Monitoring Mid-Coastal Estuaries - 2016

Final Report

by
Paul A. Montagna[1], Ph.D.
Michael Wetz[2], Ph.D.
Xinping Hu[3] Ph.D.

Texas A&M University –
Corpus Christi
6300 Ocean Drive
Corpus Christi, Texas 78412

[1]Harte Research Institute for Gulf of Mexico Studies, Unit 5869
[2]Department of Life Sciences, Unit 5860
[3]Department of Physical and Environmental Sciences, Unit

Submitted

to
Texas Water Development Board
1700 North Congress, Agency Code 580
P.O. Box 13231
Austin, TX 78701-3231
Contract # 1600011924

December 2017

Cite as:

Montagna, P.A., M. Wetz, and X. Hu. 2017. Monitoring Mid-Coastal Estuaries - 2016. Final Report to the Texas Water Development Board, Contract # 1600011924. Texas A&M University-Corpus Christi, Corpus Christi, Texas, 51 p.

Monitoring Mid-Coastal Estuaries – 2016

Table of Contents

List of Figures

List of Tables

1 Acknowledgements

Funding for the project was supplied by the Texas Water Development Board, General Revenue Fund under Interagency Cooperation Contract # 1600011924. The authors thank the program manager, Caimee Schoenbaechler. Many people helped in the field and laboratory work, this includes Leslie Adams, Ken Hayes, Larry Hyde, Richard Kalke, Elani Morgan, Terence Palmer, and Cory Staryk.

2 Abstract

In recognition of the importance that the ecological soundness of our riverine, bay, and estuary systems and riparian lands has on the economy, health, and well-being of our state, the 80th Texas Legislature enacted Senate Bill 3 (SB3, 2007), which requires an ecosystem management approach to provide environmental flows "adequate to support a sound ecological environment and to maintain the productivity, extent, and persistence of key aquatic habitats." Thus, there is a continued need for monitoring information about freshwater inflow effects on water and sediment quality and biological indicator communities to provide adaptive management of environmental inflow standards for Texas estuaries. The purpose of the present study is to extend the long-term biological collection of benthic data in San Antonio Bay (Guadalupe Estuary), Lavaca and Matagorda Bays (Lavaca-Colorado Estuary), and Nueces and Corpus Christi Bays (Nueces Estuary). Bottom-dwelling organisms are ideal bioindicators of freshwater inflow effect on bays and estuaries because they are fixed in space, and integrate ephemeral processes in the over-lying water column over long periods of time. Only benthic samples from the Guadalupe Estuary have been analyzed, and the benthic data has demonstrated that long-term hydrological cycles, which affect freshwater inflow and water quality, also regulate benthic abundance, productivity, diversity, and community structure. In addition, changes in water quality due to inflow variability were measured in all three estuaries, including chlorophyll (as an indicator of primary production), inorganic nutrients, dissolved and particulate organic matter (DOM and POM), and the carbonate system variables alkalinity, dissolved inorganic carbon (DIC), and pH. The estuaries respond rapidly to freshwater inflow variability, displaying higher inorganic nutrient and chlorophyll concentrations during high inflow, low salinity conditions. Dissolved organic carbon and nitrogen concentrations were consistently high in the estuaries, providing an important source of matter fueling microbial growth. The dissolved organic matter concentration was influenced by freshwater inflow variability in Lavaca-Colorado and Nueces Estuaries, but not in the Guadalupe Estuary. Carbonate saturation state, an important indicator for the suitability of calcifying organisms (shellfish etc.), changed with both river endmember chemical composition and the extent of freshwater inflow. The northern Lavaca-Colorado Estuary showed carbonate undersaturation following a significant freshwater discharge period, although the southern estuaries were less influenced by the hydrological change.

3 Introduction

Since the early 1970's, Texas Water Development Board (TWDB) sponsored freshwater inflow studies focused on the major bay systems of the Texas coast. These bay systems, which are influenced primarily by river inflow and exchange with the Gulf of Mexico, are now subject to greater scrutiny because of recent legislative changes. In recognition of the importance that the ecological soundness of our riverine, bay, estuary, and riparian areas has on the economy, health, and well-being of our state, the 80th Texas Legislature enacted Senate Bill 3 in 2007, which called for creation of Basin and Bay Area Expert Science Teams (BBEST) to establish environmental flow recommendations for bay and estuary inflows, and Basin and Bay Area Stakeholder Committees (BBASC) charged with balancing environmental needs with the need for water for human uses. In the past, the State methodology depended on modeling inflow effects on fisheries harvest in Texas estuaries (Longley 1994). SB 3 however, requires an ecosystem management approach to provide environmental flows adequate to support a sound ecological environment and to maintain the productivity, extent, and persistence of key aquatic habitats. SB3 also requires an adaptive management phase, in which the BBASC groups will have to review the effects of the inflow standards that were adopted by the Texas Commission on Environmental Quality (TCEQ). Thus, the BBASC groups will need monitoring information on freshwater inflow effects on water quality and biological indicator communities (Montagna et al. 2009, 2010).

Since 1986, researchers led by Dr. Montagna have been studying the effect of freshwater inflow on benthic communities and productivity (Kalke and Montagna 1991; Kim and Montagna 2009, 2012; Montagna 1989, 1999, 2000, 2013; Montagna et al. 2007; Montagna and Kalke 1992, 1995; Montagna and Li 1996, 2011; Montagna and Palmer 2009, 2010, 2011, 2012; Montagna and Yoon 1991; Pollack et al. 2009, 2011). These studies have demonstrated three main points: 1) freshwater inflow drives water quality and that regulates benthic abundance, productivity, diversity, and community structure, 2) there are salinity zone habitats within estuaries that are driven by inflow, and 3) long-term climate cycles (i.e., greater than 10 years) affects hydrology, which in turn affects biological dynamics within and among estuaries across the coast of Texas. Benthos are excellent bioindicators of environmental effects because they are very abundant and diverse, are sessile, and long-lived relative to plankton (Montagna et al. 2013). Therefore, benthos are good biological indicators of freshwater inflow effects because they integrate changes in temporal dynamics of ecosystem factors over long time scales and large spatial scales.

The benthic studies performed as part of the long-term monitoring of benthos (i.e., those listed above) have elucidated some basic principles on how inflow drives estuary and ecosystem dynamics. The Texas estuaries lie in a climatic gradient where those in the northeast receive more rainfall than those in the southwest. Consequently, freshwater inflow and nutrient loading decreases along the climatic gradient and salinity increases. In addition, there is year-to-year variation in rain and inflow that results in wet and dry years. This combination of the climatic gradient and temporal variability drives variability in estuarine communities and secondary production. Among Texas estuaries, decreased flow (and thus increased deposition and salinity) benefits deposit feeders (with increased abundance and species richness), while suspension feeders are reduced (with decreased abundance and species richness); thus there is a decrease in functional diversity when salinity is increased because of loss of a trophic guild. Within estuaries, the abundance and biomass of the upstream benthic community is reduced by reduced inflow, whereas, the downstream community increases in abundance and biomass with reduced inflow and higher salinities. This is because lower salinity regimes are required to support food production for suspension feeders, and polyhaline (i.e., between 18 and 30 psu) deposit feeding species increase during marine conditions. Overall, these studies demonstrate that freshwater inflow is important to maintain secondary productivity and functional diversity in estuaries, which is required to maintain estuarine health and sustainability (Montagna et al. 2013).

Many coastal watersheds in Texas are experiencing significant population and economic growth, resulting in increasing demands on freshwater resources (TWDB 2017). It is possible that increased water demand would reduce flow to downstream coastal waterbodies. Freshwater inflow changes the estuary water column conditions that the biological communities are all responding to. These changes are due to the materials flowing into bay borne by river water, and the dilution of salinity in the receiving water. While past studies focused on inorganic nutrients, it is increasingly clear that changes in water quality due to inflow are not limited to nutrients, but include dissolved organic matter (DOM) and particulate organic matter (POM), and changes in the carbonate system including alkalinity, dissolved inorganic carbon (DIC), and pH.

Allochthonous (i.e., transported) and autochthonous (in situ produced) organic matter are important contributors to the overall productivity of many estuaries (Day et al. 2012). However, these sources of organic matter can affect its overall lability (i.e., how bioreactive it is). Autochthonous organic matter derived from phytoplankton biomass is believed to be labile (i.e., easily degrade) under oxic conditions (Biddanda 1988; Harvey et al. 1995), as is organic matter derived from specific land use practices such as municipal wastewater discharge sites, manure-based agricultural operations, and concentrated animal waste operations (e.g., Servais et al. 1987; Abril et al. 2002; Mallin et al. 2002; Petrone et al. 2009). On the other hand, allochthonous organic matter derived from forested watersheds is typically thought to be less labile (Servais et al. 1987; Abril et al. 2002). Thus it is important to discern effects of nutrient-driven algal production versus allochthonous organic matter to project how estuaries may change in the future due to altered watershed land uses and freshwater inflows, which will affect the relative loadings of allochthonous and autochthonous organic matter, as well as the quality of the organic matter. Unfortunately, previous studies in Texas estuaries have not adequately coupled measures of organic matter components to inorganic nutrient measurements, despite studies alluding to the importance of dissolved organic matter as a key bio-reactive component of inflow (e.g., Russell & Montagna 2007; Shank et al. 2009; Bruesewitz et al. 2013).

In the case of the carbonate system, a gradual loss of alkalinity (acidification) has been observed in many Texas coastal estuaries presumably due to a reduction of alkalinity export by rivers (Hu et al., 2015), which will have important implications for calcifying organisms in these systems. The precipitation/evaporation gradient along the Texas coast creates a gradient of riverine alkalinity that shows a generally increasing trend from northeast to southwest (TCEQ, 2014; Hu et al., 2015). Data on the estuarine carbonate system in Texas estuaries are important for understanding the risks of coastal waters to acidification caused by both carbon dioxide (CO_2) uptake into seawater and estuarine alkalinity reduction caused by reduced freshwater inflow. Inorganic carbon is the dominant buffer system in estuaries, meaning it controls the acidity and alkalinity of estuaries. The inorganic carbon, or the carbonate system system (dissolved carbon dioxide or CO_2, alkalinity, carbonate saturation state, pH, and CO_2 partial pressure, or pCO_2), is controlled by river-ocean water mixing and biogeochemical processes. The river plays a dominant role in the low salinity region of estuaries because freshwater has drastically different composition than seawater (for example, much lower calcium ion (Ca^{2+}) concentration in river water than seawater and much higher pCO_2, Hu and Cai, 2013), which affects carbonate saturation state (Ω) greatly in low salinity waters. In addition, respiration and photosynthesis compete with each other in driving CO_2 release or uptake, which subsequently control pH, Ω, and pCO_2 variations. For example, respiration releases CO_2 and leads to decreases in both pH and Ω but increase in pCO_2, and photosynthesis drives these changes in opposite directions. Finally, the calcification process by calcareous organisms (typically shellfish species in estuaries) consumes alkalinity (bicarbonate ion - HCO_3^- and carbonate ion - CO_3^{2-}) and leads to decreases in both pH and Ω but increase in pCO_2. When Ω and pH are sufficiently low, carbonate preserved in estuarine sediment (and live shells) will dissolve and act as a buffer against the acidifying condition. Shellfish species heavily rely on optimal Ω conditions to survive and reproduce (Waldbusser et al., 2014), thus maintaining these conditions will require well-managed environmental flow in the semi-arid south Texas.

This study measured dissolved and particulate organic carbon/nitrogen, dissolved inorganic carbon, as well as carbonate system parameters that include pCO_2 and Ω three estuaries (Lavaca-Colorado, Guadalupe Estuary, Nueces). The goal of the study was to understand how freshwater inflow drives water quality and estuarine biogeochemistry along the Texas coast. A previously funded study captured organic and inorganic carbon/nitrogen dynamics during a period of relatively low rainfall, whereas this study corresponds with a strong El Niño period. El Niño events have been linked with higher than average rainfall (Tolan 2007). Data collected during the recent El Niño (February 2015-July 2016), coupled with data collected previously during drought (October 2012-January 2015), provided an opportunity to fully elucidate the effects of low versus high freshwater inflow. The study serves as a companion to monitoring of benthic organisms and inorganic nutrients in relation to freshwater inflow.

The ultimate goal of the long-term benthic and water column data collection is to use the data to assess ecosystem health as it relates to change in freshwater inflow by assessing benthic habitat health, and benthic productivity. However, inflow itself does not affect ecosystem dynamics; it is the change in estuarine condition primarily salinity, nutrients, DOM, POM, carbonates, and chlorophyll, which drives change in biological resources (SAC 2009). Thus, the goal here is to relate changes in water column dynamics with change in benthic dynamics. For example, it has been used to create a model of productivity based on seven years (1988 - 1995) of data in four Texas estuaries: Lavaca-Colorado, Guadalupe, Nueces, and Laguna Madre (Montagna and Li 1996, 2010). The model was used to support inflow criteria development for Matagorda Bay in the Lavaca-Colorado Estuary (Kim and Montagna 2009). Recently, the adjusted model was rerun on 20 years (1988 - 2008) of benthic and water column data and it was shown that salinity and nutrient changes (which are caused by inflow changes) drives benthic productivity and functional diversity (Kim and Montagna 2010; 2012). The data collected in this study will provide an understanding of the long-term ecosystem dynamics of the San Antonio Bay System.

4 Methods

4.1 Study Area

Sampling was performed in three estuaries in the Texas mid-coastal zone: Lavaca-Colorado Estuaries, Guadalupe, and Nueces (Figure 1). The study area is ideal to answer questions related to altered hydrology and climate variability occurring at different temporal scales (e.g., seasonal, annual, multi-annual), and different spatial scales. This is because there is a climatic gradient (among estuary) and an estuarine (within estuary) gradient. The climatic gradient is caused by precipitation decreasing from northeast to southwest, this causing an inflow gradient. The within estuary gradient is caused by freshwater inflow from rivers at one end, to tidal mixing with Gulf of Mexico waters at the other end.

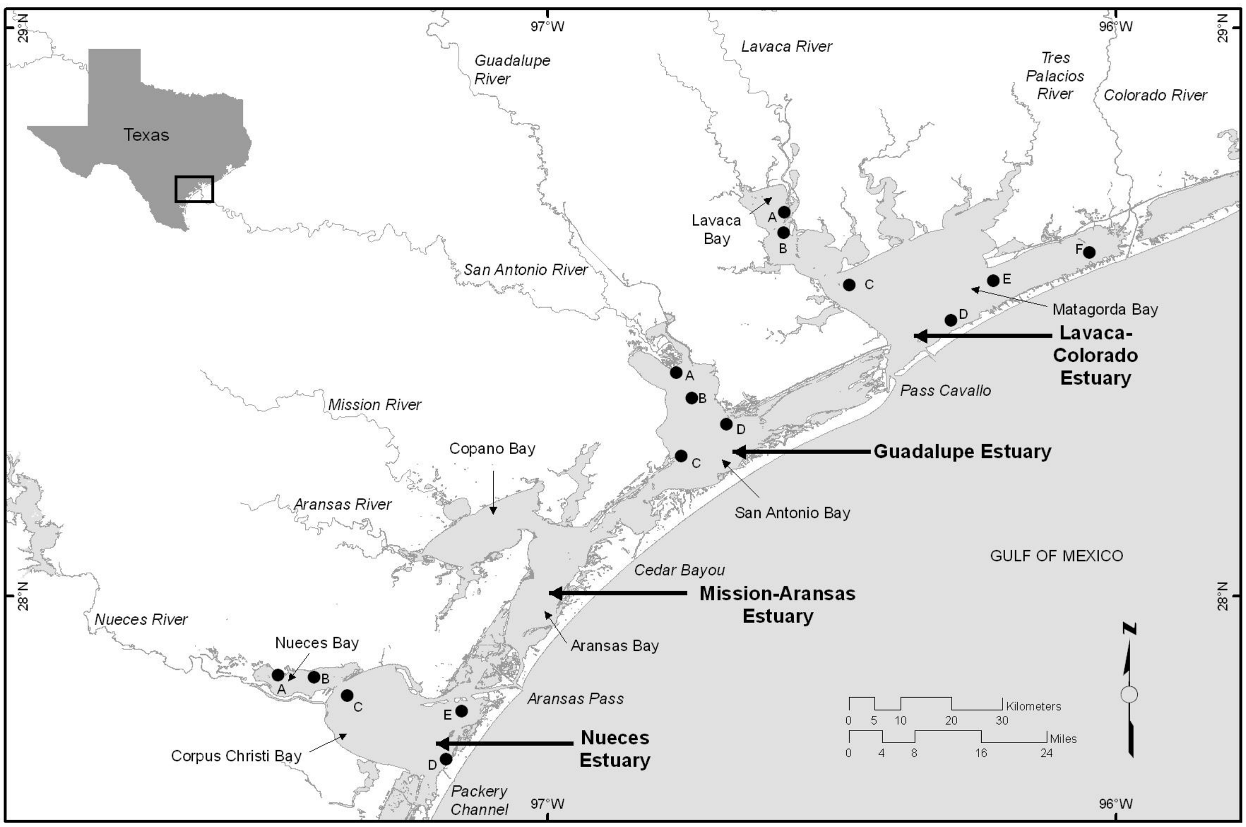

Figure 1 The three Texas Coastal Bend estuaries sampled. Station locations are along a climatic (among estuaries) and estuarine (within estuaries) gradients.

Stations were located in primary bays closer to the Gulf of Mexico exchange point, and in secondary bays closer to the freshwater inflow sources (Table 1). Four stations were sampled for macrofauna and water quality in the Guadalupe Estuary, six in the Lavaca-Colorado Estuary, and five in the Nueces Estuary.

Table 1 Locations of stations within the Guadalupe (GE), Lavaca-Colorado (LC), and Nueces (NC) estuaries.

Estuary	Bay	Station	Latitude	Longitude
GE	San Antonio	A	28.39352	-96.77240
GE	San Antonio	B	28.34777	-96.74573
GE	San Antonio	C	28.24618	-96.76488
GE	San Antonio	D	28.30210	-96.68435
LC	Lavaca	A	28.67467	-96.58268
LC	Lavaca	B	28.63868	-96.58437
LC	Matagorda	C	28.54672	-96.46894
LC	Matagorda	D	28.48502	-96.28972
LC	Matagorda	E	28.55450	-96.21550
LC	Matagorda	F	28.60463	-96.04600
NC	Nueces	A	27.86069	-97.47358
NC	Nueces	B	27.85708	-97.41025
NC	Corpus Christi	C	27.82533	-97.35213
NC	Corpus Christi	D	27.71280	-97.17872
NC	Corpus Christi	E	27.79722	-97.15083

Water column and sediment samples were collected at all stations in all estuaries. However, benthic samples were analyzed only in the Guadalupe Estuary and the benthic samples from the Nueces and Lavaca-Colorado estuaries were archived for future analysis. Only the benthos from the Guadalupe Estuary are described and discussed in this report.

Sampling occurred seven times: January 2016, April 2016, July 2016, October 2016, January 2017, April 2017, and July 2017. Results are also included from nutrients and organic matter samples collected prior to the start of the current study, going back to October 2012. A comparison with long-term benthic samples since 1987 is also included.

4.2 Water Quality

Physical water quality measurements in addition to chlorophyll and nutrients were sampled in duplicate just beneath the surface and at the bottom of the water column at all stations on every sampling date.

4.2.1 Hydrographic Measurements

Hydrographic measurements were made at each station with a YSI 6600 multi parameter instrument. The following parameters were read from the digital display unit (accuracy and units): temperature (± 0.15 ˚C), pH (± 0.1 units), dissolved oxygen (± 0.2 mg l^{-1}), depth (± 0.1 m), and salinity (psu). Salinity is automatically corrected to 25 ˚C. In addition, water salinity was also measured at the Hu's lab using a benchtop salinometer to corroborate with field measurements. Based on our observation, slight differences between field and lab salinity may occur, presumably due to density stratification. Thus in our subsequent calculation, we always used lab measured salinity.

4.2.2 Chlorophyll

Chlorophyll samples were filtered onto glass fiber filters and placed on ice (< 4.0 °C). Chlorophyll is extracted overnight and read fluorometrically on a Turner Model 10-AU using the non-acidification technique (Welschmeyer, 1994; EPA method 445.0).

4.2.3 Nutrients

Nutrient samples were filtered to remove biological activity (0.45 μm polycarbonate filters) and placed on ice (<0.4 °C).Water samples were analyzed at the Harte Research Institute using a OAI Flow-4 autoanalyzer with computer controlled sample selection and peak processing. Chemistries are as specified by the manufacturer and have ranges as follows: nitrate+nitrate (0.03-5.0 μM; Quikchem method 31-107-04-1-A), silicate (0.03-5.0 μM; Quikchem method 31-114-27-1-B), ammonium (0.1-10 μM; Quikchem method 31-107-06-5-A) and phosphate (0.03-2.0 μM; Quikchem method 31-115-01-3-A.

4.2.4 Dissolved Organic Carbon and Nitrogen

Surface water samples were collected in acid-washed amber polycarbonate bottles. Bottles were stored on ice until return to a shore-based facility where processing of samples occurred. Inorganic nitrogen (nitrate + nitrite, ammonium) was determined from the filtrate of water that passed through GF/F filters using an OI Systems analyzer (see Montagna 2014). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined in the filtrate of water that passed through precombusted (400°C for 4 hours) GF/F filters using a Shimadzu TOC-V analyzer with nitrogen module. Potassium nitrate and potassium hydrogen phthalate were used as standards, and check samples of a known C/N concentration of both Glucosamine and Consensus Reference Water (http://www.rsmas.miami.edu/groups/biogeochem/CRM.html) were routinely injected to insure proper instrument functioning. Dissolved organic nitrogen (DON) was estimated as the difference between TDN and inorganic nitrogen. Total organic carbon (TOC) and Total nitrogen (TN) were estimated as above, except the prefiltration step was omitted.

4.2.5 Carbonate Chemistry

Following the standard operating protocol in ocean CO_2 study (Dickson et al., 2007), we used pre-combusted 250 ml Pyrex® ground borosilicate bottles for sample collection. One bottle volume overflow was ensured to completely flush the sampling bottle. 100 µL saturated mercuric chloride ($HgCl_2$) was added into each sample before the bottle was stopped with Apiezon® grease and secured with a rubber band and a nylon clamp to ensure gas tightness.

Dissolved inorganic carbon (DIC) in water was analyzed based on acidification, CO_2 extraction, and infrared quantification on an Apollo DIC analyzer. Total alkalinity was analyzed by open cell Gran titration on an Apollo alkalinity titrator. Certified Reference Material purchased from Dr. Andrew Dickson's lab at Scripps Institution of Oceanography was used ensure the accuracy of both analyses. For each sample with added purified m-cresol purple (mCP) solution, absorbance at 434 and 578 nm (after correcting for baseline shift at 730 nm) in a custom-made 10 cm water-jacketed flow-through glass cell (Hellma Optics) was measured on an Agilent 8453 UV-Vis spectrophotometer under strict temperature control using the setup as described in Carter et al. (2013); pH was then calculated using the equation in Liu et al. (2011). Because of the applicable salinity limitation in Liu et al. (2011), pH of all samples with lower than salinity 20 were measured using an Orion® Ross combination glass electrode, which was calibrated using NBS pH buffers (4.01, 7.00, 10.01) at 25°C. Ca^{2+} concentration was analyzed using EGTA titration on a Metrohm automated titrator, and a Ca^{2+} ion-selective electrode was used to determine the titration endpoint (Anderson and Granéli, 1982). Derived carbonate system parameters ($p$$CO_2$, carbonate saturation state with respect to aragonite or $\Omega_{aragonite}$) were calculated using a computer program CO2SYS (Lewis and Wallace, 1998). Carbonic acid dissociation constants in Millero (2010) were used in the calculation. $\Omega_{aragonite}$ obtained from CO2SYS was corrected using measured Ca^{2+} concentration.

4.3 Sediment Quality

Sediment samples were collected using cores deployed from small boats. The position of all stations is established with a Global Positioning System (GPS) with an accuracy of ±3 m. Macrofauna were sampled with a 6.7-cm diameter core tube (35.4 cm^2 area). The cores were sectioned at 0-3 cm and 3-10 cm depths to examine vertical distribution of macrofauna. Three replicates are taken per station. Organisms were extracted on a 0.5 mm sieve, and enumerated to the lowest taxonomic level possible. Biomass is determined for higher taxonomic groupings by drying at 55 ºC for 24 hours.

4.4 Analytics

Community structure of macrofauna species was analyzed by non-metric multidmensional scaling (MDS) and cluster analysis using a Bray-Curtis similarity matrix (Clarke 1993, Clarke and Warwick 2001). Prior to analysis, the data was $\log_{10}$ transformed. Log transformations improve the performance of the analysis by decreasing the weight of the dominant species. MDS was used to compare numbers of individuals of each species for each station-date combination. The distance between station-date combinations can be related to community similarities or differences between different stations. Cluster analysis determines how much each station-date combination resembles each other based on species abundances. The percent resemblance can then displayed on the MDS plot to elucidate grouping of station-date combinations. The group average cluster mode was used for the cluster analysis.

Multivariate analyses were used to analyze how the physical-chemical environmental changes over time. The water column structure was each analyzed using Principal Component Analysis (PCA). PCA reduces multiple environmental variables into component scores, which describe the variance in order to discover the underlying structure in a data set (Clarke and Warwick 2001). In this study, only the first two principal components were used.

5 Results

5.1 Guadalupe Estuary During the Study Period: Water Quality

Of the 21 water quality variables measured, only 11 (temperature, salinity, dissolved oxygen, pH, phosphate, silicate, ammonium, nitrite+nitrate, chlorophyll, dissolved inorganic carbon, and total alkalinity) were measured at the surface and bottom. A multivariate analysis of variance was run to determine if there was a difference between the surface and bottom overall all variables, and there was not ($P = 0.1811$). Therefore, the surface and bottom values were treated as replicates and the average was used in further spatial and temporal analysis.

Principal Components Analysis explained 70 % of the variation within the water quality data set (Figure 2). Principal Component (PC) 1 explained 50 % of the variation while PC2 explained 20 % of the variation. PC1 represents inflow changes in water quality because low salinity values are inversely correlated to inorganic nutrients such as Nitrite+Nitrate (NO2+3), phosphate (PO4), and silicate (SiO4). Also, the high nutrient concentrations are correlated to high chlorophyll (Chl) concentrations (Figure 2A). Interestingly, salinity is also inversely correlated with pH, which means when salinity is high, the bays are becoming more acidic. PC2 represents a seasonal, temporal gradient because the lowest temperature values are correlated to highest dissolved oxygen (DO) values.

A result of PC1 being an inflow indicator is that Stations A and B, nearest the Guadalupe River mouth, occur at the top of the sample scoring scale with positive (Figure 2B). There is a gradient where station B and station C scores are in the middle of the PC1 axis near zero values, and station D scores are generally negative.

The seasonal sampling months have a tendency to group together as well (Figure 2C). The gradient is from the coldest month January, which has positive scores to April with lower positive scores, to July and October, which both have similar negative scores. However, the salinity between January 2013 and January 2015 was always above 20 and therefore, this was a dry period overall (Figure 3).

A)

B)

C)

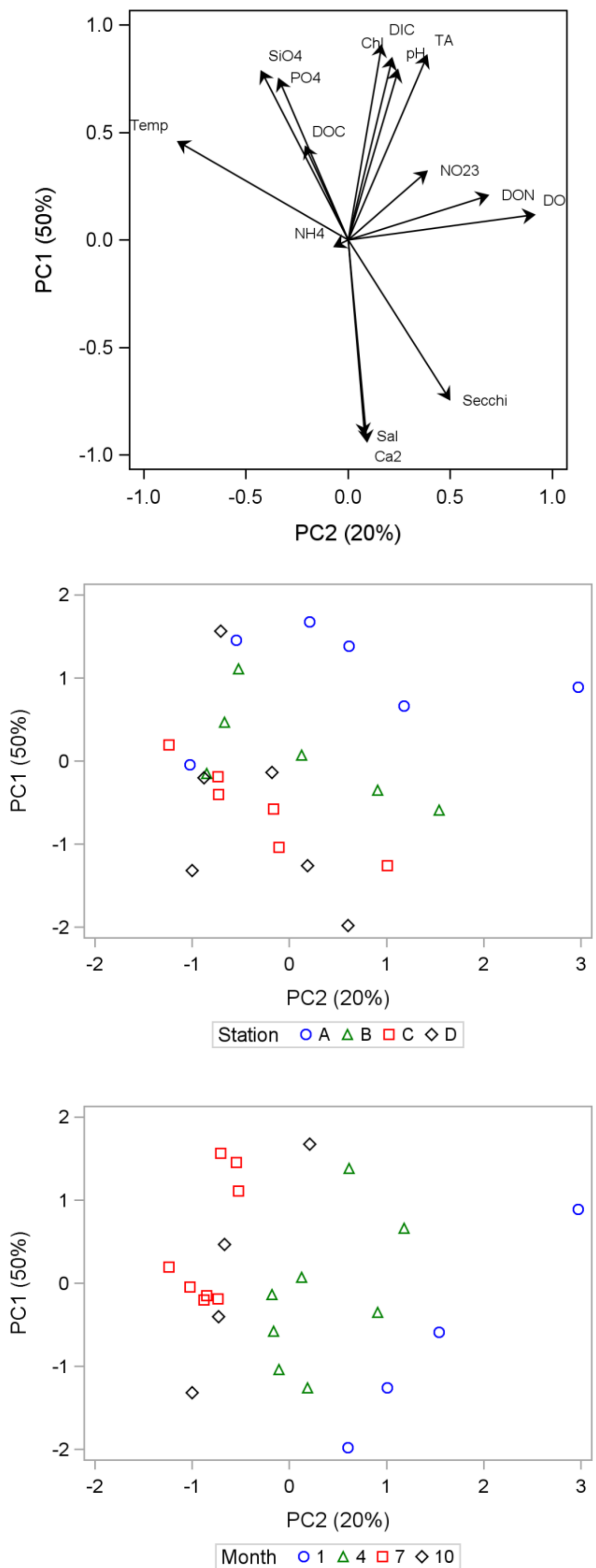

Figure 2 **Principal Components Analysis (PCA) of water quality variables in the Guadalupe Estuary from January 2014 through to July 2017. A) Variable loading plot. B) Station scores labeled by station (B). C) Month number.**

The lowest average salinity and highest average concentrations of all nutrients (silicate, phosphate, ammonia, and nitrate+nitrite), and chlorophyll concentrations occur at Stations A and B, and this is an indicator of river flow from the Guadalupe River into San Antonio Bay (Table 2). Ammonium concentrations are below detection limits for many samples, so the overall average is only near 1 umol/L. Mean chlorophyll concentrations are the highest at stations A and B, and decrease along the salinity gradient from station C to Station D. Mean dissolved oxygen concentrations are also highest at station A, and decline along the salinity gradient.

Table 2 Overall average (for both top and bottom and over the sampling period) mean water quality values for each station. Standard deviation (STD) for all samples at each station are in parentheses. Abbreviations: DO = dissolved oxygen, NH4=ammonium, NO2+3=nitrate+nitrite, PO4=phosphate, SiO4=silicate, and Chl=chlorophyll.

Water Quality Variable (units)	Station (number of samples)							
	A (45)		B (51)		C (47)		D (58)	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD
DO (mg/l)	8.90	(4.41)	8.94	(4.31)	7.78	(2.04)	7.53	(2.02)
Salinity (psu)	16.69	(8.97)	21.06	(8.98)	24.90	(8.71)	26.71	(8.14)
Temperature (°C)	24.00	(7.56)	22.29	(8.23)	24.03	(7.47)	23.14	(7.78)
NH4 (umol/L)	2.06	(2.43)	0.97	(0.63)	1.55	(2.26)	1.84	(3.24)
NO2+3 (umol/L)	20.14	(44.75)	1.98	(3.06)	1.17	(1.55)	1.42	(1.95)
P04 (umol/L)	3.40	(4.37)	2.09	(3.14)	1.68	(2.43)	1.48	(2.50)
Si04 (umol/L)	136.73	(92.18)	100.42	(74.10)	79.61	(50.55)	72.79	(65.67)
Chl (mg/l)	27.48	(20.78)	17.50	(7.85)	9.43	(3.55)	9.10	(7.60)
pH	8.45	(0.27)	8.36	(0.28)	8.20	(0.23)	7.96	(0.30)

The sampling period was characterized by generally dry conditions from January 2013 to February 2015, which maintained high salinities near 28 psu over the entire estuary for the entire period, and wet conditions from April 2015 to July 2017 (Figure 3). The high salinities were maintained even though there were 7 high inflow events where flows were greater than 1900 cfs per day because none of the events were for long periods of time. In contrast, nearly continuous flows occurred from March 2015 to July 2015, which caused salinity to average near zero over the entirety of San Antonio Bay by July 2015 (Figure 3).

As expected, secchi disk depths correlated with salinity (Figure 3). As flow is high, turbidity increases, so that the secci depth decreases. There does not appear to be a long-term trend with dissolved oxygen, but there are seasonal trends where dissolved oxygen is always lower in summer and higher in winter (Figure 3).

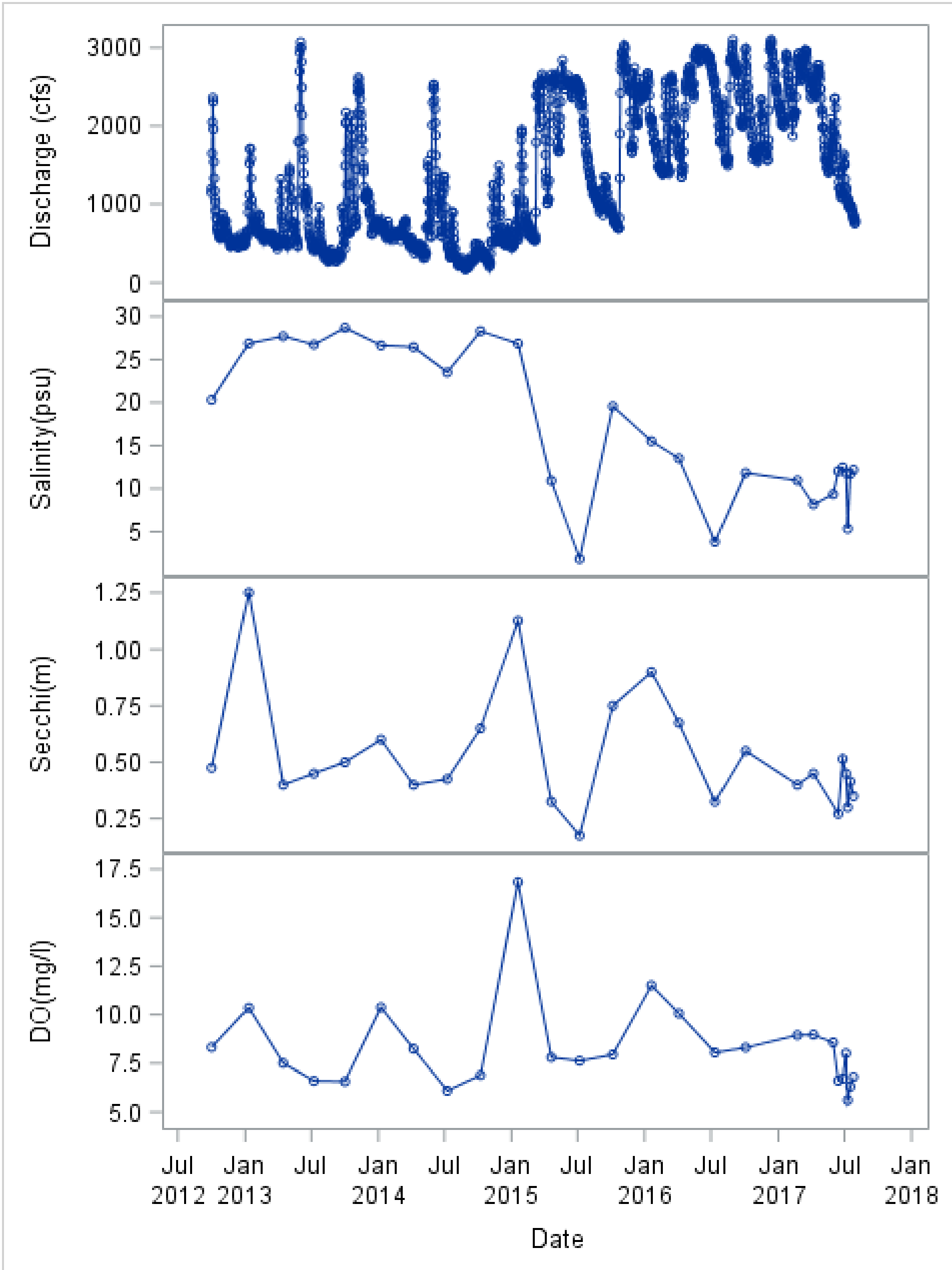

Figure 3 River flow and San Antonio Bay water quality. Daily flow at gage USGS 08188800 Guadalupe River near Tivoli, TX and mean estuary-wide salinity, Seechi disk depth, and dissolved oxygen.

5.2 Guadalupe Estuary During the Study Period: Benthos

The four stations (A through D) in San Antonio Bay lie along a gradient from river to marine end at the Gulf Intracoastal Waterway (Figure 1) and that is reflected in the differences in salinity among the stations as well where salinity increases from A to B, B to C, and C to D (Figure 4A). Analysis of variance showed that the stations were all significantly different for salinity ($P < 0.0001$, Table 3A).

The four stations had different abundances ($P < 0.0001$, Table 3B), biomasses ($P = 0.0002$, Table 3C), and diversity ($P < 0.0001$, Table 3D). Station A, closest to the river, had the highest macrofauna abundance (Figure 4B), biomass (Figure 4C), and diversity (Figure 4D). Station D, closest to the Gulf connection had the second highest biomass in 2016, but station B had the second highest biomass in 2017 (Figure 4C). All stations had similar trends over time where there were peaks in spring 2016 and spring 2017, and low values in summer of 2016 and 2017. The flooding in May 2015, which caused salinity to go to near zero that led to a loss of benthos, and then increased rapidly during the summer of 2015 as salinities increased following the flood.

There were a total of 42 species found over the study period (Table 4A). The capitellid polychaete *Mediomastus ambiseta* was the most abundant species overall and was especially dominant at station A. Overall, *M. ambiseta* made up about 62 % of the total number of organisms found. Another polychaete *Streblospio benedicti* was the second most dominant species and it made up about 21% of the organisms. The bivalve species *Mulinia lateralis* was the third dominant species at about 3%. This species was found predominantly at stations A and B, which are the stations closest to the river mouth. Two more bivalves, *Mulinia lateralis* and *Rangia cuneata* made up about 3% of the community, and were also found primarily in stations A and B. Together the five most dominant species made up 90% of all organisms found. Only six species occur at all four stations. The high diversity found in San Antonio Bay is made up of rare organisms. For example 19 species were found in only one station, and 7 species were found only two stations. Together these 26 rare species made up only 0.6% of all species found. Rare species were more common in the marine parts of the bay, because 12 occurred in stations A and B, and 18 occurred in stations C and D.

More organisms were found in spring and winter than in summer and fall (Table 4B). Even though more organisms were found in spring, higher diversity was found in winter. Only the top most abundant 12 species were found year-round, and 17 species were found in only one season.

Table 3 **A 2-way analysis of variance (ANOVA) of salinity, abundance, biomass, and diversity in the Guadalupe estuary during the study period (October 2012 – July 2017).**

A. Salinity(psu)					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	10	5.91E+03	5.91E+02	529.99	<.0001
Station	3	1.12E+03	3.73E+02	334.08	<.0001
Date*Station	30	3.49E+02	1.16E+01	10.43	<.0001
Error	44	4.91E+01	1.12E+00		
Corrected Total	87	7.43E+03			
B. Abundance (n/m^2)					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	10	2.78E+09	2.78E+08	12.1	<.0001
Station	3	3.17E+09	1.06E+09	45.92	<.0001
Date*Station	30	3.06E+09	1.02E+08	4.44	<.0001
Error	88	2.02E+09	2.30E+07		
Corrected Total	131	1.10E+10			
B. Biomass (g/m^2)					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	10	2.45E+04	2.45E+03	1.32	0.2318
Station	3	3.93E+04	1.31E+04	7.08	0.0003
Date*Station	30	7.44E+04	2.48E+03	1.34	0.1486
Error	48	2.50E+04	5.21E+02		
Corrected Total	71	4.17E+04			
C. Diversity (S/core)					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	10	1.72E+01	1.72E+00	16.89	<.0001
Station	3	2.21E+01	7.36E+00	72.14	<.0001
Date*Station	30	1.07E+01	3.56E-01	3.49	<.0001
Error	87	8.88E+00	1.02E-01		
Corrected Total	130	5.86E+01			

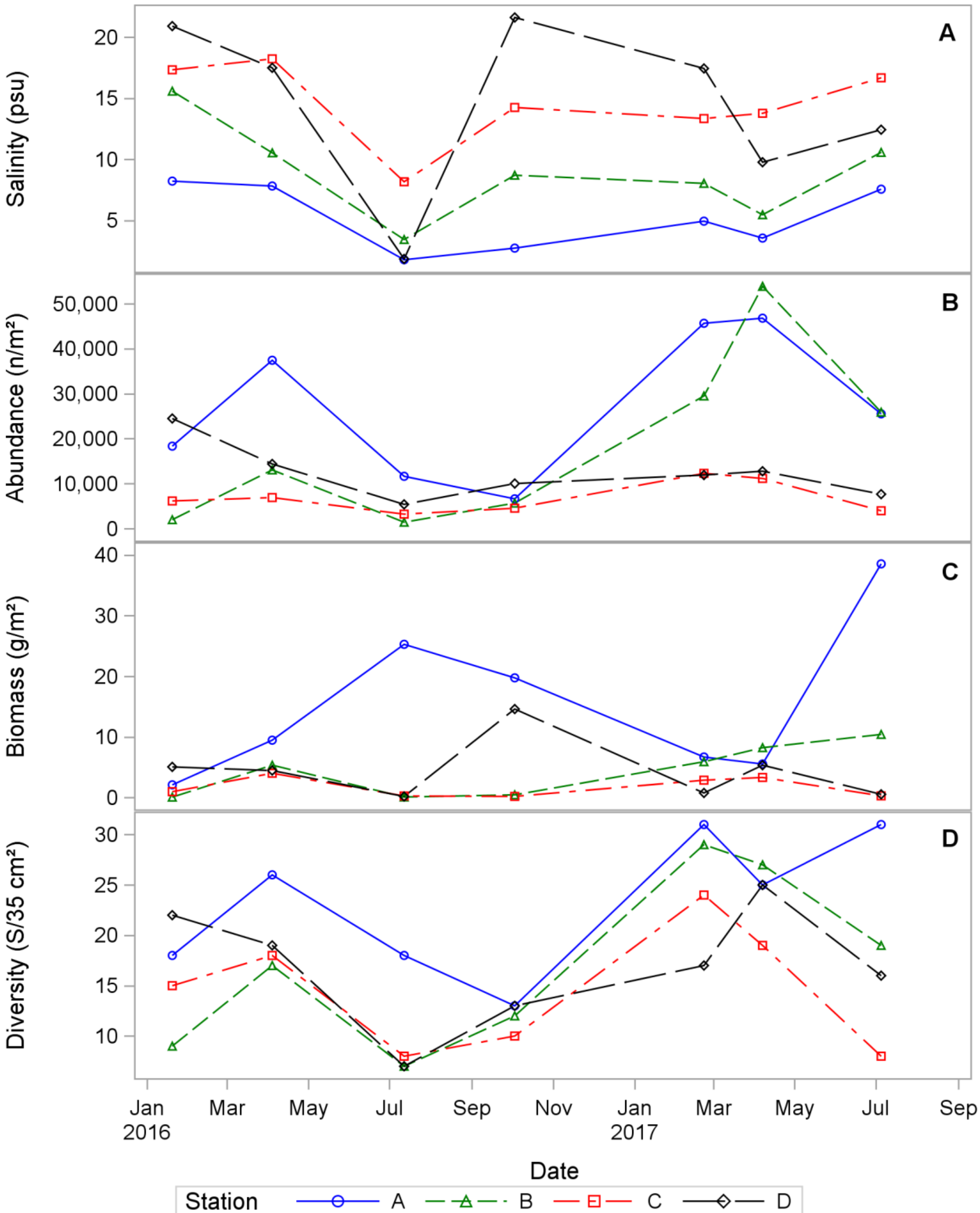

Figure 4 Macrofauna characteristics in the Guadalupe Estuary, average by station over the sampling period. Subfigures: A) Salinity, B) Abundance, C) Biomass, and D) Diversity.

Table 4 Species average abundance (n m-2) in Guadalupe Estuary over the period January 2016 to July 2017 period. A) By stations. B) By season.

A.		Station					
Rank	Species Name	A	B	C	D	Mean	Cum%
1	*Mediomastus ambiseta*	15,776	11,670	4,322	8,793	10,140	61.96%
2	*Streblospio benedicti*	7,050	4,336	1,094	1,418	3,475	21.23%
3	*Mulinia lateralis*	851	959	297	135	561	3.43%
4	*Capitella capitata*	594	675	0	41	328	2.00%
5	*Rangia cuneata*	999	81	0	0	270	1.65%
6	*Macoma mitchelli*	122	338	365	41	216	1.32%
7	*Texadina sphinctostoma*	554	257	14	0	206	1.26%
8	*Glycinde solitaria*	0	68	419	284	192	1.17%
9	Nemertea (unidentified)	338	176	41	108	165	1.01%
10	*Hermundura ocularis*	68	14	108	378	142	0.87%
11	Oligochaeta (unidentified)	486	27	0	0	128	0.78%
12	*Hobsonia florida*	392	41	0	0	108	0.66%
13	*Acteocina canaliculata*	0	0	68	311	95	0.58%
14	*Tellina texana*	0	0	0	243	61	0.37%
15	*Hemicyclops sp.*	0	0	14	176	47	0.29%
16	*Cyclaspis varians*	0	0	27	95	30	0.18%
17	*Polydora cornuta*	81	0	0	0	20	0.12%
18	*Paraprionospio pinnata*	0	14	54	0	17	0.10%
19	*Gyptis brevipalpa*	0	0	41	27	17	0.10%
20	*Spiochaetopterus costarum*	0	0	0	68	17	0.10%
21	Chironomidae (larvae)	54	0	0	0	14	0.09%
22	Ostracoda (unidentified)	0	54	0	0	14	0.09%
23	Turbellaria (unidentified)	0	14	14	27	14	0.09%
24	*Pectinaria gouldii*	0	0	0	54	14	0.09%
25	*Polydora websteri*	27	0	0	0	7	0.04%
26	*Monoculodes* sp.	14	0	0	14	7	0.04%
27	*Eulimastoma* sp.	0	27	0	0	7	0.04%
28	*Alitta succinea*	0	0	0	27	7	0.04%
29	*Dipolydora caulleryi*	0	0	0	27	7	0.04%
30	*Hypereteone heteropoda*	14	0	0	0	3	0.02%
31	*Edotia triloba*	14	0	0	0	3	0.02%
32	*Paranaitis speciosa*	0	14	0	0	3	0.02%
33	*Americamysis almyra*	0	14	0	0	3	0.02%
34	*Haploscoloplos foliosus*	0	0	14	0	3	0.02%
35	*Lepidophthalamus louisianensis*	0	0	14	0	3	0.02%
36	*Amygdalum papyrium*	0	0	0	14	3	0.02%
37	*Ensis minor*	0	0	0	14	3	0.02%
38	*Xanthidae* (unidentified)	0	0	0	14	3	0.02%
39	*Phoronis architecta*	0	0	0	14	3	0.02%
40	*Xenanthura brevitelson*	0	0	0	14	3	0.02%
41	*Tagelus plebeius*	0	0	0	14	3	0.02%
42	*Oxyurostylis* sp.	0	0	0	14	3	0.02%
	Total	27,434	18,779	6,906	12,365	16,365	
	Number of Species	17	18	16	26	42	

B.		Season				
Rank	SpName	Winter	Spring	Summer	Fall	Mean
1	*Mediomastus ambiseta*	11,381	15,447	6,713	3,900	9,360
2	*Streblospio benedicti*	4,385	5,271	1,631	1,749	3,259
3	*Mulinia lateralis*	638	768	544	24	493
4	*Capitella capitata*	355	544	225	47	293
5	*Rangia cuneata*	106	496	319	47	242
6	*Macoma mitchelli*	485	225	35	24	192
7	*Texadina sphinctostoma*	47	425	236	24	183
8	*Glycinde solitaria*	260	272	95	95	180
9	Nemertea (unidentified)	177	236	83	165	165
10	*Hermundura ocularis*	106	189	130	142	142
11	Oligochaeta (unidentified)	130	47	260	24	115
12	*Hobsonia florida*	95	35	177	142	112
13	*Acteocina canaliculata*	83	248	0	0	83
14	*Hemicyclops* sp.	24	24	0	236	71
15	*Tellina texana*	213	0	0	0	53
16	*Cyclaspis varians*	12	71	24	0	27
17	*Polydora cornuta*	24	0	47	0	18
18	*Paraprionospio pinnata*	47	12	0	0	15
19	*Gyptis brevipalpa*	12	47	0	0	15
20	*Spiochaetopterus costarum*	0	59	0	0	15
21	Turbellaria (unidentified)	0	35	0	24	15
22	Ostracoda (unidentified)	47	0	0	0	12
23	*Pectinaria gouldii*	24	12	12	0	12
24	Chironomidae (larvae)	0	0	47	0	12
25	*Alitta succinea*	12	0	0	24	9
26	*Dipolydora caulleryi*	24	0	0	0	6
27	*Polydora websteri*	24	0	0	0	6
28	*Monoculodes* sp.	12	12	0	0	6
29	*Eulimastoma* sp.	0	24	0	0	6
30	*Tagelus plebeius*	0	0	0	24	6
31	Xanthidae (unidentified)	0	0	0	24	6
32	*Amygdalum papyrium*	12	0	0	0	3
33	*Ensis minor*	12	0	0	0	3
34	*Haploscoloplos foliosus*	12	0	0	0	3
35	*Paranaitis speciosa*	12	0	0	0	3
36	*Phoronis architecta*	12	0	0	0	3
37	*Americamysis almyra*	0	12	0	0	3
38	*Edotia triloba*	0	12	0	0	3
39	*Lepidophthalamus louisianensis*	0	12	0	0	3
40	*Xenanthura brevitelson*	0	12	0	0	3
41	*Hypereteone heteropoda*	0	0	12	0	3
42	*Oxyurostylis* sp.	0	0	12	0	3
	Total	18,783	24,547	10,602	6,715	15,162
	Number of Species	30	26	18	17	42

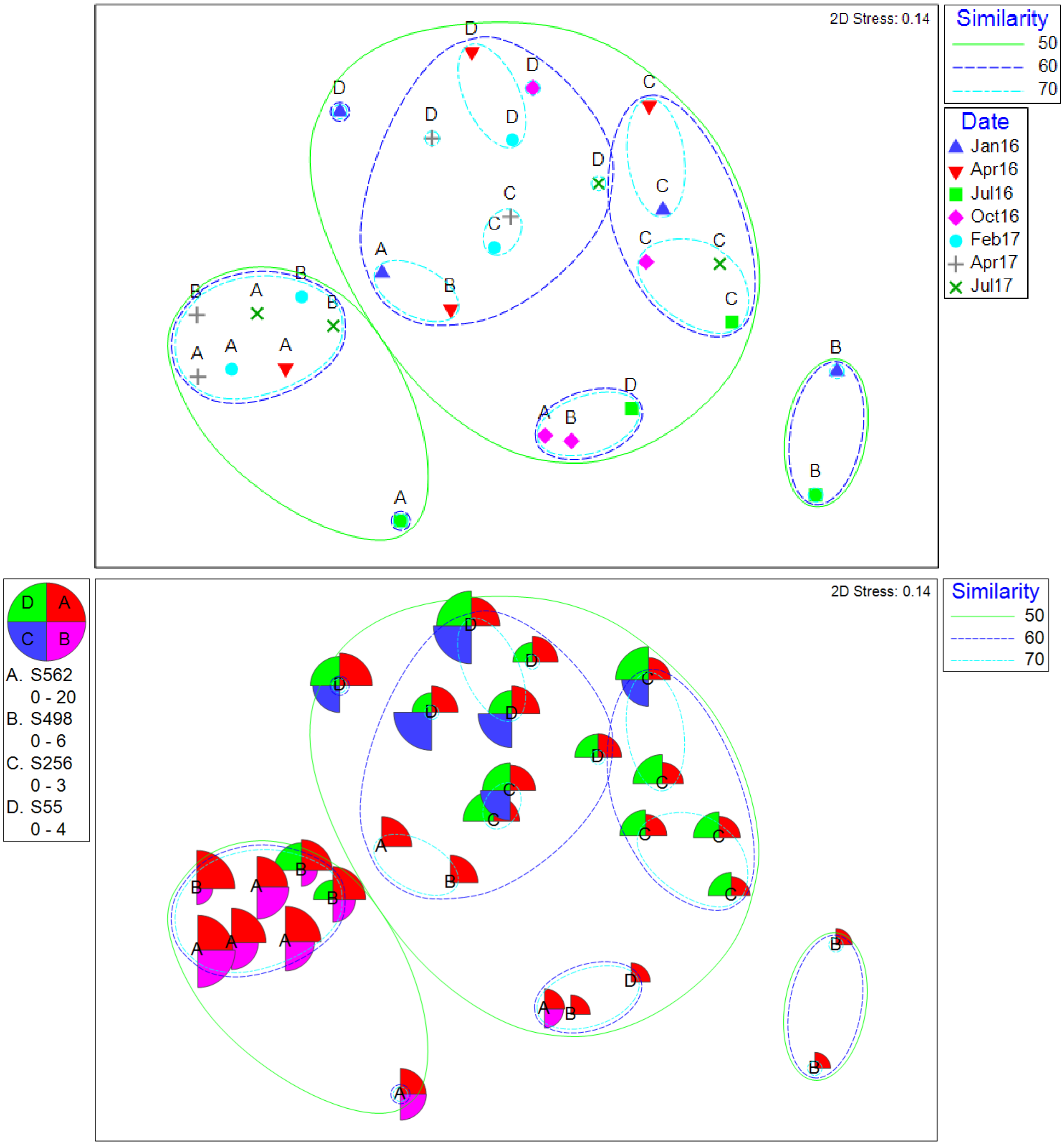

Figure 5 **Multidimensional Scaling plot of macrofaunal community structure in the Guadalupe Estuary. Top: symbols are labeled by date-station combination. Bottom: Symbols are bubble plots of indicator species abundances. S562 = *Mediomastus ambiseta*, S498 = *Rangia cuneata*, S256 = *Acteocina canaliculata*, and S55 = *Glycinde solitaria*. Lines indicate percent similarity of samples from a cluster analysis.**

Macrofauna community similarity for each station-date combination is depicted in a multidimensional scaling plot (MDS, Figure 5). Significant clustering of communities are represented by similarity contours that are overlaid on the MDS plot. In general, there is a trend in the plot from right to left of communities over time, and from bottom to top for the fresher stations A and B to the marine stations C and D (Figure 5 Top). These represent changes in salinity over time and space.

The station changes over time are illustrated by overlaying the relative abundance of indicator species (Figure 5 Bottom). Four indicator species were chosen based on distribution and dominance in Table 4: the polychaete *Mediomastus ambiseta* (S562) and the bivalve *Rangia cuneata* (S498) are indicator species for freshwater inflow effects; whereas the bivalve *Acteocina canaliculata* (S256) and the polychaete *Glycinde solitaria* (S55) are indicators of more marine conditions in stations C and D.

5.3 Long-term Analyses of the Guadalupe Estuary: Benthos

Benthic data has been collected in the Guadalupe Estuary since 1987 (Figure 6). The recent period between April 2015 and July 2017 was a relatively wet period where salinity averaged 10.8 psu over the whole estuary. This contrasted with the period between July 2013 and January 2015, which was one of the most extended dry periods in the record, averaging about 22.6 psu over the entire estuary-bay system for the entire (Figure 6A). The highest estuary-wide average salinity however, reaching an average of 35 psu among all stations, occurred in October 2011. The other months when salinity was also high were October 1988 (25 psu), October 1996 (29 psu), October 1999 (25 psu), October 2008 (27 psu), and July 2009 (29 psu). So the dry period in 2013-2015 was typical of past dry periods, it was just longer. However, prior to 2011, the highest recorded average salinity was 6 psu less than observed that October 2011, which was the most acute dry period.

There has been a long-term decline in abundance over the entire range of sampling dates, and this continued during the current sampling period. Biomass has fluctuated, sometimes high biomass occurs during high salinity periods as it did between 1994 – 1996, 2000, 2005 – 2006, 2008-2009, and 2014. But high biomass always occurs following low salinity periods, indicating a lagged effect. The biomass was relatively low over the current sampling period compared to the long-term trends, but there was an anomalous peak in April 2014 when the highest biomass ever recorded (50 g/m^2) was observed. Diversity fluctuates with salinity, being higher during high salinity periods. Diversity and biomass have the highest correlation with salinity over time, Spearman Correlation Coefficients are 0.48 ($P < 0.0001$) and 0.45 ($P < 0.0001$) respectively. Abundance is not correlated with salinity (Spearman = 0.09, $P = 0.3279$).

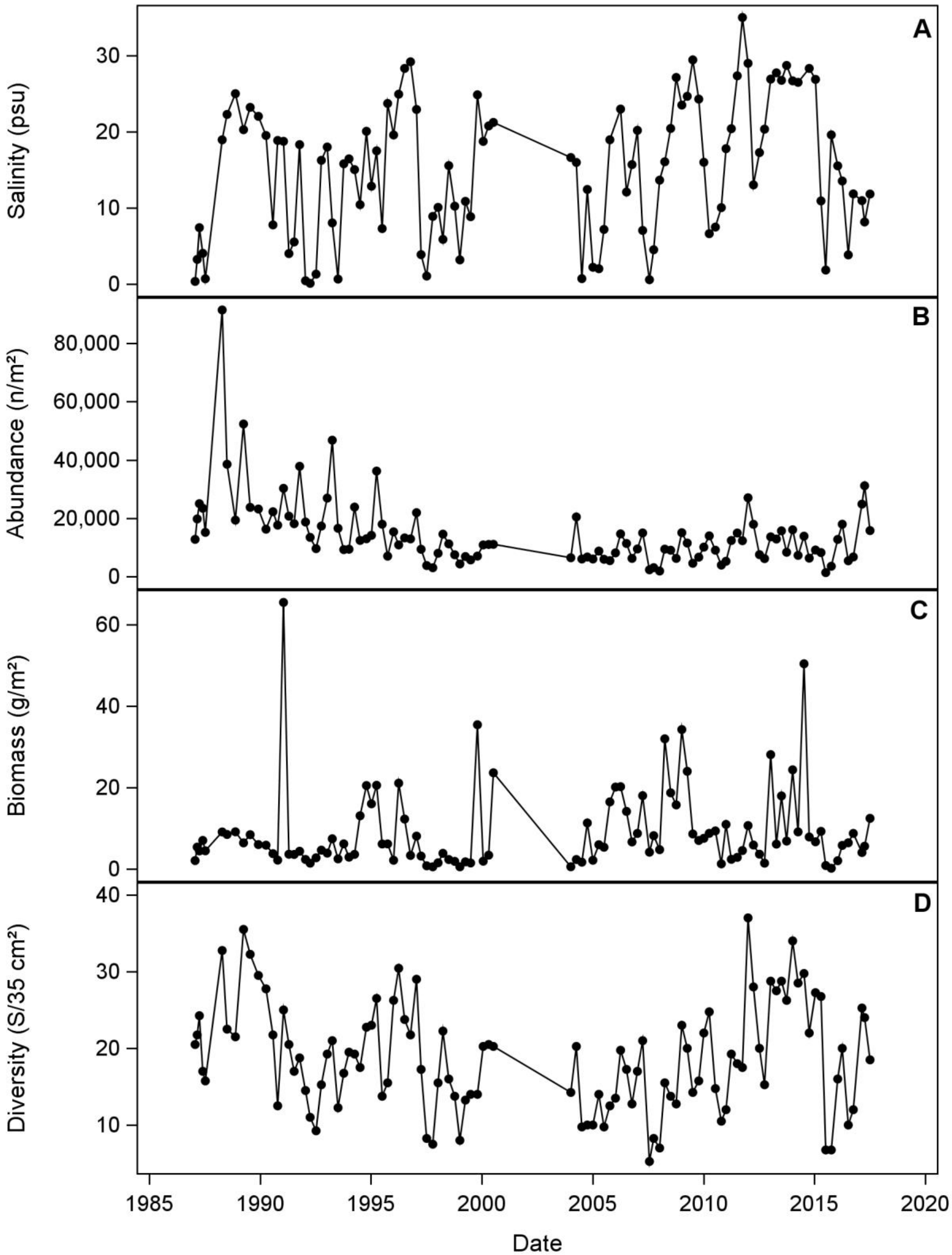

Figure 6 Long-term estuary-wide times series. A) salinity, B) abundance, C) biomass, and D) diversity, number of species (S).

5.4 Water Column Conditions in Mid-Coastal Estuaries: Inorganic Matter

Water quality measurements were also made in the Nueces (NC) and Lavaca-Colorado (LC) estuaries. The salinity change over time is largely parallel among the three estuaries (Figure 7). The wet period in April 2015 to July 2015 can be seen as lower salinities in all three estuaries. For the period April 2013 to October 2015, the Guadalupe Estuary GE) has the lowest mean salinity 14.5 psu, the Lavaca-Colorado Estuary has an average salinity of 22.5 psu, and the Nueces Estuary had the highest average salinity 30.2 psu.

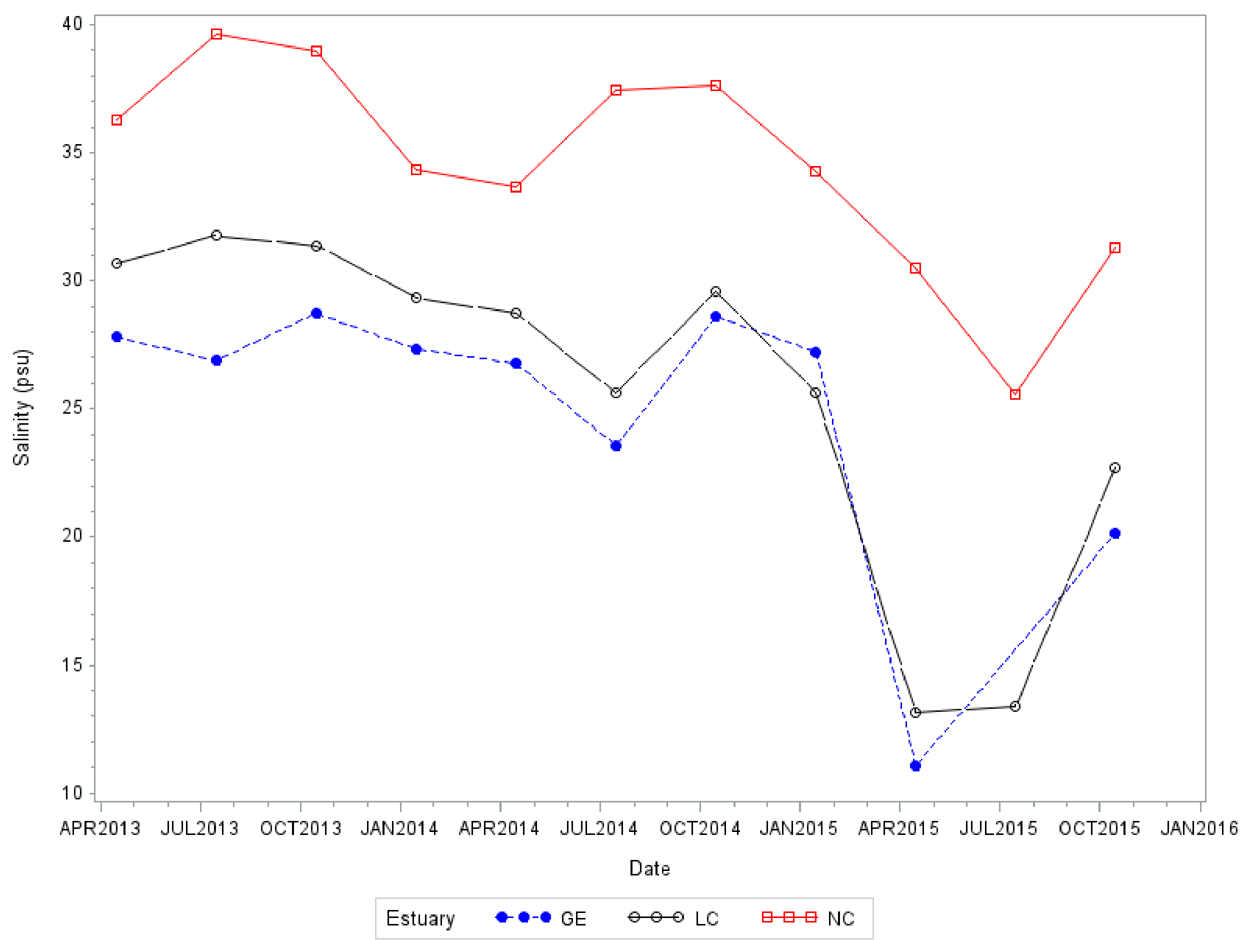

Figure 7 Average estuary-wide salinity for each sampling period during the current study in three mid-coast estuaries.

Salinity at stations generally follows the expected gradient of lower values near the freshwater input source relative to the point of exchange with the Gulf of Mexico. In the Guadalupe Estuary, station C was higher than D in July in all three years (2013 – 2015) (Figure 8).

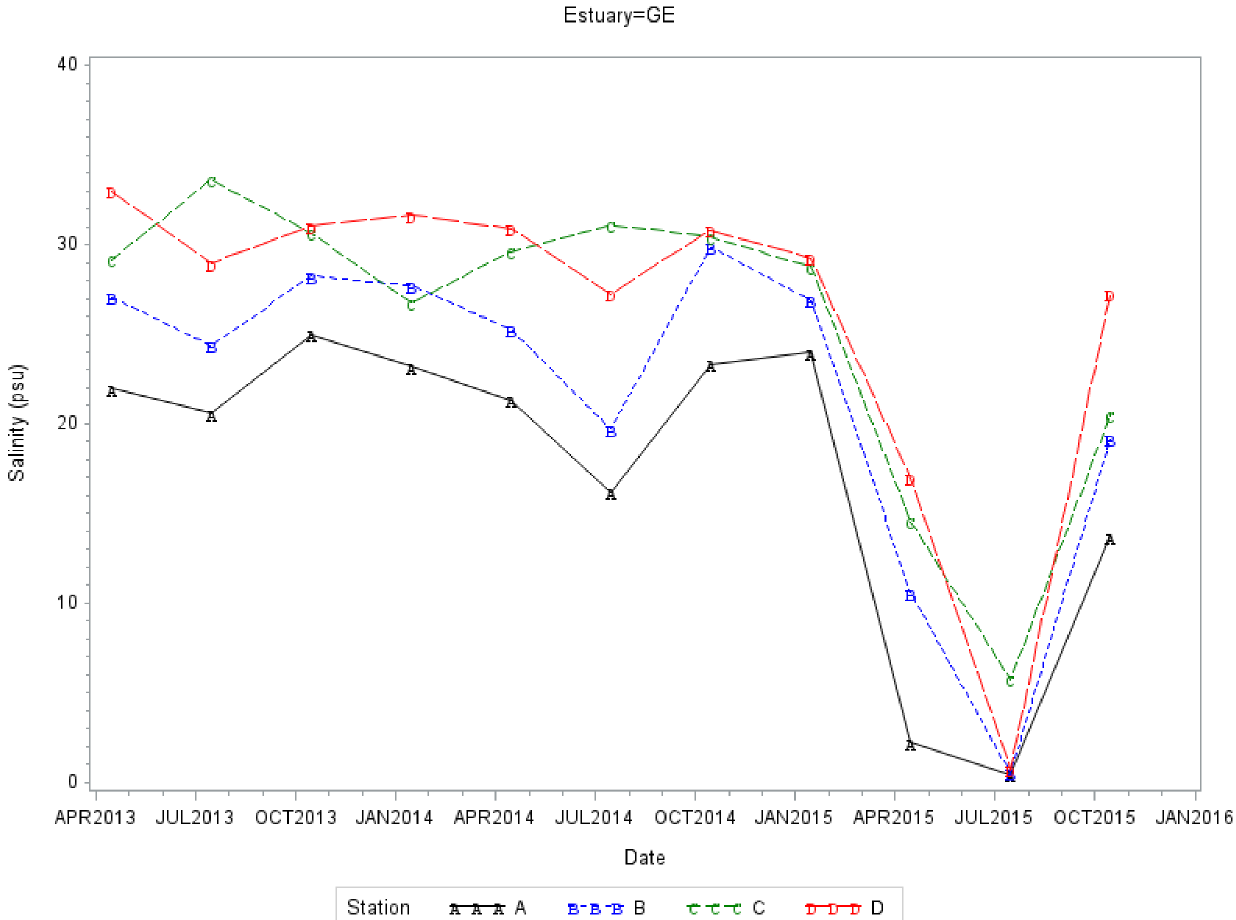

Figure8 Salinity at stations within the Guadalupe Estuary (GE).

The Nueces Estuary is a "reverse estuary" so in dry periods the highest salinities are at stations A and B near the mouth of the Nueces River as it was in April 2013 and October 2013 (Figure 9). The Nueces River was running in July 2014 and July 2015 so salinities were lower at stations A and B during that time.

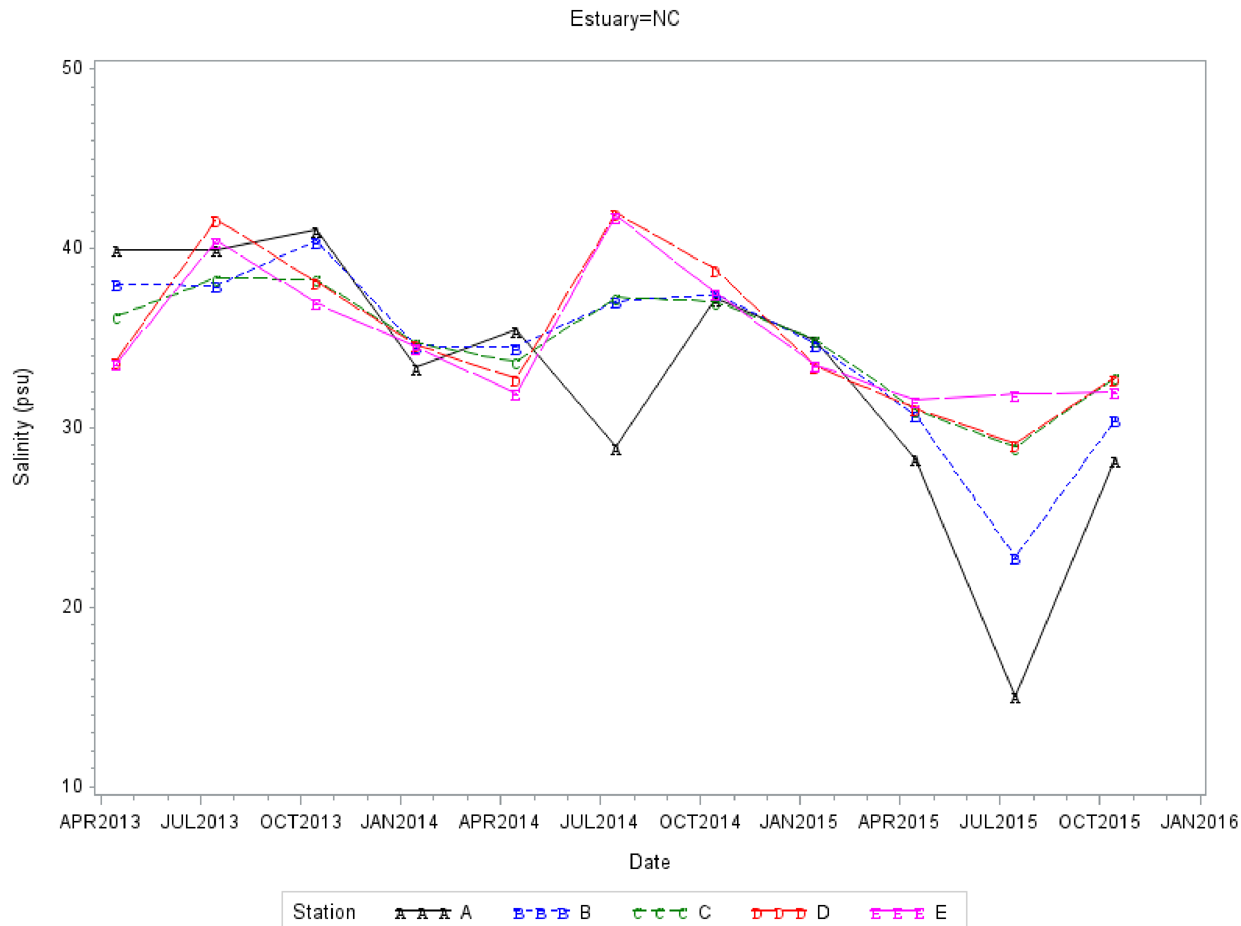

Figure 9 Salinity at stations within the Nueces Estuary (NC).

The Lavaca-Colorado Estuary has two river sources the Lavaca River, which enters Lavaca Bay (near stations A and B); and the Colorado River, which enters Matagorda Bay (near stations E and F) (Figure 1). Because of the two inflow sources, station F in Matagorda Bay sometimes takes on characteristics of stations A and B in Lavaca Bay when flows are high, as it did in April and July 2015 (Figure 10). However, when flows are lower, as in July 2014, insufficient flow goes down the Colorado River and salinity at stations E and F remain high. On only two occasions, January 2014 and January 2015, Station F had the lowest salinity values, which might indicated that more flow was coming in from the Colorado River than the Lavaca River at those times. The period between April 2013 and April 2014 was so dry that all stations had uniformly high salinities, and thus there was no estuarine gradient in the system during that period.

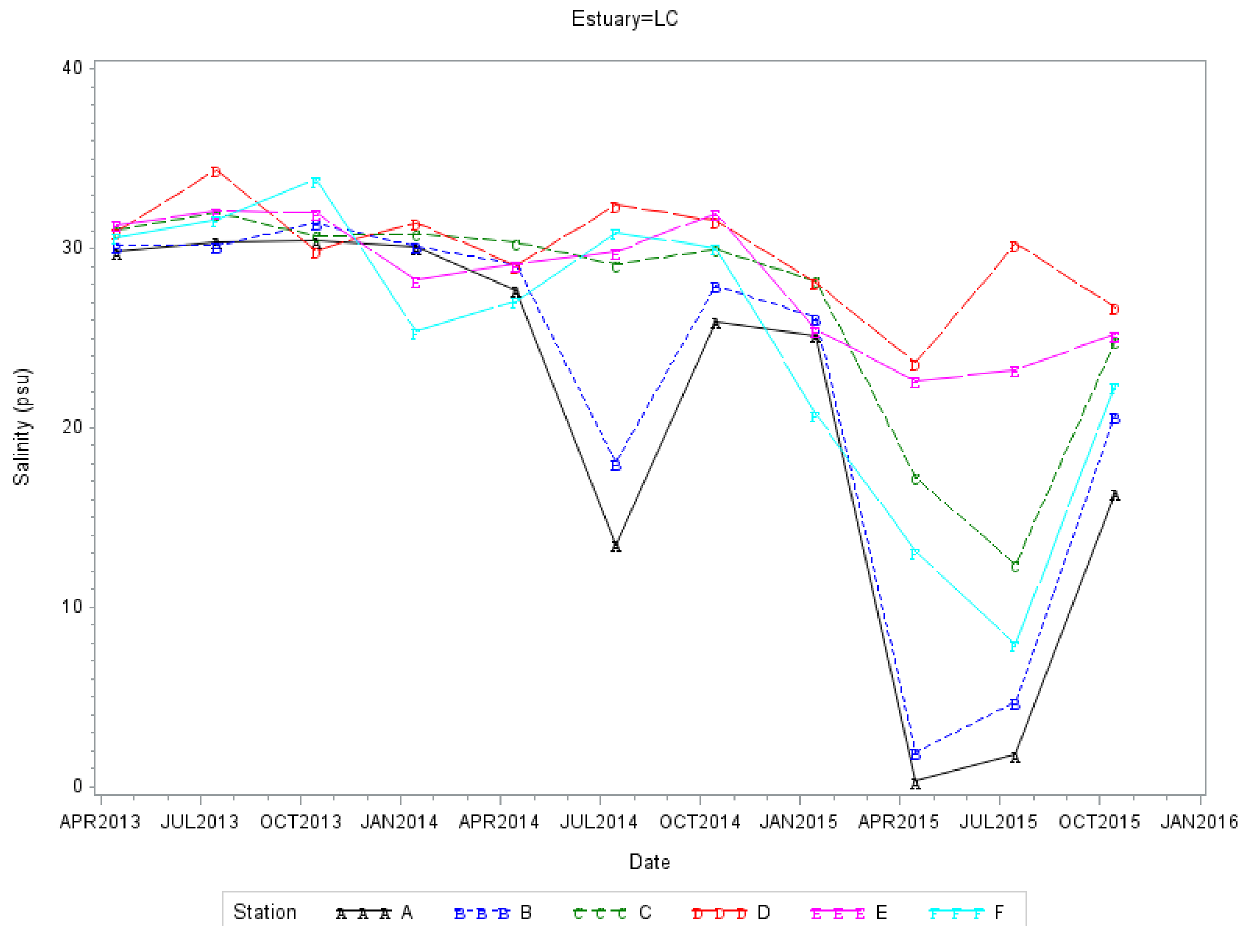

Figure 10 Salinity at stations within the Lavaca-Colorado Estuary (LC).

There is a relationship between the overall average salinity and nutrient concentrations during the study period, because the Guadalupe Estuary had the lowest average salinity and the highest average nitrate+nitrite (NO_{2+3}), phosphate (PO_4), and silicate (SiO_4) concentrations (Table 5). Concomitantly, chlorophyll a (Chl) concentrations were highest in the Guadalupe Estuary as well. These trends are true over the long-term, i.e., Guadalupe has lowest salinity (14.5 psu), highest nitrate+nitrite (14.3 uM), and highest chlorophyll (11.3 ug/L); in contrast the Nueces has highest salinity (30.2 psu), lowest nitrate+nitrite (1.43 uM), and lowest chlorophyll (5.7 ug/L) (Table 5). The same trends seen in the current study period are also true for the entire long-term record (Table 6).

Table 5 Estuary-wide average (over all quarterly sampling dates, stations, and depths) concentrations for water quality variables for the period April 2013 - October 2015. Number station-date combinations: LC=36, GE=24, NC=30. Abbreviations: Est=estuary (LC=La vaca-Colorado, GE=Guadalupe, NC=Nueces), n = number of observations, DO=dissolved oxygen, Temp=temperature, NH4=ammonium, NO2+3=nitrate+nitrite, PO4=phosphate, SiO4=silicate, Chl=chlorophyll, pH = acidity, and turbidity (NTU).

Est	n	DO (mg/L)	Salinity (psu)	Temp (°C)	NH_4 (µmol/L)	NO_{2+3} (µmol/L)	PO_4 (µmol/L)	SiO_4 (µmol/L)	Chl (µg/L)	pH	Turbidity (NTU)
GE	220	8.3	23.1	23.3	1.67	5.32	1.97	89.70	15.4	8.22	40
LC	309	7.7	25.5	23.3	1.54	3.43	1.02	38.70	8.4	8.16	16
NC	256	7.1	34.9	23.0	1.25	1.06	1.10	46.00	7.7	8.12	13

Table 6 **Long-term, estuary-wide, average concentrations for all quarterly samples for water quality variables. Period of record: Lavaca-Colorado (LC) Estuary= April 1988 – October 2015, Guadalupe (GE) Estuary = November 1986 – October 2015, Nueces (NC) Estuary = October 1987 – October 2015. Abbreviations: DO=dissolved oxygen, Temp=temperature, NO2+3=nitrate+nitrite, PO4=phosphate, SiO4=silicate, Chl=chlorophyll, and N = number of sampling observation periods.**

	Lavaca-Colorado					Guadalupe					Nueces				
Variable (unit)	**N**	**Mean**	**STD**	**Min**	**Max**	**N**	**Mean**	**STD**	**Min**	**Max**	**N**	**Mean**	**STD**	**Min**	**Max**
DO (mg/l)	108	7.34	1.58	4.60	13.33	94	7.99	1.96	5.05	17.10	111	6.80	1.50	4.19	11.00
Salinity (psu)	111	22.85	6.74	6.19	39.22	103	15.36	8.86	0.10	34.96	111	30.65	6.06	12.34	42.94
Temperature (C)	111	22.18	6.60	7.10	31.54	103	22.50	6.54	7.69	31.45	111	22.61	6.30	8.44	32.50
NH4 (umol/L)	97	2.27	2.89	0.00	23.77	93	2.56	3.90	0.00	33.46	99	1.83	2.21	0.00	13.00
NO2+3 (umol/L)	97	3.53	5.67	0.02	32.23	92	13.29	15.86	0.11	90.23	101	1.40	1.77	0.00	8.64
P04 (umol/L)	97	1.43	2.74	0.09	26.70	92	2.44	2.12	0.06	10.51	101	1.10	0.83	0.14	5.58
SiO4 (umol/L)	97	70.19	48.46	4.57	247.42	92	120.56	88.81	1.63	713.46	101	65.89	44.49	0.33	229.80
Chl (mg/L)	68	7.68	4.16	0.92	21.59	61	12.17	7.46	1.04	35.86	67	6.01	3.70	0.82	16.27
pH	104	8.14	0.37	6.76	10.31	88	8.25	0.33	7.01	9.58	102	8.12	0.21	7.63	9.27
Turbidity (NTU)	16	15.14	17.02	-12.16	52.28	16	32.73	44.30	-10.18	140.68	15	16.82	22.84	-6.11	74.74

Over the current study period, ammonia concentrations generally had parallel responses in all stations within an estuary, except for when large inflow events occurred (Figure 11). Large inflow events occurred in July 2014 and April to July April 2015, which caused spikes in concentrations. The large peak in ammonia found in station A of the Guadalupe estuary in April 2014 was replicated in Stations A of the Nueces estuary, but not in the Lavaca-Colorado estuary. In the Lavaca-Colorado estuary, the highest peaks of ammonia occurred in July 2015 in stations A and B near the Lavaca River mouth, and stations E and F near the Colorado River mouth. Typically ammonia is highest near river sources in all estuaries.

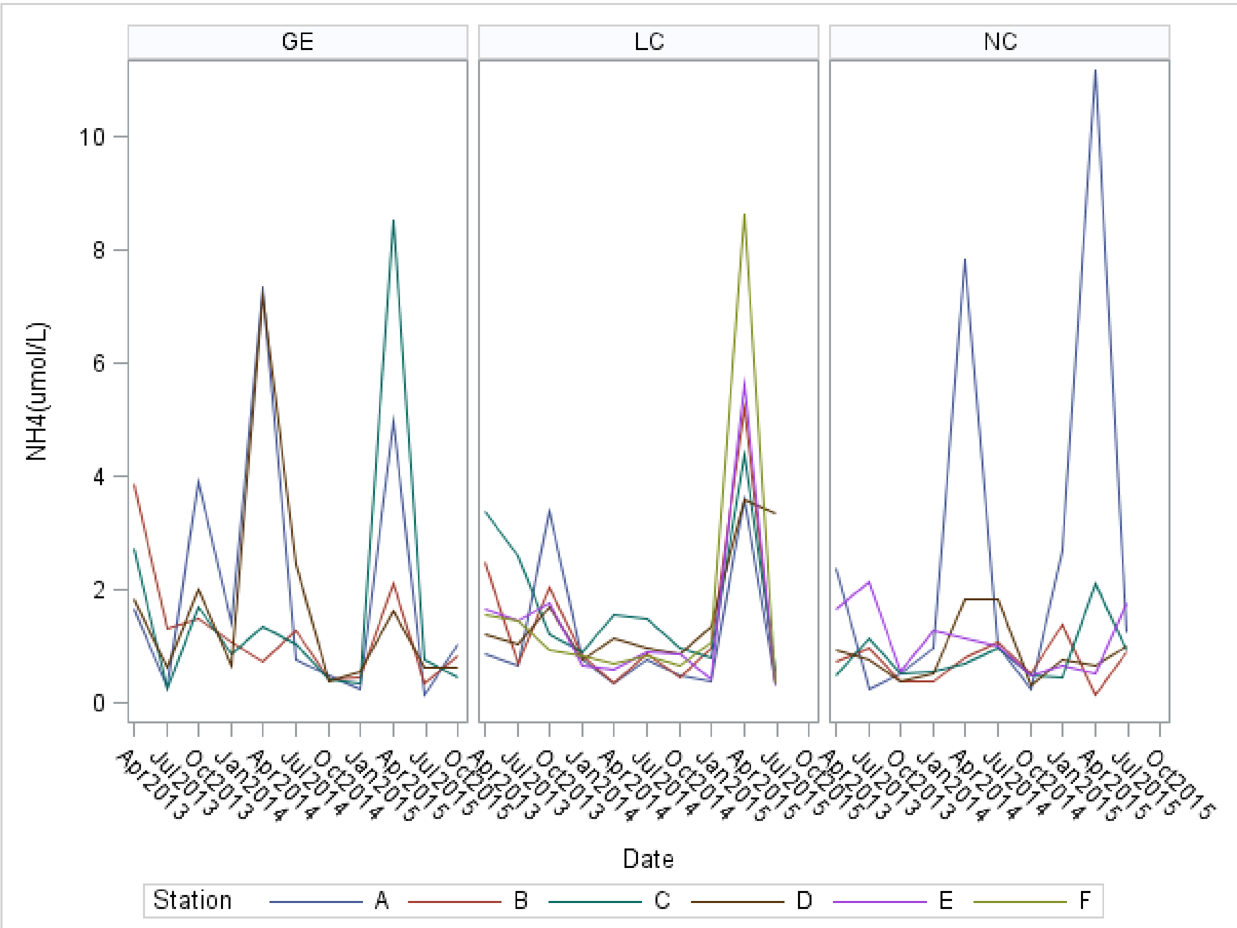

Figure 11 **Ammonia concentrations in three estuaries over the reporting period. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.**

The large inflow event in April 2015 caused a large spike in nitrate+nitrite concentrations at Station A in the Guadalupe Estuary (Figure 12). Over time, nitrate+nitrite concentrations generally had parallel responses in all stations within an estuary (Figure 13).

The large inflow event in April 2015 manifested differently in the Lavaca-Colorado estuary with spikes in nitrate+nitrite in stations A, B, E, and F (Figure 12). The Nueces estuary, with the lowest inflow rates always had the lowest nitrate+nitrite concentrations.

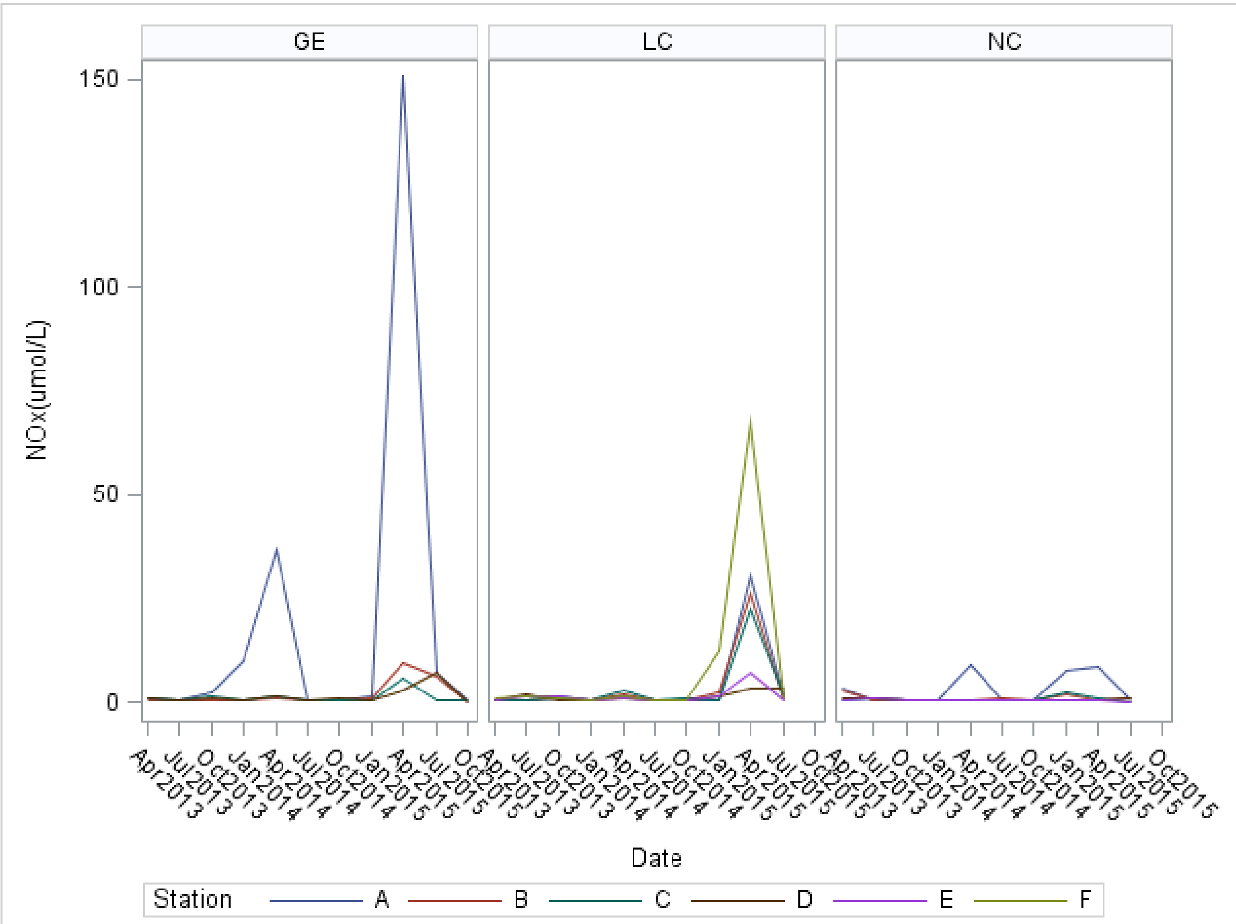

Figure 12 Nitrate+Nitrite (NOx) concentrations in three estuaries over the reporting period. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.

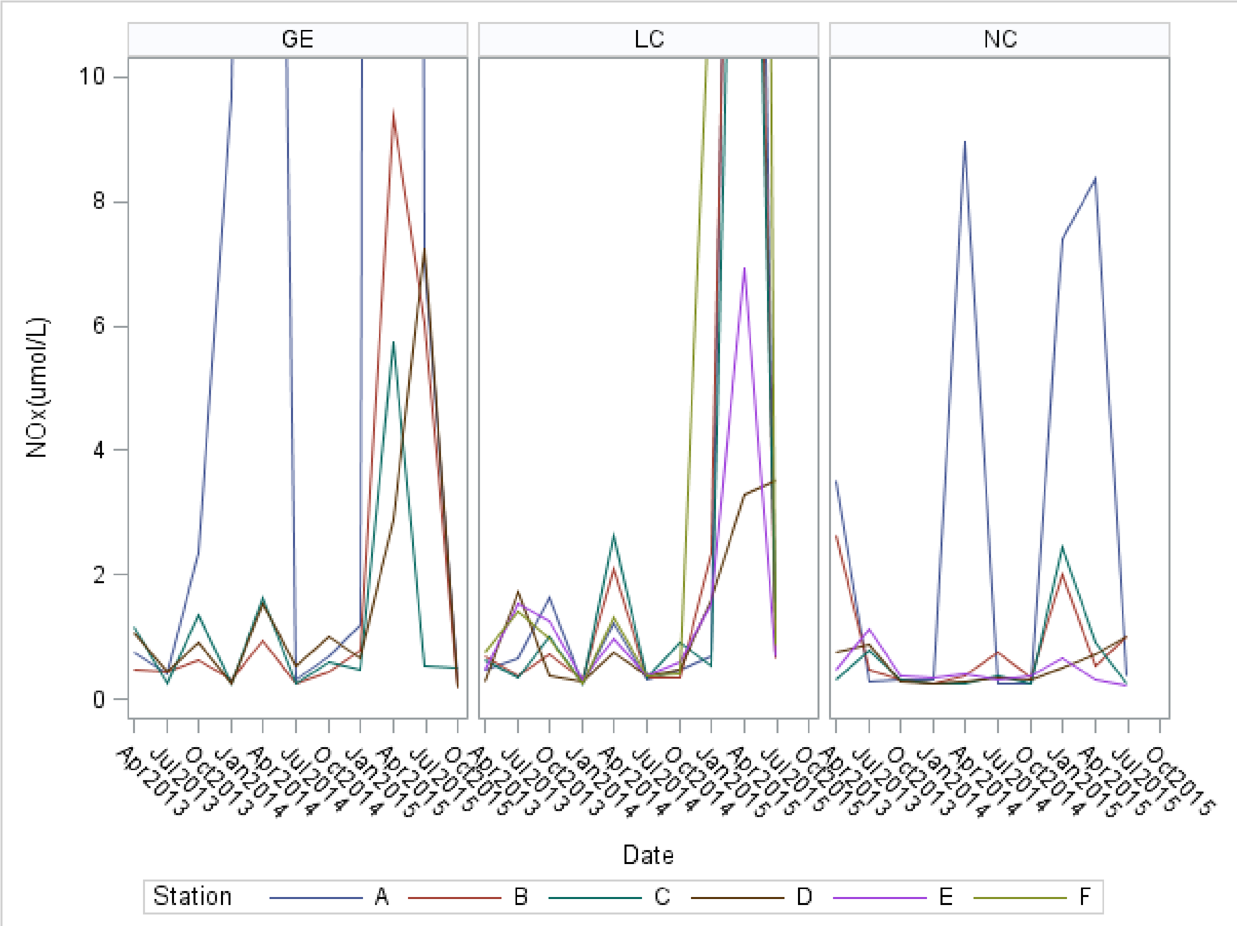

Figure 13 **Nitrate+Nitrite (NOx) concentrations in three estuaries over the reporting period. Concentration maximum is 10 umol/L to show detail for low concentrations. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.**

Over time, phosphate concentrations generally had similar rising and falling responses in all stations within an estuary (Figure 14). The parallel responses were especially evident in the Guadalupe Estuary. In the Nueces Estuary, there were distinct station differences with A and B higher than C and D. In the Lavaca-Colorado Estuary, the highest phosphate concentrations were found in station F, closest to the Colorado River mouth. The Guadalupe Estuary generally had a higher concentrations than Lavaca-Colorado and Nueces estuaries.

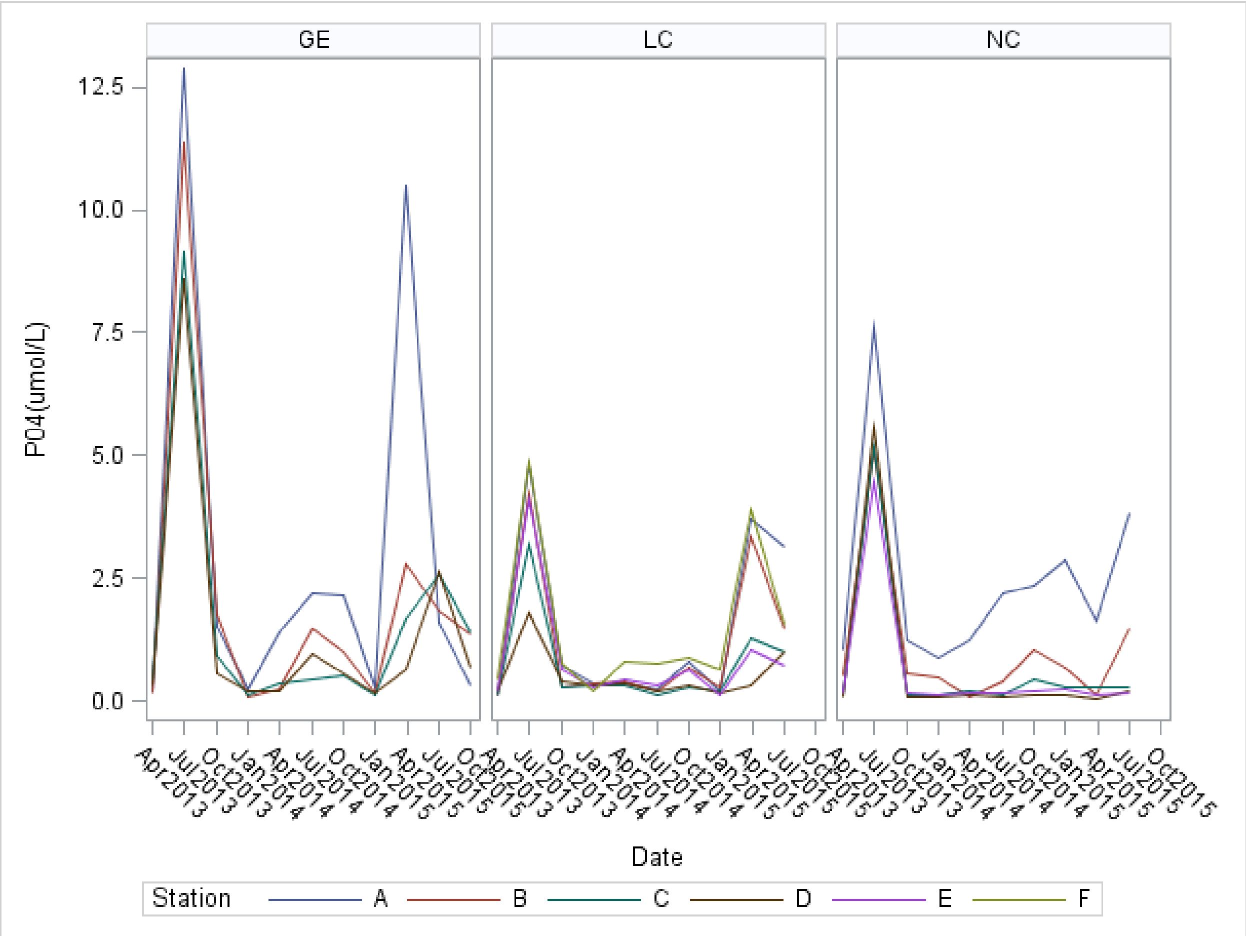

Figure 14 Phosphate (PO4) concentrations in three estuaries over the reporting period. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.

Over time, silicate concentrations generally had parallel responses in all stations within an estuary (Figure 15). Concentrations at Stations A and B in the Nueces were higher than C and D. The temporal pattern of silicate concentrations was very similar at all stations in all estuaries with higher concentrations near the river source and lower concentrations farthest from the river source. The Guadalupe estuary had the highest concentrations.

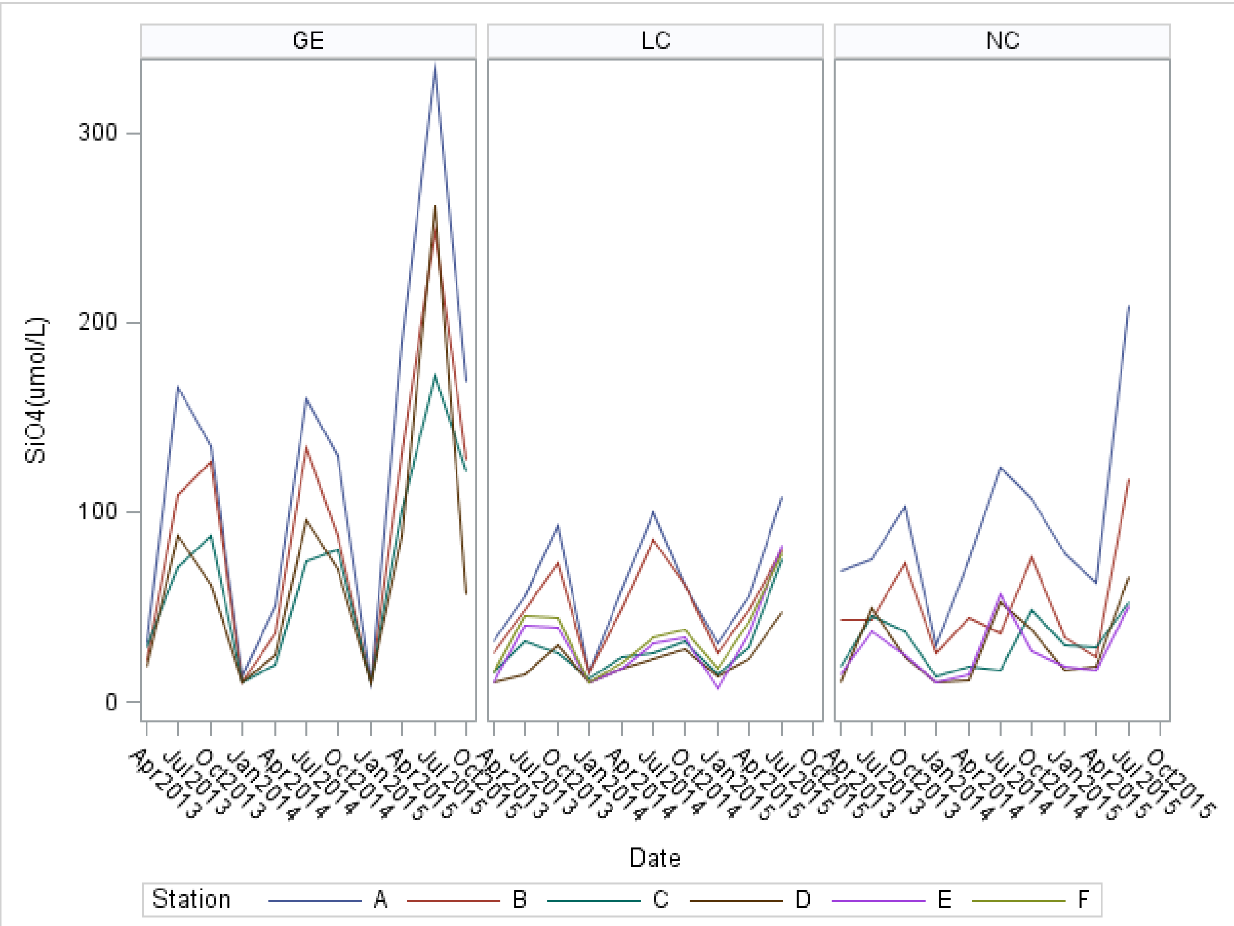

Figure 15 Silicate concentrations in three estuaries over the reporting period. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.

Over time, chlorophyll concentrations generally had parallel responses in all stations within an estuary (Figure 16). Low values were recorded in January 2014 and 2015 and higher values were recorded in April, July and October in all estuaries. In the Guadalupe estuary, stations A and B had the highest concentrations. The highest chlorophyll values in the Lavaca-Colorado and Nueces estuaries occurred in station F for LC and B for NC.

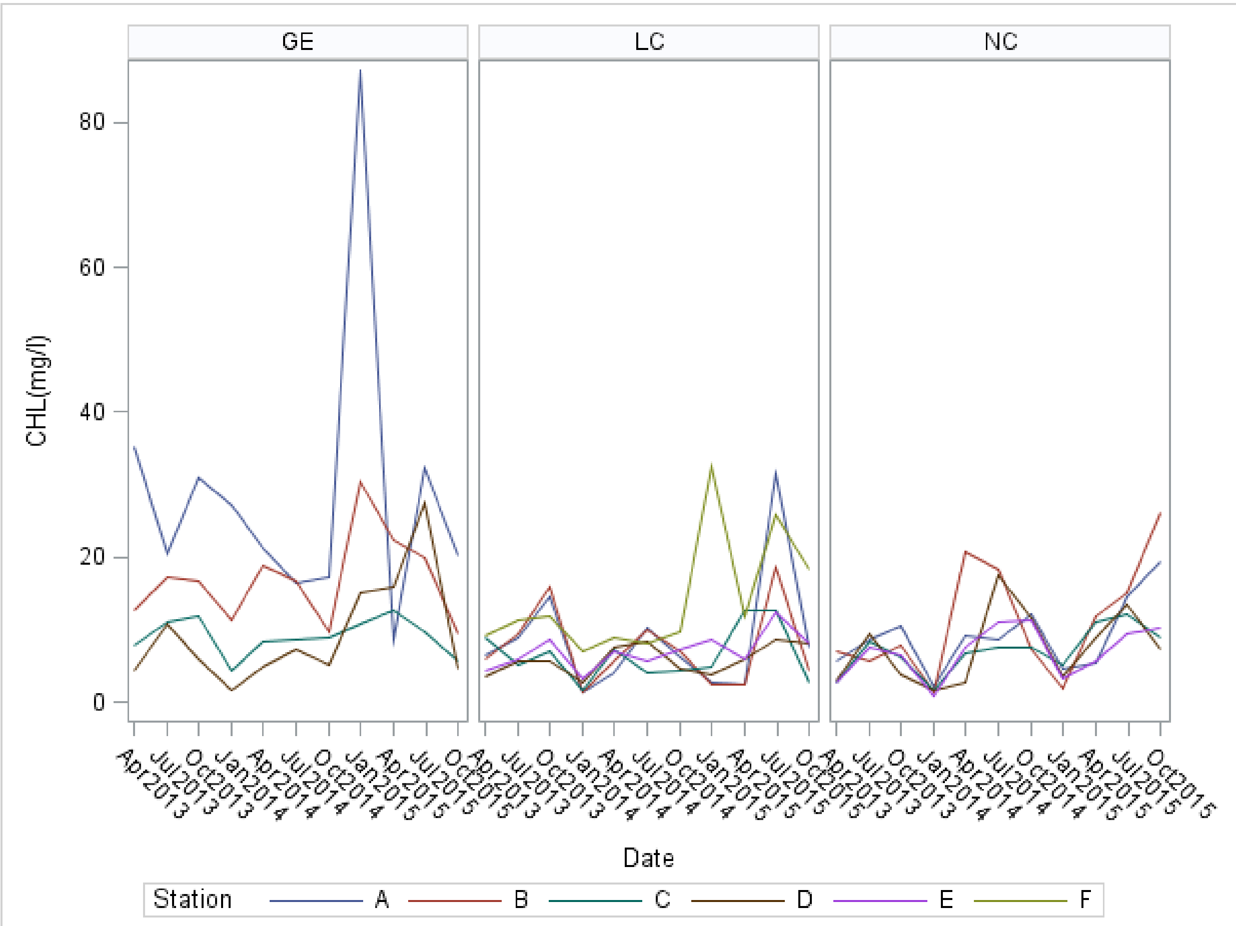

Figure 16 Chlorophyll a concentrations in three estuaries over the reporting period. Abbreviations: LC=Lavaca-Colorado, GE=Guadalupe, NC=Nueces.

Because stations typically have the same patterns over time (Figures 11-16), the estuary-wide average concentrations were calculated and plotted for each variable at each time point for all three estuaries (Figures 17-19). A common pattern is decrease in salinity, increases in nutrients followed by increases in chlorophyll.

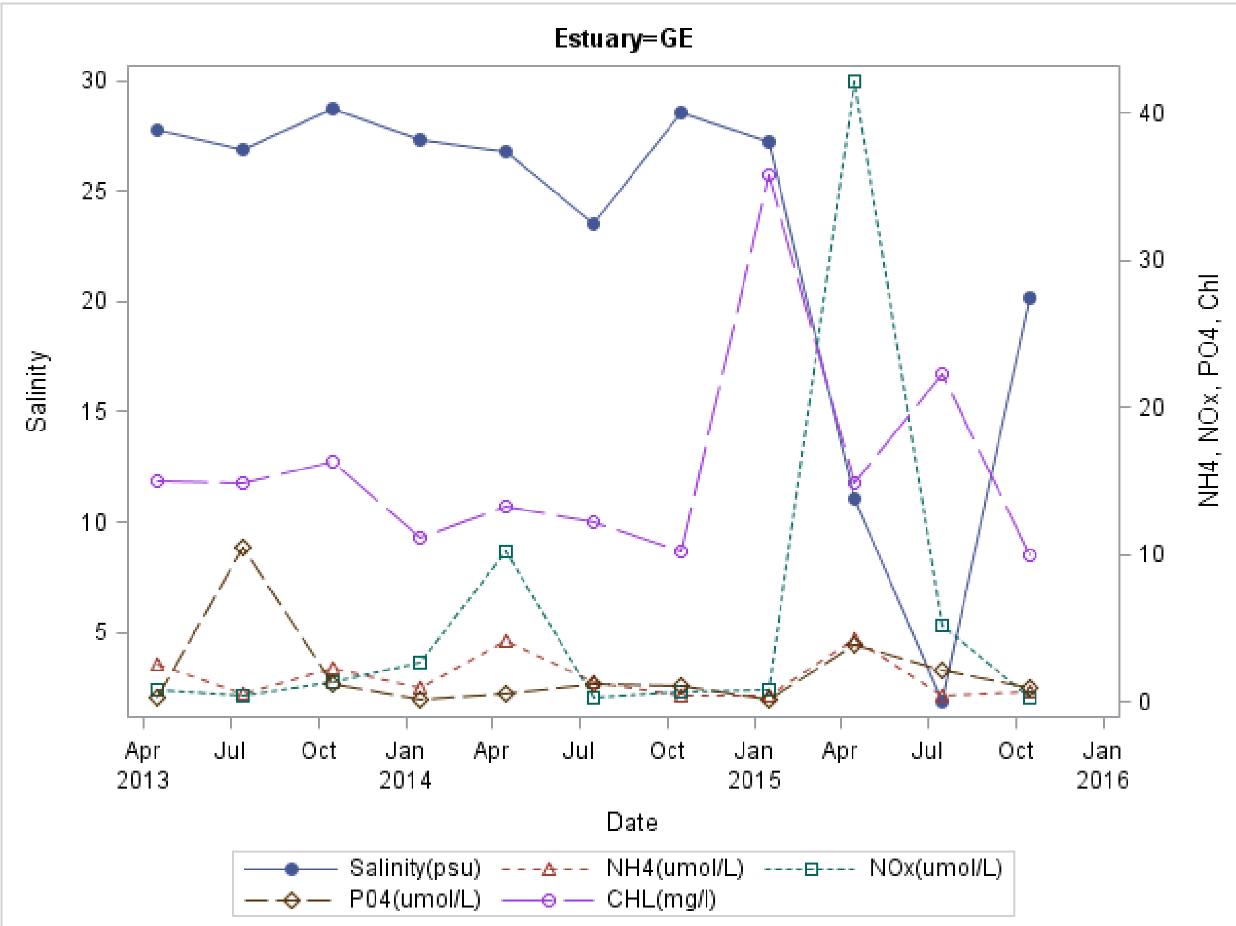

Figure 17 Estuary-wide average water quality variables in the Guadalupe Estuary (GE) over the study period.

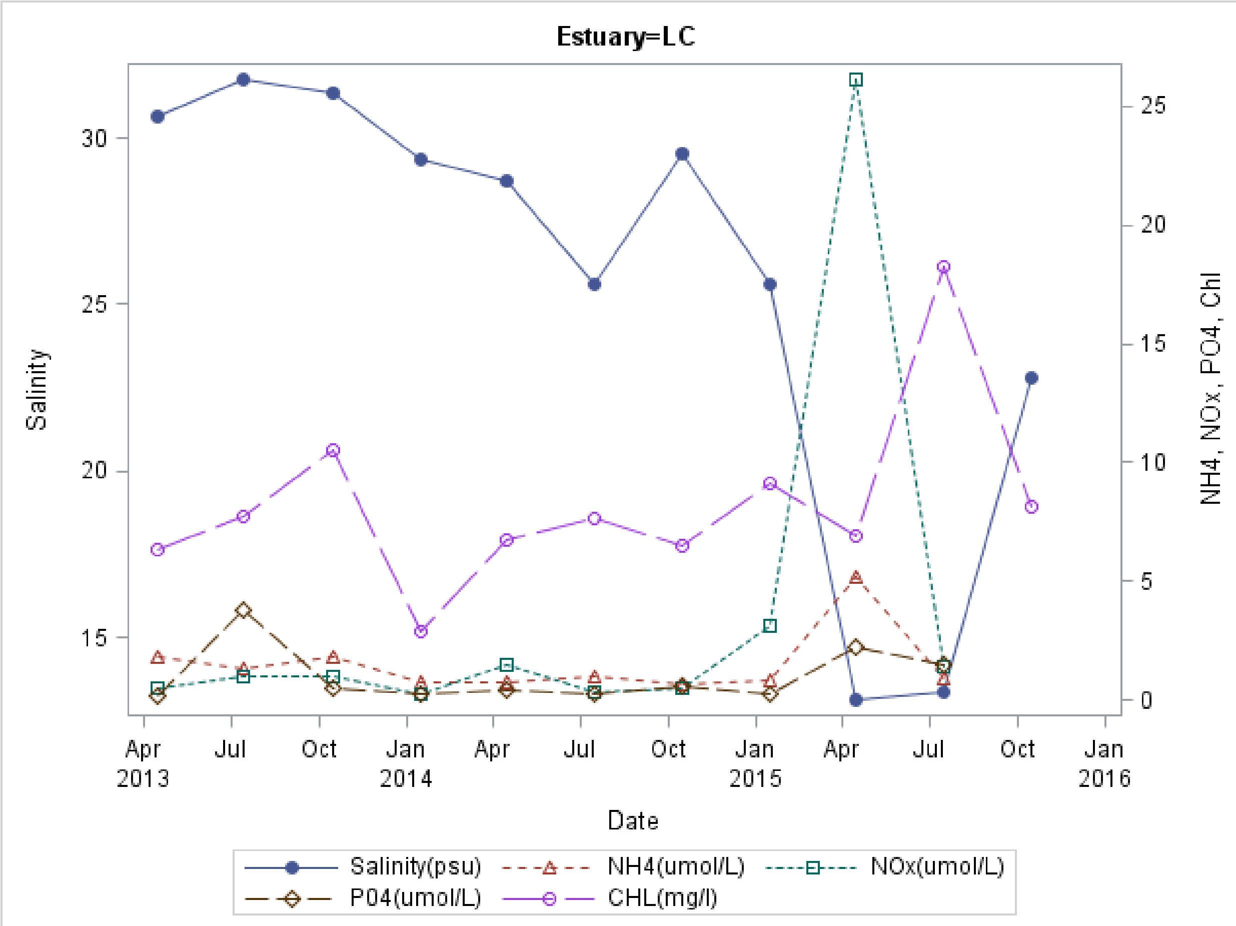

Figure 18 Estuary-wide average water quality variables in the Lavaca-Colorado Estuary (LC) over the study period.

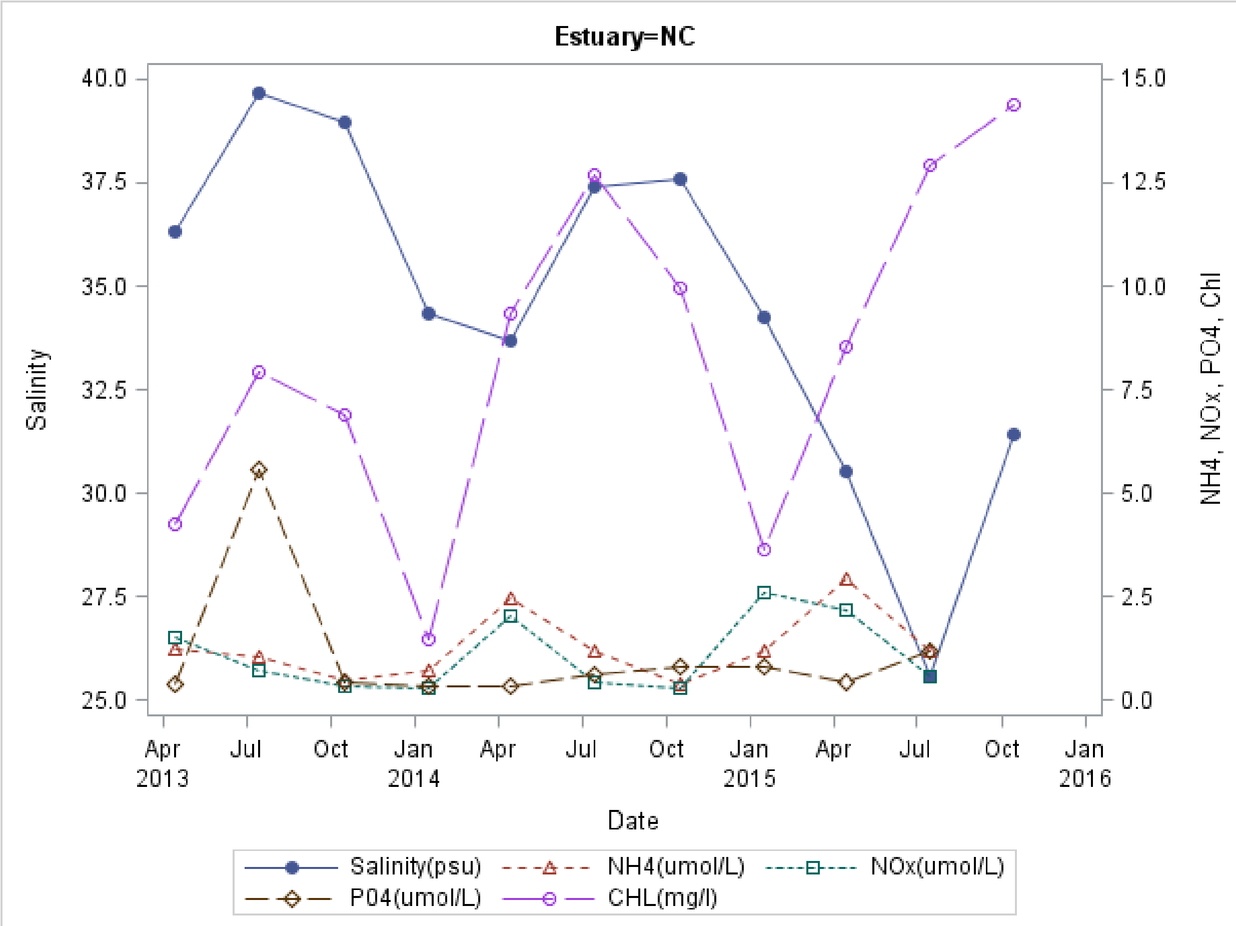

Figure 19 Estuary-wide average water quality variables in the Nueces Estuary (NC) over the study period.

5.5 Water Column Conditions in Mid-Coastal Estuaries: Organic Matter

Dissolved organic carbon (DOC) and nitrogen (DON) concentrations were similar between Lavaca-Colorado, Guadalupe, and Nueces-Corpus Christi estuaries (Figure 20). To estimate the river endmember DOC concentration, salinity-DOC relationships were extrapolated to zero salinity. The estimated zero salinity DOC concentrations were 920 μM for Nueces Estuary during the wet period, 702 μM for Lavaca-Colorado, and 446 μM for Guadalupe Estuary (data not shown).

We observed a positive relationship between DOC and chlorophyll in Nueces-Corpus Christi Bay (R^2 = 0.35-0.37), which indicates a contribution from autochthonous sources (i.e., seagrass, phytoplankton). This was not observed in the other estuaries, however (Table 7).

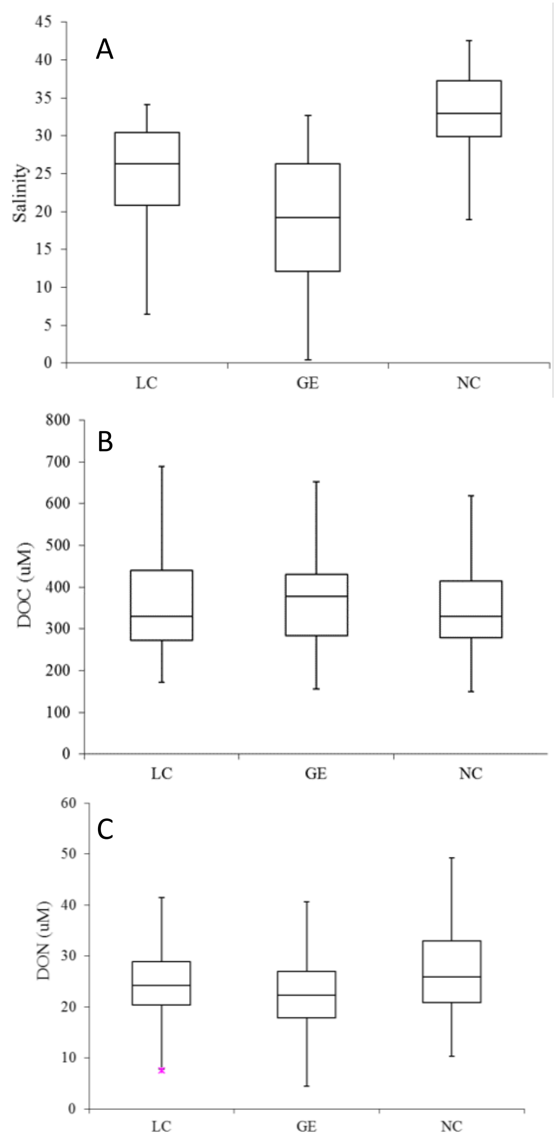

Figure 20. Average water column condition in each estuary (GE = Guadalupe, LC = Lavaco-Colorado, NC = Nueces) from October 2012-April 2017. A) Salinity. B) Dissolved organic carbon.C) Dissolved organic nitrogen.

Table 7 Correlation analysis of DOC concentrations with relevant environmental parameters.

Estuary	Salinity	Temperature	Chlorophyll
Lavaca-Colorado	m = -14.3, r^2 = 0.68	m = 4.9, r^2 = 0.05	m = 8.5, r^2 = 0.13
Guadalupe	m = -4.2, r^2 = 0.15	m = 9.3, r^2 = 0.30	m = 1.5, r^2 = 0.03
Nueces (dry)	m = 19.9, r^2 = 0.25	m = 11.2, r^2 = 0.35	m = 12.9, r^2 = 0.35
Nueces (wet)	m = -19.4, r^2 = 0.53	m = 6.4, r^2 = 0.12	m = 13.2, r^2 = 0.37

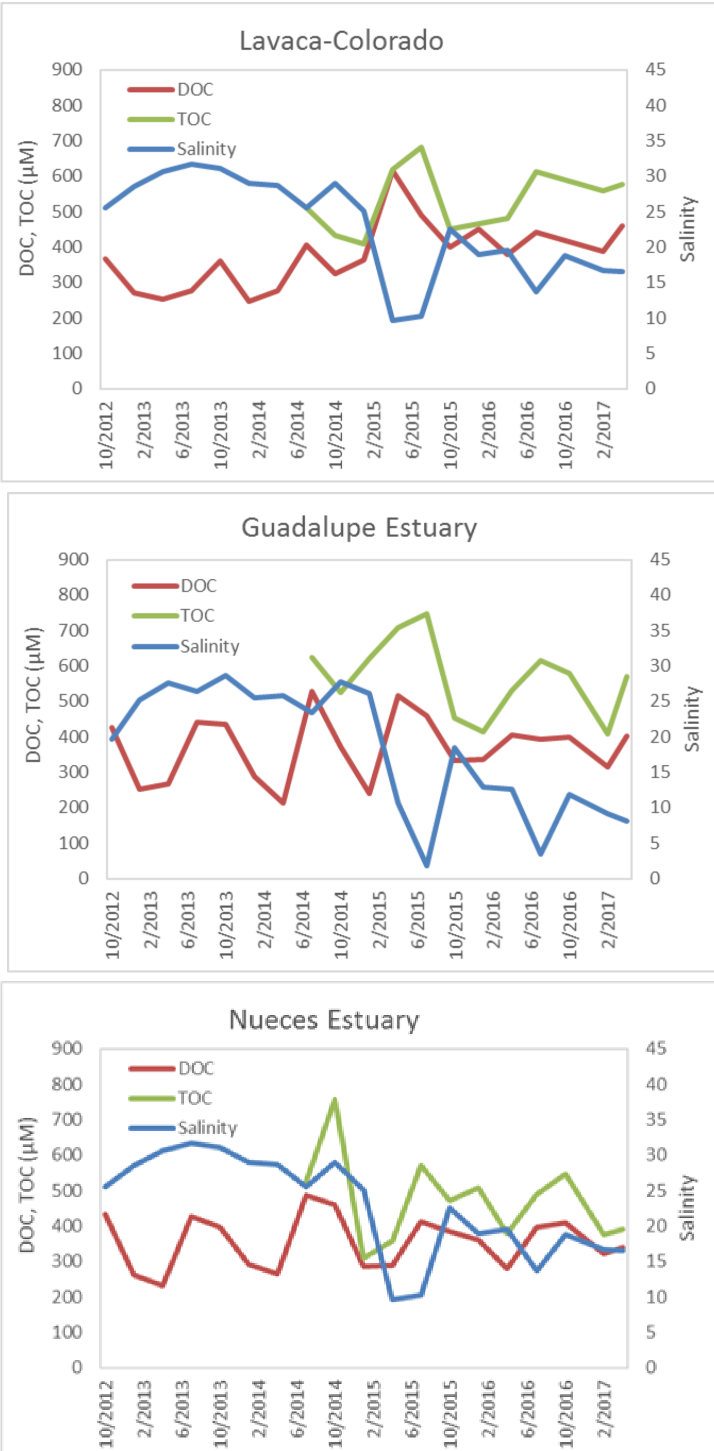

Figure 21 Temporal distribution of DOC, TOC and salinity in Lavaca-Colorado, Guadalupe, and Nueces Estuaries from October 2012-April 2017.

Dissolved organic carbon displayed a seasonal cycle in Guadalupe Estuary and also during the dry period Nueces Estuary, with higher concentrations observed during the spring-summer (Figure 21). In these systems, DOC concentration was positively correlated with water temperature (Table 7). It is likely that DOC is produced internally during the warmer months via degradation of organic matter, thereby adding to the existing DOC pool. A strong inverse relationship between DOC and salinity was observed in Lavaca-Colorado (Table 1) and during the wet period in Nueces Estuary (Table 7; Figure 21). This indicates that freshwater inflow exerts an important control on DOC in these systems. A much weaker relationship was observed for Guadalupe Estuary and during the dry period in Nueces Estuary, suggesting that freshwater inflows may is either too ephemeral to have a noticeable effect given the timescale (quarterly) of our sampling, or simply that the freshwater inflows that occurred were not large enough to have an estuary-wide effect. In Nueces and Corpus Christi bays, a positive relationship was observed between DOC and chlorophyll (Table 7), with chlorophyll also being highest in summer (data not shown). Thus it is likely that phytoplankton produce some of the additional dissolved organic matter observed during the warmer months.

With the exception of phytoplankton bloom periods, the ratio of DOC to TOC averaged 73-83% (Figure 22A), which indicates that dissolved organic carbon is an important driver of ecosystem metabolism (oxygen and CO_2 dynamics, and productivity) in these estuaries of the Texas coast. Furthermore, it is important to note that 90-95% of the dissolved nitrogen in these estuaries was in organic form as opposed to nitrate and ammonium (Figure 22B), which (organic nitrogen) has been shown to favor brown tide phytoplankton over ecologically healthy phytoplankton.

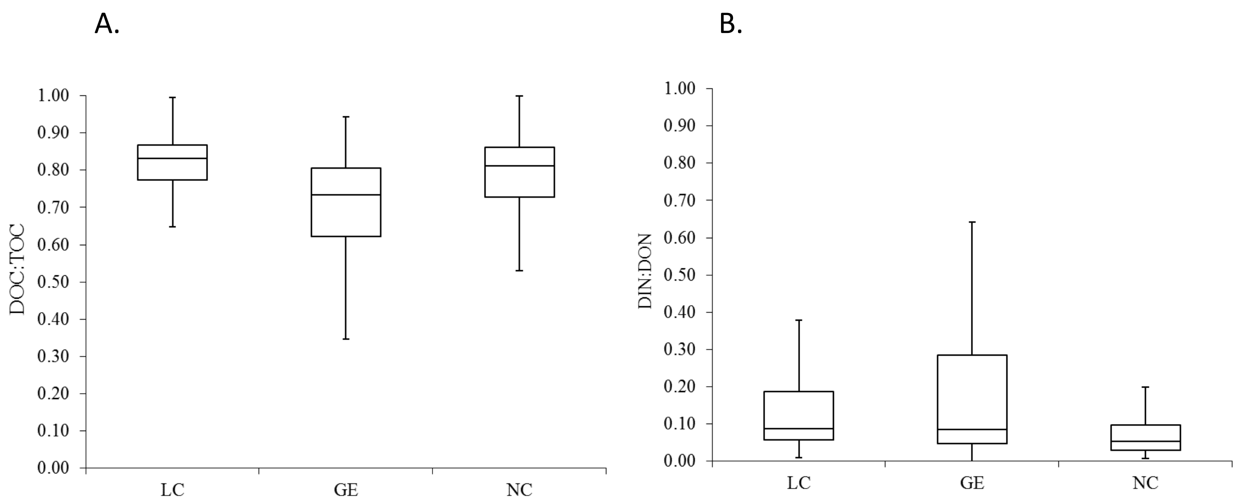

Figure 22 Average ratio of dissolved matter in each estuary from October 2012-April 2017. A) Dissolved organic carbon (DOC) to total organic carbon (TOC). B) Dissolved inorganic nitrogen (DIN: ammonium plus nitrate, nitrite) to dissolved organic nitrogen (DON).

5.6 Water Column Conditions in Mid-Coastal Estuaries: Carbonate Chemistry

In general, the water bodies had large variability in terms of carbonate system dynamics both among these different systems and across time (Table 8, Figs. 23 - 24).

Table 8 Estuarine carbonate system characteristics during our surveyed period (01/2014-04/2017). DIC represents measured total dissolved inorganic carbon, TA is titrated total alkalinity.

Bay System	T (˚C)	Salinity	DIC (µmol/kg)	TA (µmol/kg)	$\Omega_{aragonite}$	pCO$_2$ (µatm)
San Antonio	22.2±6.7	16.0±9.6	2710+388	3030±357	4.86±2.29	540±375
Matagorda	22.7±6.6	19.7±8.4	2185±376	2421±428	3.16±1.66	575±544
Corpus Christi	23.2±6.4	31.2±4.2	2249±150	2578±186	3.86±1.01	418±109
Rincon Bayou	24.7±6.2	10.4±10.6	3301±714	3700±670	8.71±3.67	769±703

Both pCO_2 and $\Omega_{aragonite}$ appeared to have been significantly affected by both seasonal changes and freshwater inflow, as well as the salinity increase after the freshwater inflow (Figure 23). The dry-wet cycle in 2014-2015 had a significant imprint on the carbonate chemistry in these estuaries. During the entire 2014, which was a dry year, estuarine pCO_2 started from slightly below the atmospheric value (~300 µatm) in winter, then increased to moderately higher than air pCO_2 in spring and summer (up to ~600 µatm) before it decreased to below air pCO_2 level again in winter (Figure 23A). From 2015 onward, pCO_2 exhibited much larger spatial variations presumably caused by the more abundant freshwater in the latter years and associated respiration of allochthonous organic matter. Despite the large variations, seasonal cycle of pCO_2 was still visible, higher in summer and lower in winter for all sampled years (Figure 23A).

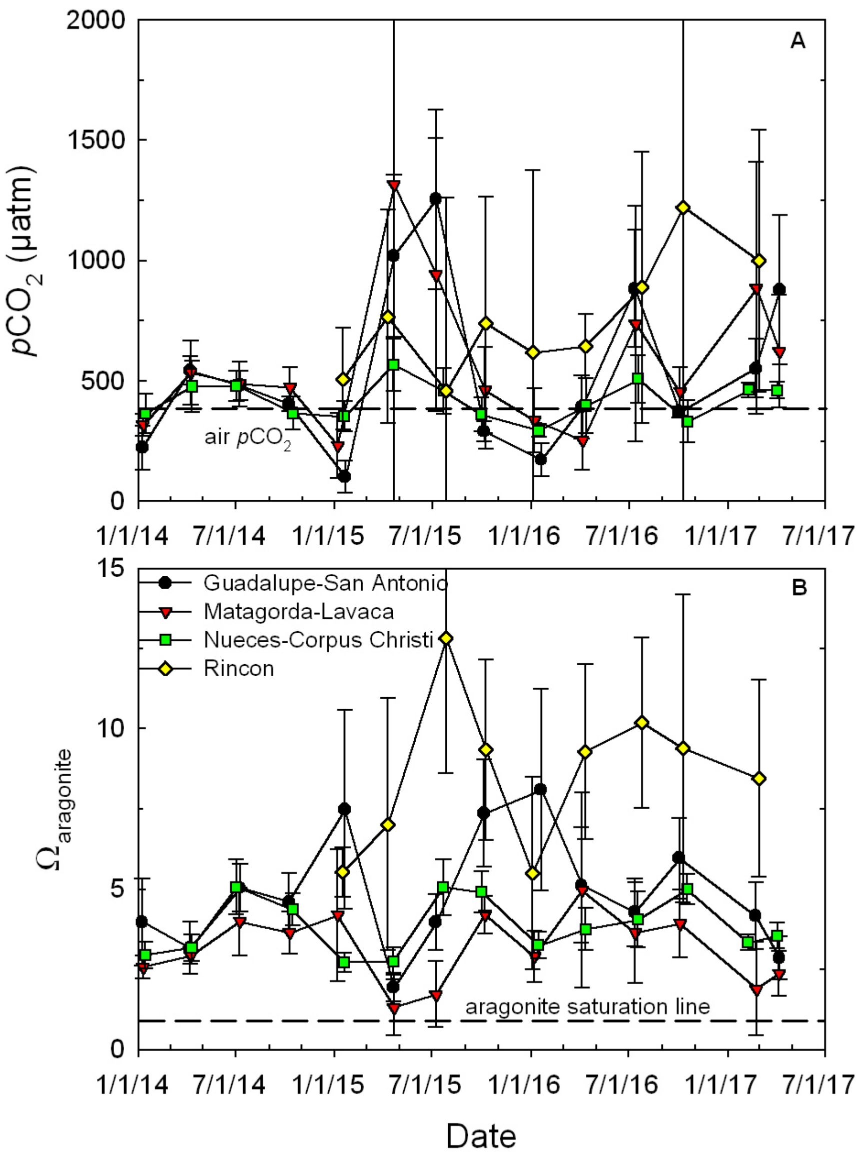

Figure 23 Temporal changes in pCO2 and $\Omega_{aragonite}$ in the three bay systems and Rincon Bayou.

The three estuaries had relatively stable $\Omega_{aragonite}$ during 2014 (Fig. 23B). Beginning in 2015, with significantly higher freshwater inflow, two estuaries (San Antonio and Matagorda) first experienced an increase in $\Omega_{aragonite}$, presumably caused by nutrient delivery from the rivers and enhanced estuarine productivity, which consumed CO_2 and led to a decrease in pCO_2 and moderate increase in $\Omega_{aragonite}$ (01/2015). However, as freshwater continued to strongly affect these estuaries and temperature increased, the respiration signal dominated the carbonate equilibria, as reflected by depressed $\Omega_{aragonite}$ and elevated pCO_2 (starting from May, 2015, Fig. 23B). Furthermore, because of low levels of alkalinity in the freshwater endmember of Lavaca-Colorado estuary, the decrease in $\Omega_{aragonite}$ was such that the estuarine water briefly became undersaturated with respect to aragonite (Fig. 23B), implying that these waters could become stressful for shellfish, especially larvae and juveniles. Similar to pCO_2, 2015-2017 $\Omega_{aragonite}$ data also showed large spatial variation but lacked seasonality as in 2014. In contrast, DIC and TA were relatively invariant over time in all the systems (Fig. 24).

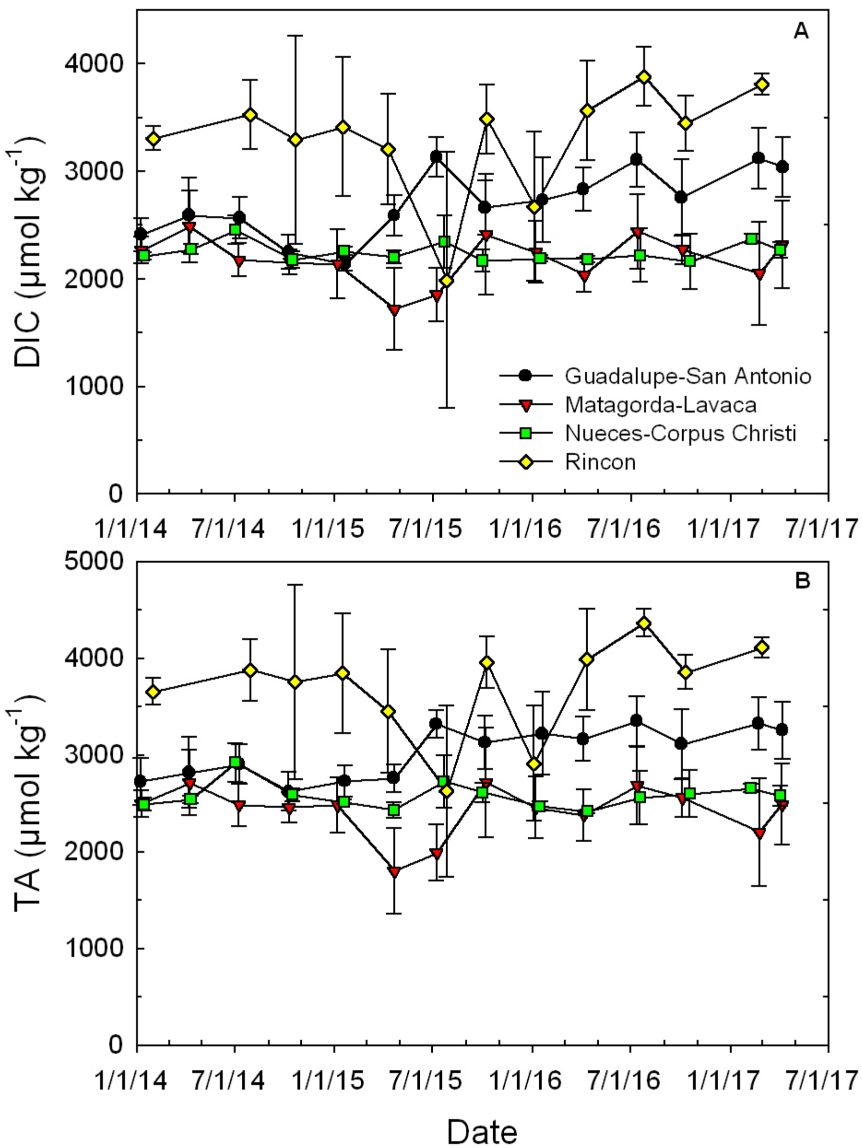

Figure 24 Changes of DIC (A) and TA (B) in the three bay systems and Rincon Bayou.

Salinity appeared to exert the dominant control on the distribution of alkalinity and Ca^{2+} (Fig. 25). However, the two parameters vs. salinity relationships showed distinct patterns. The alkalinity vs. salinity relationships (Fig. 25A) indicate varying freshwater endmember alkalinity levels from north to south, which represent the levels of weathering product (mostly bicarbonate) in the freshwater drainage basin. River endmember alkalinity increased in the order of Lavaca-Colorado, Guadalupe, Nueces (Rincon Bayou receives freshwater inflow from Nueces River), which is consistent with the TCEQ data record (Hu et al. 2015). In addition, both Guadalupe and Nueces Rivers have significantly higher alkalinity levels than their respective receiving estuaries and the coastal Gulf of Mexico (2200-2400 μmol kg^{-1}). On the other hand, calcium concentration has a linear relationship with salinity in all individual water bodies including Rincon Bayou, the latter had significantly higher slope (Fig. 25B). Again, freshwater endmember showed an increasing [Ca^{2+}] in the same order as observed for alkalinity. Note the two panels have different units that differ by a factor of 1000 (μmol kg^{-1} for alkalinity vs. mmol kg^{-1} for Ca^{2+}).

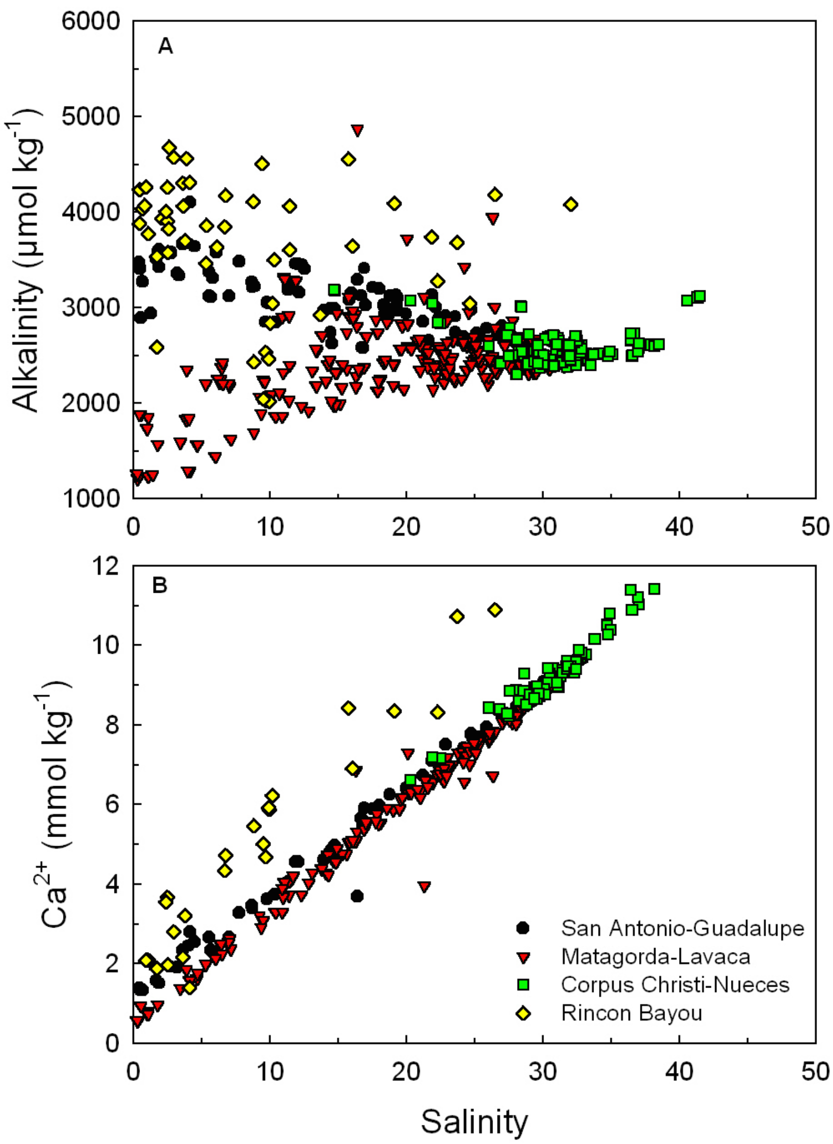

Figure 25 Alkalinity and calcium along the salinity gradient in the three bay systems and Rincon Bayou.

pCO_2 and $\Omega_{aragonite}$ along the salinity gradient reflected a more complicated pattern that corresponded to river inflow, primary production, and respiration (Fig. 26). In general, low salinity waters had much higher pCO_2 than the atmospheric value, consistent with observations in others rivers and river influenced estuaries (Butman and Raymond, 2011; Jiang et al., 2008). In mid salinity waters (10-30), however, pCO_2 was much more variable, ranging from significant CO_2 undersaturation to significant supersaturation in Matagorda (Lavaca-Colorado) and San Antonio (Guadalupe), respectively. In higher salinity waters (mostly in Corpus Christi (Nueces)), the air-water pCO_2 gradient was much smaller (Fig. 26A). In comparison, carbonate saturation state remained high except in low salinity (less than 10) waters of Matagorda (Lavaca), where the water became undersaturated with respect to aragonite. $\Omega_{aragontite}$ values in both San Antonio (Guadalupe) and Rincon Bayou were all higher than 1 (Fig. 26B). Extreme $\Omega_{aragonite}$ values (up to 18) were observed in Rincon Bayou at salinity of ~10. These observations were largely consistent with the previously reported results during 2014-2015 (Wetz and Hu, 2015).

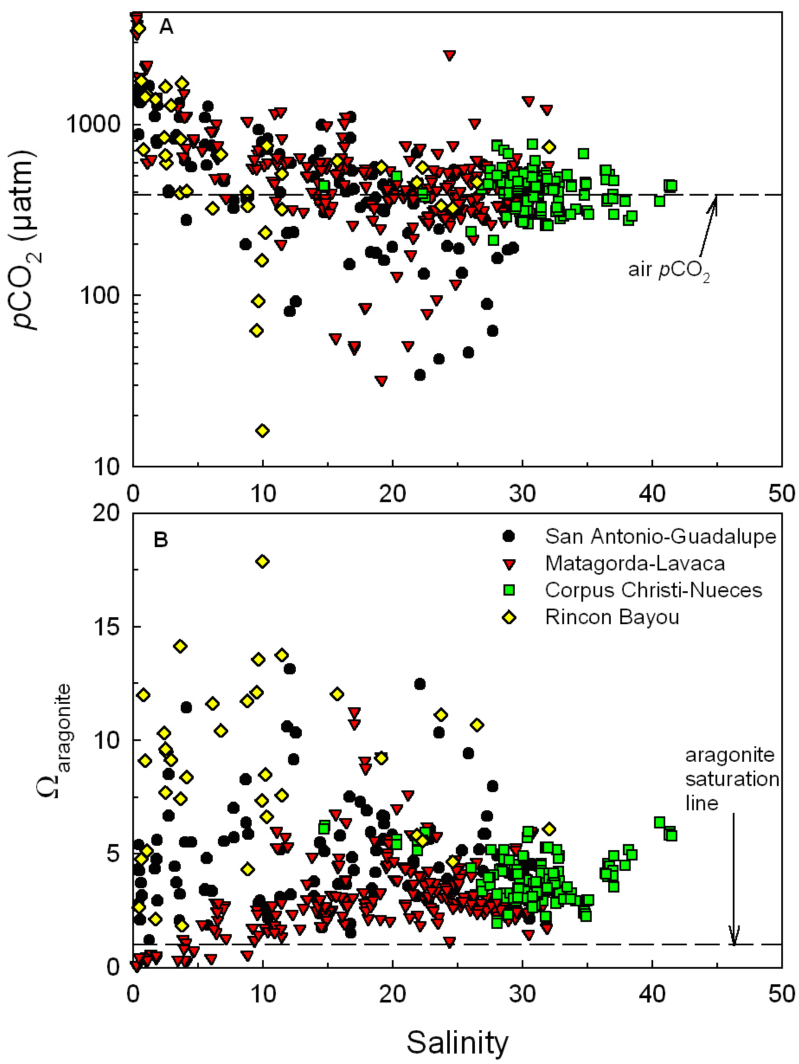

Figure 26 ***p*CO2 and $\Omega_{aragonite}$ along the salinity gradient in the three bay systems and Rincon Bayou. Note *p*CO2 is on logarithmic scale.**

pCO_2 in all water bodies exhibited significant correlation with temperature ($p<0.01$, Fig. 27A), indicating temperature control on CO_2 levels in these estuaries. This is consistent with the seasonality of pCO_2 variations as warm weather enhanced respiration and large pulses of freshwater inflow also coincided with the warm periods. However, the correlation between temperature and $\Omega_{aragonite}$ was only significant in Nueces-Corpus Christi Bay ($p<<0.001$, Fig. 27B) but not in others. The fact that freshwater had the smallest influence in this area as its salinity being the highest but with smallest variation (31.2±4.2, Table 8) determined that this estuarine was more of "marine" type compared to other estuaries, so that temperature control on $\Omega_{aragonite}$ was mostly thermodynamic, i.e., via controlling carbonic acid dissociation constants and aragonite solubility constant.

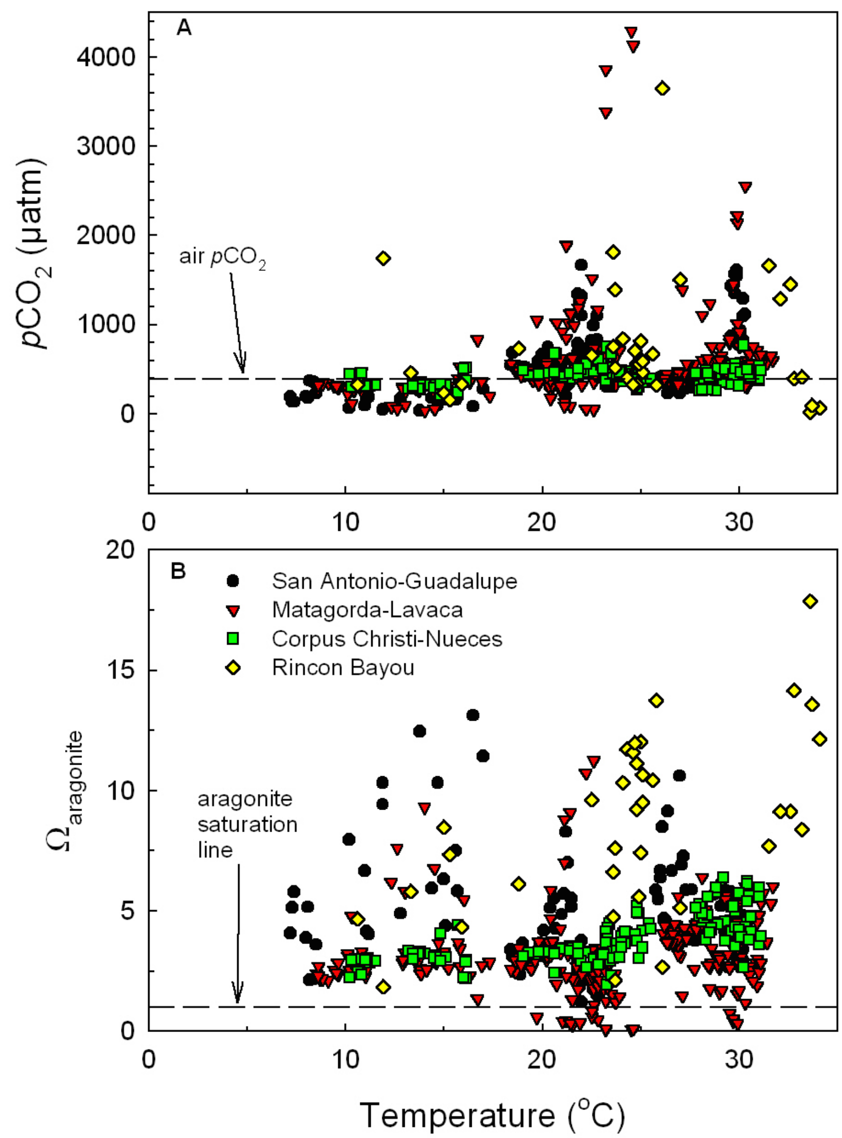

Figure 27 Relationships between temperature and inorganic carbon. A) *p*CO2 and B) $\Omega_{aragonite}$.

6 Discussion

6.1 Guadalupe Estuary Benthos Dynamics

Water quality in the Guadalupe Estuary trends of station-date combinations separate stations both by season and by amount of freshwater inflow that each station receives (Figures 2b and 2c). Temperature is inversely proportional to dissolved oxygen and the separation of the station-date combinations along this gradient represents seasonal changes in water quality. It is well known that the solubility of oxygen is limited when salinity and temperatures are high (Applebaum et al. 2005). The spatial difference in freshwater inflow that each station receives is represented by the inverse relationship between salinity and nutrients. Station A is the closest of the stations to the Guadalupe River mouth so had the highest nutrient concentrations and lowest salinity values. Typically the salinity gradient in San Antonio Bay is for A to have the lowest salinity, then B, then C, and then D, because station D is closest to the Matagorda Ship Channel, and tidal flows move south via the Intracoastal waterway. However, one unusual occurrence is that, station C was higher than D in July in all three years (2013 – 2015) (Figure 8). This reversal of the usual pattern could be caused by either winds, or variability in either circulation patterns or tidal flows. The most important trend during the current sampling period was a transition from an extended dry period to a wet period, which occurred from April 2015, but lowest salinities were recorded in July 2015 (2 psu) and July 2016 (4 psu), even though July is usually a dry month (Figs. 16 – 18).

Macrofauna communities have characteristics that are both multivariate (i.e., species differences as presented in Table 4 and Figure 5), and univariate (i.e., summary values of abundance, biomass and diversity as presented in Figures 4 and 6). There is a clear difference between macrofauna communities in environments with high and low salinities because samples from Station D always cluster together, and are distinct from other stations (Table 4 and Figure 5). Stations B and C are similar all of the time. However, station A can be like stations C and B, or it can be distinct. Freshwater inflow into the Guadalupe Estuary travels southwest along the western side of the estuary allowing lower salinities on the southwestern side to be lower than salinities on the northeastern side resulting in long-term lower salinities at station C than D.

During the last study period (April 2013 – October 2015), there was a highly unusual community in that a tunicate species, *Molgula manhattensis*, was the third most dominant species (Montagna 2015). However, *Molgula* appears to be primarily a marine species because it disappeared once the salinities dropped, and it wasn't found at all during the current study period.

Benthic communities in San Antonio Bay are characterized by dominance.

It is also apparent that total macrofauna abundance, biomass, and diversity are quite variable over time (Figure 4). Generally, station D, the most marine station has the highest diversity, and this is because of invasion by marine species. Station D also often has the highest biomass. However, Abundance was highest at station A in 2013. The community reacted to the flood of 2015 with a large decrease in abundance, biomass and diversity; however it is apparent that abundance was beginning to recover in October 2015 in Station D.

There has been a long-term decline in macrofauna abundance in the Guadalupe Estuary since 1987, but it does not appear that there is an associated decrease in macrofauna biomass or species richness (Figure 6). Diversity follows a pattern of increasing when salinity increases and decreasing when salinity decreases, and this is because of the expansion of a more diverse marine fauna that can move into San Antonio Bay during dry periods because there is a larger area of marine water habitat. A similar decline in benthic abundance, but also biomass and diversity, in the Lavaca-Colorado estuary has been observed over the past 21 years (Pollack et al. 2011). However we do not know if this decline is a result of natural, long-term population or community cycles that span multiple decades and will reverse, or if it is due to a permanent state-shift. This decline is troubling however, because benthos are the principal food for many important commercial and recreational fishery species including shrimp, crab, red fish, flounder, black drum, and spotted seatrout. It is unknown if the disappearing benthos, which is fish forage, is affecting fish populations.

Biomass does not exhibit a clear trend, sometimes following salinity patterns, but sometimes not following salinity patterns (Figure 6). Biomass did increase following drops in salinity on six occasions: January 1991 following a 1 psu drop, October 1994 following 5 psu drop, April 1996 following a 4 psu drop, April 2007 following a 13 psu drop, and July 2009 following a 4 psu drop. However, biomass increased following a rise in salinity on six occasions: April 1995 following a 4 psu rise, October 1999 following a 16 psu rise, October 2004 following a 11 psu rise, April 2006 following a 5 psu rise, April 2008 following a 3 psu rise, January 2011 following a 7 psu rise, and January 2013 following a 5 psu rise.

Mean estuary-wide salinity in October 2011 (35 psu) was the highest it has ever been and is 2 times the long-term average salinity of 15.4 psu (Figure 6). Some of the benthic metrics are much lower than average. Average abundance in October 2011 was 12,291 n/m^2, which is 82% of the long-term average abundance of 14,899 n/m^2. Average biomass in October 2011 was 4.53 g/m^2, which is a little more than half (55%) of the long-term average biomass of 8.23 g/m^2. Average species richness is about the same, because in October 2011 it was 10.5 species/0.01 m^2, which is 4% more than the long-term average richness of 10.1 species/0.01 m^2.

The spring of 2015 had high flows continuously beginning in March 2015. The Memorial Day flood of May 2015 was one of the largest and most damaging on record. Additional rain in June and July, plus the time it takes for river water to move down to the coast, led to the lowest salinities in July 2015 (Figure 13). The large floods washed away nearly all the estuarine and marine species, and replaced them with freshwater and oligohaline species.

6.2 Mid-Coastal Estuaries Water Column Dynamics

The three Texas mid-coast estuaries share a connection via large lagoons. Matagorda Bay is connected to San Antonio Bay via Espiritu Santo Bay. San Antonio Bay is connected to Corpus Christi Bay via Aransas Bay and Lydia Ann Channel. The Intracoastal Waterway enhances these connections and further facilitates water exchange among these Texas lagoons. However, because of the strong climatic gradient along the Texas coast, the three estuaries have different inflow regimes and consequently different patterns in water quality and benthic responses. The most important result of the current study is the recognition of the importance of dissolved organic matter as a constituent of freshwater inflow. The carbon component of this dissolved organic matter has been shown elsewhere to stimulate the microbial food web, with propagating effects to higher trophic levels (Pomeroy 1974). As for the nitrogen component, prior studies of nutrient-phytoplankton dynamics have tended to ignore the DON pool, yet its importance is becoming increasingly clear (Seitzinger and Sanders 1997), and itis clearly important in Texas bay systems. This novel, important information on organic matter concentrations, speciation (i.e., dissolved vs.

particulate) and controls upon its estuarine distributions for several vital mid-coast Texas estuaries provides a basis for future work to understand how variability in freshwater inflow and watershed land use may affect the reactivity of the organic matter. Through other funding, these results are being combined with data from the hypersaline Baffin Bay system to provide the first ever analysis of effects of freshwater inflow on estuarine biogeochemistry across the full spectrum of inflow balances (positive-neutral-negative estuaries). An added advantage is that the 2016 - 2017 collections correspond with a study of ecosystem metabolism and hypoxia formation on the Texas coast (NOAA-funded study by Montagna, Wetz, Hu), providing the most comprehensive opportunity to date to link this bioreactive pool to estuarine biogeochemistry.

It was expected that organic matter concentrations would be higher in the northern, lower salinity estuaries due to input of allochthonous organic matter, yet dissolved organic carbon and nitrogen concentrations were similar between Lavaca-Colorado, Guadalupe, and Nueces-Corpus Christi bays. There are several possible explanations for this deviation from the expected relationship. One possibility is that the riverine endmember DOC concentration was higher for Nueces-Corpus Christi Bay than the other two northern bays. The reason for these differences is unclear, although prior studies have suggested that watershed land use coverage can play a significant role in determining riverine DOC concentrations (Servais et al. 1987; Abril et al. 2002; Mallin et al. 2002; Petrone et al. 2009). Another possibility is that the DOC in Lavaca-Colorado and Guadalupe Estuary is simply more labile (i.e., more easily degraded) than Nueces-Corpus Christi and is more rapidly removed from the system. One indicator of this would be the ratio of DOC to DON, which in our dataset shows no differences between sites. Additional indicators of organic matter lability are currently being explored by collaborators at UTMSI.

The carbonate chemistry parameters collected to date provided a strong first-order view of estuarine metabolism. For example, estuarine pCO_2 levels can be used to study aquatic ecosystem metabolism (Cai, 2011; Crosswell et al., 2012). During the drought conditions of 2014, the estuaries were a small CO_2 sink during cooler months and a moderate CO_2 source (to the atmosphere) in warmer months. In contrast, during the high rainfall period in early-mid 2015 the estuaries became a large source of CO_2 to the atmosphere because of large air-water pCO_2 gradient (Fig. 9A). This increase in CO_2 source condition is presumably due to significant terrestrial organic matter inputs and subsequent respiration. The dissolved organic matter inputs to estuaries that drive ecosystem metabolism can be strongly affected by climate patterns, as well as water resource and land use changes in coastal watersheds, thus this database provided critical baseline information for these important biogeochemical constituents.

River endmember alkalinity has been shown to affect the buffering capacity of estuarine water itself (Hu and Cai, 2013; Salisbury et al., 2008). Based on our observation, lower alkalinity rivers (such as Lavaca and Colorado) are undersaturated with respect to aragonite while lower latitude rivers (Guadalupe and Nueces) are not. Therefore, river inflow increase due to precipitation increase may have different impacts on these three estuaries (Figs. 10B and 11B). For example, in Lavaca Bay, a decrease in salinity could potentially lead to corrosive conditions for shell-forming organisms in wet seasons, while there will likely be no such condition in Guadalupe and Nueces Bays, the latter derived from Rincon Bayou data. Additional data analysis and modeling studies are planned to allow us to make projections on the potential effects of long-term variability in freshwater inflows to the area.

7 References

Abril G., M Nogueira, H. Etcheber, G. Cabecadas, E. Lemaire, and M.J. Broguiera. 2002. Behaviour of organic carbon in nine contrasting European estuaries. *Estuarine, Coastal and Shelf Science* 54: 241-262

American Society for Test Methods. ASTM D5176 - 08 Standard test method for total chemically bound nitrogen in water by pyrolysis and chemiluminescence detection. (Online Reference)

Anderson, L. and Granéli, A. 1982. A compact automated titration system, applied to a high precision determination of calcium in sea-water. *Journal of Automatic Chemistry* 4: 75-78.

Applebaum, S., P. A. Montagna, and C. Ritter. 2005. Status and trends of dissolved oxygen in Corpus Christi Bay, Texas, U. S. A. *Environmental Monitoring and Assessment* 107: 297-311.

Biddanda, BA. 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. II. Microbial metabolism. *Marine Ecology Progress Series* 42: 89-95.

Bruesewitz D.A., Gardner W.S., Mooney R.F., Pollard L., and Buskey E.J. 2013. Estuarine ecosystem function response to flood and drought in a shallow, semiarid estuary: nitrogen cycling and ecosystem metabolism. *Limnology and Oceanography* 58: 2293-2309.

Butman, D. and Raymond, P.A., 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geosciences* 4: 839-842.

Cai, W.-J. 2011. Estuarine and coastal ocean carbon paradox: CO_2 sinks or sites of terrestrial carbon incineration? *Annual Reviews of Marine Science* 3: 123-145.

Carter, B.R., Radich, J.A., Doyle, H.L. and Dickson, A.G. 2013. An automatic system for spectrophotometric seawater pH measurements. *Limnology and Oceanography Methods* 11: 16-27.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117-143.

Clarke, K.R. and R.M Warwick. 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. 2nd Edition. PRIMER-E: Plymouth, United Kingdom.

Crosswell, J.R., Wetz, M.S., Hales, B. and Paerl, H.W. 2012. Air-water CO2 fluxes in the microtidal Neuse River Estuary, North Carolina. *Journal of Geophysical Research* 117: C08017.

Day, J.W., and 3 others. 2012. Estuarine Ecology, 2nd edition. Wiley-Blackwell, 568 pp.

Dickson, A.G., Sabine, C.L. and Christian, J.R., 2007. Guide to Best Practices for Ocean CO2 Measurements. PICES Special Publication 3.

Harvey HR, JH Tuttle, and JT Bell. 1995. Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. Geochimica et Cosmochimica Acta 59: 3367-3377

Hu, X., Beseres Pollack, J., McCutcheon, M.R., Montagna, P.A. and Ouyang, Z. 2015. Long-term alkalinity decrease and acidification of estuaries in Northwestern Gulf of Mexico. *Environmental Science and Technology* 49: 3401–3409.

Hu, X. and Cai, W.-J. 2013. Estuarine acidification and minimum buffer zone - A conceptual study. *Geophysical Research Letters* 40: 5176-5181.

Jiang, L.-Q., Cai, W.-J. and Wang, Y. 2008. A comparative study of carbon dioxide degassing in river- and marine-dominated estuaries. *Limnology and Oceanography* 53: 2603-2615.

Kalke, R.D. and P.A. Montagna. 1991. The effect of freshwater inflow on macrobenthos in the Lavaca River Delta and Upper Lavaca Bay, Texas. *Contributions to Marine Science* 32: 49-72.

Kim, H-C. and P.A. Montagna. 2009. Implications of Colorado River freshwater inflow in the benthic ecosystem dynamics and bay health: a modeling study. *Estuarine, Coastal and Shelf Science* 83: 491-504.

Kim, H.-C. and P.A. Montagna. 2010. Effect of Climatic Variability on Freshwater Inflow, Benthic Communities, and Secondary Production in Texas Lagoonal Estuaries. Final report to the Texas Water Development Board, contract number 08-483-0791.Harte Research Institute for Gulf of Mexico Studies, Texas A&M University-Corpus Christi, Corpus Christi, Texas.119 pages.

Kim, H.-C. and P.A. Montagna. 2012. Effects of climate-driven freshwater inflow variability on macrobenthic secondary production in Texas lagoonal estuaries: A modeling study. *Ecological Modelling* 235– 236: 67– 80.

Lewis, E. and Wallace, D., 1998. Program Developed for CO_2 System Calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Liu, X., Patsavas, M.C. and Byrne, R.H., 2011. Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements. *Environmental Science and Technology* 45: 4862-4868.

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

Mallin, M.A., M.H. Posey, M.R. McIver, D.C. Parsons, S.H. Ensign and T.D. Alphin. 2002. Impacts and recovery from multiple hurricanes in a Piedmont-coastal plain river system. *Bioscience* 52: 999-1010.

Millero, F.J. 2010. Carbonate constants for estuarine waters. *Marine and Freshwater Research* 61: 139-142.

Montagna, P.A. 1989. Nitrogen Process Studies (NIPS): the effect of freshwater inflow on benthos communities and dynamics. Technical Report No. TR/89-011, Marine Science Institute, The University of Texas, Port Aransas, TX, 370 pp.

Montagna, P.A. 1999. Predicting long-term effects of freshwater inflow on macrobenthos and nitrogen losses in the Lavaca-Colorado and Guadalupe Estuaries. Final Report to Texas Water Development Board. Technical Report No. TR/99-001, Marine Science Institute, The University of Texas, Port Aransas, TX, 68 pp.

Montagna, P.A. 2000. Effect of freshwater inflow on macrobenthos productivity and nitrogen losses in Texas estuaries. Final report to Texas Water Development Board, Contract No. 2000-483-323, University of Texas Marine Science Institute Technical Report Number TR/00-03, Port Aransas, Texas. 78 pp.

Montagna, P.A. 2013. Macrobenthos Monitoring In Mid-Coastal Estuaries - 2013.Final Report to the Texas Water Development Board, Contract # 1248311357.Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas, 34 pp.

Montagna, P.A. 2014. Macrobenthos Monitoring In Mid-Coastal Estuaries - 2014. Final Report to the Texas Water Development Board, Contract # 1448311638. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas, 39 p.

Montagna, P.A., E.D. Estevez, T.A. Palmer, and M.S. Flannery. 2008. Meta-analysis of the relationship between salinity and molluscs in tidal river estuaries of southwest Florida, USA. *American Malacological Bulletin* 24(1-2):101-115.

Montagna, P.A. and R.D. Kalke. 1992. The effect of freshwater inflow on meiofaunal and macrofaunal populations in the Guadalupe and Nueces Estuaries, Texas. *Estuaries* 15:266-285.

Montagna, P.A. and R.D. Kalke. 1995. Ecology of infaunal Mollusca in south Texas estuaries. *American Malacological Bulletin* 11:163-175.

Montagna, P.A, E.M. Hill, and B. Moulton. 2009. Role of science-based and adaptive management in allocating environmental flows to the Nueces Estuary, Texas, USA. In: Brebbia, C.A. and E. Tiezzi (eds.), Ecosystems and Sustainable Development VII, WIT Press, Southampton, UK, pp. 559-570.

Montagna, P.A., and J. Li. 1996. Modeling and monitoring long-term change in macrobenthos in Texas estuaries. Final Report to the Texas Water Development Board. University of Texas at Austin, Marine Science Institute, Technical Report No. TR/96-001, Port Aransas, Texas, 149 pp.

Montagna, P.A. and J. Li. 2010. Effect of Freshwater Inflow on Nutrient Loading and Macrobenthos Secondary Production in Texas Lagoons. In: Coastal Lagoons: Critical Habitats of Environmental Change, M. J. Kennish and H. W. Paerl (eds.),CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 513-539.

Montagna, P.A., and T.A. Palmer. 2009. Effect of Freshwater Inflow on Macrobenthos Productivity in the Guadalupe Estuary 2008-2009. Final Report to the Texas Water Development Board, Contract # 0904830893. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas, 12 pp.

Montagna, P.A., and T.A. Palmer. 2011. Effect of Freshwater Inflow on Macrobenthos Productivity in the Guadalupe Estuary 2009-2010. Final Report to the Texas Water Development Board, Contract # 1004831015. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas, 17 pp.

Montagna, P.A. and T. Palmer. 2012. Impacts of Droughts and Low Flows on Estuarine Health and Productivity. Final Report to the Texas Water Development Board, Project for Interagency Agreement 1100011150. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas. 142 pp.

Montagna, P.A., and T.A. Palmer. 2011. Effect of Freshwater Inflow on Macrobenthos Productivity in the Guadalupe Estuary 2009-2010. Final Report to the Texas Water Development Board, Contract # 1004831015. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas, 15 pp.

Montagna, P.A., T.A. Palmer, and J. Beseres Pollack. 2007. Effect of Freshwater Inflow on Macrobenthos Productivity. In: Minor Bay and River-Dominated Estuaries - Synthesis. Final Report to the Texas Water Development Board, Contract No. 2006-483-026. Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, Texas.

Montagna, P.A., T.A. Palmer, and J. Beseres Pollack. 2013. Hydrological Changes and Estuarine Dynamics. SpringerBriefs in Environmental Sciences, New York, New York. 94 p. doi 10.1007/978-1-4614-5833-3

Montagna, P., G. Ward and B. Vaughan. 2010. The importance and problem of freshwater inflows to Texas estuaries. In: Water Policy in Texas: Responding to the Rise of Scarcity, R.C. Griffin (ed.). The RFF Press, London.

Montagna, P.A. and Yoon, W.B. 1991. The effect of freshwater inflow on meiofaunal consumption of sediment bacteria and microphytobenthos in San Antonio Bay, Texas USA. *Estuarine and Coastal Shelf Science* 33: 529-547.

Petrone, K.C., J.S. Richards, and P.F. Grierson. 2009. Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia. *Biogeochemistry* 92: 27-40

Pollack, J.B., J.W. Kinsey, and P.A. Montagna. 2009. Freshwater Inflow Biotic Index (FIBI) for the Lavaca-Colorado Estuary, Texas. *Environmental Bioindicators* 4: 153-169.

Pollack, J.B., T.A. Palmer, and P.A. Montagna. 2011. Long-term trends in the response of benthic macrofauna to climate variability in the Lavaca-Colorado Estuary, Texas. *Marine Ecology Progress Series* 436: 67–80. doi:10.3354/meps09267

Pomeroy, L.R. 1974. The ocean's food web, a changing paradigm. *Bioscience*. 24: 499-504

Russell, M.J., and P.A. Montagna. 2007. Spatial and temporal variability and drivers of net ecosystem metabolism in western Gulf of Mexico estuaries. *Estuaries and Coasts* 30: 137-153.

Salisbury, J., Green, M., Hunt, C. and Campbell, J., 2008. Coastal acidification by rivers: a threat to shellfish? *EOS* 89: 513-514.

Seitzinger, S.P., and R.W. Sanders. 1997. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Marine Ecology Progress Series* 159: 1-12.

Servais P., G. Billen, and M.C. Hascoet. 1987. Determination of the biodegradable fraction of dissolved organic matter in waters. *Water Research* 21: 445-450.

Science Advisory Committee. 2009. Methodologies for Establishing a Freshwater Inflow Regime for Texas Estuaries Within the Context of the Senate Bill 3 Environmental Flows Process. Report # SAC-2009-03-Rev1., June 5, 2009.Available at http://www.tceq.state.tx.us/assets/public/permitting/watersupply/water_rights/eflows/fwi20090605.pdf

Shank, G.C., K. Nelson, and P.A. Montagna. 2009. Importance of CDOM distribution and photoreactivity in a shallow Texas estuary. *Estuaries and Coasts* 32: 661-677.

Standard Methods for the Examination of Water & Wastewater. 5310 Total organic carbon (TOC), B. High-temperature combustion method. (Online Reference)

TWDB (Texas Water Development Board) 2017. 2017 State Water Plan. Water for Texas. Texas Water Development Board, Austin, Texas. Accessed 12 December 2017, http://www.twdb.texas.gov/waterplanning/swp/2017/doc/SWP17-Water-for-Texas.pdf?d=1513113355867

Tolan, J.M. 2007. El Niño-Southern Oscillation impacts translated to the watershed scale: estuarine salinity patterns along the Texas Gulf Coast, 1982 to 2004. *Estuarine Coastal and Shelf Science* 72: 247-260

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and Oceanography* 39: 1985-1992.

Wetz, M.S. and Hu, X., 2015. Final Report to the Texas Water Development Board, Contract #1448311639. Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, TX, 14 pp.

8 TWDB Review Comments

Monitoring Mid-Coastal Estuaries – 2016
and
Spatial-Temporal Distribution of Organic Matter and Carbonate Chemistry Parameters in Texas Mid-Coast Estuaries: 2012-2017

Paul Montagna, Ph.D., Michel Wetz, Ph., D, and Xinping Hu, Ph.D.

Contract #1600011924
TWDB Comments to Draft Final Report

REQUIRED CHANGES

General Draft Final Report Comments:

The purpose of this study was to extend the long-term biological collection of benthic data and water quality including chlorophyll, inorganic nutrients, dissolved and particulate organic matter, as well as to quantify the carbonate saturation state in three mid-coast Texas estuaries. The long-term analysis of benthic and water quality data for the Guadalupe Estuary demonstrates that long-term freshwater inflow cycles regulate benthic abundance, productivity, diversity, and community structure. Furthermore, results from this study elucidate factors driving estuary ecosystem metabolism and carbonate system dynamics, which together hold implications for biological production. The authors have made great strides towards understanding how freshwater inflow drives water quality, biogeochemistry, and benthic communities in Texas estuaries. This study satisfactorily meets the goals and objectives outlined in the Scope of Work, and the reviewers commend the authors for a comprehensive and insightful report.

To place a stronger emphasis on the study findings, the reviewers recommend illuminating the conclusions and take-home points in addition to adding explanatory statements where appropriate. Some comments provided within (e.g., requests for term definitions) are focused on increasing comprehension and readability across disciplines and to the layperson.

Please integrate the two reports into one comprehensive report for submission of the Final Report. When they are combined, please take note of style formatting to adopt a consistent format, as each manuscript currently has an independent style for headers, references, figures, text justification, and captions. For reference, the TWDB "Formatting Guidelines for Texas Water Development Board Reports" are located at: http://www.twdb.texas.gov/about/contract_admin/index.asp. The guide currently has a single erratum in that the TWDB logo should *not* be used on the front cover of the report.

Please check the document for grammar, spelling, typographical errors, appropriate use of commas, and randomly dispersed symbols. Reviewers suggest avoiding the use of molecular formulas and acronyms, which tend to decrease readability of the report. If acronyms (or molecular formulas) must be used, please spell out the acronym, with the acronym in parentheses, the first time it is used. Be sure to place the preposition "the" before the TWDB acronym. Additionally, please ensure the consistent convention of spelling out numbers one through nine and writing numbers 10 and above.

Specific Draft Final Report Comments:

1. Page ii, Acknowledgements: Funding for the project was supplied by the Texas Water Development Board's General Revenue Fund. Please make the correction.

2. Pages 1 and 2, Introduction, subtitles "Benthos" and "Water": Please include subtitles that provide more context to the study or refrain from using subtitles in the introduction.

3. Page 1, Introduction: Please refer to the Senate Bill 3 (2007) mandate using the past verb tense.

4. Page 1, Introduction, second to last sentence: "Aadequate" is mis-spelled; please make the correction. Remove the "@" at the end of the sentence.

5. Page 1, Introduction, last sentence: Please expand upon the thought that BBEST and BBASC groups will need information about freshwater inflow effects on water quality and indicator communities to inform the provision for adaptive management to potentially refine the environmental flow standards.

6. Page 1, Benthos, 1st paragraph: Please define what "long-term hydrological cycles" are.

7. Page 1, Benthos, 2nd paragraph: Please change "Among Texas estuaries, increased salinity (and thus decreased inflow)…" to "Among Texas estuaries, decreased inflow (and, thus, increased salinity)…"

8. Page 2, Benthos, 1st paragraph: Please define "polyhaline."

9. Page 2, Benthos, 1st paragraph: Please delete "in" after important in the second sentence.

10. Page 2, Water, 1st paragraph: Please provide a citation for the first statement.

11. Page 2, Water, 2nd paragraph: Please define the terms "allochthonous" and "autochthonous."

12. Page 2, Water, 2^{nd} paragraph: Please provide more explanation to clarify the following statement:

    "As some fraction of the organic matter is likely to be algal-derived, it is important to discern effects of nutrient-driven algal production versus allochthonous organic matter to project how these systems may change in future due to climate and or anthropogenic drivers. Unfortunately, previous studies have not adequately coupled measures of organic matter components to inorganic nutrient measurements."

    a. Please provide specific information on why it is important to discern the effects of nutrient-driven algal production versus "allochthonous" organic matter.

    b. Also, please discuss and provide citations on how climate and anthropogenic drivers could drive changes in these systems.

13. Page 2, Water, 3^{rd} paragraph: Please ensure that all molecular formula names are written out the first time they are used.

14. Page 2, Water, 3rd paragraph: Please reword the following sentence for clarity:

    "The lack of data on the estuarine carbonate system in Texas estuaries is important, given that this determines the risks of coastal waters to acidification caused by both CO^2 invasion into seawater and estuarine alkalinity reduction caused by reduced freshwater inflow."

    a. Please clarify how "***the lack of data*** on estuarine carbonate system in Texas estuaries is important and how this determines the risks of coastal waters to acidification."

    b. Please describe "CO_2 invasion" or clarify if this terminology is in reference to "CO_2 uptake."

15. Page 2, Water, 3rd paragraph: Please define "freshwater endmember." If this is a reference to monitoring stations representing freshwater conditions or marine conditions, please refer to them as such.

16. Page 3, Approach: Please refer to the exact years being referred to in the statement:

    "...whereas this study corresponds with a strong El Niño period that has been linked with high freshwater inflow."

   a. Mention years of strong El Niño events and provide a citation to show that the higher rainfall in these years was attributable to El Niño. Else, mention that the period of data collection included two very wet years, i.e. 2015 and 2016.

17. Page 3, Methods, 1st paragraph and throughout: Please list the estuaries in order from upper coast to lower coast.

18. Page 3, Significance: Please delete, “The benthic data set has proven useful to date.”

19. Page 3, Significance: Please reword this statement: “In order to perform similar analyses and provide an understanding of the long-term ecosystem dynamics the San Antonio Bay System, data is needed, and the data collected during this study will support these efforts” to “The data collected in this study will provide an understanding of the long-term ecosystem dynamics of the San Antonio Bay System,” or other similar wording.

20. Page 5, Water Quality: Please spell out all unit acronyms, explain what symbols represent, and use the past tense when describing methods. Please ensure the use of “±” rather than the “∀” symbol.

21. Page 10, Results, 1st paragraph: Please describe whether any differences in water quality were observed between top and bottom samples.

22. Page 10, Results, 2nd and 3rd paragraphs: Please clarify that January 2013 to February 2015 was considered a dry condition. Also, please maintain consistent use of written words and their acronyms (e.g. dissolved oxygen vs. DO). The TWDB preference is to write out words rather than heavy acronym use.

23. Page 12: Table 3 is referenced in the text only with regards to salinity. Please discuss the other results from Table 3 in the body of the report.

24. Page 12, last sentence: Omit “However” from last sentence.

25. Page 15, 1st paragraph, last sentence: Please add dates to identify the period of flooding referred to in the text.

26. Page 15, 2nd paragraph: The text states that 46 species were found as noted in Table 4, but Table 4 shows only 42 species listed. Please make the appropriate correction.

27. Page 16, 1st and 2nd sentences: If there is an inference that can be drawn from the following thought, please add a clarifying statement.

"Station A, closest to the river, had the highest macrofauna abundance, biomass, and diversity. Station D, closest to the Gulf connection had the second highest biomass in 2016, but station B had the second highest biomass in 2017."

28. Page 15, 2nd paragraph: If there is an inference that can be drawn from the following thought, please add a clarifying statement. Additionally, if there is a definition for "rare species," please include it:

    "Only six species occur at all four stations. The high diversity found in San Antonio Bay is made up of rare organisms or organisms found primarily in the marine parts of the bay, especially stations C and D. For example, 22 species were found in only one station, and 10 species were found in only two stations. Together, these rare species made up 6% of all species found."

29. Page 18, Long-term analyses of the Guadalupe Estuary, 1st paragraph: The second sentence is incomplete, ending in "…for the entire." Please make the correction.

30. Page 18, 2nd paragraph, last sentence: Please clarify the statement: "Diversity trends also most clearly track salinity trends of all benthic metrics."

31. Page 21, Water Column Conditions in Mid-Coastal Estuaries, last sentence: Consider providing suggestions on why it might be the case that "in the Guadalupe Estuary, Station C was higher than D in July in all three years (2013–2015).

32. Page 27, Water Column Conditions in Mid-Coastal Estuaries, 1st paragraph: Please include any inferences as to why "…there was a second smaller spike for Station A in the Guadalupe Estuary in April 2014 during the drought, which was not related to an inflow event."

33. Page 29: Water Column Conditions in Mid-Coastal Estuaries: Please clarify what is meant by "parallel responses" and "parallel responses were especially evident." In Figure 13 (GE), the lines do not appear parallel in the mid- and latter portions of the record. Station A is much higher and stations C and D peak later than A and B in the latter portion of the record.

34. Page 32, Water Column Conditions in Mid-Coastal Estuaries, 1st paragraph: Please reconcile the statement: "Because stations typically have the same patterns" with the fact that station quantities for different variables vary based on where in an estuary the stations are located, and with the statement on page 35 (Discussion) that "Overall water quality trends of station-date combinations separate stations both by season and by amount of freshwater inflow that each station receives."

35. Page 35, Discussion, 3rd paragraph: The last sentence is incomplete: "In fact, the current period (after January 2016) had a community structure that was more typical, ." Please make the appropriate correction.

36. Pages 37-40, References: Please ensure that all references listed are cited in the text of the report. Upon a quick check, the following references are listed in the References section, but do not appear to be cited in the text: Abril et al 2002, ASTM , Butman and Raymond 2011, Crosswell et al 2012, Jiang et al 2008, Mallin et al 2002, Petrone et al 2009, Salisbury et al 2008, Seitzinger and Sanders 1997, Servais et al 1987, Standard Methods for the Examination of Water and Wastewater, Wetz and Hu 2015. Also, please list the multiple Montagna references in chronological order from most recent to less recent.

Figures and Tables Comments:

1. Table 5: Please add 'pH' to the Table caption.

2. Table 6, page 25:

    a. Please add pH and NTU to the table caption.

    b. Please omit: "There are also many missing values in the data set" in the table caption. However, please do mention this in the body of the text and briefly describe what steps were taken to account for the missing values in the calculation of the descriptive statistics provided in Table 6.

    c. Please clarify if the statistics are derived from the quarterly sampling or from annual statistics across all samples.

    d. Use subscripts formatting where necessary (e.g. NO_{2+3}, PO_4)

3. Figure 6: Please reword the figure caption to "Long-term estuary-wide time series" or "Long-term estuary-wide patterns," or "Long-term estuary-wide trends." If using the word, "change," there should be a description of the metric used to quantify "change."

SUGGESTED CHANGES

Specific Draft Final Report Comments:

1. Page 2, Water and throughout: Please consider that avoiding the use of molecular formulas and acronyms will significantly increase readability. Please consider writing out "carbonate saturation state" rather than using the omega sign "Ω."

2. Throughout: Please consider replacing the use of “changes” with “variability” or similar term where appropriate, throughout the report.
3. Page 35, last paragraph: In reference to the following sentence, “… and this is because of the expansion of a more diverse marine fauna that invades San Antonio Bay during dry periods,” please consider clarifying the statement to expand on the specific species that prosper during dry periods. The word ‘invade’ suggests invasive species or non-native species which may not be what the author is suggesting. Text in preceding paragraphs mention *Molgula manhattenis* as such an opportunistic species, but the text states this species was not found during the study period.

Figures and Tables Comments:

1. Table 6: Please consider disaggregating the statistics by season.

Spatial-Temporal Distribution of Organic Matter and Carbonate Chemistry Parameters in Texas Mid-Coast Estuaries: 2012-2017

Michael S. Wetz, Ph.D. and Xinping Hu, Ph.D.

Contract #1600011924
TWDB Comments to Final Report

REQUIRED CHANGES

General Draft Final Report Comments:

Throughout the report Rincon Bayou is described separately from the Nueces Estuary. While Rincon Bayou may have characteristics that make it distinctive, it is located in the Nueces River Delta and is typically referred to as part of the Nueces Estuary. Please make this indication throughout the report where appropriate.

Specific Draft Final Report Comments:

1. Page 1, Introduction: Please ensure that all molecular formula names are written out the first time they are used.

2. Page 1, Introduction, 1st paragraph: Please indicate that Rincon Bayou is also located in the Nueces Estuary.

3. Page 1, Introduction, 3rd paragraph: Please explain why the inorganic carbon or the carbonate system is the "dominant buffer system in estuaries."

4. Page 1, Introduction, Water, 3rd paragraph (and in other instances throughout the report): Please define "endmember." If this is a reference to monitoring stations representing freshwater conditions or marine conditions, please refer to them as such.

5. Page 3, Methods, 1st paragraph: Rincon Bayou is located within the Nueces Estuary but it is presented as separate from the Nueces Estuary. Please make the appropriate correction.

6. Page 3, Methods, 1st paragraph: Please clarify why "The study area is ideal to answer questions related to altered hydrology and climate variability occurring at different temporal, and different spatial scales of inflow along climatic and estuarine gradients." Also, please clarify what is meant by "spatial scales of inflow."

7. Page 3, Methods, 2nd paragraph 3rd sentence: Insert "occurred" after "Sampling."

8. Page 4, Methods, 1st paragraph: please omit "at the Hu's lab."

9. Page 4, Methods, 4th paragraph: Remove "Upon returning to the lab."
10. Page 5, Results and Discussion, 2nd paragraph: Please define "labile."

11. Pages 12-13, References: Please ensure that all the references listed are cited within the body of the text. A quick check shows that Salisbury et al., 2008 is not cited in the report.

12. Page 8, Results and Discussion, Carbonate chemistry spatial and temporal variations: Please provide a summary description of the data presented in Table 2 and Figure 8.

Figures and Tables Comments:

1. Please consider combining Figures 2, 3, and 4 as a single figure with subfigures (e.g., 2A, 2B, 2C). Please increase the size of the figure and format without text wrapping.

2. Please consider presenting Figure 5 as a single figure with subfigures 5A, 5B, and 5C. Please increase the size of the figure and format without text wrapping.

3. Please consider combining Figures 6 and 7 as a single figure with subfigures A and B. Please increase the size of the figure.

4. Please expand Figures 8 and 9 in size so that each set of figures (8A and B; 9A and B) is displayed on a separate page.

5. Please display Figure 10 with subfigures A and B and expanded in size.

6. Please expand Figures 11 and 12 in size so that each set of figures (11A and B; 12A and B) is displayed on a separate page.

SUGGESTED CHANGES

Specific Draft Final Report Comments:

1. Page 1, Introduction and throughout: Please consider that avoiding the use of molecular formulas and acronyms will significantly increase readability. Please consider writing out "carbonate saturation state" rather than using the omega sign "Ω."

2. Please consider developing a conceptual diagram – an effective tool for science communication – to depict the essential attributes of the carbonate system as

controlled by the river-ocean mixing and biogeochemical processes described in the text.

3. Include statements in conclusion that relate results of the study to clarify exactly how freshwater inflow drives water quality and estuarine biogeochemistry along the Texas coast.

Figures and Tables Comments:

The reviewers suggest that manuscript body text not be wrapped tightly around figures (text to the left or right) but rather formatted above or below figures for increased consistency and readability. Each figure and table should be presented on a separate page with accompanying caption to increase readability. Figure 4 in the Montagna manuscript is a good example of a full-page figure with caption. Please consider using hard breaks at the end of figures so the report text starts on the next line.