



Effects on Benthic Macrofauna from Pumped Flows in Rincon Bayou

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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 macrofauna are ideal indicators of ecological effects because of their relative immobility and longevity in contrast with plankton of comparable size. Archived samples were analyzed as well as new samples collected from the upper Rincon Bayou near Corpus Christi, TX. One historical station (466C) was sampled biweekly. Conductivity, temperature, and salinity were monitored continuously. Additional water column measurements were taken during sampling events. Macrofauna 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 *Streblospio benedicti*, during especially low and high salinity periods. These results 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). Stream flow to the Nueces River has decreased by 99% since the building of the Wesley Seale Dam and by an additional 55% since the construction of the Choke Canyon Dam (Asquith et al. 1997, 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. 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 station, C (27.89878° N, -97.60417° W) sampled since 1994 (Montagna et al. 2002), was sampled for this study (Fig. 1). Station C is near 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 (Montagna et al. 2009). The site is surrounded by dense shrubbery and grasses that grow to the shoreline. Clay and mud dominate the substrate at all stations.

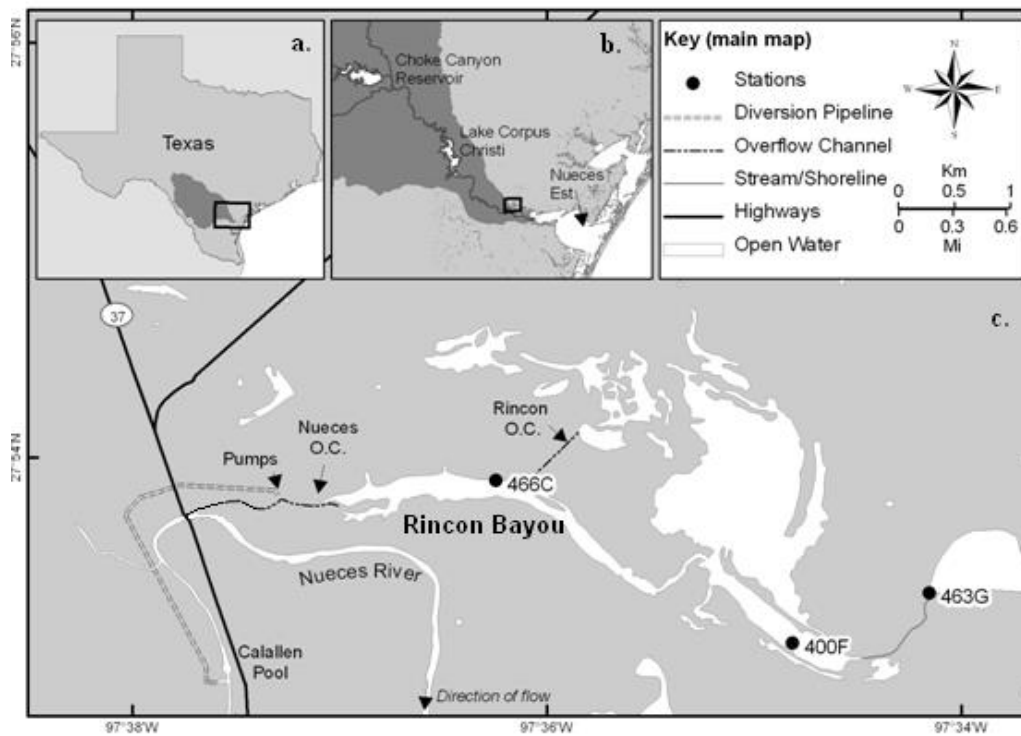


Figure 1. Map of study area.

Sampling Methods

Macrofauna samples were collected using a 6.7-cm diameter benthic core (area=35.23 cm²). 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.

Archived Samples

Archived benthic samples from previous collections in Rincon Bayou were used in addition to new samples collected during the present study period. A total of 21 archived samples (i.e., 3 replicates for 7 sampling dates) were processed (Table 1). These samples were analyzed using the method above.

Table 1. Archived samples at Station C analyzed for the current study.

Date	Station
May 2010	C
June 2010	C
January 2011	C
April 2011	C
July 2011	C
July 2012	C
October 2012	C

New Samples

Samples were taken biweekly from 25 October 2013 through 27 April 2015 at station C. Benthic samples have been collected at different sampling frequencies since 1994 (Table 2). The original method was to take samples quarterly and measure biomass by major taxa level only (Montagna et al. 2002). For the purposes of the current study, biomass was measured at the species level, or lowest taxonomic level possible. This species level biomass measurement was performed on the archived samples (Table 1) and samples collected since October 2012.

Table 2. Sampling periods and protocol differences for archived and new samples.

Study Period	Period Between Observations	Biomass Method
Oct 1994 – Oct 2008	Quarterly	Taxa
Jan 2009-Sept 2010	Monthly	Taxa
Oct 2010-Oct 2013	Quarterly	Taxa
Nov 2013-present	Biweekly	Species

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⁻¹), salinity (psu), conductivity (mS cm⁻¹), 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.

The abundance/biomass comparison (ABC) method was used to assess community response during different salinity periods. This method allows for the comparison of ranked species abundance and biomass versus an environmental variable, such as salinity, despite differing units of measurement (Clarke & Warwick 1994). Only the species specific biomass data could be used. First the salinity data for the entire period from January 2009 to April 2015 was classified into three periods representing wet, average, and dry conditions, using PROC RANK. Then the overall average abundance and biomass was calculated for each species for each period. The cumulative abundance and biomass were then ranked by species dominance using SAS programming and PROC RANK. The ABC plots for each salinity period were created using PROC SGPLOT.

Benthic community structure of macrofauna species was 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

There were a total of 80 sample dates when samples were taken at Station C (Table 3). Salinity ranged from fresh (0.34 psu) to hypersaline (79.66 psu). Dissolved oxygen (DO) never was hypoxia, i.e., less than 2 mg/L. Water elevation ranged from a depth of 13 cm to 50 cm.

Table 3. Summary of all hydrographic grab measurements at Station C from January 2009 to April 2015.

Variable	N	Mean	Std Dev	Minimum	Maximum
Salinity (psu)	80	14.26	17.11	0.34	79.66
Temperature (°C)	79	22.81	6.36	8.08	32.44
DO (mg/L)	78	8.71	2.26	4.11	17.57
pH	79	8.40	0.27	7.73	9.25
Depth (m)	80	0.18	0.13	0.01	0.50
Turbidity (NTU)	40	111	139	0	750

Salinity fluctuated over short time frames (i.e., days to weeks), and long time frames (i.e., interannually and seasonally) (Fig. 2). The salinity ranged from 0.3 psu on to 57.3 psu over the study period (Table 3). Salinity was low with nearly freshwater conditions during eight periods during the study: October 2009, January 2010, May to June 2010, October to November 2013, February 2014, June to July 2014, October 2104, and April 2015. Salinity was very high with hypersaline conditions during three periods: February to March 2009, May to July 2009, and April 2013. An alternation between generally wet and generally dry periods occurred in 2009 to 2010, 2010 to 2011, 2011 to 2013, 2013 to 2014, and in late 2014. Biomass measured at the species level occurred during a mix of wet, dry and average periods, but not at the most extreme hypersaline conditions in 2009 (Fig. 2).

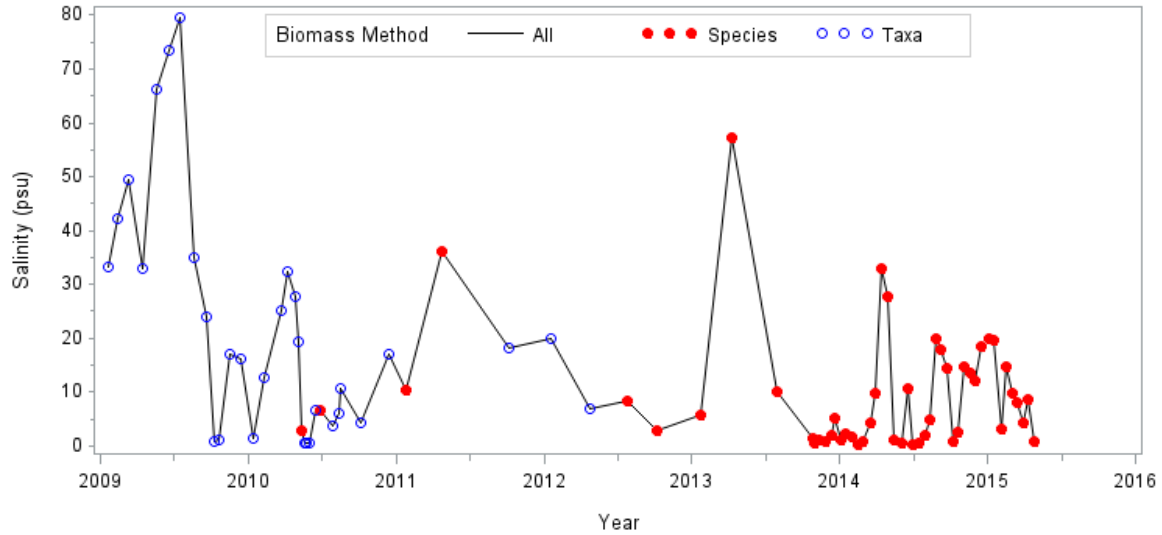


Figure 2. Salinity change in grab samples at Station C over the study period. Red dots are when biomass was measured at the species level, blue circles when biomass measured at taxa level.

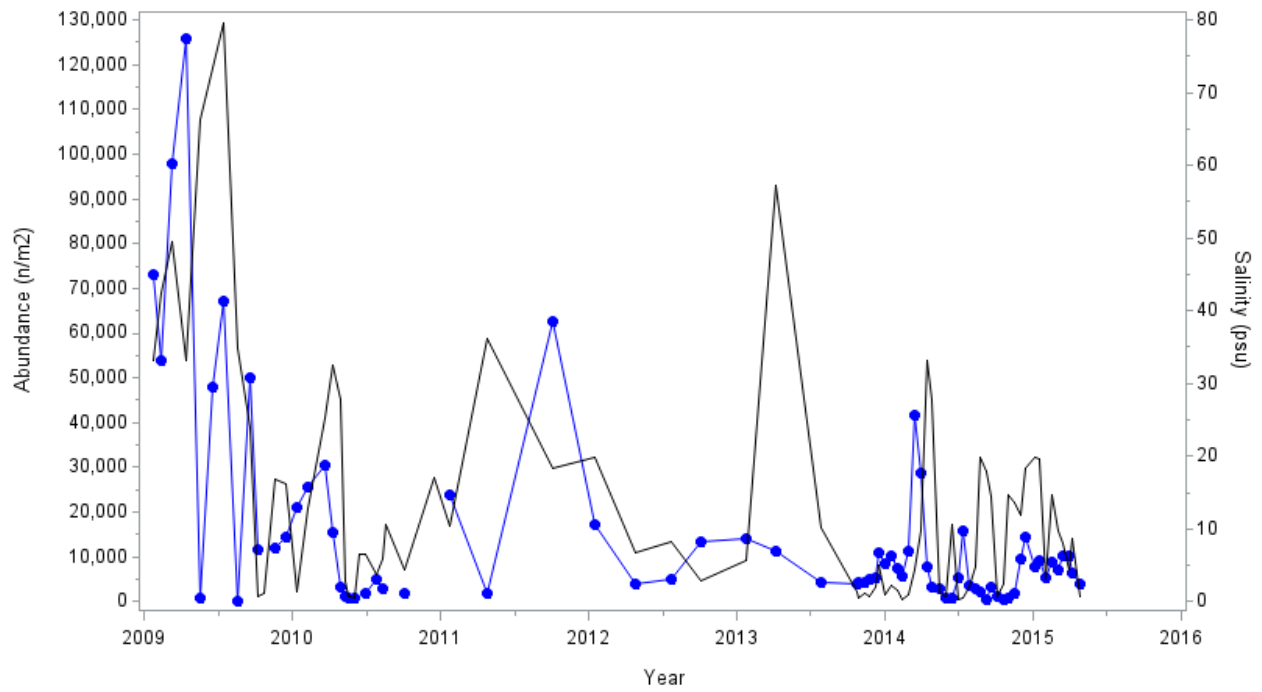
All of the salinity values from grab samples (Fig. 2) were divided into three bins based on ranks, which makes each bin about 33.3% of the samples (Table 4). The three salinity periods were: wet with a mean salinity of 0.8 psu, average with a mean salinity of 8.5 psu, and dry with a mean salinity of 18.0 (Table 3).

Table 4. Salinity parameters at Station C during three ranked periods.

Salinity Rank	N	Mean	Std Dev	Minimum	Maximum
1-Wet	26	1.2	0.8	0.3	2.9
2-Average	27	8.5	3.6	3.1	14.8
3-Dry	27	32.6	18.0	16.3	79.7

The time series of abundance, biomass, diversity, and evenness show that there is a great fluctuation over time for all the metrics of benthic biological response (Fig. 3). The benthic abundances in Rincon Bayou are high (Fig. 3A), but the biomass (Fig. 3B), diversity (Fig. 3C) and evenness (Fig. 3D) values are low (Table 5).

A)



B)

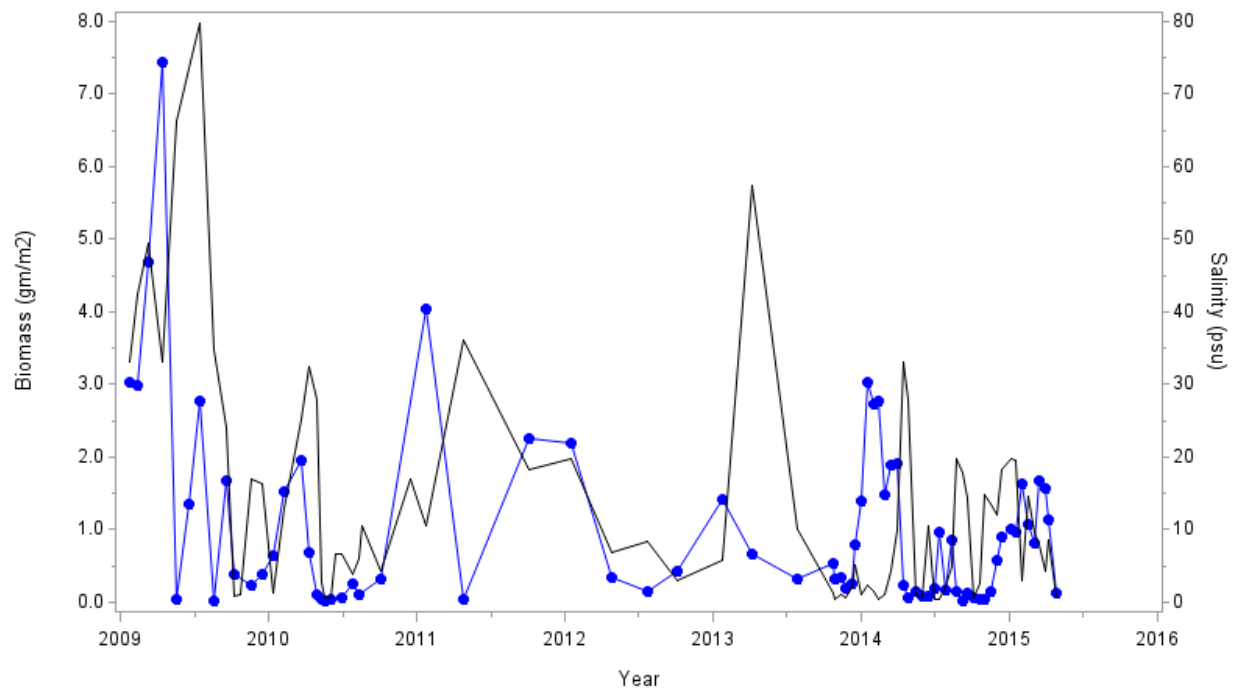
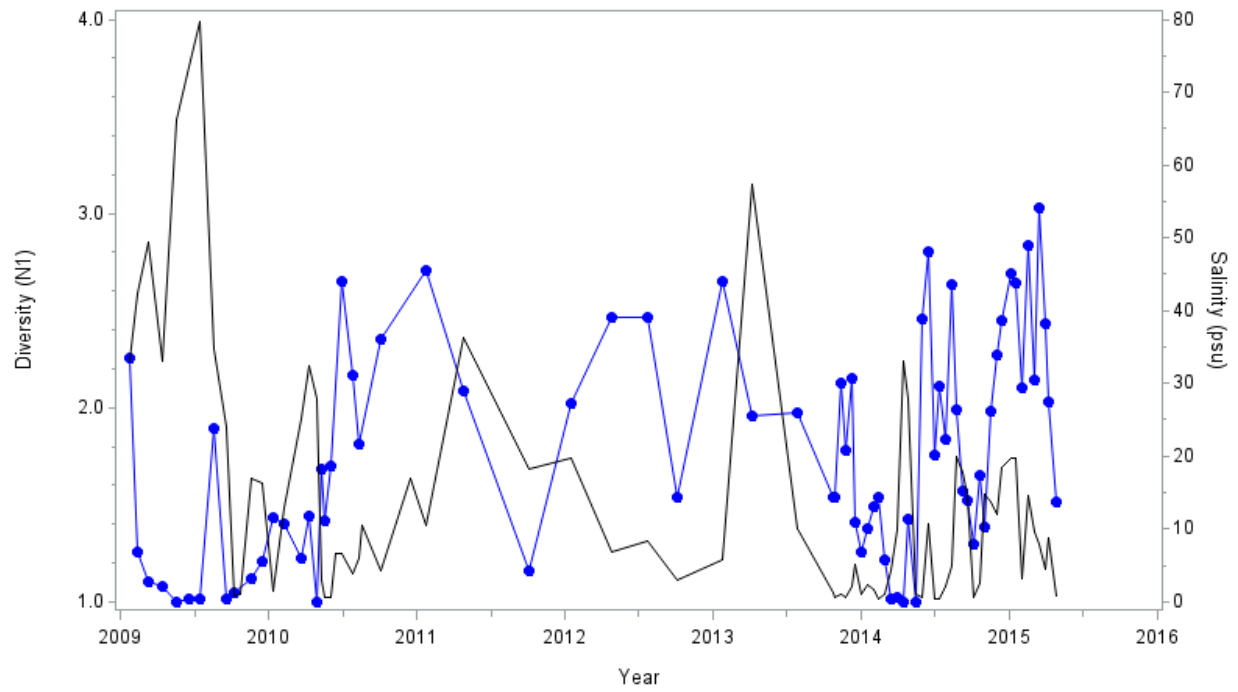


Figure 3. Time series data at Station C with salinity as black line. A) Abundance, B) Biomass, C) Diversity, D) Evenness.

C)



D)

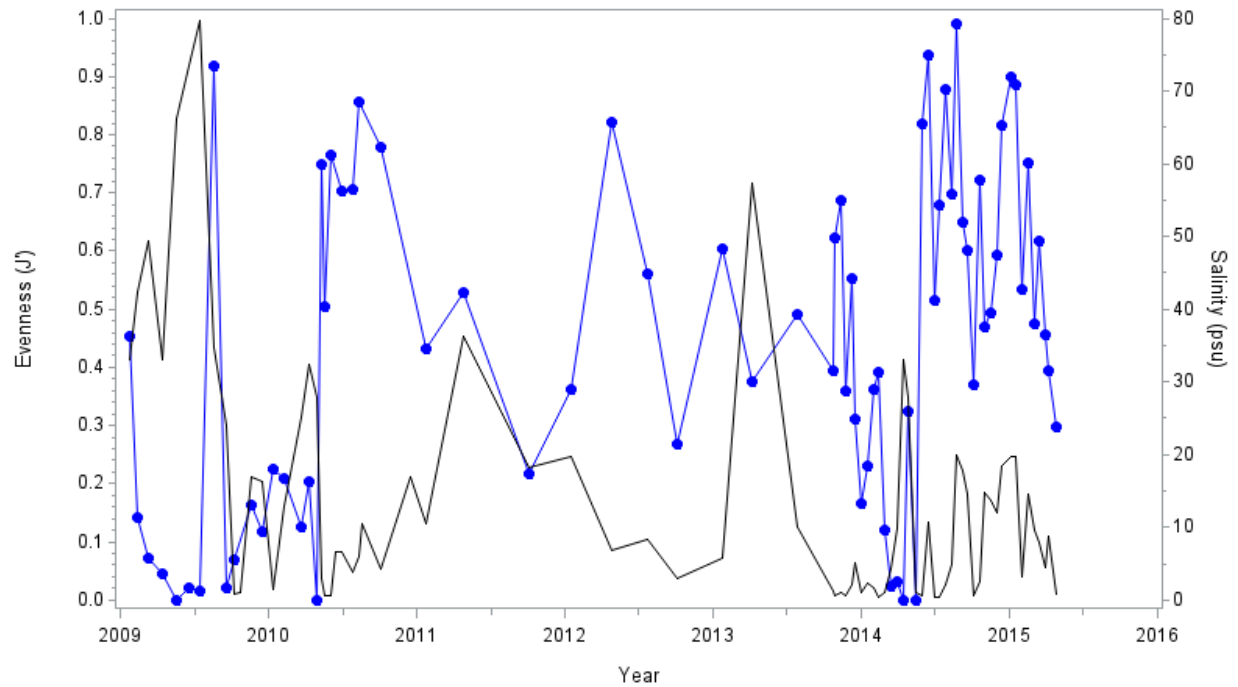


Figure 3. Continued. Time series data at Station C with salinity as a black line. A) Abundance, B) Biomass, C) Diversity, D) Evenness.

Table 5. Summary of the mean benthic parameters over 74 sampling periods at all stations.

Variable	Mean	Std Dev	Minimum	Maximum
Abundance (n/m ²)	15,081	23,035	284	125,936
Total Species (R)	3.51	1.67	1	10
Diversity (N1)	1.76	0.57	1	3.03
Evenness (J')	0.44	0.29	0	0.99
Biomass (g DryWt/m ²)	1.02	1.29	0.02	7.45

All of the biological metrics have an inverse correlation to salinity, except species richness (Table 6). Richness is inversely correlated with depth, i.e., temperature and DO concentration. Abundance is inversely correlated with depth as well.

Table 6. Correlations between physical and biological variables over 74 sampling periods. Bold values are significant at the 0.05 level. Abbreviations as in Table 5.

Physical Variable	Pearson Correlation Coefficients					
	Prob > r under H0: Rho=0					
	Biological Variable					
	R	n/m ²	H'	N1	J'	g/m ²
Depth(m)	-0.2061	-0.25017	-0.10775	-0.15186	0.04536	-0.2958
	0.0781	0.0316	0.3608	0.1965	0.7011	0.0105
Temperature(°C)	-0.38558	-0.11545	0.04887	0.04098	0.2903	-0.3242
	0.0007	0.3273	0.6793	0.7289	0.0121	0.0048
Salinity(psu)	-0.04418	0.49368	-0.32245	-0.2784	-0.35963	0.27209
	0.7086	<0.0001	0.0051	0.0163	0.0016	0.019
DO(mg/L)	0.30572	-0.18095	0.21517	0.19673	0.06188	0.07267
	0.0085	0.1255	0.0675	0.0953	0.603	0.5412

Community structure at station C changed over time (Fig. 4). Community structure is similar to the 60 level in most of the years, such as 2009, 2013, 2014, and 2015. These were predominantly high salinity years (Fig. 2). When abrupt salinity changes occur there is abrupt community structure change, such as between August (symbol lower left) and September (symbol upper right) when salinity decreased by 44.8 psu. The year 2015 is the most variable because symbols are scattered over the entire MDS space. The spring 2015 is largely in the lower right, the summer moves to the center, the fall moves to the top.

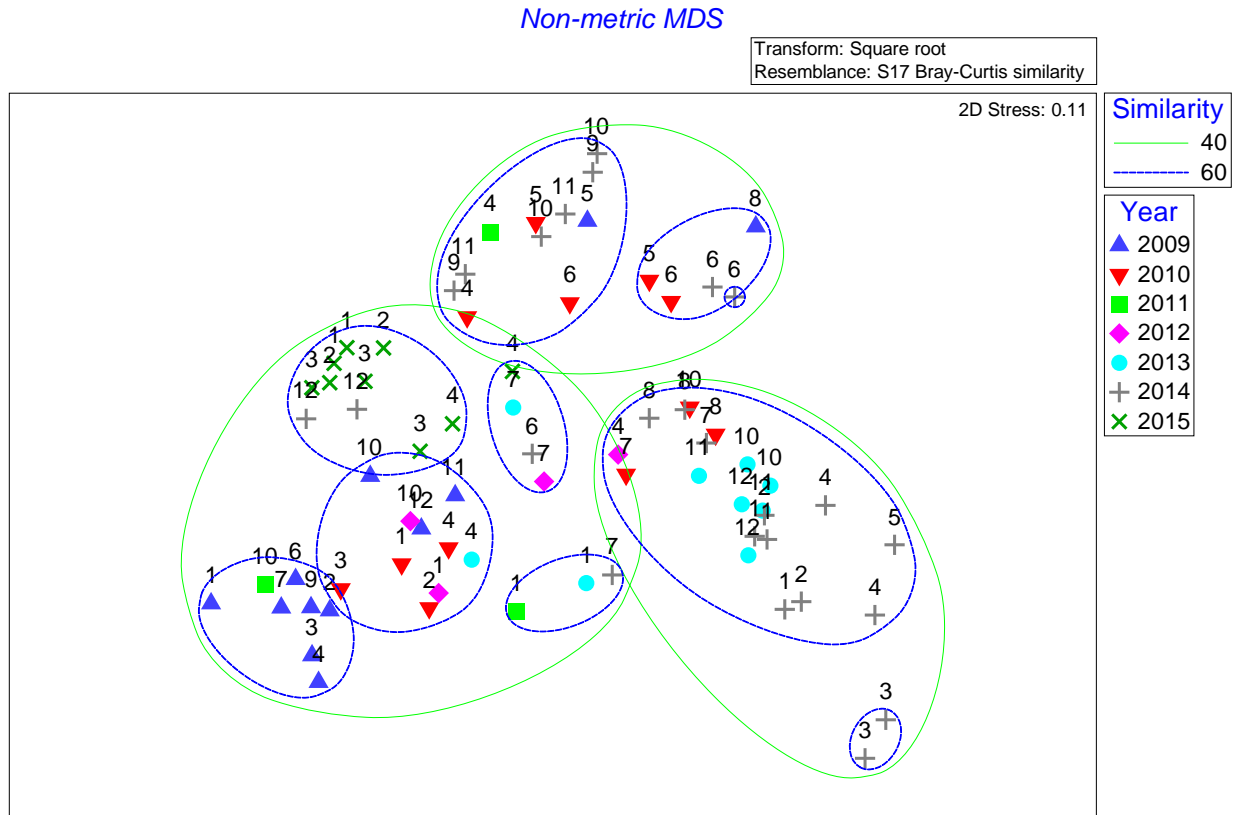
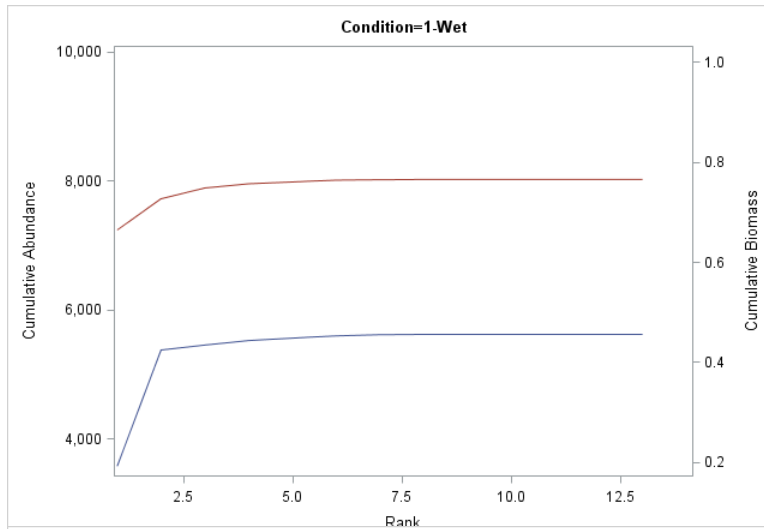


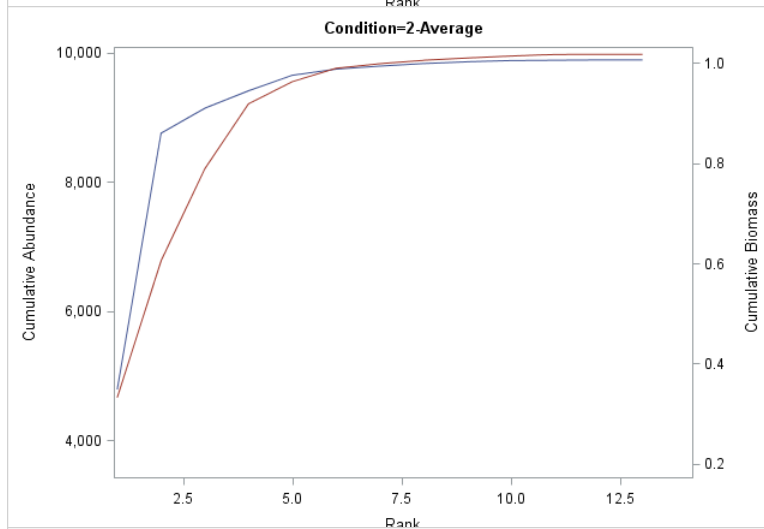
Figure 4. Multidimensional scaling plot of community structure change over time at station C. Symbol labels are months.

The ABC method was applied to three salinity groups, which roughly correlate to dry, average, and wet periods (Table 4). In low salinity or wet conditions, abundance levels are higher than biomass (Fig. 4). In average, midrange salinity, conditions both abundance and biomass are high. Dry, high salinity, conditions appear to mirror wet conditions with much higher abundance than biomass.

A.



B.



C.

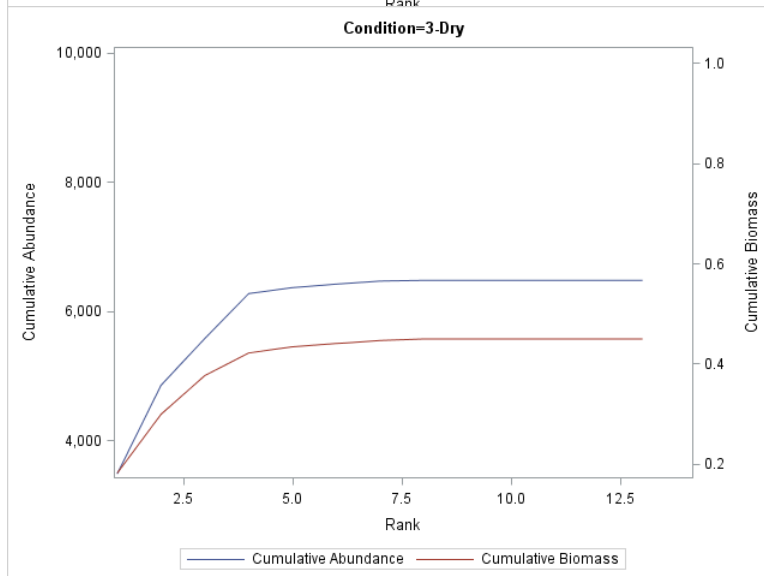


Figure 5. ABC curves for three salinity ranges. A) wet, B) average, and C) dry.

Chironomidae (larvae) and *Streblospio benedicti* are the most abundant species for all three salinity ranks (Table 7). Chironomid (larvae) has the highest biomass for each salinity rank. *Streblospio benedicti* also remained in the top five biomass contributors (Table 8). The highest abundance and biomass occur during the average salinity periods.

Table 7. Species contributions to abundance for three salinity ranks used in ABC analysis.

Species	Abundance (n/m ²)		
	Wet 0-3 psu	Average 3.1-15 psu	Dry 15.1-80 psu
Chironomidae (larvae)	3,574	4,789	1,371
<i>Streblospio benedicti</i>	1,801	3,966	3,482
<i>Mediomastus ambiseta</i>	76	47	95
Nemertea (unidentified)	71	95	11
<i>Streblospio gymnobranchiata</i>	38	388	693
<i>Hobsonia florida</i>	33	0	0
<i>Laeonereis culveri</i>	19	269	725
Oligochaeta (unidentified)	5	241	0
Ceratopogonidae (larvae)	0	38	0
<i>Mulinia lateralis</i>	0	28	53
Ostracoda (unidentified)	0	19	47
<i>Americamysis almyra</i>	0	5	0
<i>Farfantepenaeus setiferus</i>	0	5	0
Total	5,617	9,890	6,477

Table 8. Species contributions to biomass for three salinity ranks used in ABC analysis.

Species	Biomass (g/m ²)		
	Wet 0-3 psu	Average 3.1-15 psu	Dry 15.1-80 psu
Chironomidae (larvae)	0.664	0.332	0.045
<i>Streblospio benedicti</i>	0.063	0.184	0.181
Nemertea (unidentified)	0.021	0.027	0.003
<i>Mediomastus ambiseta</i>	0.008	0.003	0.006
<i>Streblospio gymnobranchiata</i>	0.004	0.044	0.077
<i>Hobsonia florida</i>	0.004	0	0
<i>Laeonereis culveri</i>	0.001	0.272	0.117
Oligochaeta (unidentified)	0.001	0.004	0
<i>Farfantepenaeus setiferus</i>	0	0.130	0
<i>Mulinia lateralis</i>	0	0.009	0.013
Ceratopogonidae (larvae)	0	0.007	0
Ostracoda (unidentified)	0	0.005	0.006
<i>Americamysis almyra</i>	0	0	0
Total	0.766	1.017	0.448

Discussion

Disturbance and stress events can cause changes in the diversity of existent communities (Menge & Sutherland 1987). By changing the species composition of a community, the ecological diversity and functionality of a system can be affected. Organisms have a specific range of conditions in which they can be successful, surviving and reproducing; as environmental parameters change only some organisms will survive. Natural and anthropogenic events can result in physical and/or physiological disturbance or stress. Systems can be affected by more than one disturbance and/or stress at a time, or sequentially, or by one disturbance or stress in succession (McFarland et al. 2013). Multiple disturbances or stresses can complicate the effects beyond that of a single stressor. In Rincon Bayou, salinity fluctuations were seen throughout the duration of the study period. Salinity variance has been characterized as a disturbance in Texas estuaries (Van Diggelin, 2014), so the wide range of salinity observed in the current study is possibly causing a disturbance within the benthic macrofaunal community. Organisms that are mobile (i.e., mobile epifauna, nekton, and plankton) are likely to migrate from the area resulting in a lower diversity following a disturbance. Sessile organisms and infauna, unable to leave the affected area, remain. Sessile organisms will suffer severe mortality if they are not adapted to a sufficiently broad range of salinity conditions to survive. Their inability to retreat from the disturbance puts sessile organisms at a higher risk of being affected by a disturbance event (Menge & Sutherland 1987).

Benthic invertebrates have been used as indicators of estuarine health in the Texas Coastal Bend area for over two decades (Mannino and Montagna 1997, Kim and Montagna 2009, Montagna and Palmer 2011, Palmer et al. 2011, Pollack et al. 2011). Numerous studies have shown benthic invertebrates are ideal indicators of freshwater inflow effects because of their sessile nature (Kalke and Montagna, 1991; Montagna and Yoon, 1991; Montagna and Kalke, 1992). Benthic macrofaunal communities respond to long term hydrological cycles (e.g. droughts) with reduced integrity just as they respond to reduced freshwater inflow on shorter time scales (MacKay et al., 2010; Palmer et al., 2011, Palmer and Montagna 2015).

Reduced diversity and an increase in smaller pioneering species is an indicator of early stages of disturbance (Rhoades et al. 1976, Pearson and Rosenberg and 1978). Thus one would expect that a graphical representation where species rank (i.e., dominance) is related to high biomass would also represent the early stages of succession, and this is what the abundance, biomass comparison (ABC) method attempts to portray (Clarke and Warwick 1994). The ABC graph is a combination of two k-dominance curves, biomass and abundance, in which the log of species rank is plotted against cumulative ranked abundance or biomass, expressed as a percentage of the total abundance or biomass. The ABC method, therefore, provides a greater depth of understanding than a simple diversity index by retaining species specific data (Clarke 1987). In the current study, the ABC plots showed higher abundance than biomass at both low and high salinity ranges indicating the presence of pioneer species (Fig. 6) (Warwick 1986). Species contributions to both biomass and abundance indicate two known pioneer species,

Streblospio benedicti and Chironomid (larvae). While Chironomids dominate Station C during low and average salinity periods, *S. benedicti* dominates during average and high salinity periods. These are the same pioneer species seen in other studies of inflow restoration to Rincon Bayou (Palmer 2002, Ritter et al. 2005).

While the ABC method can be helpful in clarifying diversity data, it cannot be relied upon as the sole analysis. Dominance curves rely heavily on the ranking of the most dominant species and may fail to accurately represent secondary and succeeding dominant species. An example of this can be seen in Table 5 and Table 6 where the dominant species do not match between abundance and biomass. Other computations, such as those suggested by Clarke (1990), are available to correct such oversights. However, because the total number of species in Rincon Bayou is so low, all the species were used in the ABC analyses presented here.

Areas affected by high frequencies of disturbance are likely to have low diversity as a result of being consistently dominated by pioneer species (Sousa 1979, Ritter et al. 2005, McFarland et al. 2013, Teuber et al. 2013). The Intermediate Disturbance Hypothesis (IDH) predicts the effects of disturbance frequency on system will be a bell-like curve where species diversity changes with differing frequencies of disturbance (Teuber et al. 2013, Sousa 1979). It has been shown that frequent salinity disturbances in Rincon Bayou caused domination by pioneer species, *Streblospio benedicti* and Chironomid (larvae), adapted to a wider range of salinities than other species within the system (Ritter et al. 2005).

The current study proves the usefulness of species specific biomass data in assessing estuary health. In contrast, past benthic studies in Rincon Bayou assessed biomass by major taxa only. Future studies should endeavor to create longer time series of biomass data to capture more interannual variability. This could inform changes to the Rincon Bayou inflow management and restoration programs.

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 naturally. It also means that there is no relief when salinities are high and the fresh water is needed the most. It is clear from this study that

the large swings from fresh to hypersaline conditions maintains this habitat in a constant disturbed state with negative consequences on the community. 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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