Jan	-	-	-	-	-	-	-	-	-
	UK D Jan	-	-	-	-	-	-	-	-	-
	UK E Jan	-	-	-	-	-	-	-	-	-
	UK F Jan	-	-	-	-	-	-	-	-	-
	UK G Jan	-	-	-	-	-	-	-	-	-
	UK H Jan	-	-	-	-	-	-	-	-	-
	UK A Feb	-	R	-	-	-	-	-	-	-
	UK A Mar	-	-	-	-	-	-	-	-	-
	UK B Mar	-	-	-	-	-	-	-	-	-
	UK C Mar	-	-	-	-	-	-	-	-	-
	UK D Mar	-	-	-	-	-	-	-	-	-
	UK A Apr	-	-	-	R	-	-	-	-	-
	UK B Apr	-	-	-	-	-	-	-	-	-
	UK C Apr	-	-	-	-	-	-	-	-	-
	UK A May	-	-	-	-	C	-	-	-	-
	UK B May	-	-	-	-	-	-	-	-	-
	UK C May	-	-	-	-	-	-	-	-	-
	UK A Jun	-	-	-	-	-	-	-	-	-

***Table 5**. Frequency of each Genus during each month at Station 6.*

		Station 6								
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**
DIATOMS	*Asterionellopsis*	ND	-	ND	-	-	-	-	-	-
	Chaetoceros	ND	-	ND	-	-	-	-	-	-
	Coscinodiscus	ND	-	ND	-	-	-	-	-	R
	Cylindrotheca	ND	-	ND	-	C	R	-	C	C
	Ditylum	ND	-	ND	-	-	-	-	-	-
	Grammatophora	ND	-	ND	-	-	-	-	-	-
	Guinardia	ND	-	ND	-	-	-	-	-	-
	Navicula	ND	-	ND	C	C	R	R	R	C
	Nitzschia	ND	-	ND	-	-	-	-	-	-
	Odontella	ND	-	ND	-	-	-	-	-	-
	Pleurosigma	ND	-	ND	R	R	C	-	A	C
	Pseudo-nitzschia	ND	-	ND	-	-	-	-	-	-
	Skeletonema	ND	-	ND	-	-	-	-	-	-
	Thalassionema	ND	-	ND	-	-	-	-	-	-
	Thalassiosira	ND	A	ND	C	R	C	R	-	-
DINOFLAGELLATES	*Akashiwo*	ND	-	ND	-	-	R	R	-	-
	Ceratium	ND	-	ND	-	-	-	-	-	-
	Dinophysis	ND	-	ND	-	-	-	-	-	-
	Noctiluca	ND	-	ND	-	C	C	A	-	-
	Prorocentrum	ND	-	ND	-	-	-	-	-	-
	Protoperidinium	ND	-	ND	-	R	-	-	-	-
UNKNOWNS	UK A Jan	ND	-	ND	-	-	-	-	-	-
	UK B Jan	ND	-	ND	-	-	-	-	-	-
	UK C Jan	ND	-	ND	-	-	-	-	-	-
	UK D Jan	ND	R	ND	-	-	-	-	-	-
	UK E Jan	ND	-	ND	-	-	-	-	-	-
	UK F Jan	ND	-	ND	-	-	-	-	-	-
	UK G Jan	ND	-	ND	-	-	-	-	-	-
	UK H Jan	ND	-	ND	-	-	-	-	-	-
	UK A Feb	ND	-	ND	-	-	-	-	-	-
	UK A Mar	ND	-	ND	-	-	-	-	-	-
	UK B Mar	ND	-	ND	-	-	-	-	-	-
	UK C Mar	ND	-	ND	-	-	-	-	-	-
	UK D Mar	ND	-	ND	-	-	-	-	-	-
	UK A Apr	ND	-	ND	C	-	-	-	-	-
	UK B Apr	ND	-	ND	C	-	-	-	-	-
	UK C Apr	ND	-	ND	C	-	-	-	-	-
	UK A May	ND	-	ND	-	-	-	-	-	-
	UK B May	ND	-	ND	-	R	-	-	-	-
	UK C May	ND	-	ND	-	R	-	-	-	-
	UK A Jun	ND	-	ND	-	-	-	-	-	-

The color coding in the tables matches that in Figure 6 with one exception. All species highlighted in orange were classified as "unknown", that is, we have not been able to identify them down to genus level. In many cases, these phytoplankton appear as coccoid shaped cells, present as individuals or in chains. As can be seen in Tables 3 to 5, these unknowns were present at all stations in Galveston Bay. We have created a digital library of all species labeled as "unknown" in hopes of identifying these cells in the future.

Consistent with the HPLC analysis (Figure 6), Diatoms and dinoflagellates made up the dominate group of phytoplankton identified at all stations (Tables 3 to 5). Many of the species identified are common euryhaline phytoplankton occurring in estuaries all over the world. Harmful algal blooms are associated with several of the species detected, these include: *Pseudo-nitzschia* and *Nitzschia.* Dinoflagellates are relatively large and have distinct plates (theca) making them relatively easy to identify – they are also known to produce harmful algal blooms in Texas bays and estuaries. Of those found, *Akashiwo, Dinophysis, Prorocentrum* and *Protoperidinium* have been implicated in fish kills and shellfish hatchery closures. We did not detect any *Karenia brevi* nor *Pfiesteria* sp. in our microscopic examination – blooms of these two species in Galveston Bay would severely disrupt the Galveston Bay ecosystem.

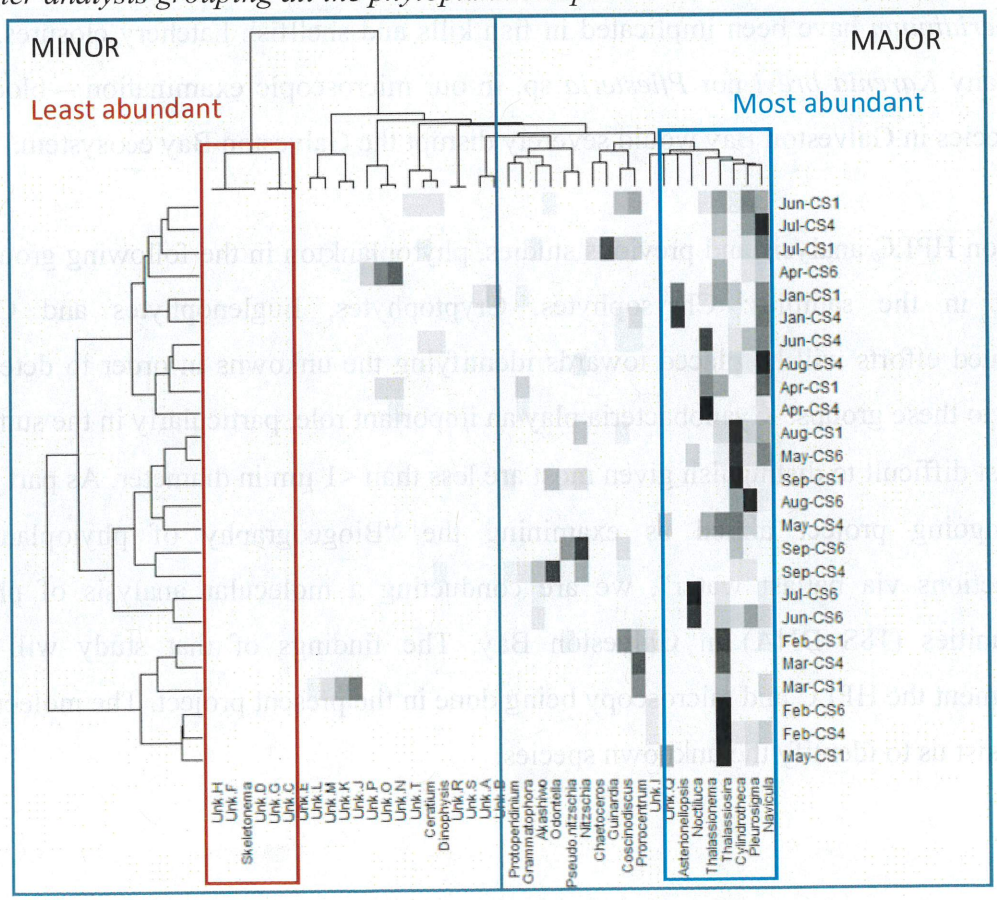
Based on HPLC analysis and previous studies, phytoplankton in the following groups were also present in the samples: Chrysophytes, Cryptophytes, Euglenophytes and Chlorophytes. Continued efforts will be placed towards identifying the unkowns in order to determine if any belong to these groups. Cyanobacteria play an important role, particularly in the summer, but are the most difficult to distinguish given most are less than <1 μm in diameter. As part of a separate and ongoing project aimed as examining the "Biogeography of phytoplankton versus introductions via ballast water", we are conducting a molecular analysis of phytoplankton communities (18S DNA) in Galveston Bay. The findings of that study will be used to compliment the HPLC and microscopy being done in the present project. The molecular analyses may assist us to identify the unknown species.

4.4 Statistical analysis

In order to find patterns which we could associate with freshwater inflows and/or the stations in which the phytoplankton were collected, we performed a series of statistical tests using the 2005 phytoplankton presented in Tables 3 to 5 above. First, a two-way cluster analysis grouping phytoplankton genera (as percent abundance calculated by dividing the number of cells of that taxa by the total number of cells a a station) and stations simultaneously is presented in Figure 7 below. The intensity of the shaded boxes reflects abundance with darker colors being more abundant genera and the lighter being least abundant, or rare, as defined in Tables 3 to 5 above. The most abundant genera clustered together on the right side of the heat map (blue box) whilst the rarer genera clustered mostly to the left (red box) (Figure 7).

Figure 7

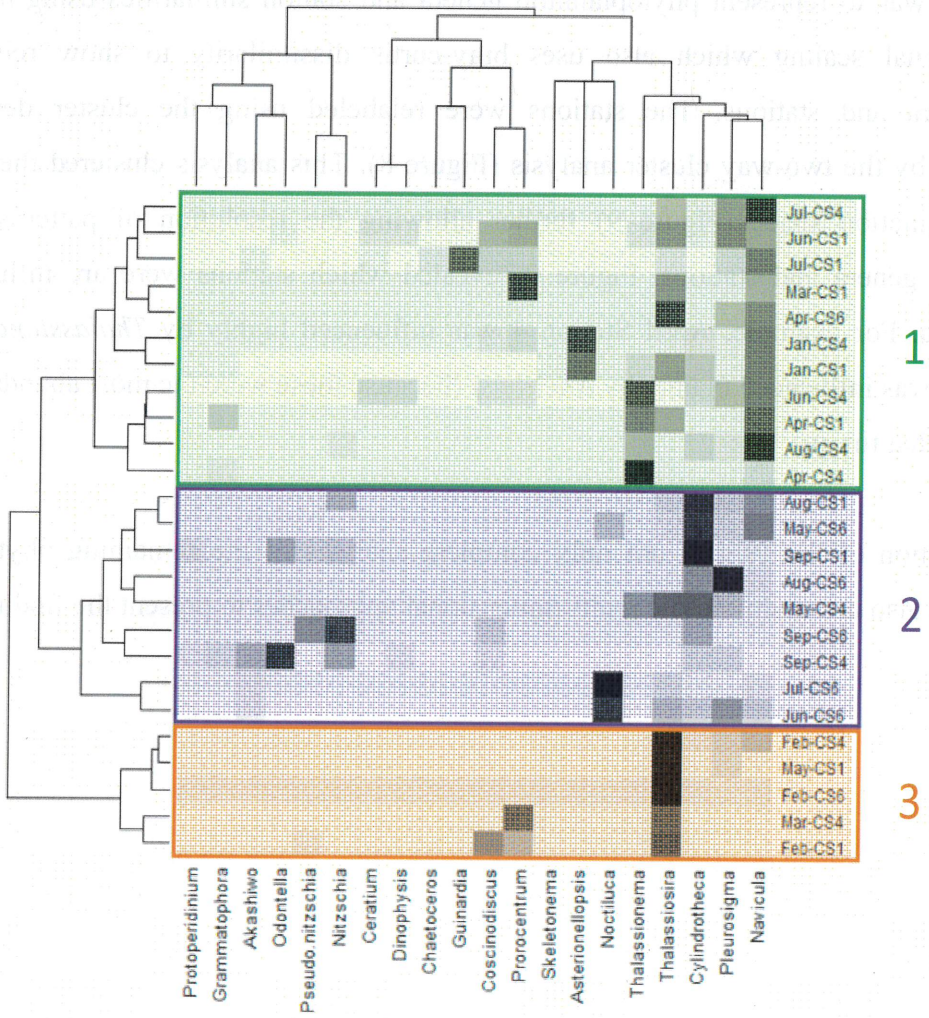
Two-way cluster analysis grouping all the phytoplankton species and stations simultaneously.



Since all the unkowns were relatively rare (Tables 3 to 5), they were not included in further analyses of the data in order to provide a more detailed picture of what was taking place with only the dominant taxa. In Figure 8, a two-way cluster analysis performed using the Ward method and Bray-Curtis similarity is presented. The data was grouped according to similarities between *dominant* genera and stations. As with Figure 7, in this heat diagram, the darker colors reflect the abundance of certain genera at certain stations. The boxes were added as a visual aid to focus the reader to the major clusters present: Cluster 1 – green, Cluster 2 – purple and Cluster 3 – orange.

Figure 8

Two-way cluster analysis grouping the dominant phytoplankton species only and stations simultaneously.



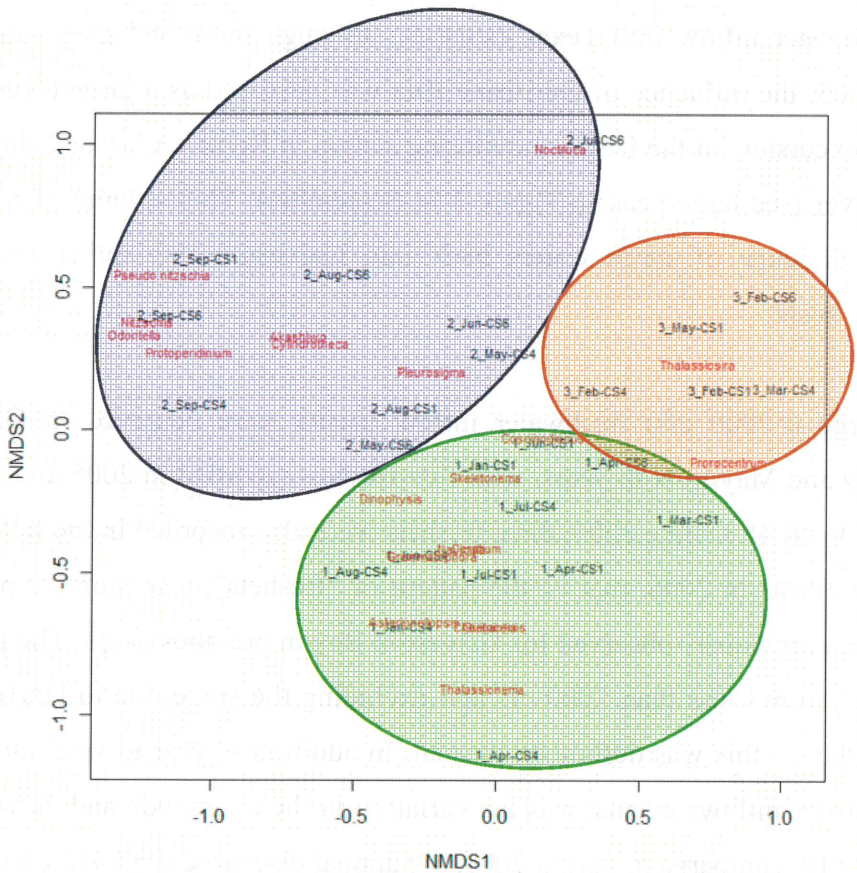
Cluster 1 in Figure 8 revealed that *Navicula*, *Thalassionema, Thalassiosira* and to a lesser extent *Pleurosigma,* were most abundant at Stations 1 and 4 (Bolivar Pass near mouth and Morgan's Point in the middle of Galveston Bay respectively) during the spring and summer months (March to August 2005). Clustering in box 2, *Cylindrotheca* and many of the other diatoms which were present mostly at Station 6 (Trinity River basin), but to a lesser extent in other parts of Galveston Bay. The diatom *Thalassiosira* dominated in Cluster 3 (orange box, Figure 8), appearing as the major player at all stations in February 2005. The diatom *Coscinodiscus* and the dinoflagellate *Prorocentrum* were also important members of the phytoplankton community at Stations 1 and 4 in February and March 2005 as shown in Figure 8. Hence, while all the diatom and dinoflagellate genera identified in Tables 3 to 5 were present at different times of the year in different parts of Galveston Bay, only a few species dominated the community at any given time or location.

The next step was to represent phytoplankton genera and station similarities using non-metric multi-dimensional scaling which also uses bray-curtis dissimilarity to show relationships between genera and stations. The stations were relabeled using the cluster designations determined in by the two-way cluster analysis (Figure 8). This analysis clustered the data into groups in ordination space (Figure 9) further allowing the resolution of patterns between phytoplankton genera and stations. Figure 9 revealed which stations were are influenced by specific genera. For example, April Station 4 was influenced highly by *Thalassionema* while July Station 6 was influenced mainly by *Noctiluca* (because these were the most abundant taxa at that station at that time).

It is the intention of the PI's to not only complete analysis of the remaining phytoplankton samples but to also use multi-variate multi-dimensional approaches to present the findings.

Figure 9

Non-metric multi-dimensional scaling using Bray-Curtis dissimilarity to show relationship between genera and stations. Stations were relabeled based on the two-way cluster analysis shown in Figure 8.



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.

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. This was certainly the case for follows during 2006 (Figure 4) but less so in 2005 (Figure 3). Periodic hurricanes and tropical storms in the summer have been known to greatly increase the freshwater inflow into Texas estuaries, although these and their impacts are not predictable. In 2005, the influence of Hurricane Rita was observed as a large (105,000 cfs) but brief (three day) excursion on the USGS monitoring station in Romayor, Texas which is situated on the Trinity River (see large peak in Figure 3 in September). This volume of water however may reflect the drainage of surge waters back into Galveston Bay rather than a genuine freshwater inflow event.

Frequent and large (>7000 cfs) freshwater inflow events were observed in Galveston Bay between February and May in both years, with five freshets recorded in 2005 and four recorded in 2006 (Figures 3 and 4). Unlike 2005, three freshets were also recorded in the fall of 2006. The pattern of inflow events in 2006, that is, the pattern of "freshets", was more "typical" in 2006 with what has been previously observed for Galveston Bay in previous years. The prolonged dry period (defined by flows less than 7000 cfs and excluding the spike due to Hurricane Rita) in 2005 lasted 257 days – this was definitely unusual. In addition to year to year variability in the number of freshwater inflows events, was the variation in the magnitude and duration of events in 2005 and 2006. By comparison, during 2006, the annual discharge (957,413 cfs) was less than half of that oberved in 2005 (2,039,564) and more recently in 2008 (Quigg et al. 2009a,b) making 2006 a relatively "dry" year.

5.2 Influence of Freshwater inflows on Phytoplankton Community Composition

Freshwater inflows carry with them important nutrient and sediment loads as well as freshwater which modifies estuarine salinity gradients. These three factors directly influence phytoplankton biomass, distrubition and on a macroscopic scale, community composition, as has been shown previously for Galveston Bay by Örnólfsdóttir et al. (2004), Pinckney (2006), Davis et al. (2007),

Quigg et al. (2007, 2009 a,b) and others. This is however the first effort in many decades to attempt to identify the specific phytoplankton, down to genera and species level, involved. The last such effort was summarized in a report published by the Texas Water Development Board in 1981 (TWDB, 1981). Unfortunately, the report only provides a synopsis of the major groups of phytoplankton present, much like the HPLC analysis presented herein (Figure 6). Consistent between all these reports and papers however was that the diatoms were the dominate group in Galveston Bay at all locations. Quigg et al. (2009 a,b) reported a seasonal ossicallation in 2008 with diatoms and dinoflagellates contributing to a larger fraction of the overall biomass in the cooler months, from fall to spring, while the cyanobacteria were more important in the summer months. Similar ossciliations were observed in 2005 and 2006, however, cyanobacteria were important from spring to summer in 2006 as seen in Figure 6. Örnólfsdóttir et al. (2004) and Pinckney (2006) also reported this pattern in these particular major groups; this finding has more to do with changing water temperatures than autochthonous factors.

Also consistent between reports was the distribution of phytoplankton biomass, with the least amount of phytoplankton present in the southern section of Galveston Bay (Station 1), adjacent to the Gulf of Mexico, and increasing amounts moving north towards the San Jacinto (Station 5) and Trinity (Station 6) river basins in both 2005 and 2006 (Figure 6). Previous studies have seen a similar trend (e.g., Davis et al. 2007; Quigg et al. 2007, 2009 a,b). The Trinity and San Jacinto Rivers are an important sources of nutrient and sediment loads to Galveston Bay, with freshwater inflows and returned flows being the two major sources (Brock 2001). Both dissolved and total particulate nutrient concentrations are highests in the river basins and decrease as one moves south towards the mouth of Galveston Bay (e.g., Davis et al. 2007; Quigg et al. 2007, 2009 a,b). The gradient depends, non-linearly, on the magnitude, duration and to a lesser degree, on the timing of the pulse event. For example, Quigg et al. (2009a) found that 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. Total particulate nutrient concentrations however, were greatest in the summer but did not vary significantly during the other seasons. What differs between years is the actual concentration of nutrients. The State of the Bay reports that nutrient concentrations in Galveston Bay continue to decline since the 1970's (Lester and Gonzalez, 2002) along with chlorophyll-a concentrations, which is used as proxy for phytoplankton

biomass. What remains unknown is which fraction, if any, of the phytoplankton community is most impacted. Changes in phytoplankton population dynamics at this level could have a significant effect on economically important and ecologically characteristic sport or commercial fish and shellfish species.

5.3 Influence of Freshwater inflows on Phytoplankton Genera

Microscopic examination of phytoplankton is important for determining which genera, and if possible, species are the major players in an estuarine system. Native populations of phytoplankton in Galveston Bay are impossible to define given the three large ports have occupied its northern (Port of Houston), mid (Port of Texas City) and southern (Port of Galveston) sectors for over 100 years. Phytoplankton have likely been "introduced" to Galveston Bay by ship's deballasting in these ports or simply as transients on vessels of all sizes utilizing the complex. Which of these species if any, is likely to be "invasive", is even more challenging to define, given that under different conditions any one species may or may not pose a threat to higher trophic levels. Hence, any sampling conducted at present will at best give the "current" community composition. This is nonetheless valuable as it provides for future studies by providing a relative baseline.

We found the examination of phytoplankton microscopically extremely challenging for many reasons, principally as there a very few classically trained taxonomist available for consultation, especially for marine species. Hence, we foscused on genera level rather than species levels unless it was obvious. Further, we focused on the larger (> 5μm) genera present. Unfortunately this meant we were not able to identify cyanobacteria which are typically less than 2 μm. This also resulted in a large number of unknowns (see Tables 3 to 5). In hopes of future resolution of these unknown species, we did however create a digital library.

Using a mutli-variate and mutli-dimensional statistical approach, we did find that specific genera of diatoms and dinoflagellates, the two major groups, occur in specific parts of Galveston Bay at specific times of the year (Figures 7 to 9). For example, the diatoms *Navicula*, *Thalassionema, Thalassiosira* and to a lesser extent *Pleurosigma,* were most abundant at Stations 1 and 4 (Bolivar Pass near mouth and Morgan's Point in the middle of Galveston Bay respectively)

during the spring and summer months (March to August 2005). *Cylindrotheca* and many of the other diatoms were present mostly at Station 6 (Trinity River basin), but to a lesser extent in other parts of Galveston Bay. These euryhaline phytoplankton are common to many bays and estuaries throughout the world and form the basis for many important trophic food webs, supporting zooplankton and fish populations.

Unfortunately, we also identified species which have been associated with harmful algal blooms. These include the diatoms *Pseudo-nitzschia* and *Nitzschia* and the dinoflagellates *Akashiwo, Dinophysis, Prorocentrum* and *Protoperidinium.* These phytoplankton species have been implicated in fish kills and shellfish hatchery closures around the world (Graneli and Turner 2006). We did not detect any *Karenia brevi* nor *Pfiesteria* sp. in our microscopic examination – blooms of these two species in Galveston Bay would severely disrupt the Galveston Bay ecosystem.

Within the constraints of the current study we were not able to determine a direct relationship between freshwater inflows and phytoplankton genera present in Galveston Bay. The next step is to select environmental parameters (temperature, salinity, dissolved oxygen, nutrients, etc…) reported in Davis et al. (2007) for 2005 and 2006 in each of the clusters and prepare histograms to examine differences plus the ranking of these and another 2-way cluster analysis. These types of analyses have not been done previously on this kind of data before (to the PI's knowledge) and require further investigation before final publication.

Given the importance to determining freshwater inflows that are adequate to maintain productivity of economically important and ecologically characteristic species in Galveston Bay, the findings presented herein will be essential for developing the next generation of predictive models relating freshwater inflow to bay health.

6. Conclusion

The objective of this program was to examine samples collected from the Galveston Bay Estuary as part of a monthly sampling program in 2005 to 2006, specifically examining plankton community composition (*Program Element 1*). To this effect we trained several research technicians (Rachel Windham and Tyra Booe), a postdoctoral research associate (Dr. Angela Capper) and graduate student (Samuel Dorado) in plankton identification and enumeration techniques. Although the aim was to examine samples collected in 2005 to 2006, we were only able to examine those from 2005 due to time constraints. For those samples examined we created a digital library of identified and "unknown" phytoplankton genera for future reference not only to the PI's but for anyone interested in examining phytoplankton communities in Galveston Bay.

The second program element (*Program Element 2)* was to examine the link between riverine inflows (and corresponding loading of materials) and spatial patterns in phytoplankton community structure by analyzing the data collected in 2005 and 2006. These data are included as tabular and graphical products. This was done for both the data processed using HPLC and that derived from microscopic analyses. In comparing findings from microscopic analysis to those performed using HPLC, both methods found that the diatoms and dinoflagellates were the two major plankton divisions in Galveston Bay over the sampling period. However, the members of the other divisions could not be reliably identified using microscopic methods. We were able to link temporal patterns in inflows to spatial patterns in estuary-wide diatom and dinoflagellate community structure through multivariate statistical analyses.

This report (*Program Element 3)* summarizes the accumulation of our efforts and includes data on plankton community structure as graphical and tabular analyses. This information is available to the GBEP, GBC and GBC subcommittees in written and electronic format (as requested). The findings presented in this report will also be presented to the GBEP, GBC and GBC subcommittees (as requested).

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