FINAL REPORT

Towards development of a nutrient budget for the Trinity-San Jacinto Estuary

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

To:

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

Interagency Cooperative Contract TWDB Contract No. 0904830894 and TWDB Contract No. 1004831016



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

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Acknowledgements

This report is the result of research partially funded by a grant (TWDB Contract No. 0904830894 and 1004831016) from the Texas Water Development Board (TWDB) to Antonietta Quigg (Principal Investigator). The views expressed herein are those of the author and do not necessarily reflect the views of TWDB. Many people contributed to the successful completion of this project. From Texas A&M University at Galveston, Tyra Booe, Jamie Steichen, Rachel Windham, Kim Janusaitis, Allison McInnes, and Sam Dorado participated in all aspects of field work and processing samples in the laboratory. From Texas A&M University, Kung-Jen Liu (Calvin) helped with nutrient analysis and from the TWDB, Carla Guthrie, Mikhail Umorin and Ruben Solis helped with all aspects of administering this grant. Also from TWDB, Caimee Schoenbaechler and Robert Burgess, are thanked for their contribution in proving comments on the draft report.

List of Abbreviations and Acronyms

DIN	dissolved inorganic nitrogen
DOM	dissolved organic matter
CCC	Coastal Coordination Council
cfs	cubic feet per sec
chl	chlorophyll
СМР	Coastal Management Program
GBEP	Galveston Bay Estuary Program
NOAA	National Oceanic and Atmospheric Administration
PAR	Photosynthetically Active Radiation
РНҮТО-РАМ	Phytoplankton Pulse-Amplitude Modulated Fluorometer
<i>rel</i> ETR	relative electron transport rate (μ mol electrons m ⁻² s ⁻¹)
RLA	Resource Limitation Assay
TAMUG	Texas A&M University at Galveston
TGLO	Texas General Land Office
TN	Total particulate nitrogen
TP	Total particulate phosphorus
TSS	Total Suspended Solids
TWDB	Texas Water Development Board
USGS	United States Geological Service

1. Abstract

The Galveston Bay 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 Trinity-San Jacinto Estuary. The major gap in the present knowledge remains a clear understanding of the downstream ecological impacts of changes to freshwater inflows and modes of nutrient loading on estuaries. Herein, water quality and phytoplankton responses were monitored in response to freshwater inflows in Trinity-San Jacinto Estuary. The project spanned a range of inflow conditions into the Trinity-San Jacinto Estuary between January and December 2009. Spatial maps generated from monthly sampling campaigns with a Dataflow unit provided a clear depiction of inflow effects on water quality in the system. Noticeable differences in the northern section (upper Bay) versus the southern section (lower Bay) of Trinity-San Jacinto Estuary in terms of water quality, nutrient load (dissolved and particulate), primary productivity and community composition were measured. In most instances, these could be related to freshwater inflow effects – from both the San Jacinto and Trinity Rivers. The magnitude and duration of flow events had observable downstream effects. The findings of resource limitation assays in this study indicate that phytoplankton communities were frequently co-limited by N (as nitrate) and P (as orthophosphate) for much of the year. In terms of developing a nutrient budget for the Trinity-San Jacinto Estuary, this study has revealed that while water column nutrient fluxes are critical to observed changes in phytoplankton (biomass, productivity, distributions); they do not provide a complete understanding of the interactions. Future studies should consider the role of nutrients in sediments, and sediment-water interactions. Given the shallow nature of the Bay and the importance of wind mixing, an understanding of processes taking place at the sediment-water boundary will be needed to full develop a nutrient budget.

Towards development of a nutrient budget for the Trinity-San Jacinto Estuary

2. Introduction

Freshwater inflows (FWI) from rivers, streams, and local runoff maintain salinity and nutrient gradients required to sustain an "ecologically sound and healthy estuary". FWI is 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 "ecologically important and characteristic sport or commercial fish and shellfish species and estuarine life upon which such fish and shellfish are dependent". The Trinity-San Jacinto Estuary also referred to as the Galveston Bay Estuary (Fig. 1), is in the largest watershed (24,000 square miles) on the Texas coast (TWDB, 2007). It is one of 22 ecosystems that are part of the National Estuary Program (see details at www.gbep.state.tx.us) because it faces serious conservation challenges. Suburban and industrial development are reducing critical wetland habitat at a faster rate than anywhere else along the Texas coast (<u>www.gbep.state.tx.us</u>). 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 and expansion of the Houston Ship channel have altered circulation patterns along with the construction of the Texas City Dike (GBEP 2001). Exotic species like Chinese tallow, giant salvinia, water hyacinth and grass carp threaten native flora and fauna. In an investigation of fish kills occurring along the Texas coast from 1951 to 2006, Thronson and Quigg (2008) found that Trinity-San Jacinto Estuary and Matagorda Bay (located further south on the Texas coast) had the highest number of fish kill events and total number of fish killed during the 50 year period of study.

The Galveston Bay Estuary Program (GBEP) 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 economically important and ecologically characteristic sport or commercial fish and shellfish species and estuarine life upon which such fish and shellfish are dependent." Hence a clear understanding of the downstream ecological impacts of changes in freshwater inflows on estuaries remains a priority for resource managers and scientists alike.

To complicate matters, Texas coastal municipalities (TWDB 2001; 2007) are undergoing rapid population growth, leaving water regulators and managers faced with the additional 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-Houston (Fig. 1) area is likely to see the largest population growth along the Texas coast, with a doubling predicted within the next 20 years (TWDB 2007). Hence, there is a need to understand how the present Trinity-San Jacinto Estuary responds to FWI in order to predict the consequences of human development on the Bay ecosystem health and its ability to sustain local fisheries and to be able to mitigate potential negative impacts of future population growth.



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

2.1 Role of nutrients

Nutrients, in the appropriate quantities, contribute positively to water quality and ecosystem function (Longley, 1994; Nixon 1995). However, if present in excessive amounts, can lead to the development of harmful algal blooms and other deleterious impacts on ecosystems health and services (Quigg et al. 2009c) including but not limited to algal blooms and fish kills (Thronson and Quigg, 2008; McInnes and Quigg, 2010). Excessive nitrogen loading to rivers and estuaries is cited as the principal causal factor of the rise and spread of eutrophication world wide (Diaz and Rosenberg, 2008). The "dead zone" which appears each summer along the Louisiana coast has long been attributed to loading of the Mississippi River upstream by the application of fertilizer to crops by farmers in the mid-west (references in Diaz and Rosenberg, 2008).

Guillen (1999) published a report indicating that primary production in Trinity-San Jacinto Estuary was phosphorus (P) limited while Örnólfsdóttir et al. (2004) reported that it was nitrogen (N as nitrate) limited. Quigg et al. (2009a) and Quigg (2009) recently reported that the response of phytoplankton communities to nutrient loading varies both with location and season in Trinity-San Jacinto Estuary. These authors found evidence of both N and P limitation, and also or co-limitation by both N and P. While Örnólfsdóttir et al. (2004) also examined nutrient limitation on spatial (transect from Trinity River into the middle of the Trinity-San Jacinto Estuary) and temporal (year long study) scales and found that N was the nutrient limiting growth of phytoplankton; these authors did not consider the San Jacinto River basin, nor the entrance to Trinity-San Jacinto Estuary at the southern most point which connects with the Gulf of Mexico (Bolivar Point).

Previous studies in Galveston bay have found phytoplankton production to be dominated by: cyanobacteria, green algae and diatoms (references in Örnólfsdóttir et al., 2004). While Örnólfsdóttir et al. (2004), Quigg et al. (2009a) and Quigg (2009) found that diatoms were the taxa that most often responded to the addition of N sources in their assays, Quigg et al. (2009a) and Quigg (2009) also observed that when populations were co-limited by N and P, cryptophytes, haptophytes, prymnesiophytes also responded significantly. The resulting shift

in phytoplankton community composition towards these taxa may not be of concerns because they are not typically associated with significant harmful algal blooms in the Bay. Nonetheless, there are a number of noxious species which reside in Texas estuaries, particularly species of *Nitzschia* and *Pseudonitzschia* (Quigg et al. 2009b), which have been associated with shellfish poisoning from eating mussels and oysters contaminated with domoic acid.

Buyukates and Roelke (2005) found that plankton assemblages receiving nutrient loads in a pulsed mode lead to 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. Hence, shifts in phytoplankton composition may change the nutritional value of phytoplankton communities to consumers, ranging from zooplankton, oysters and fish at higher trophic levels. This impact is less well studied but available literature indicates that it may be a cause for concern.

2.2 Objectives

This study builds on earlier Texas Water Development Board (TWDB) and Texas General Land Office – Coastal Management Programs (TGLO-CMP) funding (Quigg et al. 2007, 2009a, b, Quigg 2009); the latter which is managed by the Texas Coastal Coordination Council (CCC). It was conducted concurrently with new TGLO-CMP and Texas Sea Grant support. *The specific objective was to continue research aimed at developing a nutrient budget for the Trinity-San Jacinto Estuary*. Monthly water quality sampling along a tight spatial transect was performed from January to December 2009. Nutrients (dissolved and particulate), chlorophyll (proxy for phytoplankton biomass) and water quality parameters were examined at six fixed stations in the Trinity-San Jacinto Estuary as part of the monthly surveys. Resource limitation assays were performed at a station in the northern and southern section (RLA North and RLA South respectively) of the Bay; Fig. 2) to examine the role of nutrients have an effect on phytoplankton biomass, productivity and community composition. Only the major phytoplankton players (diatoms, dinoflagellates and

cyanobacteria) were considered. The data collected as part of this and other ongoing studies will be invaluable for developing the next generation of predictive models relating FWI to Bay health.

Specific objectives:

- (i) High spatial and temporal resolution mapping of Trinity-San Jacinto Estuary,
- Measure nutrients and total suspended solids in the Trinity River and Trinity-San Jacinto Estuary, and
- (iii) Determine influence of nutrient load on the phytoplankton in the Trinity-San Jacinto Estuary.

3. Methods

3.1 Water Quality

Real-time flow data (cubic feet per sec) from a USGS monitoring station (Trinity River at Romayor; USGS gauge 08066500) was used determine the freshwater inflow into Trinity-San Jacinto Estuary from January to December 2009.

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, Trinity-San Jacinto Estuary (Fig. 2). This integrated instrument system concurrently measured water temperature, conductivity, salinity, water clarity (beam transmittance), chlorophyll (chl) *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. Water quality surveys took two successive days following the transect lines (grey lines) shown in the map Fig. 2. GPS and Dataflow information was used to create highly detailed contour maps of water quality parameters in relation to physiographic features using Surfer Version 8.0

(http://www.goldensoftware.com/products/surfer/surfer.shtml). The default (kringing method) protocol was used to prepare the maps.



Fig. 2 Trinity-San Jacinto Estuary 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, chlorophyll and total suspended solids. Blue circles show locations of stations where resource limitation assays were conducted.

Station	Latitude	Longitude	Site description
1	29°21.51'	94°46.12'	Bolivar Pass
2	29°18.55'	94°52.88'	Adjacent to West Bay
3	29°32.90'	94°34.72'	East Bay
4	29°33.13'	94°48.27'	Middle of Galveston Bay
5	29°36.56'	94°55.88'	Trinity River basin
6	29°41.77'	94°51.16'	San Jacinto River basin
RLA North	29°37.01'	94°49.66'	
RLA South	29°25.75'	94°50.68'	

Table 1: Latitude and longitude of fixed sampling stations in Trinity-San Jacinto Estuary.

At six fixed stations (Table 1), water profiles will be measured with a Hydrolab: temperature, salinity, dissolved oxygen and pH will be recorded. Salinity (throughout the report) will be reported using the Practical Salinity Scale according to UNESCO (1981). The Practical Salinity Scale salinity is defined as a pure ratio, and has no dimensions or units. Further, it will not have any numerical symbol to indicate parts per thousand. Salinity will thus be reported as a number with no symbol or indicator of proportion after it. In particular, it is not correct to add the letters PSU, implying Practical Salinity Units, after the number.

In addition, dissolved nutrient (nitrate, nitrite, ammonia, and phosphate), total particulate nitrogen (TN) and phosphorus (TP), chlorophyll *a* (chl *a*), and total suspended solids (TSS) data will be collected from surface waters at the same time. Water from each of these stations was filtered (GF/F; Whatman) onto filters under low vacuum pressure (< 130 kPa). Filters were folded and frozen at -20°C for later chl *a* and phaeophytin *a* (phae *a*) analysis. Calibration and measurement techniques were performed according to Arar and Collins (1997) with some modifications described in Quigg et al. (2007, 2009).

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. Total nutrients were measured on unfiltered samples. Samples for nutrient analysis were frozen immediately until analysis was performed using analytical auto-analyzer according to Hansen and Koroleff (1999). The ratio of inorganic nitrogen (DIN) to phosphate ($P = PO_4$ -P) nutrients was calculated after summing the nitrogen inputs ($DIN = NO_3$ -N + NO_2 -N + NH_4 -N).

For measurement of total suspended solids, filters were precombusted (500°C for 5 hrs) and preweighed. After filtration of a known volume of water, filters were dried in an oven at 60 °C for no less than 48 hrs and then reweighed.

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 – August) and fall (September– November).

3.2 Nutrients and total suspended solids in the Trinity River.

A nutrient autosampler located in Romayor Texas adjacent to the USGS gauge station was established to collect water samples for nutrient (nitrate, nitrite, ammonia, phosphate, TN and TP) and TSS analysis once every two days for 12 months. During the entire period of this study, the autosampler essentially did not perform as described by the manufacturer. Despite numerous attempts to repair and collect samples from this system, it was never possible to collect any meaningful water samples from the autosampler. As a surrogate, discrete water samples were collected during visits to work on the autosampler as well as additional monthly visits to ensure a timeline of data. These were analyzed as described above.

3.3 Resource Limitation Assays

Resource limitation assays (RLA) were undertaken to identify which resource (nutrient(s) and/or light) limited phytoplankton growth at two sampling sites in Trinity-San Jacinto Estuary during the study period. Figure 2 shows the location of RLA North and RLA South; the exact latitude and longitude of these two collection sites are given in Table 1. Bioassays were carried out essentially as described by Fisher et al. (1999) with modifications as described in Quigg et al. (2007, 2009).

Essentially, surface (0 - 0.5 m) water was collected in 20 L acid washed carboys for the treatments (total thirty carboys) and an "initial control". Immediately upon returning to the laboratory, water from the control was used to measure initial water quality and phytoplankton characteristics of the sample using a Phytoplankton Pulse Amplitude Modulated (PHYTO-PAM) fluorometer (see below). Specifically, we sampled for "initial nutrient concentrations" using the method described in Section 3.1 and "initial phytoplankton community composition" using the method described in Section 3.4. These samples provide

information on the starting conditions in the assays. Triplicate carboys were then randomly selected for one of six treatments:

- (i) a control (no addition),
- (ii) $+ N (30 \mu mol L^{-1} NO_3),$
- (iii) + P (2 μ mol L⁻¹ PO₄³⁻),
- (iv) + NP (30 μ mol L⁻¹ NO₃⁻, 2 μ mol L⁻¹ PO₄³⁻)
- (v) "grazing" or G.

The nutrient concentrations above are the final concentrations of each nutrient in each treatment. For the grazing experiment, no nutrients were added (as done for the control) but the water was pre-filtered with a 330 µm filter before filling each carboy. Treatments were incubated at ambient water temperatures, turbulence and under 50% ambient sunlight in an outdoor facility. 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 for changes in phytoplankton productivity, biomass, and community composition with a PHYTO-PAM. Initially it was proposed to measure nutrient concentrations (nitrate, nitrite, ammonia, urea, phosphate, and silicate, TN and TP) at the beginning and at the end of the experiment; but given that it was found that no significant change occurred during this period, this additional step was dropped (that is, the measurement of nutrients at the end of the experiment was not performed). Carboys were collected and processed as quickly as possible either in the laboratory or outdoors in a low light (shaded) environment.

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. Given that the "initial" biomass was measured at the start of the experiment and the "maximum" biomass was that measured at the end of the experiment (one week later), PRI reflects the change in biomass over the duration of the RLA. 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.

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. 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 (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 with increasing photosynthetically active radiation (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 $0.1 \ \mu g$ 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.

As with traditional Turner type fluorometers, the PHYTO-PAM is calibrated with a chlorophyll a standard so that it can then be used to estimate the biomass of the phytoplankton community (see Arar and Collins 1997).

	Data	flow	Reso Limitatio	ource on Assavs	River Sampler
	North	South	North	South	Sumpton
J	*	*	*	*	Y
F	#	#	Y	Y	Y
Μ	Y	Y	Y	Y	Y
Α	Y	#	ns	ns	Ν
Μ	Y	Y	Y	Y	Y
J	Y	Y	Y	Y	Y
J	Y	Y	ns	ns	Y
Α	Y	Y	ns	ns	Y
S	Y	#	Y	Y	Y
0	Y	Y	ns	ns	Y
Ν	Y	Y	Y	Y	Y
D	Y	#	Y	Y	Y

 Table 2: Summary of sampling 2009 schedule:

* boat in shop for repairs; # bad weather; ns is not scheduled

4. **Results**

4.1 Freshwater Inflow into Trinity-San Jacinto Estuary during 2009

Real-time freshwater inflow measured as daily discharge to Trinity-San Jacinto Estuary from January 01 to December 31 2009 was downloaded from the USGS monitoring gauge located on the Trinity River at Romayor (08066500). Given in this study we were primarily interested in understanding the response of the Bay to major river flows from the Trinity River, we did not include estimates to total freshwater inflow to the estuary, that is, we did not include ungauged flows, and flows from the San Jacinto River.

Of the water discharged in 2009 from the Trinity River (Fig. 3), most flow occurred in the Fall from early October to late December. As can be seen in Fig. 3, three significant freshwater inflow events (>10,000 cubic feet per sec; cfs) or freshets also occurred in 2009 during the spring: first during a four day period in March (total of 51,300 cfs), a second during a seven day period in April (total of 142,900 cfs) and the third, lasting almost two

weeks early in May (total of 265,700 cfs). In 2009, there were significantly freshwater inflow events during the spring and fall months. Relative to the previous year, FWI in 2009 involved more discharge events across the year, and events of greater magnitude (Quigg 2009).



Fig. 3 Daily discharge of freshwater into Trinity-San Jacinto Estuary from January 01 to December 31 2009. 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.

Given TWDB reports inflows and water volumes in acre-feet, we used the unit conversion 1.983471 Acre-Feet = 1 cfs for 24 hours (Qingguang Lu; hydrologist TWDB) or 723.97 Acre-Feet = 1 cfs for 1 Year to report flows in terms of annual discharge. Compared to previous years in the last decade (Table 3), average discharges in 2009 were on par to those measured in typical "wet" years. While 2001 and 2007 are the wettest years in the last decade, with around 10,635,120 acre-feet (af) each year, 2000 and 2006 are the driest years with only an average of about 1,732,100 acre-feet (Table 3).

Period of record	Annual Discharge	Annual Discharge	Wet or Dry
	(cfs × 1000)	(af)	
2000	2,957	2,140,779	dry
2001	14,900	10,787,153	wet
2002	8,193	5,931,486	wet
2003	9,113	6,597,539	wet
2004	9,757	7,063,775	wet
2005	8,858	6,412,926	wet
2006	1,828	1,323,417	dry
2007	14,480	10,483,086	wet
2008	6,214	4,498,750	wet
2009	8,182	5,923,523	wet

Table 3 Annual discharge (cfs and af) measured at the USGS monitoring gauge located on the Trinity River at Romayor (08066500) from 2000 to 2009.

4.2 Nutrients and total suspended solids in the Trinity River

The Trinity River is an important source of nutrients and TSS to the Trinity River basin and potentially, the Trinity-San Jacinto Estuary. Monthly nutrient and TSS concentrations were measured in water collected from the Trinity River, taken from a site adjacent to the USGS monitoring gauge (08066500) located at Romayor. The data is summarized in Table 4 along with monthly the discharge values. TSS values ranged from 22 mg/L during periods of low flow (summer) to 76 mg/L in the spring and fall (high flow); a significant correlation was not observed ($r^2 = 0.2$; p > 0.05). Given this analysis was based on 12 monthly samples of TSS, the sample size was too low for presentation of results as being statistically significant.

While [NO3+NO2] concentrations were low for the first 9 months of the year (0.33 - 8.22 μ M from January to September), they were significantly higher during the last 3 months of 2009 - 27.7 μ M (± 1.5) (Table 4; p > 0.05). Highest discharge was also recorded during these last 3 months (Fig. 3; Table 4). On the other hand, ammonium and phosphate concentrations varied 4-5 fold during the course of 2009 ($0.96 - 4.39 \mu$ M and $0.39 - 1.81 \mu$ M respectively) and showed no clear relationship to discharge (Table 4; p < 0.05). Hence, any

changes in DIN concentrations and DIN:P ratios were associated with changes in [NO3+NO2] rather than in NH4 concentrations.

	Monthly	Salinity	TSS	[NO3+NO2]	[NH4]	[PO4]	DIN	DIN:P	Potentially
	discharge								limiting
	(cfs)	ppt	mg/L	uM	uM	uM	uM		nutirent
J	41515	1	53	2.89	2.09	0.39	4.98	12.77	Р
F	37186	1	26	0.38	1.67	0.63	2.05	4.10	Ν
М	127295	1	76	8.22	3.69	0.63	11.91	18.90	Р
Α	234280	n.d	n.d	n.d	n.d			n.d	-
М	365400	3	49	n.d	n.d			n.d	-
J	110070	0	40	0.39	1.37	0.77	1.76	2.29	Ν
J	34673	2	22	0.35	4.09	0.66	4.44	6.73	Ν
Α	55710	0	27	0.57	4.39	1.40	4.96	3.54	Ν
S	153840	0	34	0.33	1.64	0.69	1.97	2.86	Ν
0	602940	1	33	29.67	0.96	1.06	30.63	28.90	Р
Ν	888800	0	72	27.63	1.59	1.81	29.22	16.14	Р
D	342870	0	28	26.02	2.36	0.63	28.38	45.05	Р

Table 4 Monthly summed discharge (cfs) measured at the USGS monitoring gauge located on the Trinity River at Romayor (08066500) in 2009. Monthly TSS and nutrient concentrations measured at the same location on the Trinity River.

In general, a DIN: P ratio in the range of 7:1 to 12:1 by mass is associated with plant growth being limited by neither phosphorus nor nitrogen. If the DIN:P ratio is greater than 12:1, phosphorus tends to be limiting, and if the DIN:P ratio is less than 7:1, nitrogen tends to be limiting (Wetzel, 2001). Based on this relationship, the DIN:P ratios for Trinity River water was calculated (Table 4). For most of the summer (July – September), DIN:P ratios were significantly less than 7.1 indicating the potential for N limitation of phytoplankton growth while in the fall (October – December), DIN:P ratios were significantly greater than 12.1 suggesting the system had switched to P-limitation. The high concentrations of [NO3+NO2] during this same period would allow rapid growth of phytoplankton which would then be curtailed due to insufficient concentrations of phosphorus. In the spring, DIN:P concentrations predict that phytoplankton populations would oscillate between N and P limitation (Table 4).

4.3 Temporal and spatial distributions of water quality parameters in Trinity-San Jacinto Estuary

The physio-chemical parameters mapped in Trinity-San Jacinto Estuary include water temperature, conductivity, salinity, water clarity, chl *a*, and dissolved organic matter (DOM). After sensor calibration and blank correction, data was imported into Surfer, a 3D contouring and surface plotting program. Spatial characteristics of temperature, conductivity, chl *a* and DOM for August and November 2009 are shown in Fig. 4 below. These months were chosen as they represent "dry" and "wet" periods respectively (all months sampled are included in Appendix A). There is no data available for January and February in 2009 due to boat repairs and poor weather (high winds) respectively preventing sampling (see Table 2). Due to incremental weather in November, the transect was modified so as to complete sampling earlier.

During August, water temperatures averaged 30°C ±1°C (Fig. 4A). By November 2009, temperatures had fallen significantly to $18^{\circ}C \pm 2^{\circ}C$. These temperature ranges are typical for this ecosystem (Davis et al. 2007; Quigg et al. 2007; 2009). While conductivities (and salinities) were significantly higher across Trinity-San Jacinto Estuary in August relative to November 2009 (Fig. 4 below), they were also typical for these times of year (Davis et al. 2007; Quigg et al. 2007; 2009). Average conductivities were 45 mS cm⁻¹ and the corresponding salinities were 27 ± 7 . On the other hand, conductivities (and salinities) were significantly lower across the entire Trinity-San Jacinto Estuary in November (Fig. 4). It can be seen that the large influx of freshwater inflows from the Trinity River (Fig. 3) had pushed the higher salinity waters out of the estuary towards the Gulf of Mexico (Fig. 4). This is also reflected by the range of conductivities - 0 to 1.7 mS cm^{-1} (salinities of 0 - 1) in the Trinity River basin, to 7.5 to 10.1 mS cm^{-1} (salinities of 5 -7) in the middle of the estuary, 29.4 to 33.9 mS cm⁻¹ (salinities of 23 - 27) near the mouth estuary – Bolivar Pass (Fig. 4). Conductivities (and so salinities) were generally higher on the west side of the Bay than on the east side reflecting the circulation patterns of the Bay. The magnitude of freshwater entering Trinity-San Jacinto Estuary early in the year had a long and significant influence of the system's salinity gradient (refer to Appendix A).



Fig. 4 Temporal (August and November 2009) and spatial patterns of temperature (°C) and conductivity (mS cm⁻¹) as measured with the Dataflow in Trinity-San Jacinto Estuary.



Fig. 4 Continued.

Temporal (August and November 2009) and spatial patterns of in vivo chlorophyll a (ug L^{-1}) and dissolved organic matter (ug L^{-1}) and as measured with the Dataflow in Trinity-San Jacinto Estuary.

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. 4).

Chl a concentration was measured as a proxy for the biomass of phytoplankton. In August (summer) and November (fall), chlorophyll concentrations were variable across the Trinity-San Jacinto Estuary (Fig. 4). Chlorophyll concentrations were not appreciably different between these two months - reflecting differential responses of phytoplankton to light availability (both in and out of the water column) and nutrients (dissolved and total particulate). Aquatic ecosystems vary in the relative contribution of DOM from the catchment (allochthonous) and DOM produced within the system (autochthonous). The distribution of DOM 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 less DOM in Trinity-San Jacinto Estuary in August (< 0.25 μ g l⁻¹) relative to November 2009 (0.35 to 0.7 μ g l⁻¹) (Fig. 4). This finding indicates that allochthonous sources of DOM were the primary source after large freshwater inflow, while autochthonous maybe more important during low freshwater inflows. This finding is consistent with results from 2008 (Quigg, 2009). Further in November, and during other periods of high flows (see Appendix B), highest DOM concentrations are typically measured in East Bay.

4.4 Temporal and spatial changes in temperature and salinity measured at the six fixed stations in the Trinity-San Jacinto Estuary

Given the shallow nature (average depth of 2 m) of the San Jacinto-Trinity Estuary, there was no temperature gradients observed when examining vertical profiles of the water column at each station from surface to bottom at anytime during the year (the full data set of temperatures measured is given in Appendix B). Hence, average temperatures measured at all six stations and at all depths were calculated. Natural oscillations followed annual cycles with summer highs of 30°C from June to August and winter lows of 12°C from December to March as seen in Fig. 5.



Fig. 5 Trinity-San Jacinto Estuary water temperatures changes during 2009 – average across all stations and depths.

Patterns for salinity were far more complex (Fig. 6). Again, the water column was found to be well mixed such that no halocline was observed at any station (the full data set of salinities measured is given in Appendix B). Salinities in the San Jacinto (station 6) and Trinity River (station 5) basins were very much lower (11 and 17 – annual averages) year round (Fig. 6) compared to those stations in the southern sections of the estuary. Lowest salinities were more often recorded in the San Jacinto River basin (station 5) than in the corresponding Trinity River basin (station 5) which was an unexpected finding. One possible explanation for this is the potential for higher *returned* flows coming down the San Jacinto River as a result of the expanding Dallas/Fort Worth metroplex. Returned flows are those from waste water treatment facilities, power plants and other forms of industry. Stations 1 (Bolivar Pass) and 2 (adjacent to West Bay) had year round salinities, on average, of 26 to 28 (Fig. 6) with highs often around 34 to 36, particularly in the summer months, reflecting the importance of Gulf of Mexico waters in this part of the estuary complex. As expected, intermediate salinities were frequently measured in the middle of the San Jacinto-Trinity Estuary (Station 4) year round (Fig. 6).



Fig. 6. Trinity - San Jacinto Estuary water salinity was dependent on the location in which measurements were made. Findings for salinity at the six fixed stations during 2009 are presented as the average of the vertical profile measured at each station.

4.5 Temporal and spatial changes in distributions of total sediment loading at the six fixed stations in the Trinity-San Jacinto Estuary

Total sediment loading into Trinity-San Jacinto Estuary was estimated from measurements of total suspended sediment (TSS) concentrations (Fig. 7). Data collected from the six fixed stations was averaged into seasonal bins of winter (December – February; \blacktriangle), spring (March – May; \blacktriangle), summer (June – August; \blacktriangle) and fall (September– November; \bigstar). TSS was greatest at all stations during the spring (46% of all TSS in 2009) and lowest in the summer (6% of all TSS in 2009). The winter data is biased by the lack of available data in January and February (see Appendix C).





The Trinity and San Jacinto Rivers are important sources of nutrients to Trinity-San Jacinto Estuary, with freshwater inflows and returned flows being the two major sources. On the other hand, the Gulf of Mexico is generally a poor nutrient source to the Bay. These contentions are supported by the data collected in 2009. Dissolved nitrite plus nitrate concentrations (\blacksquare) ranged between 0.12 (close to detection limit) and 41 µM while dissolved phosphate concentrations (\blacksquare) ranged from 0.18 and 4.8 µM (Fig. 8). A log scale was necessary given the great range in measured nutrient concentrations in the northern versus southern sections of the Bay.





Fig. 8 Monthly dissolved nitrite (μM) k nitrate green) $(\mu M;$ orthophosphate orange) measured in Trinity-San Jacinto Estuary during 2009 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 Trinity River was frequently a greater source of dissolved nutrients to Trinity-San Jacinto Estuary than the San Jacinto River (Station 5 and 6 respectively, Fig. 8). Typically, lowest nitrate and phosphate concentrations (10- to 100-fold lower) were measured closest to the Gulf of Mexico (Stations 1 and 2) as is illustrated in Fig. 8. Specific dissolved nutrient concentrations are summarized in Appendix C). Similar such nitrogen and phosphate concentrations and distribution patterns were reported by Pinckney (2006) and Quigg et al. (2007; 2009) for Trinity-San Jacinto Estuary.

Given DIN:P ratio's greater than 12:1 suggest phosphorus will be limiting to growth, DIN:P ratio's less than 7:1 suggest nitrogen will tend to be limiting for growth Wetzel (2001), these ratios were examined in samples collected across 2009. In most instances (76%), at most stations, DIN:P ratios were less than 7.1 indicating a strong potential for N limitation (Fig. 9). On the other hand, only 10% of measurements indicated a potential for P-limitation.



Fig. 9 DIN:P ratios calculated using dissolved nutrient concentrations measured at the six fixed stations in Trinity-San Jacinto Estuary.

While dissolved nutrient concentrations are those most bioavailable to phytoplankton, total particulate nutrient concentrations are nonetheless an important component of the water quality characteristics of any system and maybe available to some fraction of the community. TN and TP concentrations measured at the six fixed stations are summarized in Appendix C. Consistent with our understanding that different processes regulate the different nutrient fractions, patterns observed for total particulate nutrients were not identical to those observed for dissolved nutrients.

The total particulate nitrogen (TN) concentrations were particularly high (about double) in the winter and spring (Fig. 10) relative to summer and fall (Appendix C). Total particulate phosphorus (TP) concentrations were variable during the year, but lowest concentrations were generally measured in the spring (about half of what was present the rest of the year (Fig. 10; Appendix C). Consistent with these measurements, TN:TP ratios suggest a strong potential



for P-limitation of phytoplankton predominantly in the spring (ratios > 30 in March and April) whilst in the fall, TN:TP ratios (\leq 7) there was the possibility for N-limitation (Table 5).

Fig. 10 TP versus TN ratios measured at the six fixed stations – comparison of seasonal patterns and determination of the potentially limiting nutrient.

4.7 Temporal and spatial distributions of chlorophyll *a* in Trinity-San Jacinto Estuary

Chlorophyll (chl) *a* is often used as a proxy for phytoplankton biomass and so it is likely to vary on both temporal and spatial scales across the Trinity-San Jacinto Estuary. Data collected from the six fixed stations was averaged into seasonal bins of winter (December – February; \blacktriangle), spring (March – May; \bigstar), summer (June – August; \bigstar) and fall (September– November; \bigstar). Chl *a* (µg/l) was highest at all stations during the summer (41% of all Chl *a* in 2009) and lowest in the winter (5% of all Chl *a* in 2009) (Fig. 11). Although the winter data is biased by the lack of available data at several stations and during several months (see Appendix C), the general patterns still hold. Further, Chl *a* was typically greater in stations 3, 4, 5 and 6, which were those in the river basins, middle of the Bay and in east Bay, and lower at stations 1 and 2, adjacent to West Bay and Bolivar pass respectively (Appendix C).



4.8 **Resource Limitation Assays**

Based on findings with the Dataflow and in previous studies (Quigg et al. 2007, 2009), the Bay can qualitatively be divided into two sectors in terms of the influence of freshwater inflows on the phytoplankton community: North and South. Hence, resource limitations assays (RLA's) were undertaken to identify which resource (nutrient(s) and/or light) limited phytoplankton growth at two representative sites in Trinity-San Jacinto Estuary (Fig. 2 shows the location of RLA North and RLA South; latitude and longitude are given in Table 1). RLA's were conducted in February, March, May, June, September, November and December 2009 in order to capture variations in freshwater inflow as well as seasonal changes in phytoplankton responses to nutrient and sediment loading. In Fig. 12, the phytoplankton response index (PRI) was presented on a scale of 0 to 3000 for February and March and of a scale of 0 to 900 for the other months. In each case the average PRI have been presented with error bars calculated as standard deviations for triplicate treatments.

In all RLA's were a significant PRI was measured (>140%), it was always in the treatments in which nitrate was added (+N) and/or in treatments in which both nitrate and phosphate (+NP) were added together (Fig. 12). Hence, nitrogen as nitrate was primarily limiting phytoplankton growth at such stations. However, co-limitation of phytoplankton populations was important given the PRI's in such treatments (+NP) were typically twice that measured in the +N treatments alone. For example, in February 2009, the PRI for the +N treatment was 640 and 1270 in RLA North () and RLA South () respectively while in the +NP treatments, the PRI was 1240 and 2570 in RLA North and RLA South respectively (Fig. 12). In March, the response in the +NP treatment for RLA South was actually four-fold greater than that in the +N alone treatment and 50-fold greater than in the control (Fig. 12).



In several instances, phytoplankton responded more significantly in the +N and +NP treatments in RLA South than in RLA North – this occurred in February, May, June, September and December. In RLA-North conducted in May and June, there was no significant response to any treatment (Fig. 12). The addition of P as phosphate (+P) only elicited a significant response in RLA North in March and November and in RLA South in February (Fig. 12). In only one case - December - was a significant response observed in the control (Fig. 12); this occurred in both treatments. Given the only observed significant response of the phytoplankton in the grazing treatment also only occurred in both the December RLA's (Fig. 12); phytoplankton growth in this month was likely to be light limited, that is, a significant response was measured in all treatments including the control.

4.9 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. As with findings from previous studies (Quigg et al. 2007, 2009; Quigg 2009), the PHYTO-PAM did not detect Chlorophytes and Prasinophytes (green algae) during 2009 in Trinity-San Jacinto Estuary. This is now understood to reflect that concentrations of these groups are below the detection limits of this instrument rather than due to the absence of green algae from this ecosystem.

As part of this study, the principal use of the PHYTO-PAM was to measure the change in phytoplankton productivity, biomass, and community composition in the RLA's. Given changes in phytoplankton biomass in the RLA's have already been presented using direct chl *a* measurements (see section 4.8 above); similar such data from the PHYTO-PAM will not be presented herein. That measured with the PHYTO-PAM was qualitatively but not quantitatively similar; this is a function of the measuring principal of the instrument. While the relative electron transport rate (*rel*ETR) will be used as a proxy for productivity, changes in community composition will be limited to the activities of the major players in the Trinity-

San Jacinto Estuary, that is, the diatoms, dinoflagellates and cyanobacteria (see Örnólfsdóttir et al. 2004; Pinckney 2006; Quigg, et al. 2007; 2009).

The PHYTO-PAM generated an enormous amount of data describing the interplay between the major phytoplankton groups in the RLA's. Upon closer examination, the highlights only have been presented below in Fig. 13. Based on results of the biomass changes (shown in Fig. 12), the most significant response observed was that to the addition of both N and P, that is the, +NP treatments. Hence, a comparison between the control (no addition) and +NP treatments from each of the RLA's is given. In all cases, diatoms and dinoflagellates (orange bars;) were dominant in terms of biomass over the cyanobacteria (blue bars;) (Fig. 13). Typically diatoms and dinoflagellates are more dominant in the cooler months while cyanobacteria are more dominant in the warmer months (e.g., Örnólfsdóttir et al. 2004; Quigg, et al. 2007; 2009). While this is apparent for the cyanobacteria in the controls, it is not clear for the diatoms and dinoflagellates (Fig. 13 – top two panels).



Fig. 13 Response of diatoms and dinoflagellates (orange bars) and cyanobacteria (blue bars) in the RLA's conducted across 2009; top panels – controls (no addition), bottom panels – +NP treatment. The y-axis is the relative biomass (F) of each group measured with the PHYTO-PAM. The units for relative biomass (F) are fluorescence units.

Interestingly, in the +NP treatments (Fig. 13 – bottom two panels), this patterns is much more evident. Hence, while seasonal oscillations were the primary factor regulating these populations, secondarily, was the addition of nutrients.

Spatially significant responses were also observed. In the RLA's conducted at the northern station (RLA North; Fig. 13 – left two panels), cyanobacteria, when present, responded similarly in both the control and the +NP treatments. However, in the RLA's conducted in May and June at the southern station (RLA South; Fig. 13 – right two panels), cyanobacteria showed a greater response (2- to 4-fold) in the +NP treatments compared to the controls. In November, the opposite was true (this is also the month of the highest flow for 2009 – see Fig. 3). When examining the response of the diatoms and dinoflagellates, there were generally less present in the southern station relative to the northern station in both the control and the +NP treatments (Fig. 13) with a few exceptions (e.g., March -- RLA South > RLA North).

Given the findings already presented, and in order to further simplify so as to present only the major outcomes, the results for the measurement of relative electron transport chain (*rel*ETR) presented are those for only the control (no addition) and NP treatments (Fig. 14). A comparison of the outcomes for the major players was performed: diatoms and dinoflagellates (orange bars; \blacksquare) and cyanobacteria (blue bars; \blacksquare) (Fig. 14). If you consider that the diatoms and dinoflagellates dominate the water column (Fig. 13) in the cooler months, the three-fold greater phytoplankton biomass measured in the RLA's in February and March (Fig. 12) was supported by greater *rel*ETR's at the northern (Fig. 14 – top, left) and southern stations (Fig. 14 – top, right) when examining the control treatments. However, this finding was not obvious in the +NP treatments (Fig. 14 lower panels). *rel*ETR's of 140-150 µmol electrons m⁻² s⁻¹ where measured for the diatoms and dinoflagellates (\blacksquare) in RLA North in February and March while in September and November, *rel*ETR's of 45-70 µmol electrons m⁻² s⁻¹ where measured, which are significantly lower (Fig. 14, top, left). A similar scenario was found at RLA South, with *rel*ETR's of 60-90 µmol electrons m⁻² s⁻¹ measured for the diatoms and dinoflagellates in February and March while in September was found at RLA South, with *rel*ETR's of 60-90 µmol electrons m⁻² s⁻¹ measured for the diatoms and dinoflagellates in February and March while in September and November, *rel*ETR's of 30-50

 μ mol electrons m⁻² s⁻¹, almost half (Fig. 14, top, right). Consistent with the findings presented in Fig. 13 above, cyanobacteria () were more important in the summer and fall. While at the northern station (Fig. 14, top, left), cyanobacterial *rel*ETR's ranged from 120-140 µmol electrons m⁻² s⁻¹, those at the southern station were only significant in November (120 ± 15 µmol electrons m⁻² s⁻¹) as seen in Fig. 14 (top, right).



Fig. 14 Response of diatoms and dinoflagellates (orange bars) and cyanobacteria (blue bars) in the RLA's conducted across 2009; top panels – controls (no addition), bottom panels - +NP treatment. The y-axis is the relative electron transport rate (relETR) of each group measured with the PHYTO-PAM. The units for relETR are μ mol electrons m⁻² s⁻¹

In the NP treatments, *rel*ETR's were either lower (RLA North) or similar (RLA South) (Fig. 14, bottom, left and right, respectively) suggesting a complex interaction effect where phytoplankton responded to both the increase in nutrients but also to the decrease in space and light availability as populations increased in the NP treatments. Also apparent, is that the

major players responded differently to the nutrient additions – on both the spatial and temporal scales. To really understand these findings, they will have to be considered in the context of the water quality and other parameters measured. That *rel*ETR's were either lower in the RLA North NP treatments relative to the control treatments may also support the contention that phytoplankton in the northern section of the Trinity-San Jacinto Estuary are accustomed to pulses of nutrients associated with freshwater inflows, and so respond less strongly. Phytoplankton populations in the southern section receive such nutrient pulses less frequently and so respond more strongly. Complicating this response is the competition between different phytoplankton present at different times. This finding is particularly interesting and will be the focus of future research efforts.

5. Discussion

Understanding of the downstream ecological impacts of changes to freshwater inflows on estuaries is important in the development of nutrient budgets, particularly for nitrogen and phosphorus. In ecosystems such as the Trinity-San Jacinto estuary, this is further complicated by its size (1456 km²), shallow nature (average depth of 2 m), small tidal range (average of 30 cm or 1 foot), all of which have been altered by decades of urbanization and industrialization. Most dramatically altered however, is the circulation of the Trinity-San Jacinto estuary – the Texas City Dike which has reduced circulation to West Bay dramatically and deepening and widening of the Houston Ship Channel that transects the entire length of the Bay, continues to change water movement. Unlike other estuaries to its south in Texas, the Trinity-San Jacinto estuary experiences relative large freshwater inflows (in terms of volume) – annual average since 2000 is 8,448,000 cfs (Table 3) and has a relatively fast flushing rate of 112 days (references in Thronson and Quigg, 2008). To further complication matters, human induced changes such which are becoming more important included redirection of flows and the introduction of returned flows from waste water treatment facilities, particularly on the San Jacinto River side of the system. Nonetheless, 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". Herein, efforts were focused towards on understanding the source and fate of nutrients in the Trinity-San Jacinto estuary, with the intention of gathering information to be used towards developing a nutrient budget.

5.1 Freshwater inflows

In Texas, natural freshwater inflows are known to vary in magnitude and duration, with most significant flow events occurring in Fall and Spring and little or no significant flow occurring in the summer. This was certainly the case in 2009 with three major flow events or freshets (>7000 cfs) in the spring and one in the fall. Of the water discharged in 2009, a freshet of 1.67 million cfs entered the Bay from early October to late December (Fig. 3). Compared to previous years this decade, 2009 could be considered a "wet" year (Table 2). The influence of a freshet of this magnitude was seen across the northern and the upper sections of the southern portions of Bay, pushing out previously higher salinity waters towards the Gulf of Mexico (Fig. 4). The findings of Dataflow mapping in the Trinity-San Jacinto Estuary after a period of significant low flow (August 2009) and high flow (November 2009) are presented in Fig. 4 revealing further the complex system level response (all maps are presented in Appendix A). In general, salinity decreased in response to large pulses of freshwater inflow whilst chlorophyll a and dissolved organic matter increased (Fig. 4). 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, very large and long freshwater inflow events, such as that observed in the Fall of 2009, had a bigger influence on the downstream water quality characteristics than any of the smaller individual events. Chl a concentrations, measured as a proxy for the biomass of phytoplankton, did not respond linearly to freshwater inflows – reflecting differential responses of phytoplankton to light availability (both in and out of the water column) and nutrients (dissolved and total particulate) (see below for more detail). Patterns of DOM were similar to those seen in previous years (Davis et al. 2007; Quigg et al. 2007, 2009), with highest DOM concentrations are typically measured in East Bay. Conventional thought is that freshwater inflows (an allochthonous source) deliver dissolved organic matter to the bay; however, East Bay is far

from the Trinity River source and itself has no significant source of freshwater inflow. We hypothesize that the expansive wetlands along East Bay may be contributing this DOM into the Bay. However, we will need to investigate this further in order to test this hypothesis. Multivariate multi-dimensional statistical approaches will also be required to elucidate general patterns which may point the most important factors affecting spatial and temporal responses in water quality.

5.2 Nutrients in the Trinity-San Jacinto Estuary

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. 2007). The Trinity and San Jacinto Rivers are important sources of nutrient and sediments to Trinity-San Jacinto Estuary (Brock 2001). Whilst the sediment loading is important, the effort of the current study was on the source and fate of nutrients. The findings of the current study have therefore been summarized in Table 5 which includes the DIN:P ratios in the riverine and estuary waters, TP:TN in estuary waters and the response of phytoplankton to nutrient additions in the resource limitation assays.

Seasonal patterns of dissolved nutrients were clear in the northern section of Trinity-San Jacinto Estuary (Fig. 8. 9, 10; Table 5); these were related to both the magnitude and duration of freshwater inflow events. On the other hand, the total particulate nitrogen (TN) concentrations were almost double in the winter and spring relative to summer and fall. This is similar to the previously reported patterns for this ecosystem (Quigg et al. 2007, 2009; Quigg 2009). 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 Trinity-San Jacinto Estuary with the Gulf of Mexico at the southern most end of the Bay.

If the DIN:P ratio is greater than 12:1, phosphorus tends to be limiting, and if the DIN:P ratio is less than 7:1, nitrogen tends to be limiting (Wetzel, 2001; Howarth and Marino, 2006). In general, in riverine (Trinity River at Romajor) and estuarine waters in the northern portion of the Bay, DIN:P ratios (and to a lesser extent, TN:TP) indicated nitrogen limitation of phytoplankton from May to October while the P limitation was prevalent for the rest of the year (Table 5).

Table 5. Summary of major findings for the source and fate of nutrients to the Trinity-San Jacinto estuary as part of the current study.

NORTH	DI	N:P	Potentially limiting nutrient	DIN:P	Potentially limiting nutrient	TN:TP	Potentially limiting nutrient		RLA	Potentially limiting nutrient
January	1	3	Р							
February		4	N						*	NP and N
March	1	9	Р	11	D	35	Р		*	NP and N
April				25	Р	35	Р		*	
May		`	N	3	N	14	-		* *	no response
June		2	N		N	10	-		r	no response
July		/	N	2	N	/	-			
August		3	N N	2	N	11	- N		*	
September		3	IN D	3 7	N N	2	IN N		4.	no response
Nevember	1	.9	P	22	IN D	4	IN		*	ND and N
December		15	F P	0 0	г	10	- D		*	light N NP
Detember		5	1)		17	1			iigiit, iv, ivi
					Potentially		Potentially			Potentially
SOUTH				DIN:P	limiting nutrient	TN:TP	limiting nutrient		RLA South	limiting nutrient
January										
February									*	NP and N
March				6	Ν	102	PP		*	NP and N
April										
May				9		39	Р		*	N and NP
June				4	Ν	4	Ν		*	NP and N
July				2	Ν	25	-			
August				2	Ν	13	-			
September				11					*	N and NP
October				6	Ν	15	-			
November						20	-		*	NP and N
December									*	light, N, NP

P limitation was measured during periods when freshwater inflows were of greatest magnitude and duration, while N limitation was measured in the warmer months, when there was very little freshwater flows into the Trinity-San Jacinto estuary. These findings are consistent with the observations of many studies that phosphorus is the proximal limiting nutrient element of concern in fresh waters, while nitrogen is the proximal nutrient limiting productivity in marine systems (Nixon, 1995; Howarth and Marino, 2006). Similar such patterns are also consistent with earlier similar studies for Trinity-San Jacinto Estuary (Örnólfsdóttir et al. 2004; Pinckney 2006; Quigg et al. 2007, 2009; Quigg 2009).

In the southern portion of the Bay, DIN:P ratios, when available, always indicated nitrogen limitation. This finding is consistent with the high salinity waters in the lower section of the Trinity-San Jacinto Estuary (Appendix A; Table 5) and has been reported previously. Interestingly, in this part of the Bay, TN:TP, when available suggested possible P limitation or no limitation.

A more direct mechanism to examine the relationship between nutrients and phytoplankton is by using resource limitation assays (Fisher et al. 1999). The RLA's suggested co-limitation of by both N and P was widespread and frequent (Table 5). In the RLA's performed in the northern part of the Trinity-San Jacinto Estuary in February, March, November and December, the response to the addition of NP always elicited a stronger response than the addition of N alone (Fig. 12). On the other hand, there was no significant response in RLA's conducted from May to September. This typically only occurs when phytoplankton are neither light nor nutrient limited. Given that the nutrient ratios and RLA's in this part of the Bay do not provide entirely the same conclusions (not unexpected based on previous published studies), possible alternative explanations were examined. Diatoms and dinoflagellates dominate in the cooler months while cyanobacteria dominate in the warmer months. Hence, the findings in these RLA's may also reflect seasonal cycles associated with phytoplankton communities. This conclusion fell however, when examining the findings of the RLA's in the southern section of the Bay. In those, limitation by NP and N were observed year round. The additional explanation for this finding is that given these waters are mostly dominated by inputs from the Gulf of Mexico, they have lower overall nutrient concentrations

leaving phytoplankton nutrient limited all year. This is supported by the nutrient data collected (see Appendix C). Previous studies have also reported that different phytoplankton groups have different affinities for the major nutrients; thus, taxon specific trends have been observed. For example, Tilman et al. (1986) and Sommer (1989) reported that diatoms dominate in ecosystems with high N:P or when phosphate concentrations are low while cyanobacteria outcompete other groups under low NP ratios. Our findings are consistent with these generalities from earlier studies.

Elser et al. (2009) proposed an alternative protocol for interpreting RLAs. These authors considered the possibility of sequential nutrient limitation in order to more carefully tease out phytoplankton responses in assays classified as nutrient co-limited. In this approach, "sequential limitation by X" occurs when X produced a significant pairwise contrast with the control. For example, $PRI_{(NP)} > PRI_{(N)} > PRI_{(control)} = PRI_{(P)}$ would be interpreted as a RLA showing sequential co-limitation by N. When looking at the findings presented in this report (Fig. 12), it appears that in all RLAs there is evidence for sequential limitation by nitrogen in this system. In the enriched bottle replicates for May through December particularly for the southern section, there were responses to one nutrient (N), which was shown as an increase over the control, and there was no significant response to the other nutrient (P) compared to control. This suggests that there was not simultaneous scarcity of both nutrients (colimitation) but rather scarcity of a single nutrient (*i.e.*, a Liebig limitation). Alternatively, this suggests that there was significantly more of one nutrient (P) relative to the other nutrient (N) such that this then limited growth. These scenarios are both feasible in the Trinity-San Jacinto Estuary and likely vary on spatial and temporal scales. This ecosystem is not balanced for both nutrients at the same time. This is further supported by the findings in the NP treatments, where the primary limitation by N appears to be alleviated by the addition of the second nutrient, P, because biomass increases were greater in magnitude. Thus, perhaps at least at certain times of the year, phytoplankton are not co-limited by nutrients, but rather sequentially limited.

While in 2008 there was also wide spread limitation of phytoplankton production by nitrogen and phosphorus (Quigg 2009); there was also the observation the greatest phytoplankton

response indices were always measured in RLA South. This clear pattern was not observed in 2009 – the simplest rationale perhaps is the difference in flow patterns between years and hence the distribution and magnitude of nutrient loading. However, a clearer understanding will required multivariate statistics, and more importantly, several more years of data to determine which responses are seasonal, annual versus those which can be truly related to freshwater inflow events.

6. Conclusions and future directions

This study contributes to the improved understanding how the present Trinity-San Jacinto Estuary 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. In terms of developing a nutrient budget for the Trinity-San Jacinto Estuary however, the study is incomplete. While revealing greater details on the interactions of water column nutrient fluxes with changes in phytoplankton (biomass, productivity, distributions); this study identified further knowledge gaps. Future studies should consider the role of nutrients in sediments, and sediment-water interactions. Given the shallow nature of the Bay and the importance of wind mixing, an understanding of processes taking place at the sediment-water boundary will be needed to full develop a nutrient budget. The transfer of carbon derived from phytoplankton can either mediate or amplify the effects of nutrient loading and eutrophication as the material is exported or remineralized, respectively (Pinckney, 2006; Howarth and Marino, 2006). Hence, we need to gain an understanding of all the steps in the loop before a nutrient budget can truly be developed.

As part of Senate Bill 3, a committee was established to determine the importance of freshwater inflows in Galveston Bay watershed. The findings of the committee are summarized in the report by Espey et al. (2009). Pertinent to this and future studies, was development of the concept of antecedent conditions in understanding the downstream ecological impacts of freshwater inflows. Hence, whilst in the present and many other studies, direct correlations are sort between nutrients and other water quality parameters to

phytoplankton responses, what may be more pertinent is to examine antecedent conditions. The question however, remains, would one consider the week prior, the month or some other time line? Or rather than considering time lines, would it be more penitent to consider magnitude and duration of the preceding freshwater inflows. At present, there is insufficient information to attempt such a characterization, but this should be the focus of future studies.

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Appendix A:

Temporal and spatial patterns of water quality parameters measured with the Dataflow in Trinity-San Jacinto Estuary.

Temperature (°C)





















-95.00

-95.10

-94.80

Longitude (^{OW})

-94.90

-94.70

-94.60

-94.50

-94.50

-95.10

-95.00

-94.90

-94.80

Longitude (^OW)

-94.70

-94.60









Dissolved organic matter (DOM) (ug L⁻¹) (Refer to Table 2 for sampling campaign details)





Water Clarity (volts) (Refer to Table 2 for sampling campaign details)











Galveston Bay, October 09

Appendix B:

Water Quality data collected from the six fixed stations (Table 1, Figure 2) during 2009 – vertical profiles of salinity and temperature.

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14.94	14.94	13.99	14.31		15.9	14.16	14.16		1535	1535	15.48	ß		15.25	15.36	153	1577	15.77	1576	1576	16.22	16.2	16.5	16.33	16.3	(%) (%)	4
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	1941	19.6	1965	1974	1981	20.17	20.15		1984	1976	1978	1979		12	an M	8		112	113	m	12	112	112	MA	ma	(%) (%)	4
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2341	229	26.14	26.19	25.0	2432	24.43	24.45	2437	24.02	2389	2452	2491		2396	24.45	24.5		2529	25.42	25,44		2541	2543	2361	2561	("Temp	9
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8		m	12	28.75	28.87	28.88	28.88		28.88	289	2891	28.94		302	30.72	30,71	868	2999	3023	213		37.8	2885	293	368	(°C)	-
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709	709	709	709	18.12	18.13	18.14	17.28		22.65	26	22.62	22.83		18.07	17.84	17.81	36.36	36.14	36.17	36.95		36.36	35.24	34.15	34.26	(international states)	a.
36	30.75	30.84	188	QC	300	N.C.	291		306	90	RC	56		2034	306	9AC	3027	206	30,49	200		31.05	3025	DT.	381	("C)")	

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	룅	35.23	1116	月	II	ŝ	14m	26.07	21.79	Ę	19 64	181	見	या	113
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	뤙	26.8	906	周	20.82	27.78	周	21.41	2135	5	669	19.14	界	12.67	1134
	冑	2681	30.5	周	21.45	27.68	易	22.05	21.5	周	2017	19.18	喂	12.98	1127
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	룅	1493	3043	đ	1673	27.38	周	10.38	20.4						

Appendix C:

Temporal and spatial distributions of total suspended solids (mg ml⁻¹) in Trinity-San Jacinto Estuary.

Station	March	April	May	June	July
1	0.1233	na	0.0573	0.0100	0.0113
2	0.1733	na	0.0380	0.0227	0.0787
3	0.1493	na	0.0333	0.0913	0.03
4	0.118	0.06	0.0253	0.0193	0.0153
5	0.1587	0.0533	0.0360	0.0387	0.0333
6	0.0147	0.0267	0.0240	0.0413	0.0527

Station	August	September	October	November	December
1	0.0113	na	0.0260	0.1760	na
2	0.0267	na	0.0387	0.1187	na
3	0.0327	na	0.0500	0.0167	na
4	0.0147	0.0007	0.0173	0.0300	0.0233
5	0.0107	0.0060	0.0127	0.0167	0.0187
6	0.0180	0.0053	0.0153	0.1313	0.0173

** n.a. refers to data not collected because field work was not possible due to inclement weather

			March					April					May		
Station	$[NO_3+NO_2]$	[NH ₄]	$[PO_4]$	DIN	DIN:P	$[\mathrm{NO}_3 + \mathrm{NO}_2]$	[NH ₄]	$[PO_4]$	DIN	DIN:P	$[NO_3+NO_2]$	[NH ₄]	[PO ₄]	DIN	DIN:P
1	1.87	2.76	1.04	4.63	4.5	na	na	na	na	na	9.41	2.22	0.53	11.63	21.9
2	1.42	4.92	0.64	6.34	9.9	na	na	na	na	na	0.22	1.21	0.49	1.43	2.9
3	0.25	1.90	0.82	2.15	2.6	na	na	na	na	na	0.39	1.53	0.84	1.92	2.3
4	36.93	16.91	4.77	53.84	11.3	3.08	3.20	0.43	6.28	14.6	0.04	1.15	1.20	1.19	1.0
5	7.12	8.06	1.29	15.18	11.8	6.59	3.72	0.18	10.31	57.3	1.76	1.95	3.06	3.71	1.2
6	0.93	7.09	0.91	8.02	8.8	0.40	1.39	0.39	1.79	4.6	7.24	8.53	2.13	15.77	7.4
			June					July					August		
Station	[NO ₃ +NO ₂]	[NH ₄]	[PO ₄]	DIN	DIN:P	[NO ₃ +NO ₂]	[NH ₄]	[PO ₄]	DIN	DIN:P	[NO ₃ +NO ₂]	[NH ₄]	[PO ₄]	DIN	DIN:P
1	3.35	2.09	0.48	5.44	11.3	0.30	2.49	0.59	2.79	4.7	0.48	3.05	1.36	3.53	2.6
2	0.26	2.05	1.13	2.31	2.0	0.20	1.55	1.34	1.75	1.3	0.59	2.02	1.11	2.61	2.4
3	0.09	1.29	0.65	1.38	2.1	0.12	0.43	0.93	0.55	0.6	0.29	1.54	1.36	1.83	1.3
4	0.18	1.13	2.84	1.31	0.5	0.30	1.97	0.52	2.27	4.4	0.45	1.65	0.94	2.1	2.2
5	0.38	1.62	2.48	2	0.8	0.35	1.78	0.50	2.13	4.3	0.31	1.90	1.34	2.21	1.6
6	0.17	1.80	3.16	1.97	0.6	0.28	1.08	0.19	1.36	7.2	0.45	1.16	0.54	1.61	3.0
			September					October]	Vovember		
Station	[NO ₃ +NO ₂]	[NH ₄]	[PO ₄]	DIN	DIN:P	[NO3+NO2]	[NH ₄]	$[PO_4]$	DIN	DIN:P	[NO3+NO2]	[NH ₄]	$[PO_4]$	DIN	DIN:P
1	na	na	na	na	na	10.95	2.76	0.86	13.71	15.9	7.91	3.23	0.94	11.14	11.9
2	na	na	na	na	na	8.97	8.01	1.12	16.98	15.2	2.65	2.82	1.30	5.47	4.2
3	na	na	na	na	na	0.15	1.32	1.07	1.47	1.4	0.17	0.06	0.35	0.23	0.7
4	4.96	4.88	3.41	9.84	2.9	0.46	1.35	3.03	1.81	0.6	2.47	2.43	2.09	4.9	2.3
5	21.80	4.01	5.37	25.81	4.8	116.45	3.01	6.61	119.46	18.1	7.31	5.21	3.64	12.52	3.4
6	0.40	1.07	4.78	1.47	0.3	2.24	0.14	2.38	2.38	1.0	21.38	2.17	0.26	23.55	90.6

Temporal and spatial distributions of dissolved nutrients (μM) in Trinity-San Jacinto Estuary.

	December								
Station	$[NO_3 + NO_2]$	[NH ₄]	[PO ₄]	DIN	DIN:P				
1	na	na	na	na	na				
2	na	na	na	na	na				
3	na	na	na	na	na				
4	14.89	5.35	2.85	20.24	7.1				
5	40.75	14.62	4.51	55.37	12.3				
6	23.68	3.87	4.34	27.55	6.3				

Temporal	and spatial	distributions	of total	particulate	nutrients	(µM)	in	Trinity-San
Jacinto Est	tuary.							

	March		April		May		June		July	
Station	TN	TP	TN	TP	TN	TP	TN	TP	TN	TP
1	125.06	0.71	na	na	55.32	0.82	0.23	1.67	104.67	1.87
2	59.3	1.30	na	na	45.56	1.79	4.95	2.89	57.17	2.39
3	129.01	1.05	na	na	56.61	1.38	24.42	2.88	19.98	3.08
4	162.82	6.05	101.35	2.02	48.63	2.70	57.30	4.50	13.86	5.41
5	98.80	1.97	91.48	3.72	71.41	6.30	53.76	5.40	69.37	6.48
6	71.39	1.50	64.23	1.65	56.78	3.67	44.46	6.40	57.94	7.91

August		September		October		November		December		
Station	TN	TP	TN	TP	TN	TP	TN	TP	TN	TP
1	30.04	1.98	na	na	48.32	3.32	57.78	2.82	na	na
2	27.63	1.66	na	na	31.09	2.08	34.77	2.51	na	na
3	58.98	5.06	na	na	55.54	3.38	58.49	2.19	na	na
4	80.37	7.53	3.25	5.30	7.04	5.94	41.67	4.63	64.58	3.95
5	65.64	5.16	20.91	9.45	48.06	10.11	47.83	5.66	100.19	5.59
6	74.54	7.2	12.75	7.84	27.92	4.61	77.03	6.77	81.12	3.21

** n.a. refers to data not collected because field work was not possible due to inclement weather

Temporal and spatial distributions of chlorophyll ($\mu g \Gamma^1$) in Trinity-San Jacinto Estuary.

Station	March	April	May	June	July
1	8.61	na	12.40	8.42	12.98
2	2.77	na	16.44	22.50	14.98
3	7.93	na	9.44	16.60	12.49
4	3.38	4.69	18.86	12.44	11.68
5	2.24	13.00	24.34	11.86	8.84
6	3.28	10.00	6.48	16.59	13.61

Station	August	September	October	November	December
1	9.85	na	7.71	5.87	na
2	18.37	na	7.38	4.00	na
3	35.46	na	19.69	13.33	na
4	21.70	6.90	17.41	14.42	5.48
5	20.31	22.81	13.16	19.05	3.57
6	25.94	17.90	18.41	5.99	2.70

** n.a. refers to data not collected because field work was not possible due to inclement weather