



## **Effects on Benthic Macrofauna from Pumped Flows to Rincon Bayou**

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The views expressed herein are those of the authors and do not necessarily reflect the views of CBBEP or other organizations that may have provided funding for this project.

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## Abstract

Decreased inflow due to damming of the Nueces and Frio rivers has resulted in increasing salinity in Nueces Bay and caused Rincon Bayou to become a reverse estuary disturbing the overall hydrology of the adjacent Corpus Christi Bay. Adaptive management to perform hydrological restoration began in 1994 and continues today. The objectives of the present study are to determine to what extent salinity fluctuates within Rincon Bayou and what effects these fluctuations have on estuary health. Benthic infauna are ideal indicators of ecological effects because of their relative immobility and longevity in contrast with plankton of comparable size. Nearly all past studies focused on benthic infauna, here we add measurements of benthic epifauna, which are larger, more mobile invertebrates and represent a higher trophic level. Archived samples were analyzed as well as new samples collected from the upper Rincon Bayou near Corpus Christi, TX. For macroinfaunal, one historical station (C) was sampled biweekly and two historical stations (F and G) were sampled quarterly. For epifauna, all three stations were sampled biweekly. Conductivity, temperature, and salinity were monitored continuously. Additional water column measurements were taken during sampling events. Macrofauna and epifauna biomass, abundance, and diversity were recorded and analyzed. High inflow reduces salinity and introduces nutrients. Large and haphazard salinity fluctuations result in an often disturbed system populated by pioneer species, such as chironomid larvae and the polychaete *Streblospio benedicti*, during especially low and high salinity periods. Epifaunal organisms are mobile and capable of escaping unsuitable conditions, so the more immediate results of fluctuations in water quality is the lack of higher trophic marine organisms following pumping events. Results of time lags indicated that variance in diversity variables in response to changes in salinities occurred within the first few weeks after pumping. Immediate responses to salinity were not identified in abundance and biomass. Positive relationships between abundance and biomass, in response to salinity fluctuations, were evident after 6 to 8 weeks. The results of the infaunal and epifaunal analyses indicate that further changes need to be made to the Rincon Bayou restoration and management programs in order to reestablish a reasonably undisturbed ecosystem.

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## Introduction

The Wesley Seale Dam was built on the Nueces River in 1958 and the Choke Canyon Dam was built on the Frio River in 1982 (Montagna et al. 2002). In 1996, stream flow to the Nueces River had decreased by 55% since the building of the Wesley Seale Dam and the Choke Canyon Dam compared to the pre-1983 era (Asquith et al. 1997). In the same period, flow from the Nueces River to the Nueces Delta via Rincon Bayou decreased 99% (Irlbeck and Ward 2000, Ward et al. 2002). Decreased flow has increased salinity in Nueces Bay and Rincon Bayou resulting in a reverse estuary, where salinity is higher upstream than downstream, disturbing the overall hydrology of the estuary (Palmer et al. 2002). The Nueces Overflow Channel was built in 1995 by the U.S. Bureau of Reclamation in an effort to restore ecological value to the bayou by allowing increased freshwater inflows to the area (Montagna et al. 2002). The channel was closed in 2000, but reopened in 2002 (Palmer et al. 2002).

The effects on benthos of the altered freshwater inflows via the channel reached six kilometers downstream, but failed to affect restoration in the lower reaches of the bayou or in Nueces Bay (Palmer et al. 2002). However, within the affected area, organismal response to moderate inflow was positive, producing higher abundance, diversity, and biomass of benthic macrofauna (Montagna et al. 2002). Following floods, pioneer species, such as *Streblospio benedicti*, were found in high abundance (Palmer et al. 2002; Ritter et al. 2005). The presence of pioneer species indicates that Rincon Bayou is likely an area of high disturbance following floods (Connell and Slayter 1977). In fact, Rincon Bayou is likely in a constant state of early to intermediate succession because of the highly variable environmental conditions (Ritter et al. 2005).

It is possible that reducing the great fluctuation in flow could help improve the ecological state of Rincon Bayou. To improve hydrological conditions in Rincon Bayou, a pipeline and pumping station was completed in fall 2008, but first used in 2009, from the Calallen Pool to Rincon Bayou. The pumping facilitates additional freshwater inflows that do not depend upon overflow from the Calallen Dam (Adams and Tunnell 2010). The salinity and benthos were monitored for one year after initial pumping began, but that study was during relatively wet periods; average salinity was 17 psu from between 28 September 2009 and 11 August 2010 (Barajas 2011). Because salinity did not vary much during this study period, there was little correlation between abundance and species composition to salinity (Barajas 2011). Thus, it is not known what optimal pumping strategy would improve the ecology of Rincon Bayou and the surrounding systems.

The purpose of the current study is to determine the effects of salinity changes on benthic macrofauna. Salinity decreases within days when the river flows or pumping begins (Adams and Tunnell 2010, Barajas 2011), so salinity is a proxy for inflow. Macrofauna are ideal indicator organisms of habitat quality because of their relative immobility and longevity in contrast with plankton of comparable size (Diaz et al. 2004). New samples were collected for one year from

the upper Rincon Bayou and added to a time series of archived samples. The relationship between salinity and benthic metrics were analyzed to determine the effects of salinity changes on the abundance, biomass and diversity of benthic macrofauna. Biomass was measured at the species level so that species-specific responses could be observed and evaluated.

## Materials and Methods

### Site Description

The study took place in Rincon Bayou near Corpus Christi, Texas, USA. Rincon Bayou flows east from the Nueces River to Nueces Bay and is the main stem of the Nueces Marsh (Figure 1). The two main sources of freshwater input to Rincon Bayou are the Nueces River Overflow channel and the Calallen pump station that pumps water from the Calallen Pool directly into Rincon Bayou. The historical stations, C ( $27.89878^{\circ}$  N,  $-97.60417^{\circ}$  W) and F ( $27.87760^{\circ}$  N,  $-97.57873^{\circ}$  W) sampled since October 1994 (Montagna et al. 2002), and an additional station G ( $27.88992^{\circ}$  N,  $-97.56910^{\circ}$  W) sampled since October 2002, were sampled for this study (Fig. 1). Station C is nearest to the pump outfall and overflow channel in the upper Rincon Bayou and has been shown to be the most affected by previous attempts to restore freshwater inflow to the area (Palmer and Montagna 2002). Station C is also known as 466C, F as 400F, and G as 463G (Montagna et al. 2009). The sites are surrounded by dense shrubs and marsh grasses that grow to the shoreline. Clay and mud dominate the substrate at all stations.

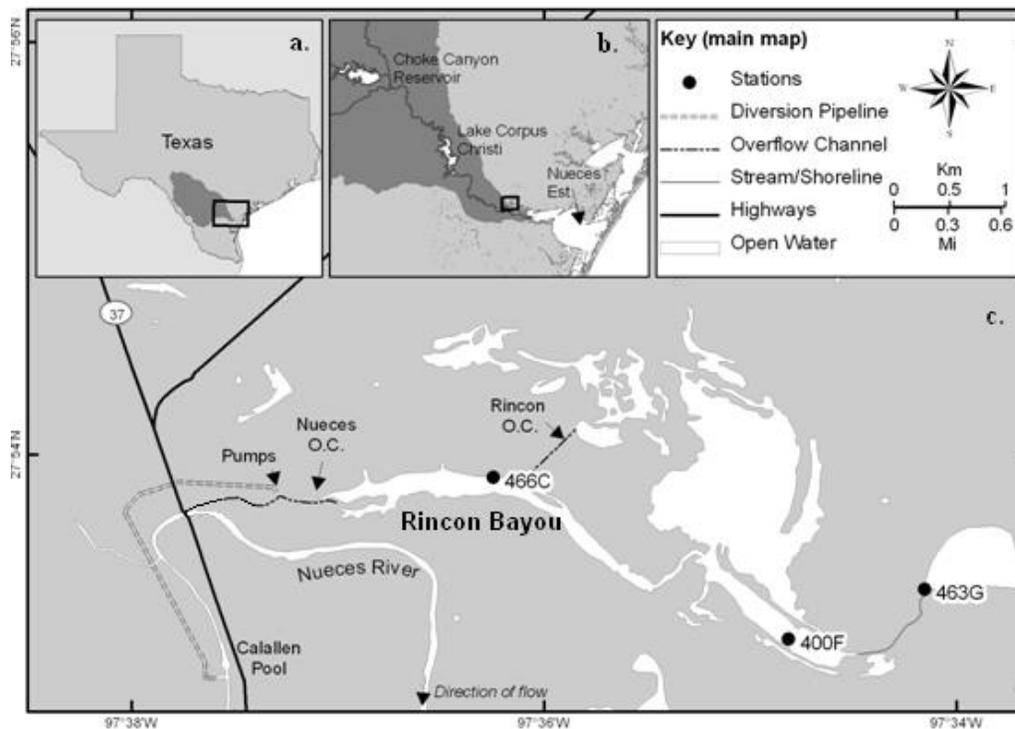


Figure 1. Map of study area. a) State of Texas with the Nueces Basin highlighted. b) Location of Choke Canyon Reservoir, Lake Corpus Christi and Nueces Estuary (Nueces Bay) within the Nueces Basin. c) Location of the Nueces Delta marsh containing Rincon Bayou.

## **Sampling Methods**

Macroinfauna samples were collected using a 6.7-cm diameter benthic core (area=35.23 cm<sup>2</sup>). Three replicates were taken by hand at each station. The cores were divided into 0-3 cm and 3-10 cm vertical sections and preserved in 10% buffered formalin. Samples were washed through a 500 micron steel sieve and sorted under a dissecting microscope to the lowest taxonomic level possible. Specimens were stored in 75% ethanol until biomass measurements were performed. Organisms were then placed on pre-weighed aluminum pans and dried in an oven for a minimum of 24 hours at 55 °C. Organism weight was recorded to the nearest 0.01 mg. Specimens weighing less were assigned a weight of 0.01 mg. Mollusk shells were dissolved in 1 N HCL prior to biomass measurements.

Epifauna samples were collected using a push net measuring 1.0 m x 1.0 m with a 5.0 mm mesh. The push net was used to collect one sample by pushing towards the shore, in 5 m tows. Samples were taken from the northern bank of station C, and the southern bank of station F. At station G, a small channel flows under an adjacent train trestle so samples were taken on the northern side and one on the southern side of the bridge. All samples were preserved in 5% buffered formalin, sieved (5.0 mm mesh), sorted, and each specimen identified to the lowest taxonomic classification possible. Fish standard lengths were measured (mm) and individual wet weights (mg) recorded. Penaeid shrimp lengths were measured (mm) from the tip of the rostrum to the tip of the telson. Blue crab lengths were measured (mm) from the width, i.e., spine to spine. Lengths of bivalves were measured across the widest portion of the shell. Gastropod shells were measured from the apex across the aperture. Shells were dissolved in 10% HCl (typically 5-10 minutes) and organism wet weights were recorded.

## **Archived Samples**

Archived epibenthic samples from previous collections in Rincon Bayou were used in addition to new samples collected during the present study period. A total of 40 archived sample dates between 28 April 2010 and 24 August 2015 were processed. These samples were analyzed using the method above.

## **New Samples**

Epibenthic samples were collected biweekly from 09 September 2015 through 25 April 2015 at all stations.

Macroinfaunal samples were collected biweekly from 09 September 2015 through 25 April 2015 at Station C, and quarterly three times (09 September 2015, 04 January 2016, and 11 April 2016) at stations F and G.

Water quality measurements were taken at each station per sampling event with a YSI 6920 multiparameter sonde at 0.1 m and at the bottom depth. Temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), salinity (psu), conductivity (mS cm<sup>-1</sup>), depth (m), and pH were measured using two YSI

600LS sondes. Calibrations were made using known standards for pH, conductivity, salinity, depth, turbidity, and dissolved oxygen (DO) concentration and percent saturation.

### Data Analysis

Data base programming, calculations, and statistical analyses were performed using SAS 9.4 software (SAS Institute Inc. 2013). Diversity was calculated using Hill's N1 diversity (Hill 1973), which is a measure of the effective number of species in a sample, and indicates the number of abundant species. It is calculated as the exponentiated form of the Shannon diversity index:

$$\text{Shannon diversity index: } H' = -\sum_{i=1}^R p_i \ln p_i$$

$$\text{Hill's N1 diversity: } N1 = e^{H'}$$

As diversity decreases N1 will tend toward 1. The Shannon index, H', is the average uncertainty per species in an infinite community made up of species with known proportional abundances (Shannon and Weaver 1949). Richness is an index of the number of species present, which is simply the total number of all species found in a sample regardless of their abundances. Hill (1973) named the richness index N0.

Evenness was calculated using Pielou's evenness index (Pielou 1975) which indicates the how numerically equal the species are within the community. This index is based on the Shannon diversity index:

$$\text{Pielou's evenness index: } J' = \frac{H'}{H'_{\max}} = \frac{H'}{\log S}$$

Correlations were calculated using the Pearson product-moment correlation which determines the strength of linear relationships between variables:

$$\text{Pearson product-moment correlation: } r = r_{xy} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}}$$

PROC CORR was used to calculate the Pearson product-moment correlation coefficients and probabilities for Hill's N1 diversity, Pielou's evenness index, biomass, and abundance.

Community structure of macrofauna and epifauna species were analyzed in PRIMER-e software by non-metric multidimensional scaling (MDS) and cluster analysis using a Bray-Curtis similarity matrix (Clarke and Warwick 1994). Prior to analysis, the data was natural logarithm 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.

## Results

### Hydrography

Rincon Bayou hydrographical variables Salinity (PSU), Conductivity (uS/cm), Temperature (C), DO (mg/L and %), pH, and Water Depth (m) were observed biweekly from September 9, 2015 to April 25, 2016 for stations C, F, and G (Table 1). Salinity (PSU), Conductivity (uS/cm), Temperature (C) were also measured hourly at station C beginning September 1, 2015 and ending February 16, 2016.

Salinity at station C was consistently lower than stations F and G, with mean salinity at 8.4 (PSU), 14.3 (PSU), and 15.77 (PSU) respectively (Table 1 and Figure 2). Temperature was similar at all stations (Figure 3). However, water depth (m) was consistently higher at station G (0.28 m) while stations C and F were similar at 0.14 (m) and 0.15 (m) respectively (Table 1 and Figure 4). Dissolved Oxygen (mg/L and %) was consistently high (> 6 mg/L) at all stations with the minimum Dissolved Oxygen 83% and maximum 203.1% (Table 1 and Figure 5). The pH averaged above 8 pH but with two low periods of < 8 pH observed in January 2016 and April 2016 (Table 1 and Figure 6).

Table 1. Summary statistics of hydrological parameters collected biweekly at stations C, F, and G from September 9, 2015 to April 25, 2016. Observations at each collection were measured at surface (0.1 m water depth) and at bottom depth (within 0.1 m of sediment). Variable water depth (m) is the reported total depth at each station per observation period.

Variable	Station	Mean	Std Dev	Minimum	Maximum
Salinity(psu)	ALL	12.98	7.85	0.83	26.45
	C	8.40	5.82	0.83	22.47
	F	14.30	7.83	1.44	25.25
	G	15.77	7.83	4.60	26.45
Conductivity(uS/cm)	ALL	21.25	12.02	1.64	41.31
	C	14.20	9.16	1.64	35.69
	F	23.27	11.98	2.77	39.76
	G	25.54	11.81	8.32	41.31
Temperature(C)	ALL	23.86	4.66	15.04	33.77
	C	23.09	4.80	15.04	32.76
	F	23.80	4.75	15.25	33.77
	G	24.56	4.50	15.87	31.96
DO(mg/l)	ALL	9.58	2.01	6.48	15.79
	C	9.31	2.06	6.48	15.13
	F	9.70	1.76	7.54	13.16
	G	9.71	2.20	6.65	15.79
DO(%)	ALL	121.00	20.68	83.00	203.10
	C	113.70	23.01	83.00	203.10
	F	123.22	17.76	102.50	172.10
	G	125.33	19.78	95.10	170.00
pH	ALL	8.13	0.17	7.74	8.49
	C	8.11	0.17	7.74	8.49
	F	8.10	0.18	7.75	8.48
	G	8.17	0.14	7.85	8.43
Water Depth (m)	ALL	0.19	0.09	0.02	0.35
	C	0.14	0.06	0.02	0.25
	F	0.15	0.07	0.03	0.25
	G	0.28	0.05	0.20	0.35

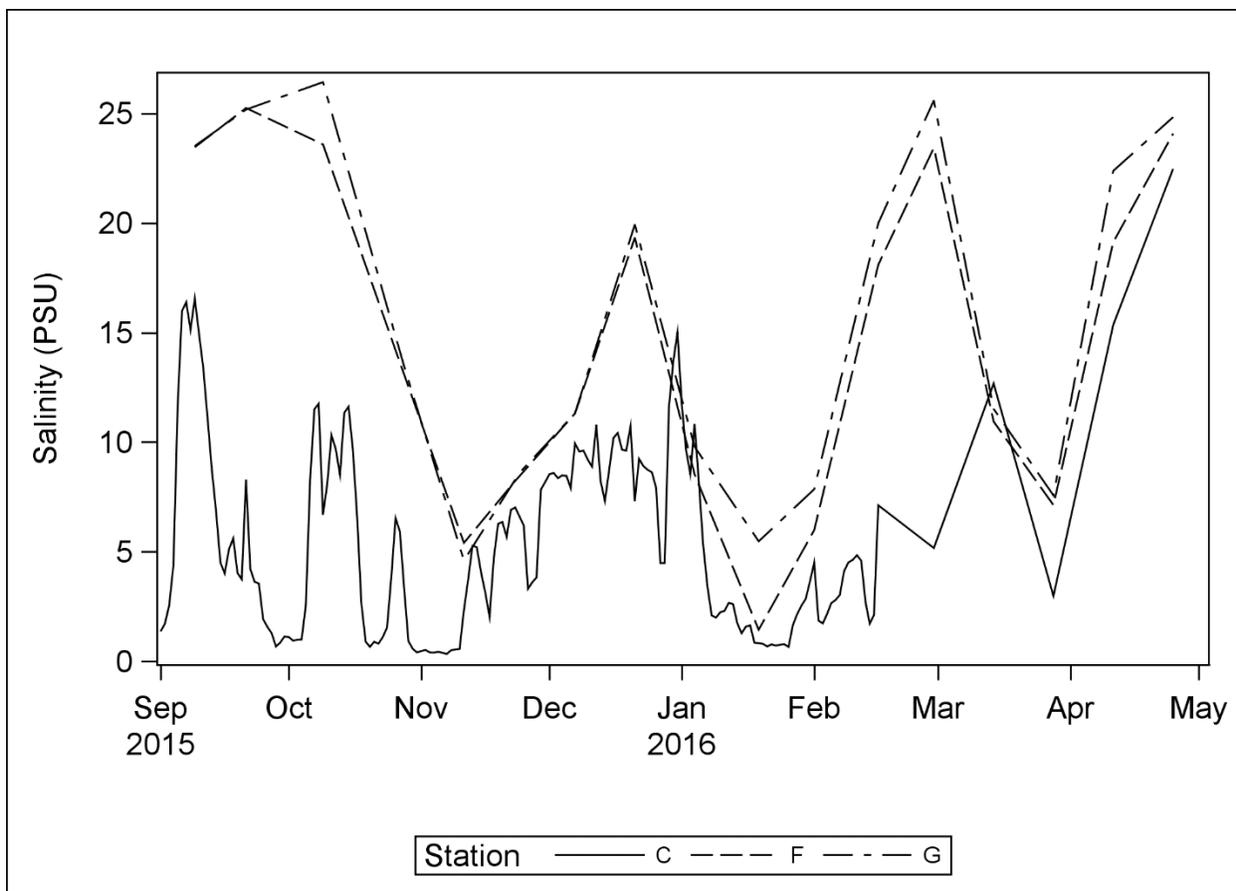


Figure 2. Rincon Bayou mean sonde salinity (PSU) observations from September 1, 2015 to April 25, 2016 for stations C, F, and G. Mean daily salinity (PSU) observations for station C beginning September 1, 2015 and ending February 16, 2016 are joined to the biweekly observations over the entire study period.

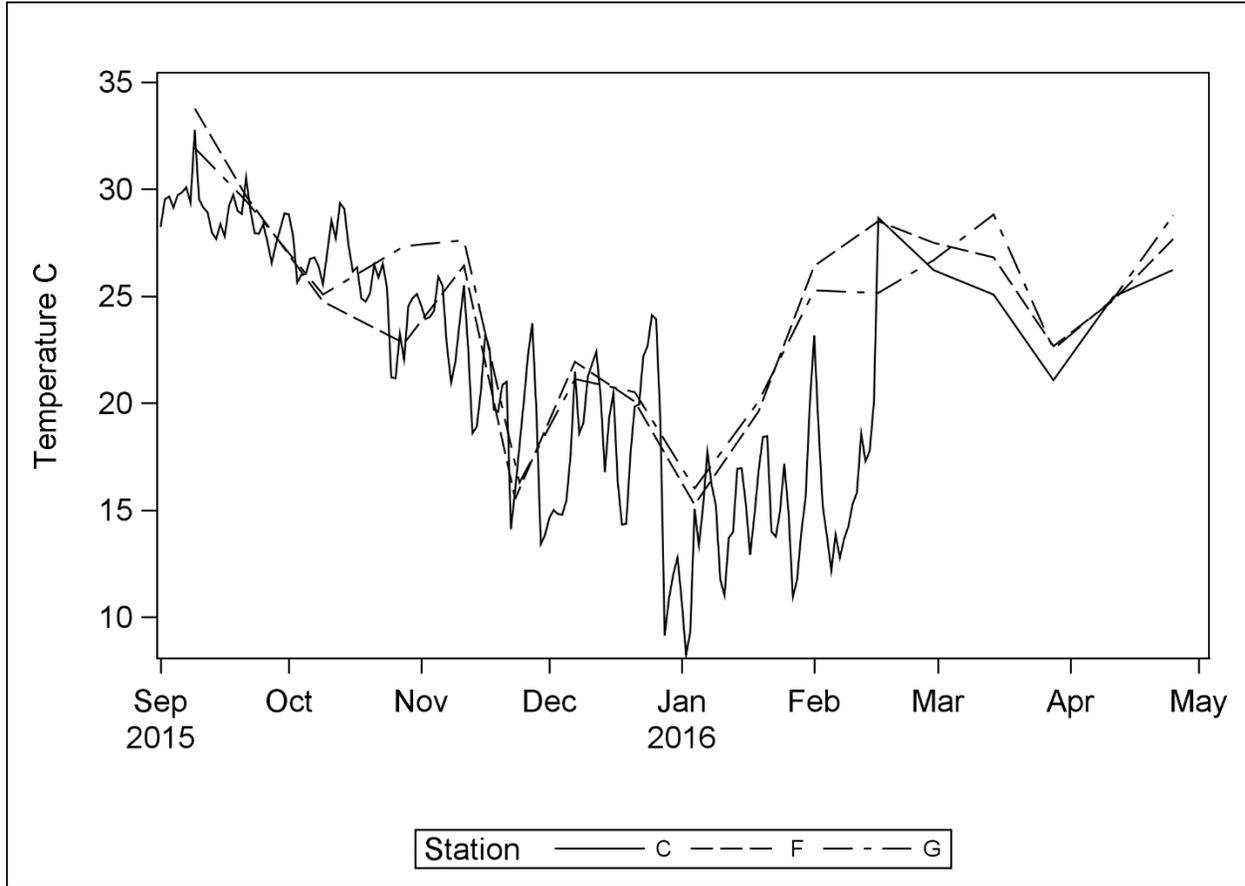


Figure 3. Rincon Bayou mean sonde temperature (C) observations from September 1, 2015 to April 25, 2016 for stations C, F, and G. Mean daily temperature (C) observations for station C beginning September 1, 2015 and ending February 16, 2016 are joined to the biweekly observations over the entire study period.

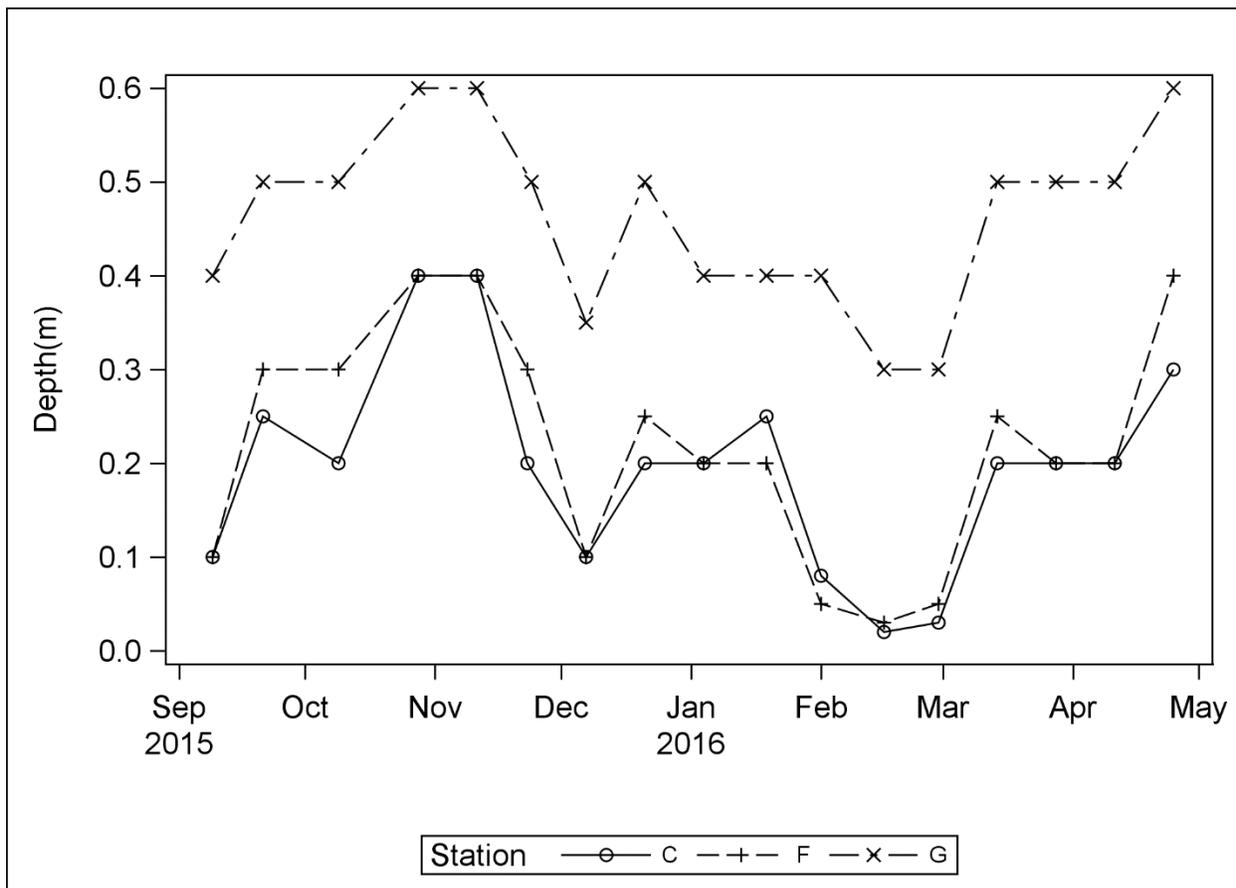


Figure 4. Rincon Bayou water depth (m) biweekly observations from September 9, 2015 to April 25, 2016 for stations C, F, and G. Water depth (m) is the total depth recorded by hand using a ruler.

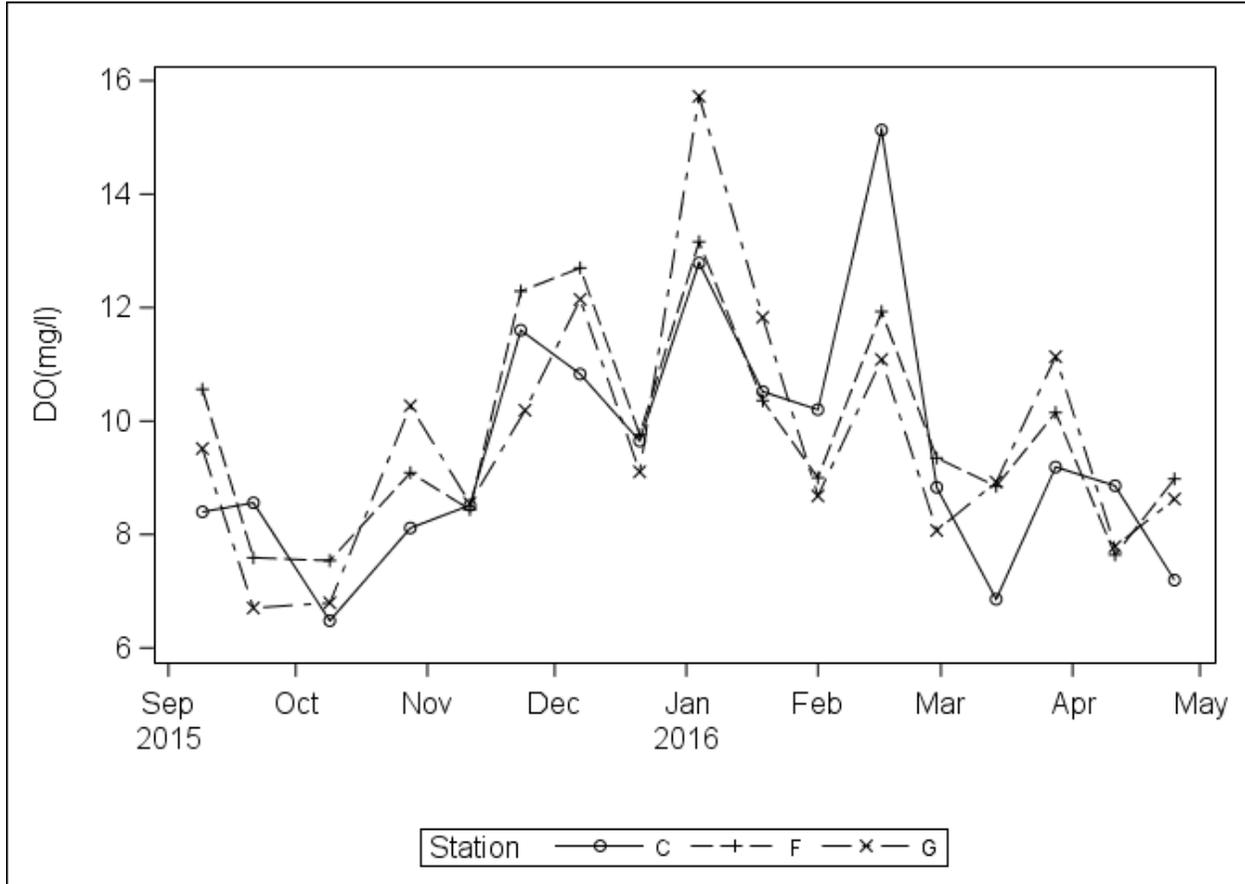


Figure 5. Rincon Bayou dissolved oxygen (DO) biweekly sonde observations from September 9, 2015 to April 25, 2016 for stations C, F, and G. Dissolved oxygen (mg/L) was recorded using a YSI 6920 V2 multiparameter sonde.

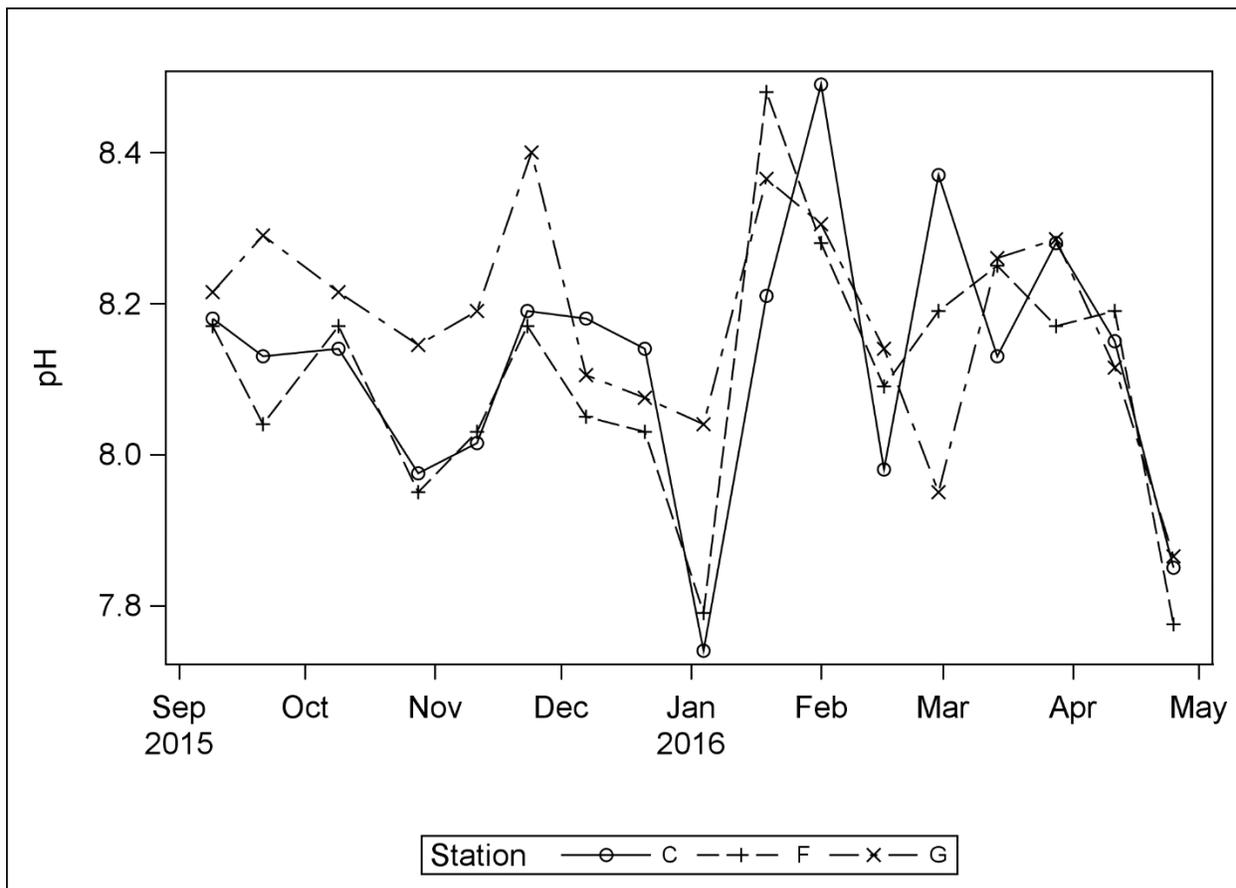


Figure 6. Rincon Bayou pH biweekly sonde observations from September 9, 2015 to April 25, 2016 for stations C, F, and G. pH was recorded using a YSI 6920 V2 multiparameter sonde.

## Grain Size

Grain size of sediment was found to increase downstream along Rincon Bayou from station C, to F, to G (Figure 7). Silt comprises the majority of sediment at station C from 0 – 10 cm in depth while station F sediment comprised primarily sand from 0 – 10 cm in depth (Table 2). Finally station G, 0 – 3 cm sediment was 47 % sand while 3 – 10 cm below the surface was 40% rubble (Table 2).

Table 2. Mean sediment grain size observations per station. Two replicate cores were sampled at each station on October 9, 2015 and each replicate sectioned 0 - 3 cm and 3 - 10 cm from the surface. Percentages in bold indicate the predominant grain size per section.

Station	Section	Sand	Silt	Clay	Rubble
C	0 - 3 cm	17.75%	<b>45.25%</b>	22.42%	14.58%
C	3 - 10 cm	18.74%	<b>49.07%</b>	20.57%	11.62%
F	0 - 3 cm	<b>37.92%</b>	28.16%	14.11%	19.80%
F	3 - 10 cm	<b>45.81%</b>	16.86%	8.95%	28.38%
G	0 - 3 cm	<b>46.76%</b>	19.65%	8.97%	24.61%
G	3 - 10 cm	39.21%	13.35%	7.33%	<b>40.12%</b>

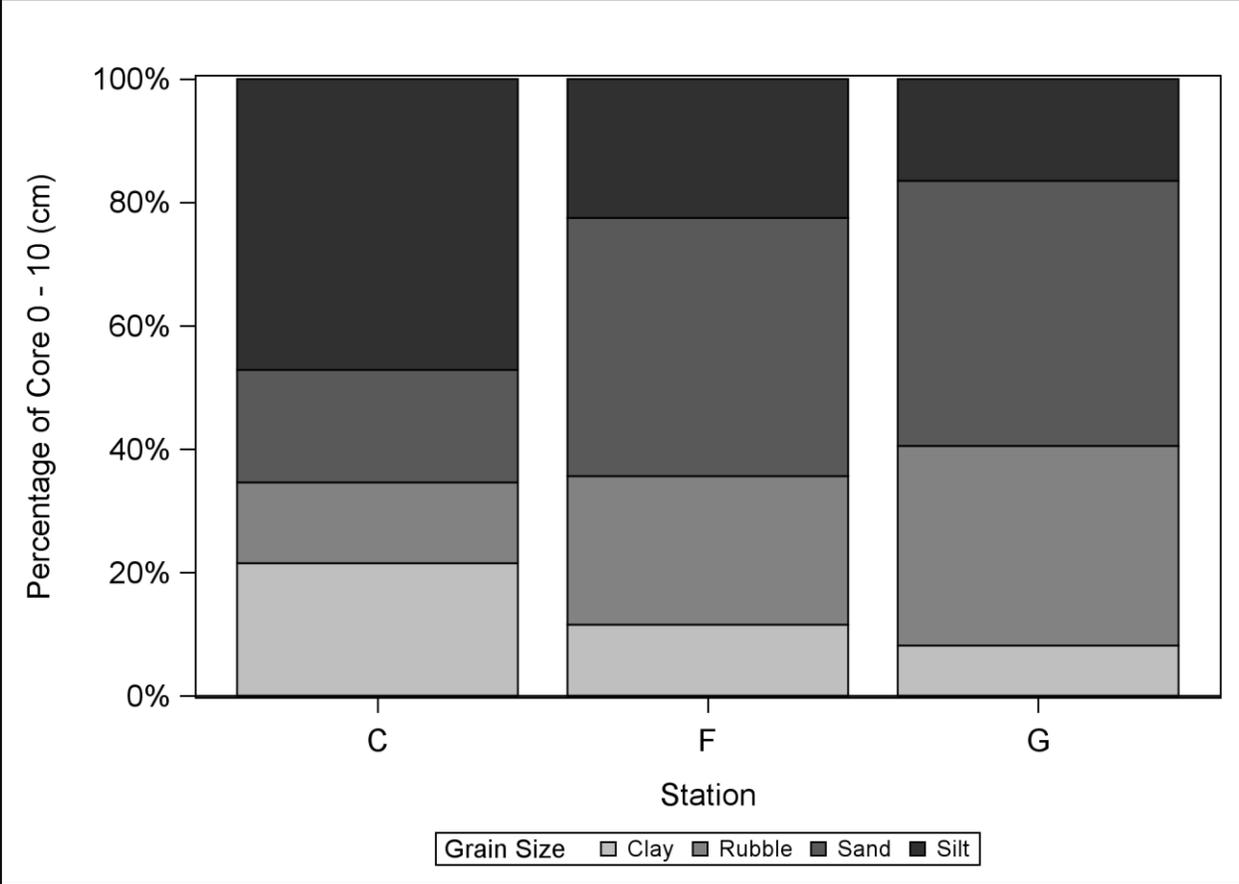


Figure 7. Average sediment grain size composition for stations C, F, and G for 0 to 10 cm in depth as observed on 9 October 2015.

## Macroinfauna

Station C exhibited both the highest (10,873 n/m<sup>2</sup>) and lowest (94.5 n/m<sup>2</sup>) mean benthic infaunal abundance (Figure 8 and Table 3). The other two stations ranged from the 1,986 n/m<sup>2</sup> to 8,226 n/m<sup>2</sup> at station G and 2,553 n/m<sup>2</sup> to 4,349 n/m<sup>2</sup> at station F in mean abundance. Stations F and G exhibit similar trends in abundance while station C's trend is independent of the other stations. Mean abundance peaked at station C in the spring while station F and G exhibited peak mean abundance in the middle of the winter months.

Station C exhibited both the highest (30.81 g/m<sup>2</sup>) and lowest (0.01 g/m<sup>2</sup>) mean benthic infaunal biomass (Figure 9 and Table 3). The other two stations ranged from the 0.09 g/m<sup>2</sup> to 0.39 g/m<sup>2</sup> at station F and 0.27 g/m<sup>2</sup> to 2.58 g/m<sup>2</sup> at station G with station G's maximum mean biomass being the second largest mean biomass. The largest biomass at station C occurred during the middle of winter while the second largest mean biomass at station G was recorded in the spring.

The highest mean benthic infaunal diversity (4.67 N1) was found at station F (Figure 10, Table 3). The other two stations ranged from the 1 N1, the lowest mean diversity, to 4.61 N1 at station C and 1.15 N1 to 3.33 N1 at station G with station C's maximum mean diversity being the second largest mean diversity. The largest mean diversity was observed in the spring while the second largest was observed in the late winter.

The highest mean benthic infaunal Shannon diversity index (1.54 H') was found at station F (Figure 11, Table 3). The other two stations ranged from the 0 H', the lowest mean diversity index, to 1.53 H' at station C and 0.14 H' to 1.20 H' at station G. The largest mean diversity index was observed in the spring while the second largest was observed in the late winter.

Station C had the highest mean benthic infaunal richness (7 species) and the lowest (1 species) (Figure 12, Table 3). The other two stations ranged from the 2 species to 6 species at station F and 3 species to 5 species at station G in mean species richness. The highest mean species richness was observed in later winter, the second highest in spring, and the lowest in fall.

Abundance appears to have a mostly inverse relationship with salinity; as salinity peaks abundance plummets and vice versa (Figure 13). Diversity appears to have a similar relationship with salinity; as freshwater is pumped in, diversity drops (Figure 14). Richness appears to have no significant relationship with salinity during this sampling period (Figure 15). However, there were no significant relationships between macrofauna metrics (abundance, biomass, and diversity) and salinity in the prior two to eight weeks (Table 4).

Table 3: Summary statistics of benthic infaunal variables collected biweekly at station C from 09 September 2015 to 25 April 2016 and quarterly from station F and G on 09 September 2015, 04 January 2016, and 11 April 2016.

Variable	Station	Mean	Std Dev	Minimum	Maximum
Abundance (n/m <sup>2</sup> )	ALL	3568.1	2554.5	94.5	10872.9
	C	3242.4	2513.9	94.5	10872.9
	F	31516	1037.1	2552.8	4349.2
	G	5830.4	3363.2	1985.5	8225.6
Biomass (g/m <sup>2</sup> )	ALL	1.81	6.35	0.01	30.81
	C	2.20	7.38	0.01	30.81
	F	0.27	0.16	0.09	0.39
	G	1.13	1.26	0.27	2.58
Diversity (N1/core)	ALL	2.04	1.14	1.00	4.67
	C	1.91	1.06	1.00	4.61
	F	2.69	1.80	1.17	4.67
	G	2.18	1.10	1.15	3.33
Shannon Diversity Index (H'/core)	ALL	0.58	0.52	0	1.54
	C	0.52	0.50	0	1.53
	F	0.84	0.69	0.16	1.54
	G	0.69	0.53	0.14	1.20
Richness (species/core)	ALL	3.48	1.90	1	7
	C	3.24	2.02	1	7
	F	4.00	2.00	2	6
	G	4.33	1.15	3	5

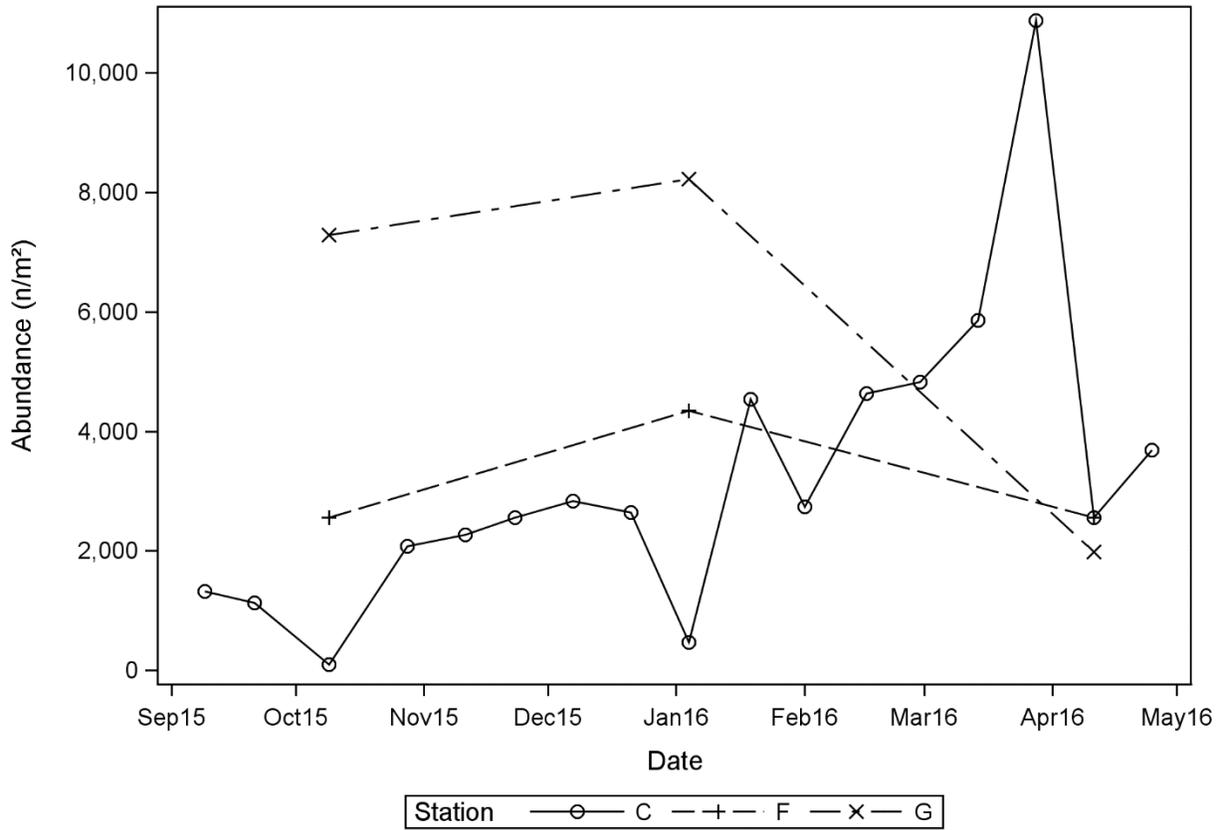


Figure 8: Mean benthic infaunal abundance over time for Rincon Bayou Stations C, F, and G from 09 September 2015 to 25 April 2016.

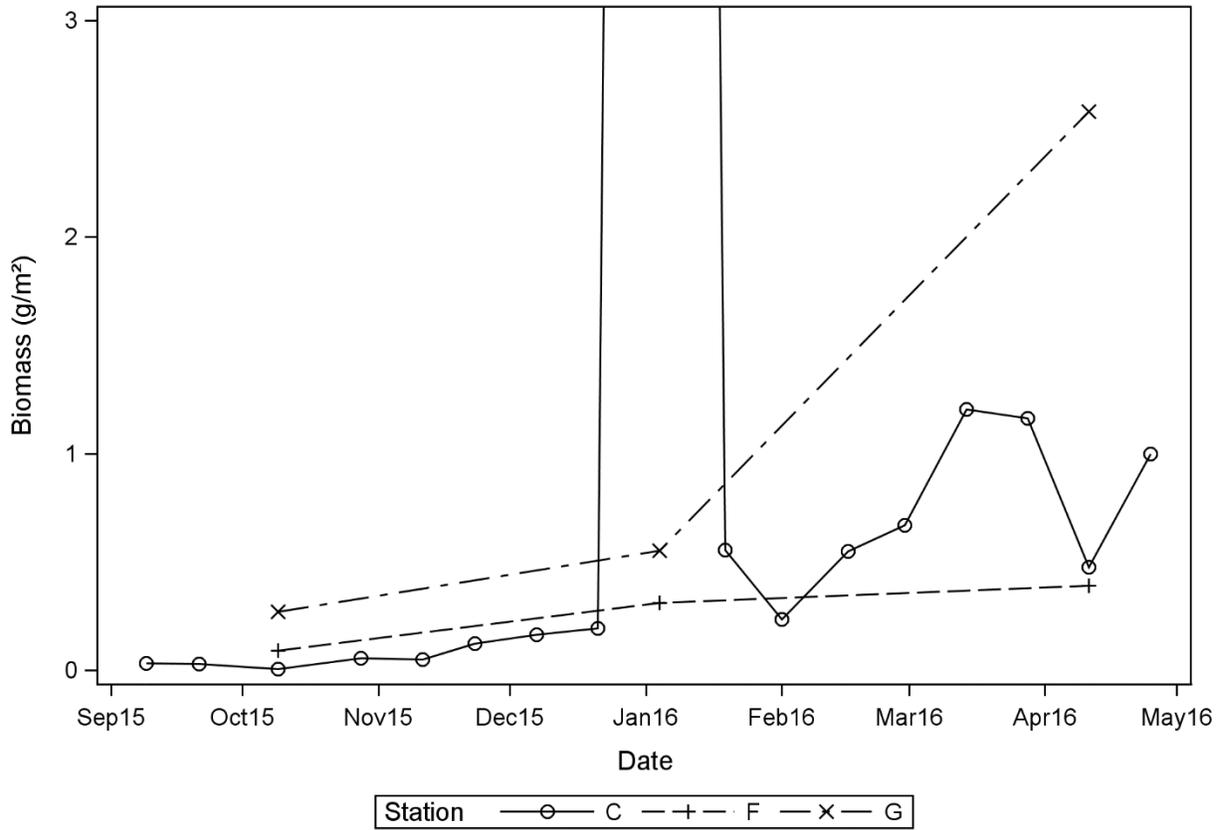


Figure 9: Mean benthic infaunal biomass over time for Rincon Bayou Stations C, F, and G from 09 September 2015 to 25 April 2016. The data point displayed off the graph was 30.81 g/m<sup>2</sup>.

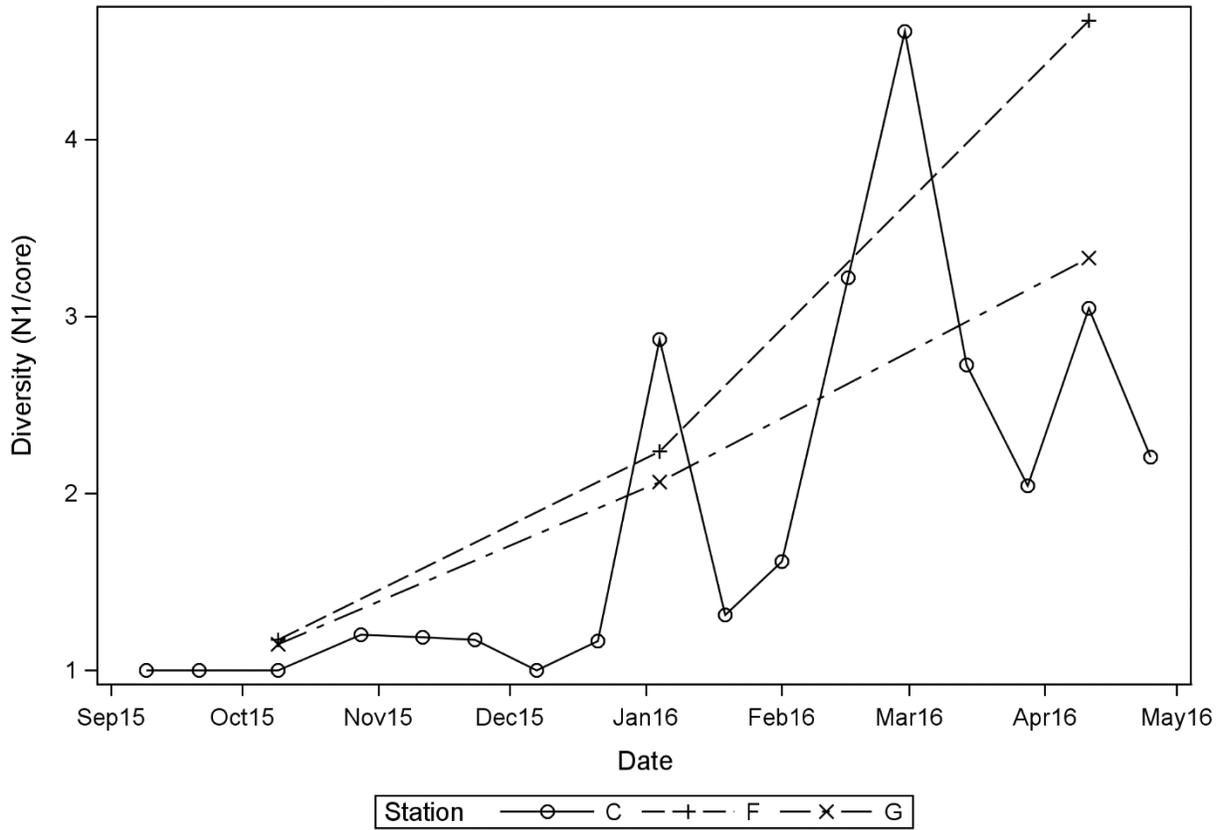


Figure 10: Mean benthic infaunal diversity over time for Rincon Bayou Stations C, F, and G from 09 September 2015 to 25 April 2016.

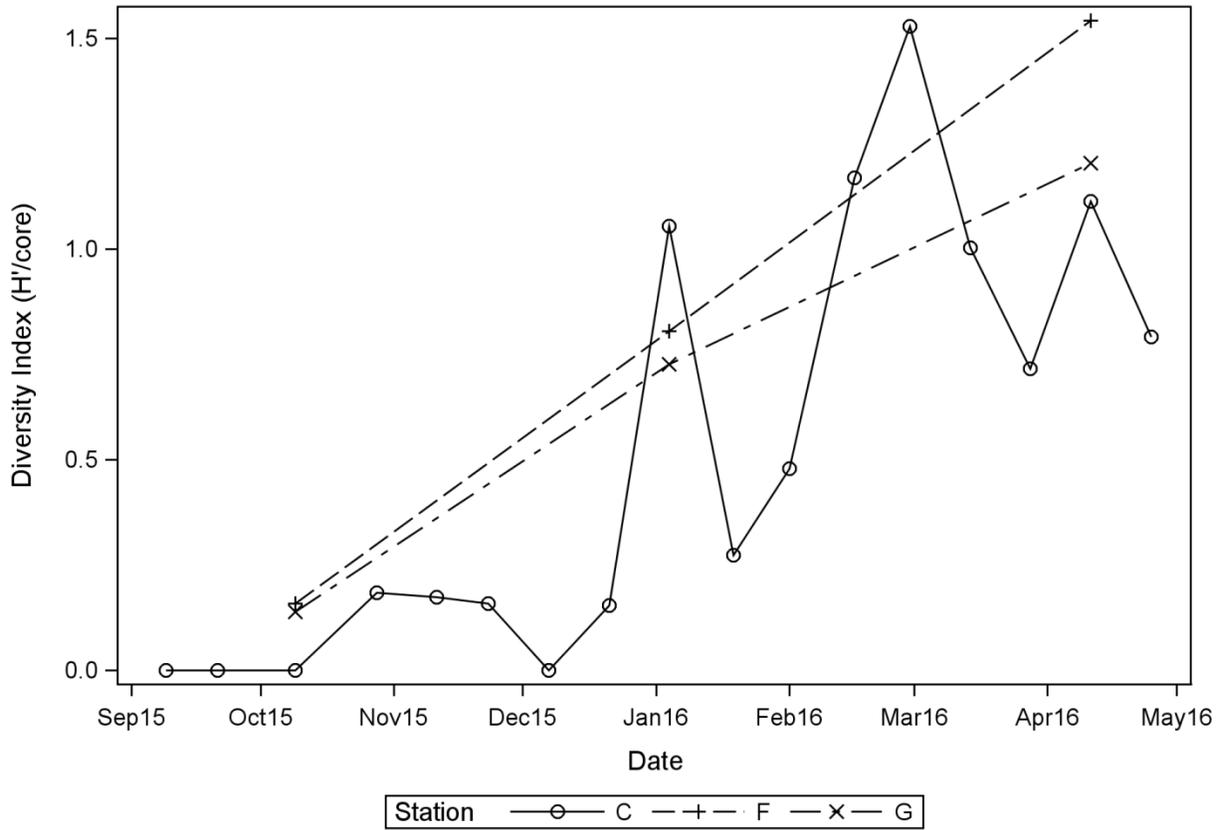


Figure 11: Mean benthic infaunal Shannon diversity index over time for Rincon Bayou Stations C, F, and G from 09 September 2015 to 25 April 2016.

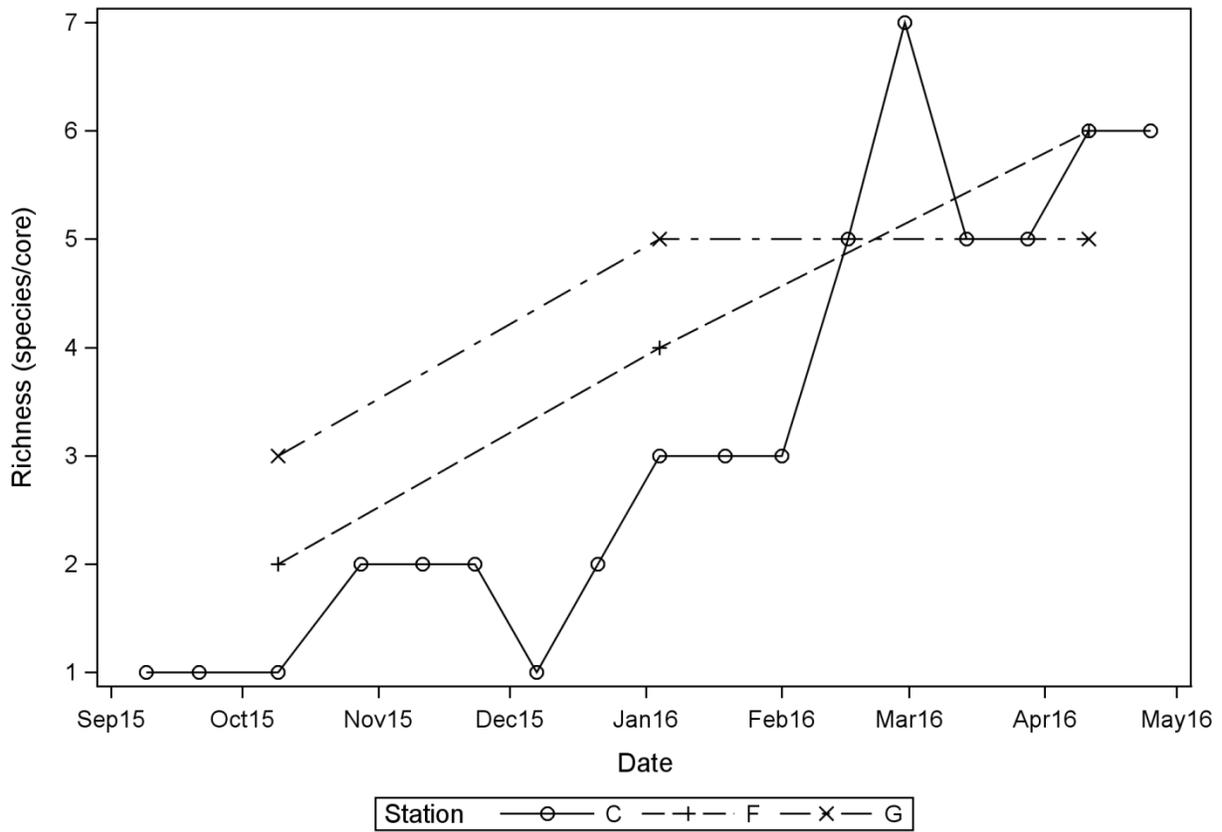


Figure 12: Mean benthic infaunal richness over time for Rincon Bayou Stations C, F, and G from 09 September 2015 to 25 April 2016.

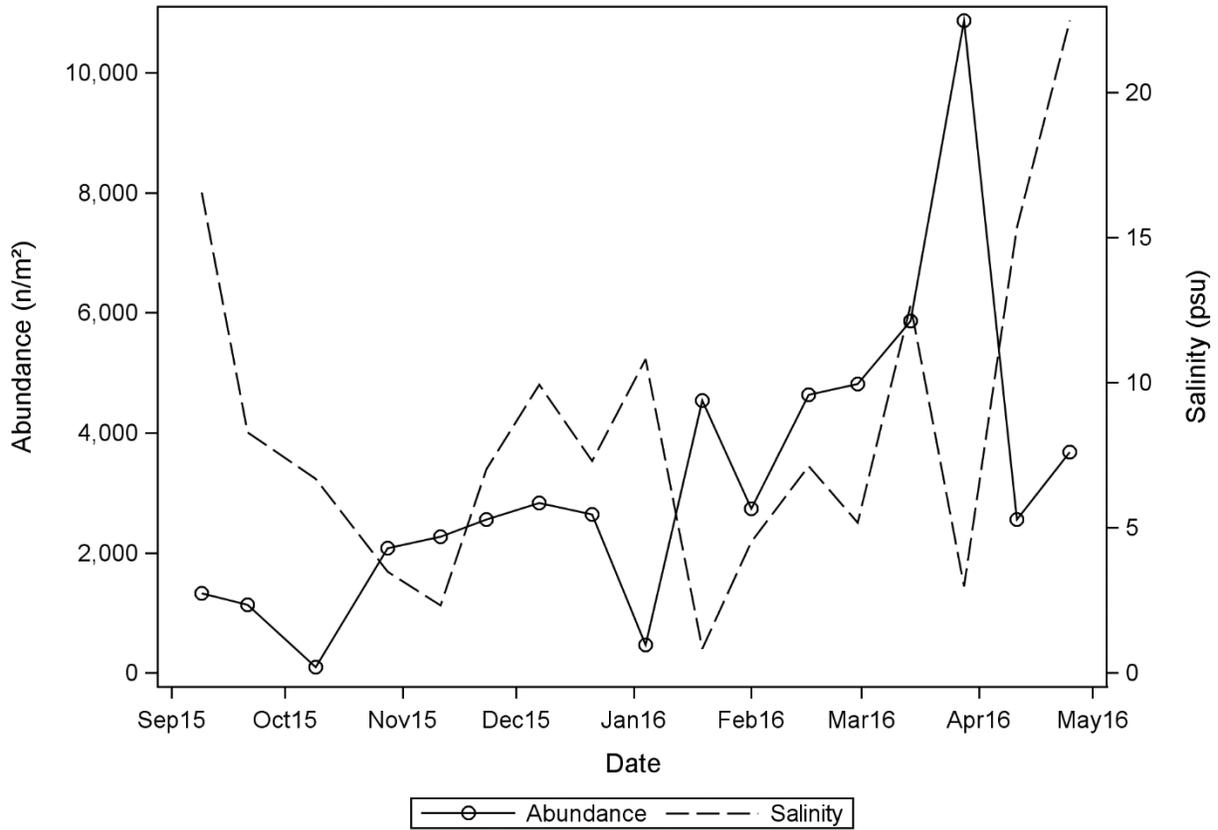


Figure 13: Mean benthic infaunal abundance and salinity at Station C from 09 September 2015 to 25 April 2016.

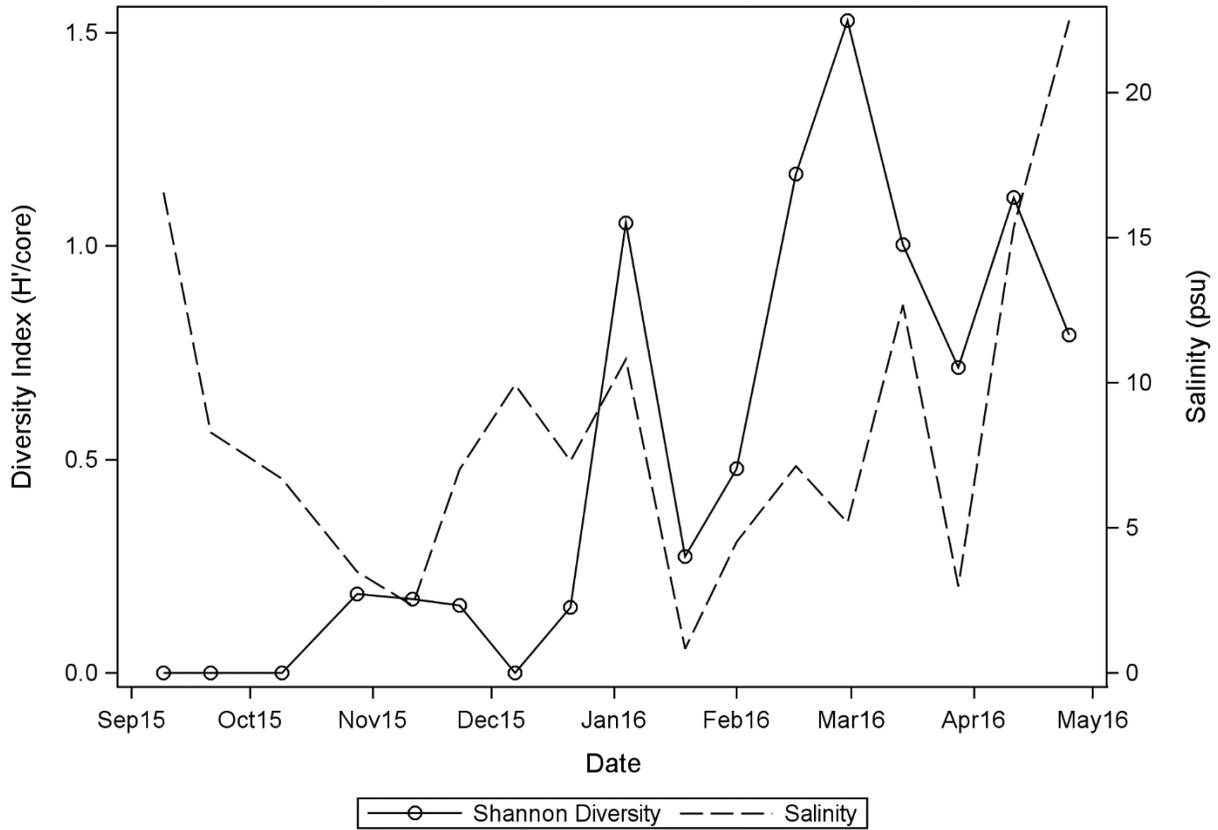


Figure 14: Mean benthic infaunal Shannon diversity index and salinity at Station C from 09 September 2015 to 25 April 2016.



Table 4: Pearson correlations for the key macrofauna metrics versus salinity for four lag periods at station C. Each lag period represents two week increments prior to sampling.

Pearson Correlation Coefficients				
Prob >  r  under H0: Rho=0				
Salinity Lag	Abundance (n/m <sup>2</sup> )	Shannon Diversity (H')	Richness (S/core)	Biomass (g/m <sup>2</sup> )
Salinity Lag 2 (2 weeks)	0.166	-0.171	-0.024	-0.002
	0.54	0.52	0.93	0.99
Salinity Lag 4 (4 weeks)	-0.456	-0.166	-0.247	0.174
	0.24	0.55	0.38	0.53
Salinity Lag 6 (6 weeks)	-0.097	-0.215	-0.121	-0.026
	0.74	0.46	0.68	0.93
Salinity Lag 8 (8 weeks)	-0.207	-0.196	-0.134	-0.366
	0.50	0.52	0.66	0.22

## Epifauna

The highest mean epifaunal abundance was identified at station G (387 n/m<sup>2</sup>), 09 October 2015 (Figure 16, Table 5). The high abundance was due almost entirely to *Americamysis almyra* (mysids), which continued into November as well (24 November 2015). The lowest epifaunal abundance was encountered at stations C and F (0 n/m<sup>2</sup>), where samples collected returned no species. The lowest abundance encountered, for samples with organisms, was observed at station C (0.6 n/m<sup>2</sup>), meaning only three organisms were found in that sample. Abundance ranged from 0 to 30.07 n/m<sup>2</sup> at station C, 0 to 250 n/m<sup>2</sup> at station F, and 0.6 to 387 n/m<sup>2</sup> at station G.

Three events occurred at station C where abundance was markedly higher: 20 October 2014 (166 n/m<sup>2</sup>), 03 November 2014 (275 n/m<sup>2</sup> – maximum abundance observed at this station), and 22 June 2015 (100 n/m<sup>2</sup>) (Figure 16). October 2014 encountered a high number of *Palaemonetes* sp (grass shrimp), accounting for greater than 85 percent of that sample. These organisms were again identified in high numbers in November, accounting for greater than 95 percent of the sample abundance.

Epifaunal biomass was found to be highest at station G, on average, but the maximum observation did occur at station C (13.01 g/m<sup>2</sup>), 03 November 2014 (Figure 17, Table 5). This was due to grass shrimp (> 80 %). The second highest observed biomass was at station G (12.96 g/m<sup>2</sup>), which occurred 16 February 2016), where grass shrimp contributed greater than 99 percent of the sample biomass. The lowest (non-zero) biomass encountered for the sampling period was at station F (0.04 g/m<sup>2</sup>). Epifaunal biomass ranged from 0 to 1.55 g/m<sup>2</sup> at station C, 0 to 2.72 g/m<sup>2</sup> at station F, and 0.11 to 12.96 g/m<sup>2</sup> at station G. Peaks in biomass typically occurred in summer and fall. The third highest biomass measurement was observed 08 November 2014, at station G, as well (12.45 g/m<sup>2</sup>). The November 2014 event returned only two organisms, grass shrimp and *Cyprinodon variegatus* (sheepshead minnow), where sheepshead minnow accounted for 90 percent of the sample biomass.

For the epifaunal community, the Shannon diversity index ranged from 0 to 2.12 H' for the sampling period (Figure 18, Table 5). The minimum (0 H') and maximum (2.12 H') values occurred at station C. Mean values for Shannon diversity did not greatly vary, and values were highest in summer 2015.

The minimum values for Hill's diversity were encountered at two stations, C and F (0 N1), and the maximum at station G (5.23 N1) (Figure 19, Table 5). Hill's diversity values for station C ranged from 0 to 8.32 N1, 0 to 4.94 N1 at station F, and 1.00 to 5.23 N1 at station G. Similar to Shannon diversity, Hill's diversity values were highest summer 2015.

Species richness in the epifaunal community peaked at 23 species identified in 22 June 2015 at station C (Figure 20, Table 5), where species richness ranged from 0 to 23 species. Species

richness ranged from 0 to 18 species at station F and 1 to 11 at station G. Peaks in species richness occurred in late spring and early summer 2015, and again in fall 2015.

Values for the evenness index of the epifaunal samples ranged from 0 to 1.00  $J'$  (Figure 21, Table 5). The minimum value of 0 was observed at all three stations, and the maximum at stations F and G. The highest evenness values occurred in spring and summer.

Table 5. Summary statistics of epifaunal community averaged over stations C, F, and G and from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

Variable	Station	N. Obs.	Mean	Std Dev	Minimum	Maximum
Abundance (n/m <sup>2</sup> )	All	144	25.12	51.96	0	387.47
	C	50	23.91	47.28	0	274.8
	F	48	18.96	43.87	0	249.67
	G	46	32.56	63.12	0.60	387.47
Biomass (g/m <sup>2</sup> )	All	144	1.89	2.53	0	13.01
	C	50	1.61	2.41	0	13.01
	F	48	0.90	1.06	0	3.88
	G	46	3.16	3.13	0.11	12.96
Shannon Diversity Index (H'/sample)	All	144	0.79	0.47	0	2.12
	C	50	0.87	0.49	0	2.12
	F	48	0.78	0.43	0	1.60
	G	46	0.72	0.47	0	1.65
Diversity (N1/sample)	All	144	2.43	1.25	0	8.32
	C	50	2.64	1.52	0	8.32
	F	48	2.35	1.06	0	4.94
	G	46	2.29	1.10	1.00	5.23
Richness (species/sample)	All	144	5.42	3.50	0	23
	C	50	5.74	4.36	0	23
	F	48	4.96	3.61	0	18
	G	46	5.18	2.30	1.00	11
Pielou's Evenness (J'/sample)	All	144	0.52	0.29	0	1
	C	50	0.52	0.25	0	0.96
	F	48	0.55	0.30	0	1
	G	46	0.47	0.31	0	1

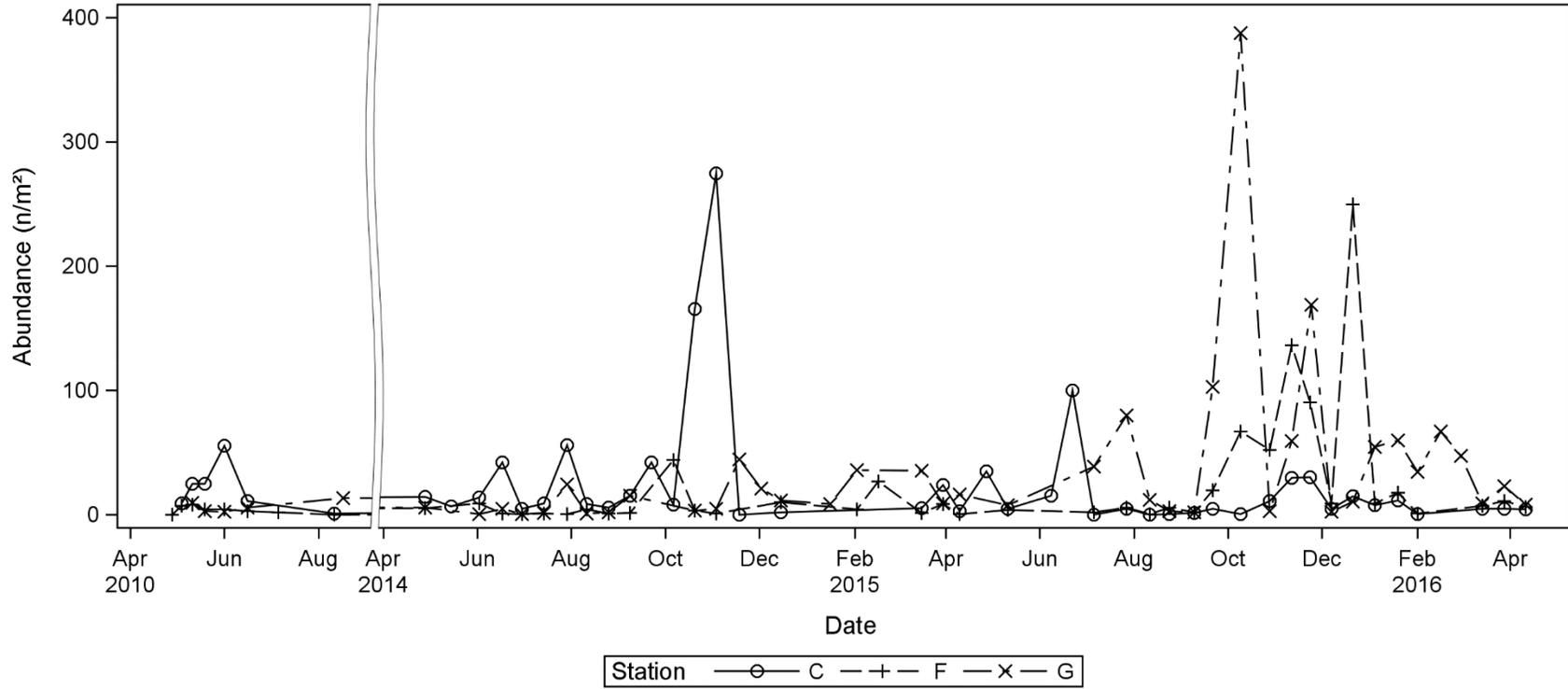


Figure 16. Mean epifaunal abundance over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

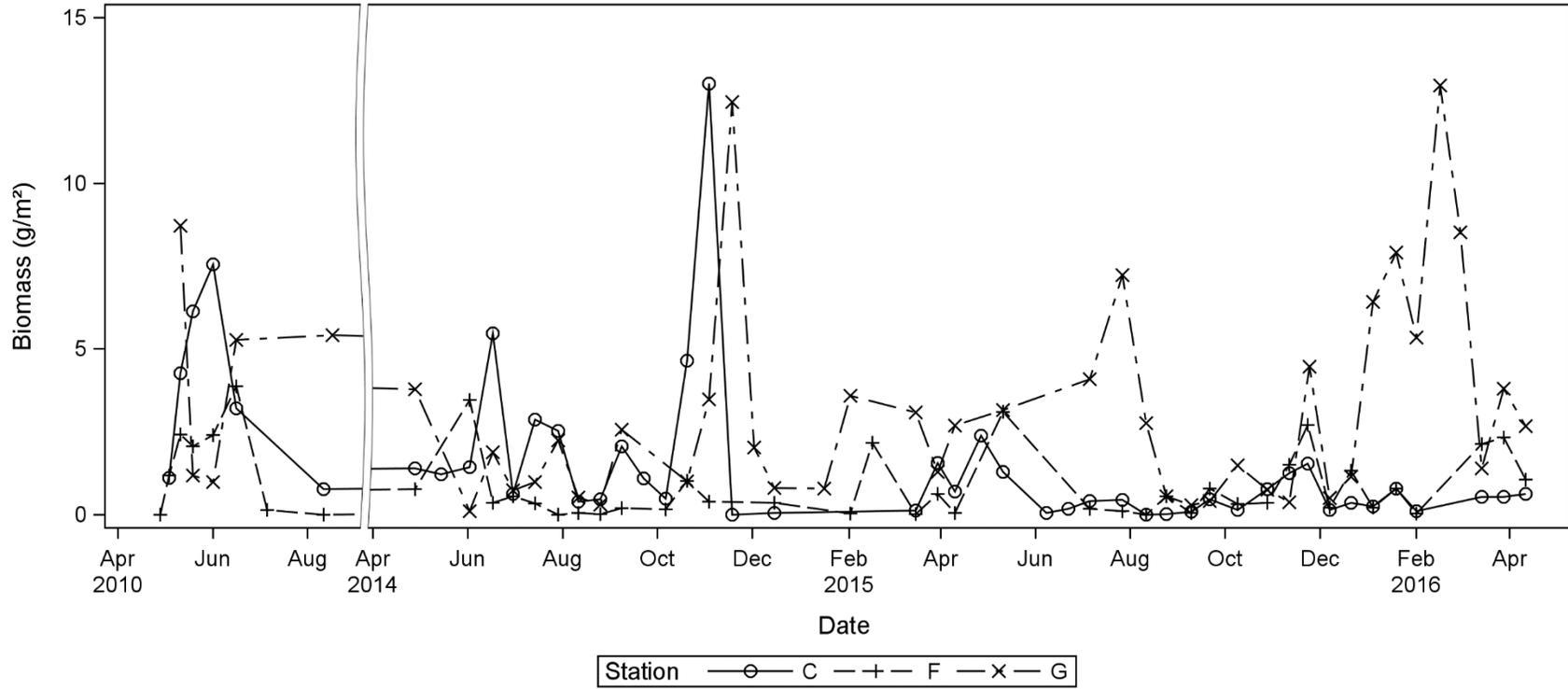


Figure 17. Mean epifaunal biomass over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

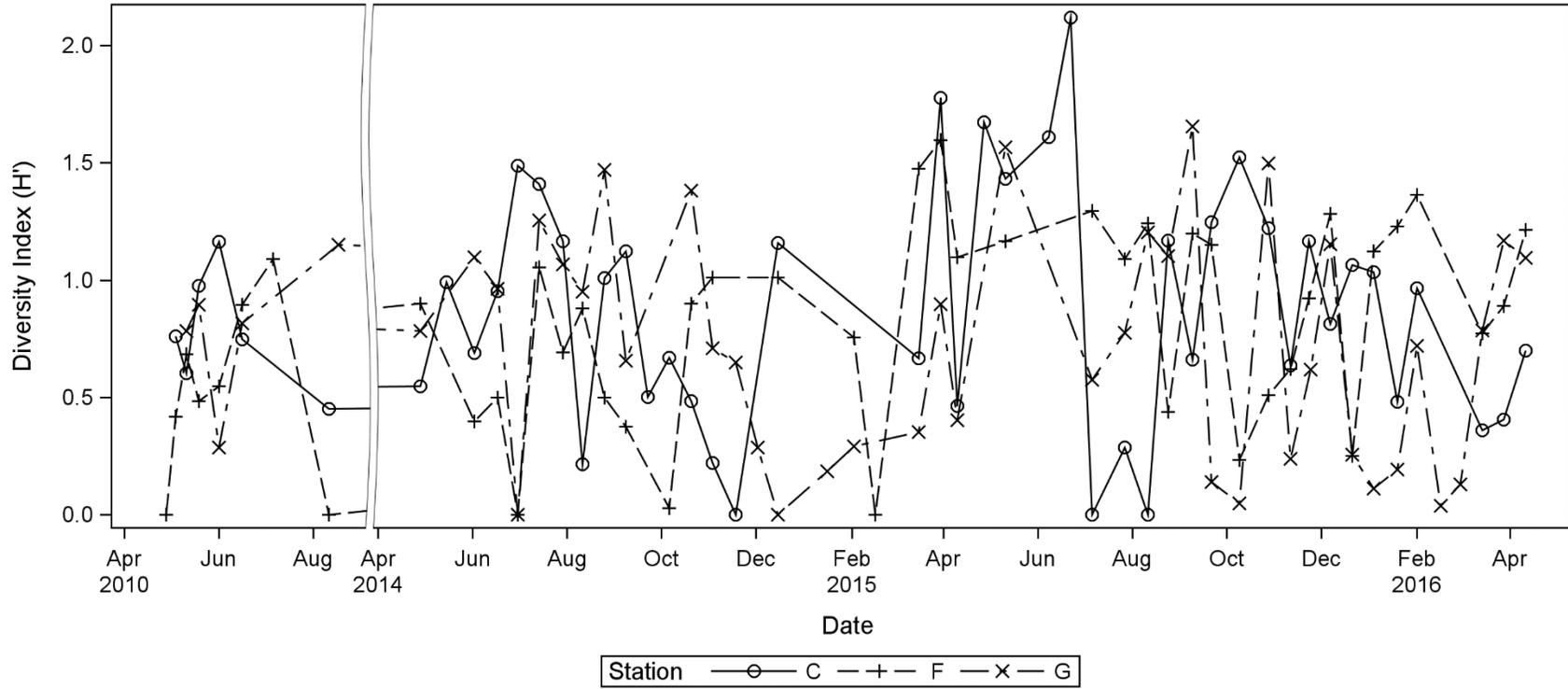


Figure 18. Mean epifaunal Shannon diversity over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

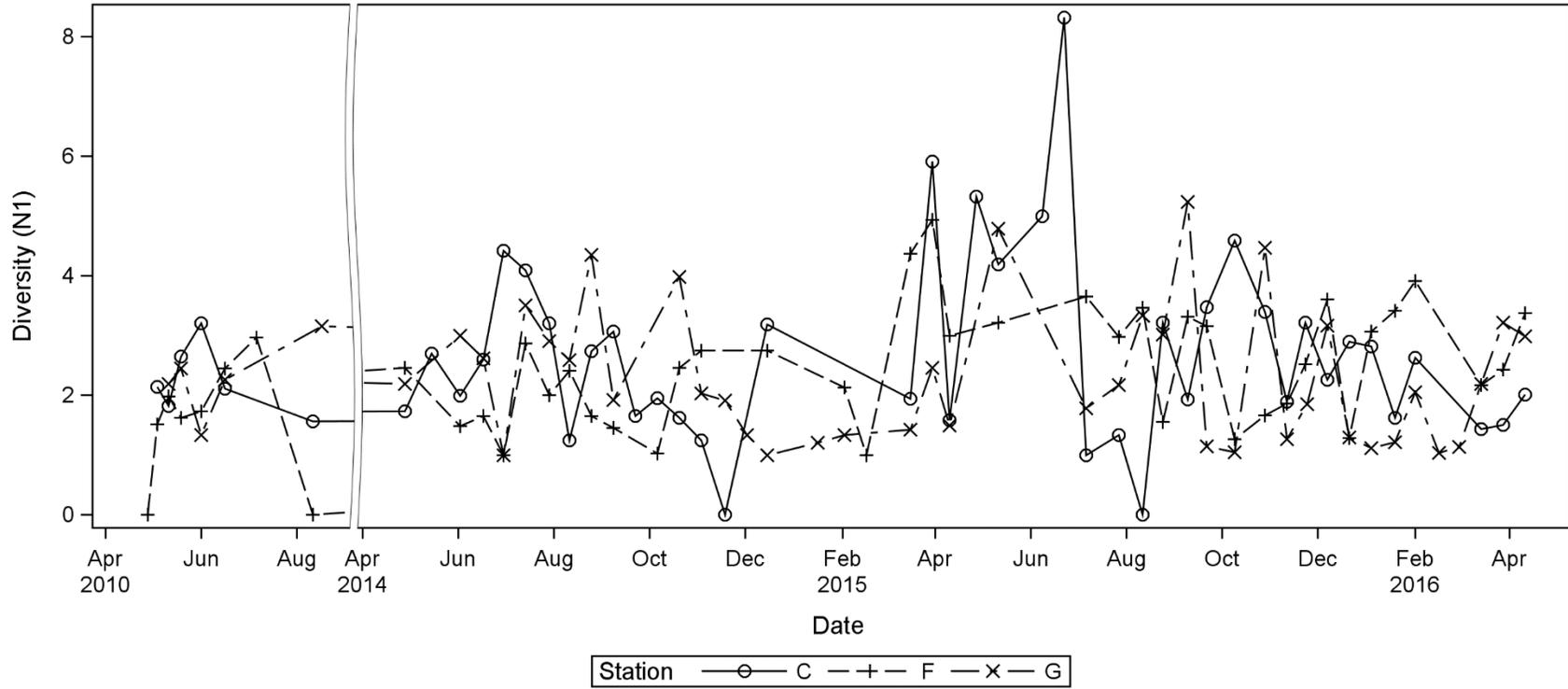


Figure 19. Mean epifaunal Hill diversity over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

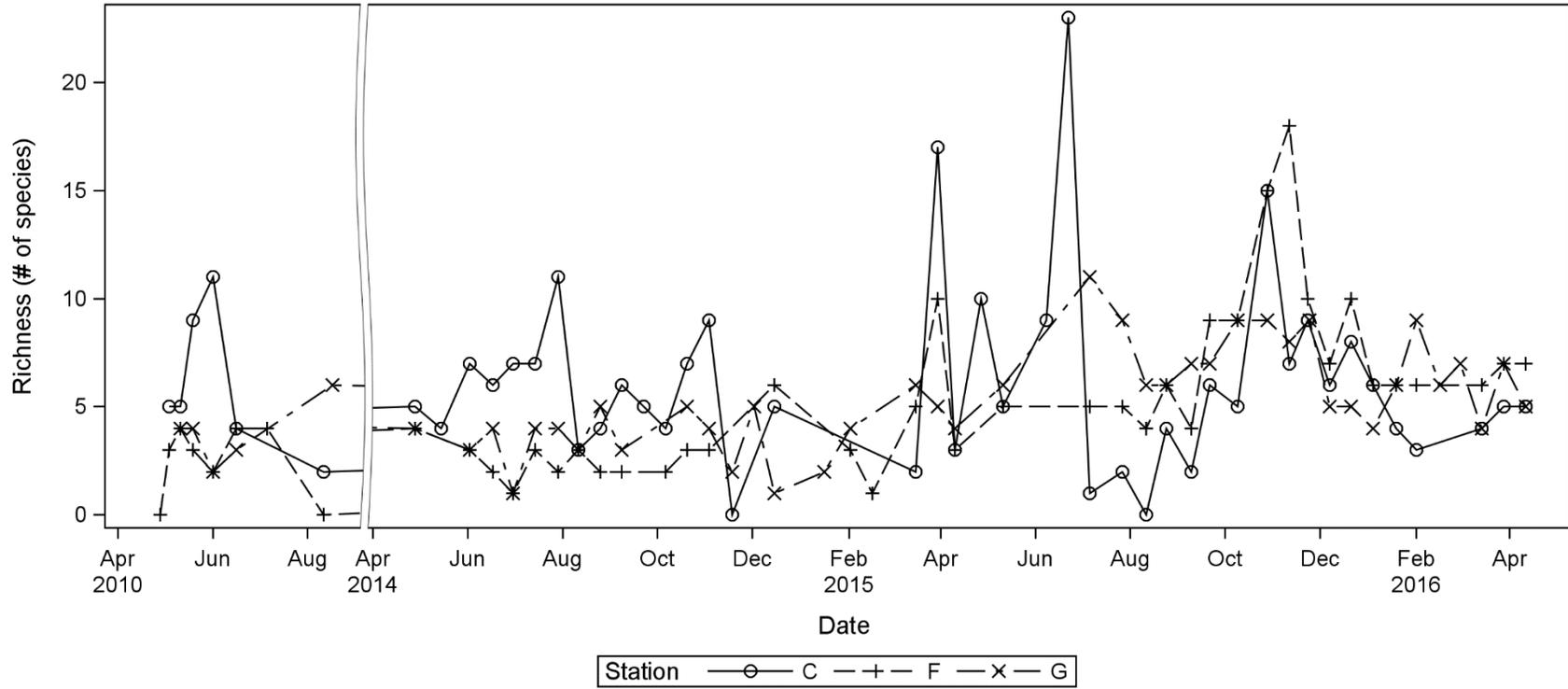


Figure 20. Mean epifaunal richness over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

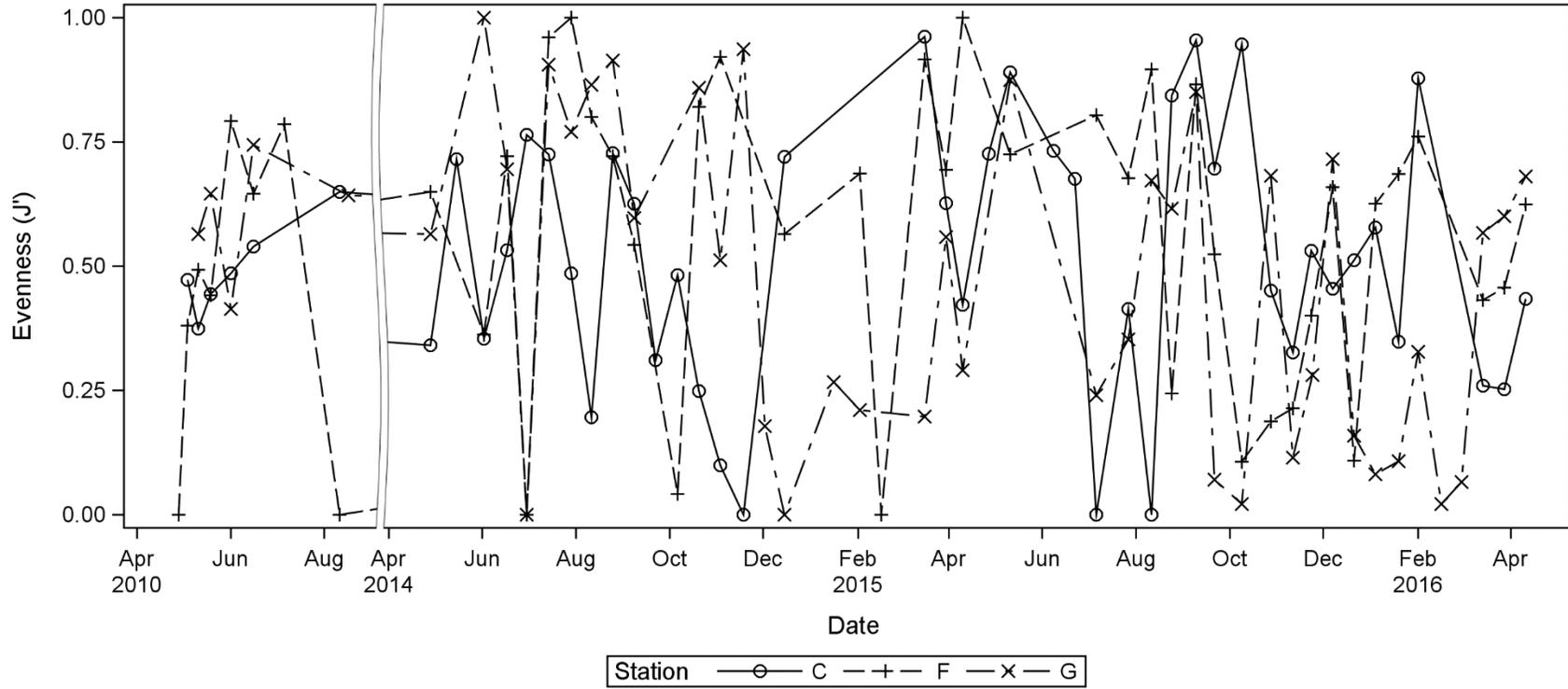


Figure 21. Mean epifaunal evenness over time for Rincon Bayou stations C, F, and G from 28 April 2010 to 17 August 2010 and 28 April 2014 to 25 April 2016.

Relative abundance did appear to increase over the study period, exhibiting consistently higher values beginning fall 2015, carrying into spring 2016 (Figure 22). Following nearly continuous pumping events in the late spring/early summer 2014, abundance stayed relatively low until late fall/early winter. Major flooding and pumping events in the late spring/early summer months 2015 resulted in a period of higher abundance June 2015. High abundance late in the year 2015 followed a period of increasing salinities, and high abundance was observed again in December following pumping events. Water levels in Rincon Bayou were extremely low in 2016 and samples could not be collected from all stations February to April 2016, and the epifaunal community exhibited decreasing trends in abundance for these months.

In general, higher biomass was observed following reduced salinities, with the exception of the February 2016 events (Figure 23). Sampling events in February and March 2016 exhibited higher biomass, even with major fluctuations in salinity. Biomass also tends to be lower when salinities are extremely high (December 2014) or low (June 2015) (i.e. floods and droughts).

Diversity (Shannon and Hill's) tended to increase directly following pumping events (Figures 24 and 25). Considering the maximum diversity period, 22 June 2015, these samples were comprised of mostly freshwater organisms, with minimal marine species. Besides the wet period of the spring and summer months in 2015, Hill's number of dominant species typically remained below 4 N1.

A peak in species richness also occurred in June 2015, following major freshwater events (Figure 26). Again, this sample was comprised of predominately freshwater organisms. Higher abundance was also observed in this sample. However, biomass for this sample was only (0.19 g/m<sup>2</sup>). Mean species richness has generally increased over the study period, where higher values occurred when salinities fluctuated between 10 and 20 PSU (except June 2015).

Mean evenness was highest in spring and summer (Figure 27). It also tended to be higher when salinities were reduced. In the spring to summer months in 2015, there was some indications that prolonged freshwater conditions led to a decline in the mean evenness index.

Time lags and Pearson correlations were also performed on the epifaunal samples. Epifaunal biotic and diversity factors were run against prior salinity at intervals of 2, 4, 6, and 8 weeks, at stations C, F, and G. At station C (Table 6), after 2 weeks, abundance, biomass, and richness were found to decrease as salinities increased. After 6 to 8 weeks, abundance and biomass were found to be positively correlated with salinity. At station F (Table 7), Shannon diversity, Hill's diversity, and evenness decreased with increasing salinities. At 4 to 8 weeks, only biomass displayed a positive correlation to salinity. At station G no correlations were discovered. A time lag of station means (Table 8) identified negative correlations at 2 weeks for both diversity

variables and evenness, and Shannon diversity displayed a negative relationship for weeks 4 to 6, as well. Biomass was positively correlated after weeks 6 to 8.

In Rincon Bayou, crustaceans are the dominant taxa, accounting for 90 percent of community abundance. The three largest contributors to community abundance are *Americamysis almyra* (mysid), grass shrimp, and *Farfantepenaeus aztecus* (brown shrimp) (Figure 28). Of the 90 percent, *A. almyra* accounts for 68.96 percent, grass shrimp 26.79 percent, and an additional 2.17 percent from brown shrimp. The MDS for dominant crustaceans in Rincon Bayou does depict periods of overlap between brown shrimp and mysids, but brown shrimp are found in lower abundance when mysid abundance is high (Figure 28).

Fish species were not collected in high abundance in push net samples. They accounted for approximately 3 percent of the overall community. The three highest contributing fish species are sheepshead minnow (1.62 %), *Menidia beryllina* (inland silversides, 0.54 %), and *Brevoortia patronus* (Gulf menhaden, 0.26 %) (Figure 29). As with the crustacean community, there is little overlap between the dominant species.

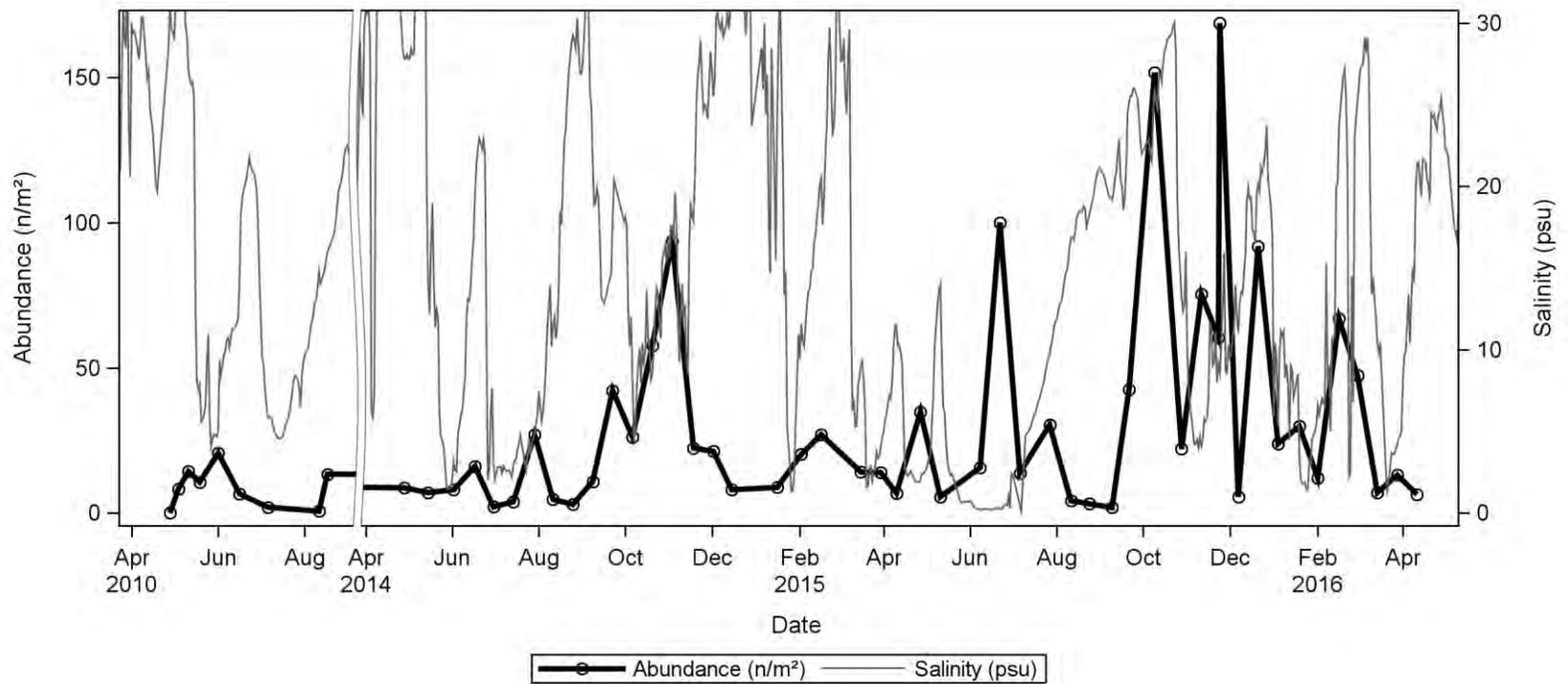


Figure 22. Epifaunal abundance (mean over all stations) and salinity over time for Rincon Bayou Stations from 28 April 2010 to 25 April 2016.

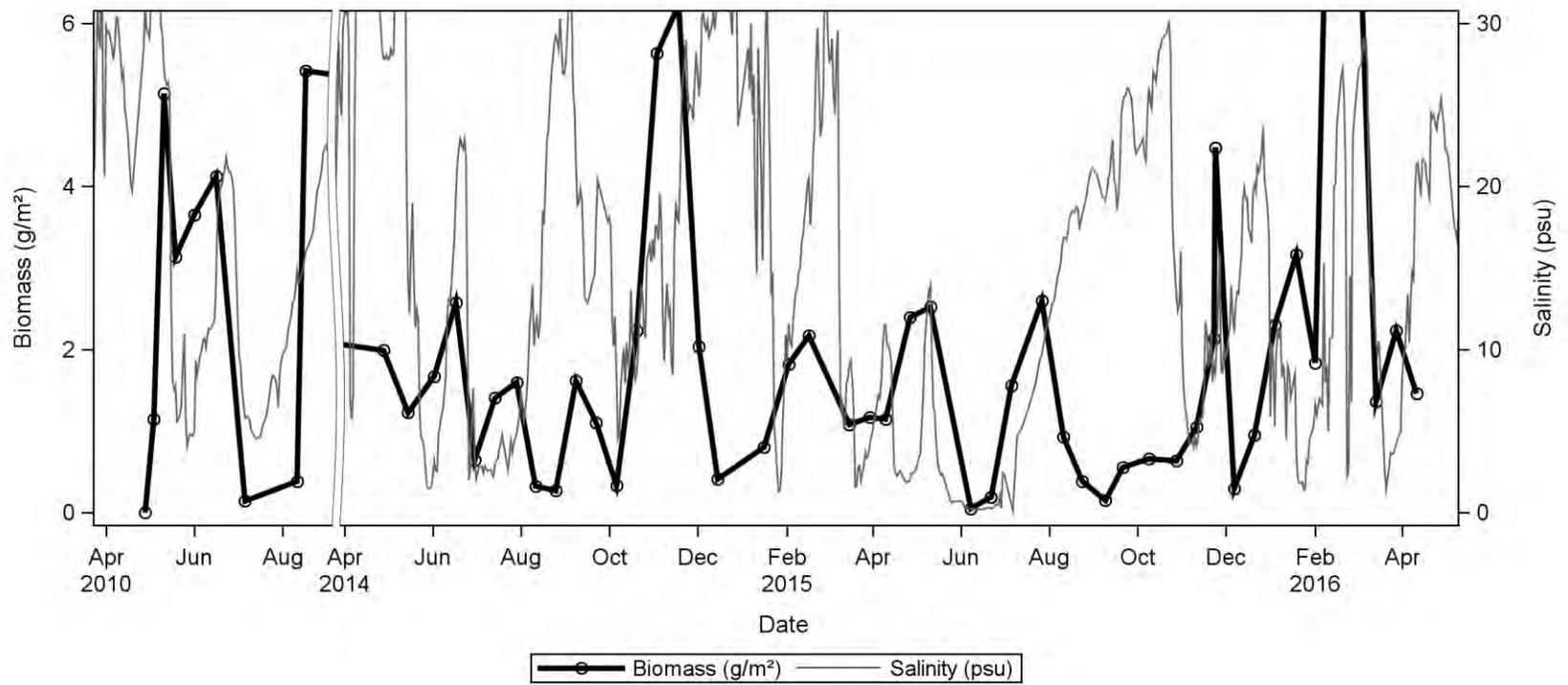


Figure 23. Epifaunal biomass (mean over all stations) and salinity over time for Rincon Bayou from 28 April 2010 to 25 April 2016.

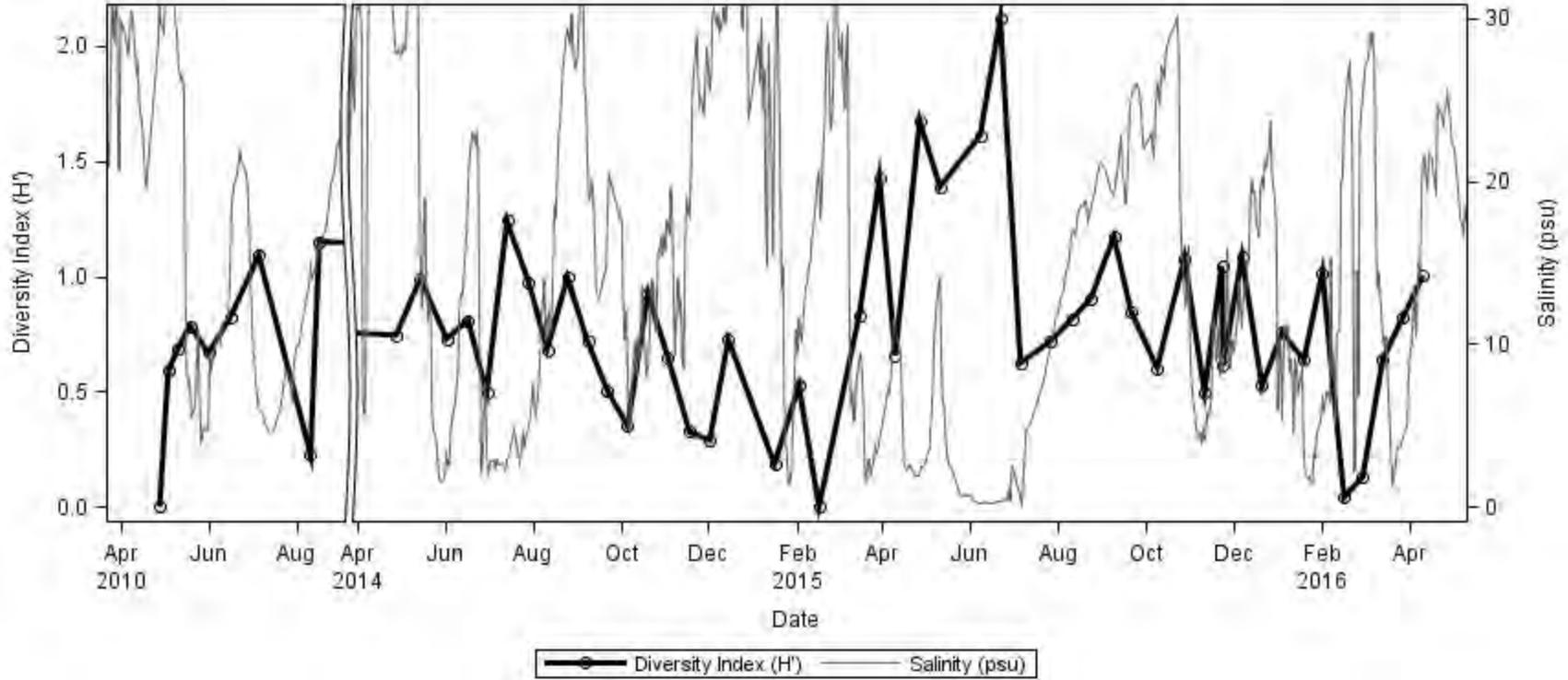


Figure 24. Epifaunal Shannon diversity (mean over all stations) and salinity over time for Rincon Bayou from 28 April 2010 to 25 April 2016.

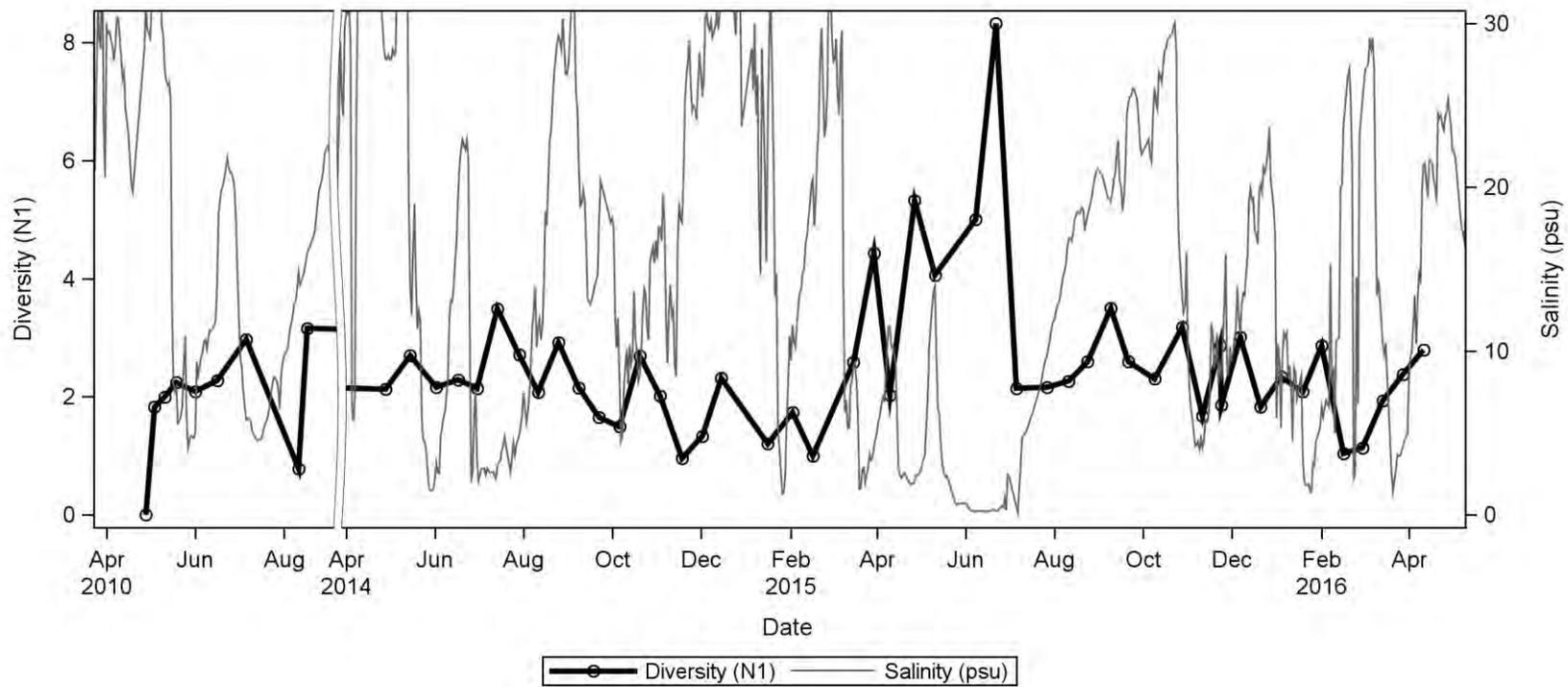


Figure 25. Epifaunal Hill's diversity (mean over all stations) and salinity over time for Rincon Bayou from 28 April 2010 to 25 April 2016.

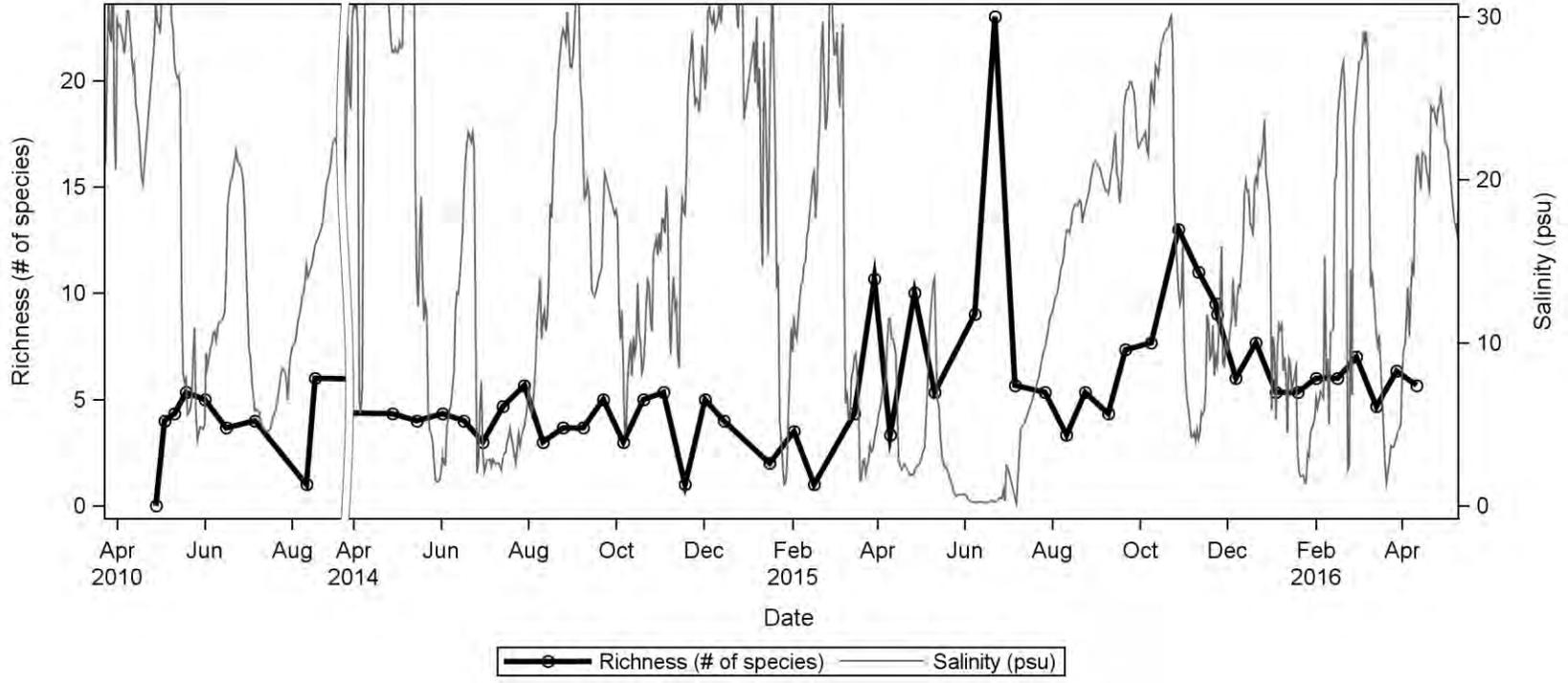


Figure 26. Epifaunal richness (mean over all stations) and salinity over time for Rincon Bayou from 28 April 2010 to 25 April 2016.

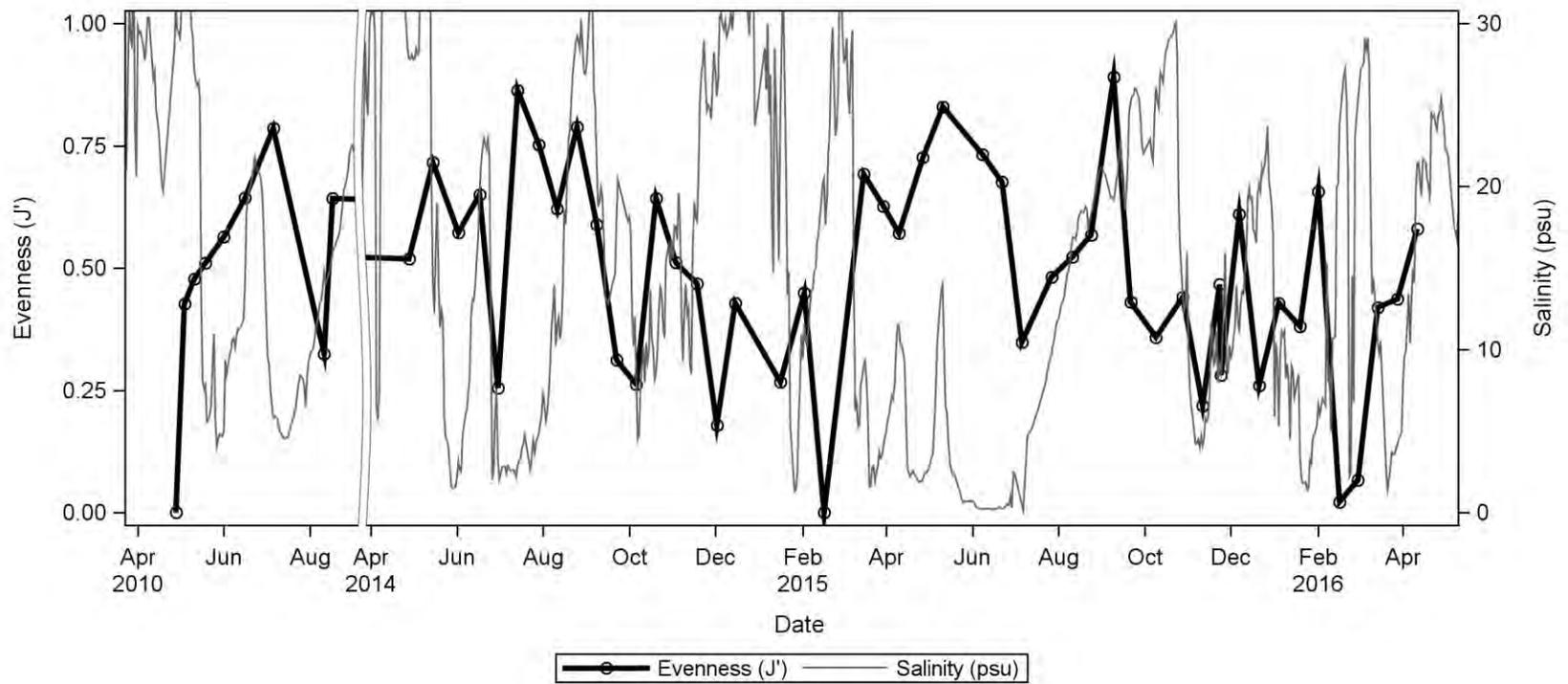


Figure 27. Epifaunal evenness (mean over all stations) and salinity over time for Rincon Bayou from 28 April 2010 to 25 April 2016.

Table 6. Pearson correlations between the epifaunal community metrics and salinity for four lag periods at station C from 28 April 2010 to 25 April 2016. Each lag period represents two weeks since sampling took place. Significant relationships ( $P \leq 0.05$ ) are bold.

Salinity Lag	Pearson Correlation Coefficients					
	Probability >  r  under H0: Rho=0					
	Abundance (n/m <sup>2</sup> )	Biomass (g/m <sup>2</sup> )	Shannon Diversity (H')	Diversity (N1)	Richness (S/sample)	Evenness (J')
Lag 2 (2 weeks)	-0.274	<b>-0.287</b>	-0.037	-0.103	-0.279	0.207
	0.06	<b>0.05</b>	0.81	0.50	0.06	0.17
Lag 4 (4 weeks)	-0.068	-0.117	0.190	0.150	0.125	0.250
	0.66	0.44	0.21	0.33	0.41	0.10
Lag 4 (6 weeks)	0.283	<b>0.397</b>	0.074	0.040	0.164	0.058
	0.06	<b>0.01</b>	0.63	0.80	0.29	0.71
Lag 8 (8 weeks)	<b>0.320</b>	0.291	0.111	0.158	0.252	-0.068
	<b>0.04</b>	0.06	0.48	0.31	0.10	0.66

Table 7. Pearson correlations for the epifaunal community metrics versus salinity for four lag periods at station F from 28 April 2010 to 25 April 2016. Each lag period represents two weeks since sampling took place. Significant relationships ( $P \leq 0.05$ ) are bold.

Salinity Lag	Pearson Correlation Coefficients					
	Probability >  r  under H0: Rho=0					
	Abundance (n/m <sup>2</sup> )	Biomass (g/m <sup>2</sup> )	Shannon Diversity (H')	Diversity (N1)	Richness (S/sample)	Evenness (J')
Lag 2 (2 weeks)	-0.015 0.87	0.128 0.42	<b>-0.375</b> <b>0.01</b>	<b>-0.368</b> <b>0.02</b>	-0.031 0.84	<b>-0.385</b> <b>0.01</b>
Lag 4 (4 weeks)	0.075 0.64	0.119 0.46	<b>-0.405</b> <b>0.01</b>	<b>-0.355</b> <b>0.02</b>	0.010 0.95	<b>-0.336</b> <b>0.03</b>
Lag 6 (6 weeks)	0.003 0.99	0.302 0.06	-0.156 0.34	0.019 0.91	0.023 0.89	-0.090 0.58
Lag 8 (8 weeks)	0.057 0.73	0.352 0.03	0.159 0.33	0.157 0.34	0.184 0.26	0.175 0.29

Table 8. Pearson correlations for the epifaunal community metrics versus salinity for four lag periods with mean biotic variables for each station C, F, and G, from 28 April 2010 to 25 April 2016. Each lag period represents two weeks since sampling took place. Significant relationships ( $P \leq 0.05$ ) are bold.

Salinity Lag	Pearson Correlation Coefficients					
	Abundance (n/m <sup>2</sup> )	Biomass (g/m <sup>2</sup> )	Shannon Diversity (H')	Diversity (N1)	Richness (S/sample)	Evenness (J')
Lag 2 (2 weeks)	-0.038	-0.064	<b>-0.209</b>	<b>-0.197</b>	<b>-0.196</b>	-0.097
	0.66	0.47	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	0.27
Lag 4 (4 weeks)	0.062	-0.051	<b>-0.156</b>	-0.124	-0.003	-0.096
	0.48	0.56	<b>0.07</b>	0.16	0.97	0.27
Lag 6 (6 weeks)	0.116	<b>0.153</b>	<b>-0.157</b>	-0.138	-0.034	-0.097
	0.19	<b>0.08</b>	<b>0.07</b>	0.12	0.70	0.27
Lag 8 (8 weeks)	0.119	<b>0.167</b>	0.037	0.041	0.095	0.060
	0.18	<b>0.06</b>	0.68	0.64	0.28	0.50

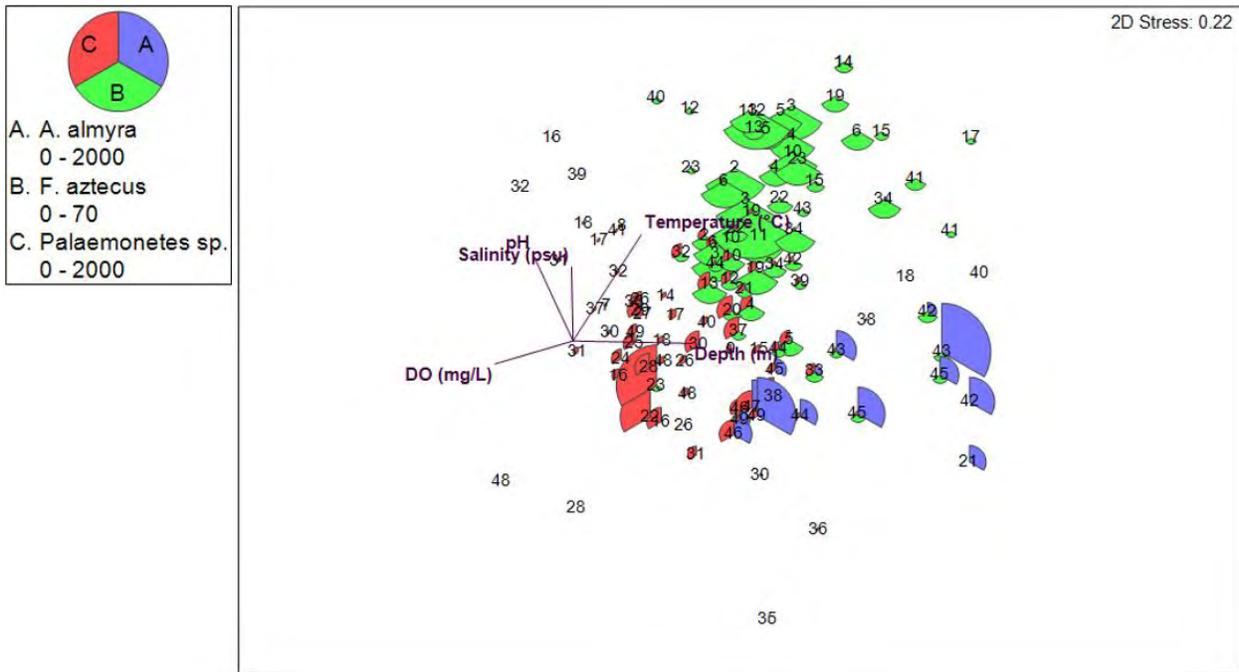


Figure 28. Non-metric MDS plots of the three dominant crustacean species of the epifaunal community where a bubble plot overlay indicates relative mean abundance of the species. Samples are labeled by period for sample dates from 28 April 2010 to 31 December 2015.

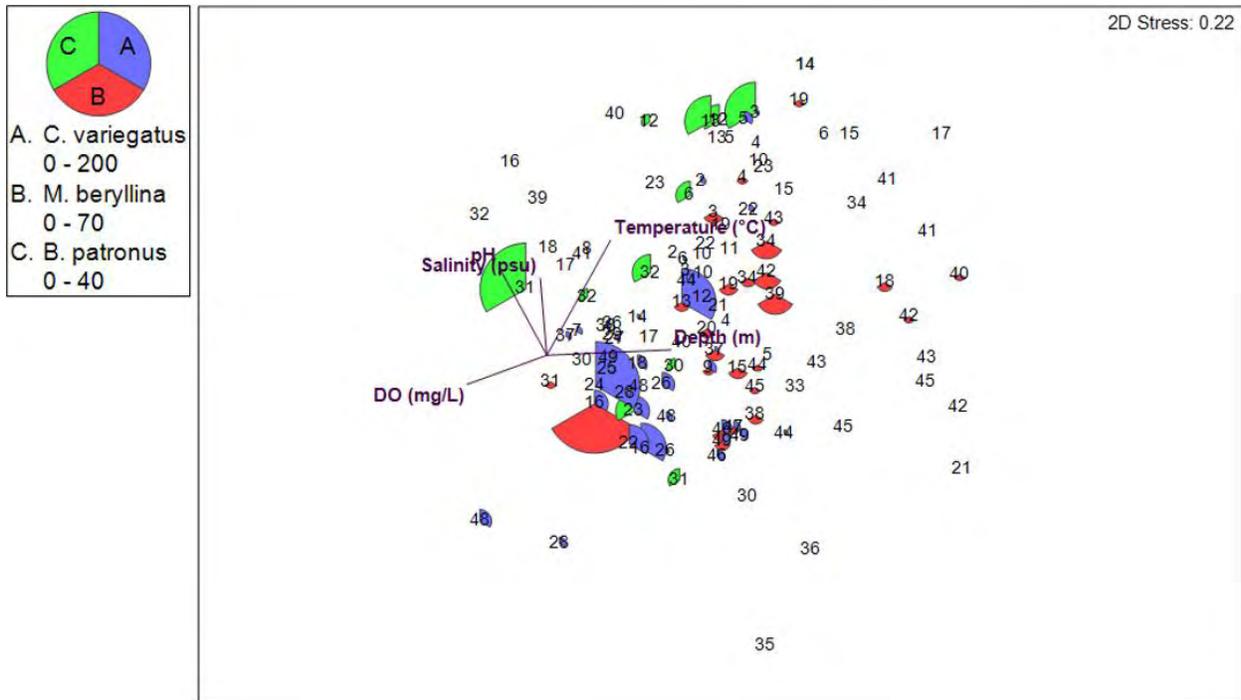


Figure 29. Non-metric MDS plots of the three dominant fish species of the epifaunal community structure where a bubble plot overlay indicates the relative mean abundance of the species. Samples are labeled by period for sample dates from 28 April 2010 to 31 December 2015 (Gordon 2016).

## Discussion

The current study follows decades of research in Rincon Bayou, which started in 1994 (Irlbeck and Ward 2000). In the first decade, research was focused on the effects of the Nueces River Overflow channel to deliver fresh water into Rincon Bayou and its effects of biological communities (Montagna et al. 2009).

More recently, focus has switched to the operation and effects of pumping from the Rincon Bayou Pipeline, which delivers water directly into Rincon Bayou from the Calallen Pool (Montagna and Herdener 2015, Montagna et al. 2015). The current study is a direct continuation of these pipeline studies. In addition to the two reports listed above, the current studies have led directly to four Master of Science theses (Herdener 2015, Chaloupka 2016, DelRosario 2016, and Gordon 2016). So, the discussion will reference the previous reports, theses, and published journal articles, to frame the results of the current study in the context of factors not measured during the current study, and dynamics beyond just one year.

### Hydrology

To discuss the relationship of measured salinity to pumped inflow, flow data was obtained from multiple sources (DelRosario 2016, Figure 30). Pumped inflow data from September 2009 to December 2016 was obtained from the Nueces River Authority (NRA) website: <http://www.nuecesra/CP/CITY/rincon/>. Flow through the Nueces River Overflow Channel into Rincon Bayou was measured at the United States Geological Survey (USGS) Rincon Bayou Channel Gage No. 08211503. Flow data from September 2009 to December 2016 was obtained from the USGS website: <http://nwis.waterdata.usgs.gov>. Salinity data from May 2009 to December 2016 was obtained from the CBI website: <http://www.cbi.tamucc.edu/dnr/station> for salinity stations Nueces Delta 2 (NUDE2) and SALT03.

The absence of a distinct elevation gradient in Rincon Bayou at the pumping outfall area allows pumped inflow to flow both upstream and downstream resulting in both positive inflow and negative discharge readings at the USGS Rincon Bayou Channel Gage (DelRosario 2016). A weir was constructed at the pumping outfall in May 2010 to reduce the amount of pumped inflow going upstream. It was replaced in July 2014 with a back-flow preventer consisting of gates, which must be manually operated. The back-flow preventer washed out in the summer flooding of 2015, which reduced negative flows back to the Nueces River while it was in place (Figure 31). Thus the back-flow preventer was not in place during the current study.



Figure 30. Map of station locations for measuring flow, salinity, and weather in Rincon Bayou. From DelRosario (2016).

The Nueces Estuary can shift between positive and negative estuarine conditions depending on the volume of inflow and precipitation (DelRosario 2016). A positive estuary is defined as a system where salinities are lower than the adjacent sea due to freshwater inflow (Bianchi, 2006). In contrast, a negative estuary is a system where salinities are greater than the neighboring sea due to the process of evaporation. In the five-month period prior to the Rincon Bayou pipeline becoming operational in September of 2009, the Nueces Estuary was negative with a mean daily salinity upstream at NUDE2 being higher than the mean daily salinity downstream in the Nueces Bay at SALT03 (DelRosario 2016, Figure 32). The Nueces Estuary oscillates between positive and negative conditions with pumping events. Pumping events coincided with periods of positive estuary conditions and the greatest difference in salinity between the bay and the upper delta happened immediately after pumping ceased (Figure 32). In summary Rincon Bayou has transitioned from a negative hypersaline estuary to a positive estuary due to pumping of freshwater to the delta.

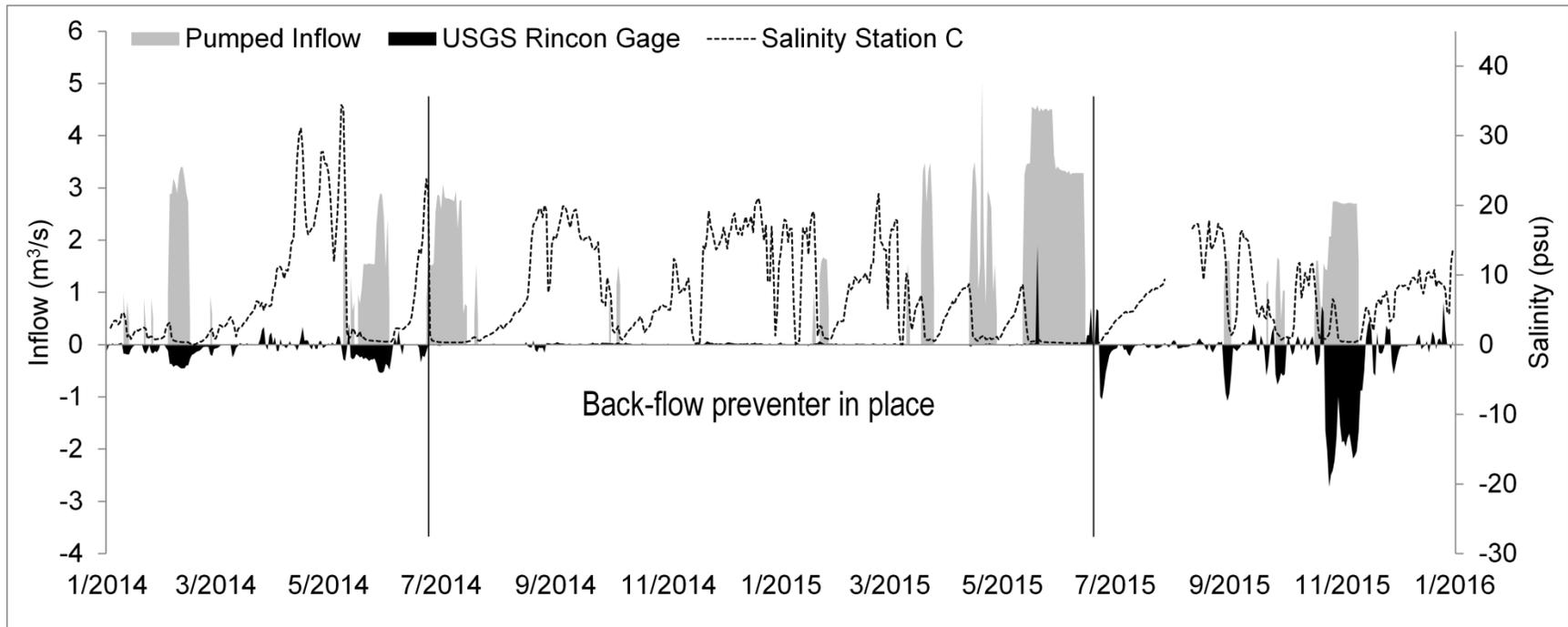


Figure 31. Salinity at Station C in Rincon Bayou TX, with inflow and discharge from the Rincon Bayou channel gage and pumped inflow, January 2014 to December 2015. From DelRosario (2016).

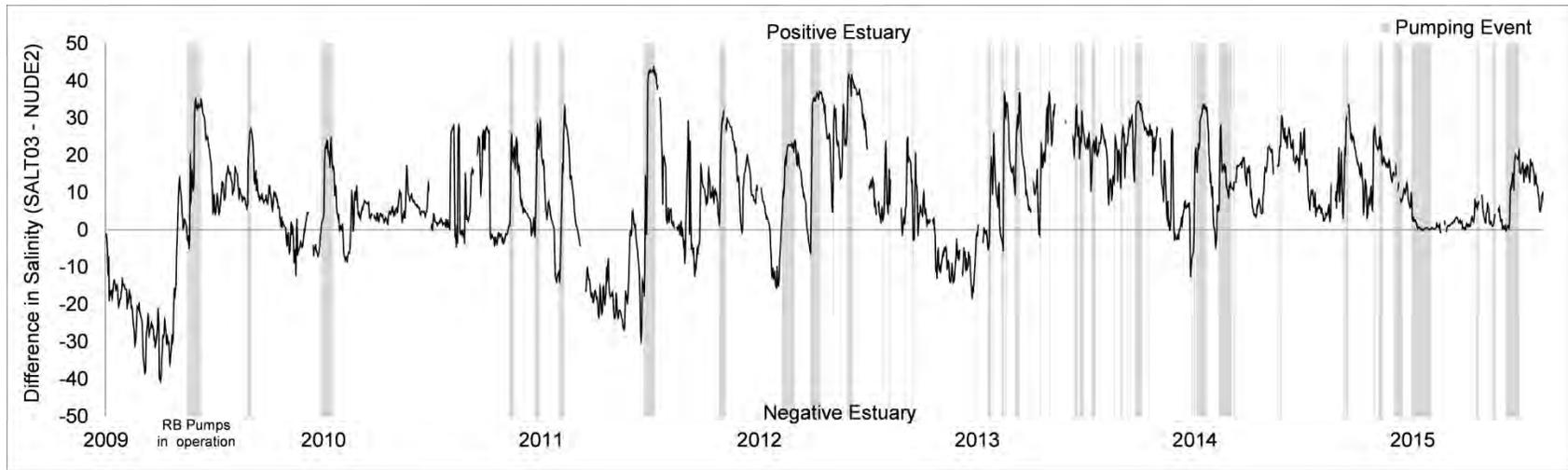


Figure 32. Salinity gradient (i.e., difference between downstream SALT03 and upstream NUDE2) and pumping event daily totals May 2009 to December 2015. From DelRosario (2016).

## Macroinfauna Response to Salinity

*Streblospio* is the dominant species in Rincon Bayou benthos and the most resilient to higher salinities and salinity changes. *Laonereis culveri* and Chironomidae larvae were predominantly found in upper Rincon Bayou Station C and are typically associated with lower salinity levels. Chironomidae larvae in particular are well documented as freshwater and water quality indicators (Rosenberg, 1992; Saether, 1979). This indicates sustained freshwater inflow to upper Rincon Bayou during the current wet period has likely altered the diversity and community structure to be favorable to freshwater indicator species such as Chironomidae.

The benthic community structure in Rincon Bayou in the current study is similar to previous studies that found *S. benedicti* and chironomid larvae were the two dominant benthic macroinfaunal species (Montagna et al. 2002, Palmer et al. 2002, Ritter et al. 2005, Montagna et al. 2015, Chaloupka 2016). In the current study, results show that when salinities peak and plummet, the benthic infaunal community exhibits state shifts in species dominance. Since 2013, there has been a clear division in salinity by macroinfauna; as expected, chironomid larvae were dominant when the salinity was low, and *S. benedicti* were dominant when the salinity was higher (Chaloupka 2016, Figure 33). Dominance by a pioneer species (*S. benedicti*) and insect larvae is typical during disturbance events (Montagna et al. 2002). Because one or both of these species were dominant on any given trip during sampling, it's possible to view the majority of the sampling period as disturbed.

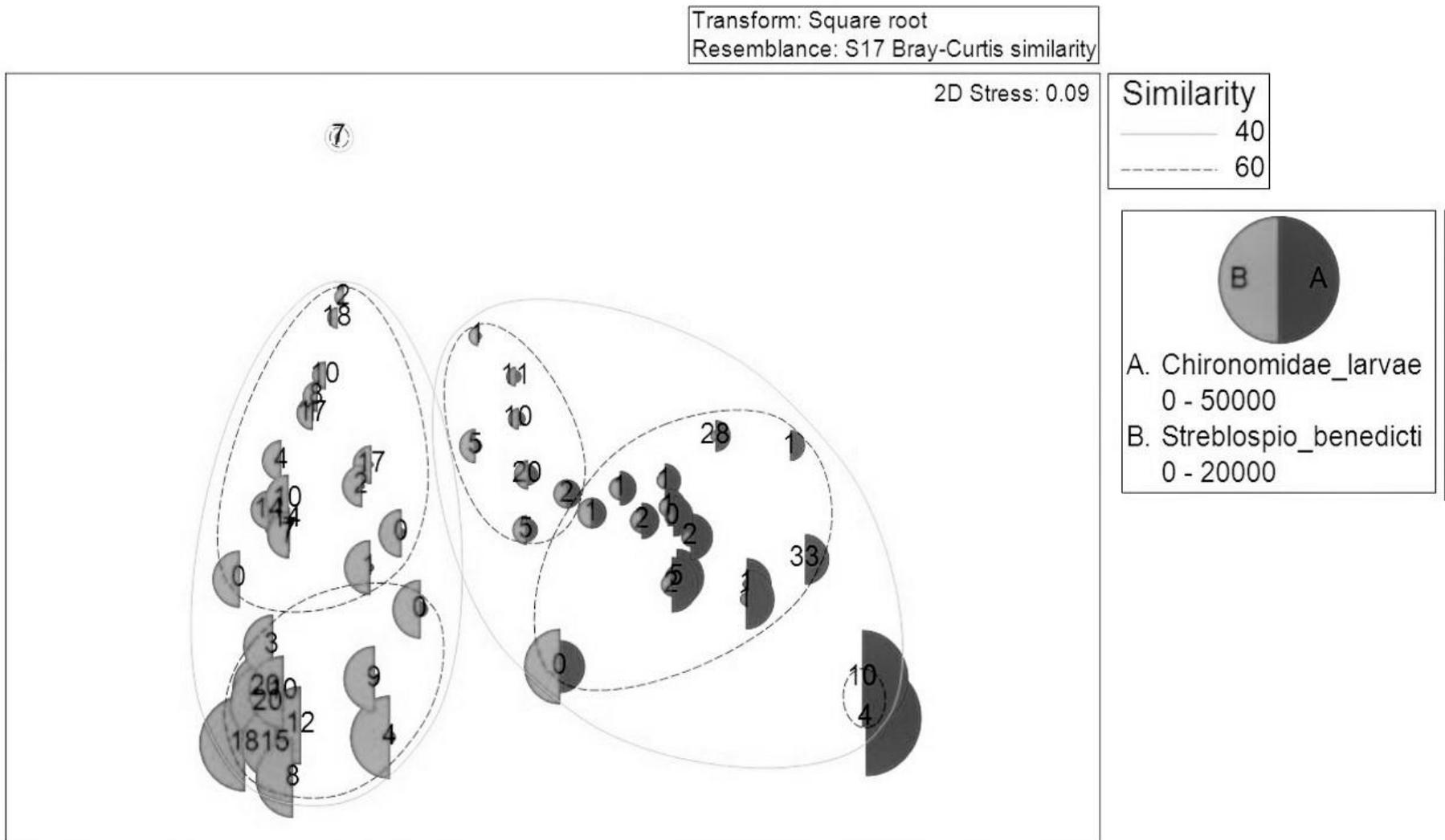


Figure 33. Non-metric MDS plots of benthic community structure where each point is overlaid with abundances (root transformed) of the two dominant species at Station C between October 2013 and December 2015. Points are labeled with salinity values at time of sampling. From Chaloupka (2016).

## Epifauna Response to Salinity

The current study is unique in that it coupled benthic epifauna and macroinfauna. Past studies have focused only on macrofauna, which live in the mud. This study focused on epifauna, which live on the mud, are larger, and are mobile. Epifauna include shrimps, crabs, and fish, and are known to feed on the smaller infauna (Flasch 2003).

Mysids were the most abundant species identified in the epifauna community. Mysids tend to enter the marsh region in higher abundance in the spring and fall and are a food source for penaeid shrimp, which migrate into the region (Lesutiene 2008, Riera et al 2000). Brown shrimp were found in lower abundance when mysid abundance was high, with little overlap. This may imply that larger shrimp (typically found in fewer numbers) are present in these periods, but post-larval and early juvenile shrimp do not overlap in these periods, reducing competition. Grass shrimp accounted for greater than 25 percent of the crustacean community in Rincon Bayou, and are a species that spend the entirety of their life in the same marsh habitat (Kneib 1985). Of the most abundant fish species identified in Rincon Bayou, sheepshead minnow and silversides would be considered resident species that contribute to the trophic food web for larger predators (Gosselink 1984, Longley 1994). Gulf menhaden are a commercially valued, migratory spring fish that enter the region as juveniles, utilizing marsh habitat during early development as refuge (Lowther and Liddel 2014).

The mobility of epifaunal organisms makes them more adaptable to fluctuating environmental conditions because they can simply relocate. The 22 June 2015 event had been identified previously as an event that was dominated by freshwater organisms, especially *S. benedicti*, Ceratopogonidae larvae, *Ephemeroptera* sp., and Lymnaeidae sp., and with minimal marine species present (DeWalt et al 2010, Gordon 2016, Palmer et al 2002). This provided some indication that inundation of freshwater into the bayou makes the environment unsuitable for marine species, whereas freshwater species are better able to exploit the region (Tolan and Newstead 2005, Turner and Brody 1983). Changes in diversity of the epifaunal community occurred within a few weeks after pumping, whereas responses to salinity were not identified in abundance and biomass right away, but positive relationships between abundance and biomass were evident after 6 to 8 weeks.

## **Recommendation**

By Texas law, beneficial inflow means a salinity, nutrient, and sediment loading regime that adequately maintains an ecologically sound environment in the receiving bay and estuary system that is necessary for the maintenance of productivity of economically important and ecologically characteristic sport and estuarine life upon which such fish and shellfish are dependent (Texas Water Code §11.147(a)). In Rincon Bayou, inflow is partially dependent on pumped inflows required by the 2001 Agreed Order from the Texas Commission on Environmental Quality. This agreement requires the city of Corpus Christi to “pass through” inflows no less than 151,000 acre-feet to the Nueces Estuary each year (TCEQ 1995). However, monthly inflows required are dependent on season, rainfall, stored levels of the reservoir system, and salinity levels in Nueces Bay (Montagna et al. 2009). The pump system has been active since 2009, but it is used during high inflow periods only because that is when pass-throughs are required. This means that pumped flows in addition to natural flooding enter Rincon Bayou and lower salinities even further than they would have occurred naturally. It also means that there is no relief when salinities are high and the fresh water is needed the most. The initial response to floods is typically reduced abundance and diversity in the first 2 to 4 weeks, then often an increase after 6 to 8 weeks. However, the large swings in salinity from fresh to hypersaline conditions maintains this habitat in a constant state of disturbance with negative consequences on the community. The disturbed nature of the community is characterized by the presence of species that are known to be early colonizers or pioneer species, and this would explain the decreased diversity immediately after a flood. Therefore we have two recommendations to ameliorate the disturbed state of the community: 1) pump when salinities are high, i.e., over 25 PSU, and 2) use one pump only to move the fresh water into Rincon Bayou in a slow trickle rather than a flood. These changes to the pumping paradigm should improve environmental conditions in Rincon Bayou.

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