MULTIPLE STRESSOR EFFECTS ON MACROBENTHIC COMMUNITIES IN CORPUS CHRISTI BAY, TEXAS, U.S.A.

A Thesis

by

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

At any moment in nature, organisms are likely being exposed to multiple stressors, the effects of which are difficult to separate. Often, however, environmental stressors are considered on an individual basis. In southeastern Corpus Christi Bay, TX, declines in benthic macrofaunal community abundance, biomass, diversity, species richness, and species evenness have largely been attributed to the occurrence of hypoxia, a condition of low dissolved oxygen (DO). This study proposes that multiple stressors contribute to these observed benthic macrofaunal declines in southeastern Corpus Christi Bay. Therefore, a 30-year time series of water quality data (salinity, temperature, DO, pH, phosphate, ammonium, nitrite+nitrate, sulfate) and benthic community data (abundance, biomass, species richness, species evenness) was analyzed to describe 1) water quality dynamics of the region and 2) relationships between water quality dynamics and benthic macrofaunal response. Principal component analysis indicated that a large variability in the water quality dataset (63%) could be summarized by three principal components representing a multiple stressor index, a nutrient index, and an acidification index. Seasonality was found to be confounded with the multiple stressor index but not the nutrient or acidification indexes. Spearman rank-order correlations indicated both the multiple stressor and acidification indexes were inversely related to benthic macrofaunal community abundance, biomass, and species richness. A stepwise multiple linear regression analysis on individual water quality variables specified DO, and possibly temperature, to be leading explanatory variables for predicting benthic abundance. Temperature, pH, and nitrite+nitrate were indicated as leading explanatory variables for predicting benthic biomass. Temperature was indicated to be the only leading explanatory variable for predicting species richness. Results demonstrate that multiple stressors, including high temperature, high salinity, and low DO concentrations, are collectively acting on benthic communities in southeastern Corpus Christi Bay.

DEDICATION

I dedicate my thesis to my parents who gave me all the support I ever could have needed.

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INTRODUCTION

Hypoxia, a condition of low dissolved oxygen (DO), is commonly defined to be in occurrence when DO concentrations fall below a threshold value of 2 mg L^{-1} (Dauer et al., 1992; Diaz and Rosenberg, 1995). Hypoxia was first documented in Corpus Christi Bay in 1988 and eventually was recognized to be a seasonal summer occurrence in bottom waters over the southeastern region of the bay (Montagna and Kalke, 1992; Ritter and Montagna, 1999; Applebaum et al., 2005). Benthic macrofaunal effects in Corpus Christi Bay were found to be occurring at DO concentrations as high as 3 mg L^{-1}, a threshold higher than the generally defined DO concentration for hypoxia of 2 mg L^{-1} and below (Ritter and Montagna, 1999). The reasoning for this higher threshold value has yet to be determined but implies a possibility that more than just low DO concentrations may be acting on the environment.

Hypoxia can have direct and indirect effects on macrofauna and macrofaunal communities (Montagna and Froeschke, 2009). Direct effects are caused by physiological mechanisms, such as, suffocation, while indirect effects are caused by interactions, such as, effects on top-down or bottom-up controls. Direct effects on benthos ascribed to hypoxia in scientific literature include reduced abundance, reduced biomass, emergence of fauna, physical inactivity and death. Indirect effects on benthos ascribed to hypoxia include higher predation pressure on emerging benthic fauna and diversion of energy flow away from benthos and towards microbes (Diaz and Rosenberg, 1995). In the southeastern region of Corpus Christi Bay, direct effects on macrofauna include changes in benthic macrofaunal community structure as well as declines in abundance, biomass, diversity, species richness, and species evenness (Montagna and Froeschke, 2009). In addition, the community's structure and species dominance have also been differentiated between hypoxic and normoxic areas of the bay (Ritter and Montagna, 1999; Montagna and Ritter, 2006; Montagna and Froeschke, 2009). The opportunistic species (typically young, small, and short-lived species that are better able to withstand stress) *Streblospio bendedicti* and oligochaeta were found to dominate in the hypoxic region of the bay only (Dauer et al., 1992; Ritter and Montagna, 1999). Thus, there is clear evidence of hypoxia acting directly on macrofauna in southeastern Corpus Christi Bay. The indirect effect of increased predation pressure on emerging fauna for the southeastern region, however, was found to be insignificant (Montagna and Ritter, 2006; Montagna and Froeschke, 2009). It does not appear that increased predation pressure on macrofauna attributed to hypoxia can clarify why macrofaunal declines in Corpus Christi Bay are observed at DO concentrations higher than the traditional definition of 2 mg L^{-1} and below. If the indirect effect of increased predation pressure cannot adequately explain the higher defined hypoxic threshold value in Corpus Christi Bay, then perhaps another variable or stressor can.

Declines in benthic community metrics and structure in southeastern Corpus Christi Bay have largely been attributed to regional hypoxia (Ritter and Montagna, 1999; Ritter and Montagna, 2006; Montagna and Froeschke, 2009). However, periods of high salinities have accompanied hypoxia occurrence were hypoxic events are more frequent and intense in the southeastern region (Montagna and Ritter, 2006). It has been found that hypoxia in Corpus Christi Bay is associated with high bottom salinity values and water column stratification; where the differences in water masses are due to differences in salinity values (Ritter and Montagna, 1999; Applebaum et al., 2005). Measured differences between bottom and surface salinity values are largest in the southeastern region where hypoxia occurs. Average difference in salinity values between surface and bottom depths measures 1.5 PSU in the hypoxic region while only 0.5 PSU in a normoxic region (Montagna and Ritter, 2006). Salinity in the bay measured during the month of July for the years 1988 to 2002 had an average of 36.0 PSU ± 6.6 in the hypoxic region and an average of 33.0 PSU ± 4.9 in the normoxic region (Montagna and Froeschke, 2009). Salinity gradients play a major role in the distribution, structuring, and abundance of benthic communities (Rosenberg et al., 2003). Because salinity gradients have an effect on benthos and high bottom salinities are correlated to low DO concentrations in Corpus Christi Bay, high bottom salinity may be a variable of interest to explain the higher observed hypoxia threshold.

The effects on benthic macrofaunal community metrics and structure attributed to DO concentrations in Corpus Christi Bay may not be isolated from the effects attributed to bottom salinity values. A stressor is any environmental factor that adversely affects an organism's and population's fitness through the impairment of an individual's structure, physiology, and or functioning (Calow, 1989). Resistant or tolerant species with traits less affected by a stressor should be favored by stressors acting as a selection pressure. At an individual level, stressor is registered through a reduction of survival or growth while at the population level, a stressor is registered through changes in population abundance, biomass, diversity, structure, and or function (Calow, 1989). Low DO concentrations during seasonal hypoxia in Corpus Christi Bay have been accredited to declines in benthic macrofaunal community abundance, biomass, diversity, species richness, and species evenness (Diaz and Rosenberg, 1995; Montagna and Froeschke, 2009). Community structure and dominance have also been found to differ between hypoxic and normoxic areas of the bay (Ritter and Montagna, 1999). At the same time, high bottom salinity values, which are correlated with low DO concentrations in Corpus Christi Bay, also have a potential to reduce benthic macrofaunal community fitness. Thus, benthic macrofaunal community declines and changes in community structure may not be responses resulting from just low DO concentrations in Corpus Christi Bay. Multiple stressor may affect benthic community metrics and structure in the southeastern region of Corpus Christi Bay.

Benthic community structure and metrics (including abundance, biomass, and species richness) have widely been used as biological indicators of ecosystem health in environmental assessment (Calabreta and Oviatt, 2008; Montagna et al., 2013). Biological indicators will assimilate to changes in an ecosystem, which makes them useful in determining the stability of an environment. Benthic macrofauna are good biological indicators of environmental change because they are relatively immobile, long lived, and utilize the detrital food chain. This means that benthic communities represent a long-term memory of sediment quality, characteristics, and general health of a specific region. In addition, a wide range of physiological tolerances for stress is represented within benthic communities (Calabreta and Oviatt, 2008). Therefore, in addition to environmental change, benthic communities have also been used specifically as indicators of stress (Merovich and Petty, 2006).

The affects of low DO concentrations on the benthic macrofaunal community in Corpus Christi Bay have been well investigated. Not as much consideration has been given to if other stressors can explain the observed declines in benthic macrofaunal community abundance, biomass, and species richness as well as account for the changes observed in community structure. It is difficult to separate benthic effects arising from multiple stressors. At any moment in nature, organisms are likely being exposed to multiple stressors. In addition, stressors could be acting synergistically amongst each other meaning the combined effect would be greater than the responses of an individual stressors acting alone (Folt et al., 1999; Vinebrooke et al., 2004). Therefore, if synergy occurs among stressors, one or both of the individual stressor effects would increase. Often effects of environmental stressors are tested individually and field studies focusing on benthic community metrics and structural responses to multiple stressors are not common (Merovich and Petty, 2007). A field environment, such as Corpus Christi Bay, is more complex than a controlled environment to study because one effect or output could be a result of more than one input. Field studies can account for both direct and indirect effects of stressors as well as greater ecosystem level effects. The use of indices for assessing ecosystem health and status for regulations such as the US Clean Water Act has increased (Ellis et al., 2015). Many univariate metrics including indicator species, indicator ratios and diversity or contaminant metrics have been proposed to address these regulations. However, multivariate models using community composition have been shown to be more sensitive to changing environmental health and therefore better at detecting smaller relative differences between sites or treatments. The study here takes advantage of a 30-year time series of benthic community and water quality data collected from a long-term field station in southeastern Corpus Christi Bay. The study aims to describe the water quality dynamics and underlying structure in southeastern Corpus Christi Bay as well as the relationships between water quality dynamics and benthic macrofaunal diversity. The study considers multiple stressors to be having effects on benthic macrofaunal communities in a way that their interaction might explain the higher threshold value of 3 mg L^{-1} at which benthic macrofauna show response. The results should provide a better understanding of the possible collective effects of multiple stressors on benthic populations in southeastern Corpus Christi Bay.

METHODS

Study Location and Design

Corpus Christi Bay, TX, USA, a leveled, shallow, semi-enclosed bay, is microtidal and subject to strong meteorological forcing (Montagna and Kalke, 1992; Ritter and Montagna, 1999). Southwestern winds dominate March through September while north-northeastern winds dominate October through March (Applebaum et al., 2005). Hydrographic models indicate that water in Corpus Christi Bay flows along the eastern shoreline to the southwest (Turner et al., 2015). Water residence times for this bay are long, being greater than five months.

As one moves south along the Texas coast, inflow balance decreases around two orders of magnitude (Montagna and Kalke, 1996). The Nueces River represents a major source of riverine input for the Nueces estuary, which is relatively small when considering bay size (Flint and Younk, 1983). Other freshwater inflow sources to Corpus Christi Bay include Oso creek (via Oso Bay) as well as watershed runoff (http://www.twdb.texas.gov/). Marine water influences on Corpus Christi Bay include the Laguna Madre to the south and the Gulf of Mexico through a limited connection via the Aransas Pass to the north as well as through a narrow ship channel running east to west across the barrier island (Montagna and Kalke, 1992). Thus, a natural salinity gradient exists within the bay extending from the Nueces river mouth (lower salinities) toward the coast (higher salinities) with small variations in salinity across this gradient. The southeastern region of the bay experiences the highest average salinities (Appelbaum et al., 2005).

Corpus Christi Bay is neither oligotrophic nor eutrophic (Turner et al., 2015). Principal point sources for nutrients include the Nueces River and Oso Bay (via a waste water treatment plant) (Nelson and Montagna, 2009; Turner et al., 2015). Wastewater dominates as a source of nutrients for the Nueces estuary (Brock, 2001). Three wastewater treatment facilities are located on Oso Creek and Oso bay and thus are possible sources of nutrients into Corpus Christi Bay (Nelson and Montagna, 2009). Still, both nitrogen and phosphorous, in Corpus Christi Bay, have been found to be limited (Appelbaum et al., 2005; Turner et al., 2015). Moreover, chlorophyll (averaging 4.19 mg L^{-1}), phosphate, and dissolved inorganic nitrogen (DIN) concentrations in the southeastern region of the bay are relatively low (averaging 4.19 mg L^{-1}, 0.7 μM L^{-1}, and 2.2 μM L^{-1} respectively) and not significantly changing over time (Appelbaum et al., 2005).

DO concentrations, although highly variable annually, are consistently lower in the southeastern region of Corpus Christi Bay relative to other parts of the bay (Montagna and Froeschke, 2009). Hypoxia was originally documented in Corpus Christi Bay in 1988 (Montagna and Kalke, 1992). Later it was determined that the area of hypoxia was larger than previously thought as it was observed to extend over the southeastern region of the bay during the summertime season (Ritter and Montagna, 1999). The southeastern region of the bay has the lowest average DO concentrations which steadily decline during the months of July and August when water circulation is low, wind speeds are low, and evaporation rates are high (Applebaum et al., 2005). In addition to season, hypoxia occurrence in the southeastern region of the bay has also been associated with high bottom salinity values as well as water column stratification influenced by salinity (Ritter and Montagna, 1999).

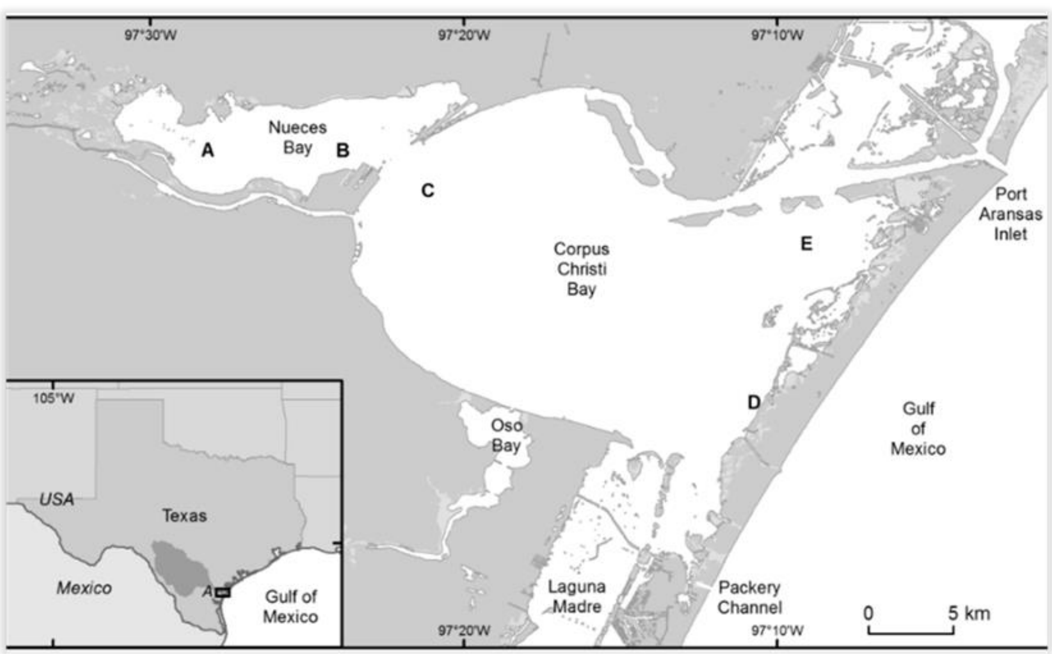


Figure 1: Long-term monitoring stations A-E; Corpus Christi Bay, Texas, U.S.A.

Since October 1987, benthic macrofauna in Nueces and Corpus Christi Bay have been sampled (using cores) alongside water quality data on a quarterly basis (January, April, July, and October) at permanently defined stations A-E (Figure 1). These five fixed stations lie along the natural salinity gradient of the bay. Stations A and B, the less saline (more freshwater) stations, are located nearest to the Nueces River mouth while stations D and E, the more saline stations, are located nearest to Laguna Madre and Aransas Pass respectively.

Station D lies within the study's area of interest: the southeastern region of Corpus Christi Bay. Previous studies have demonstrated Station D to adequately represent a region of hypoxic occurrence in the bay (Ritter and Montagna, 1999; Applebaum et al., 2005; Montagna and Ritter, 2006; Montagna and Froeschke, 2009). At the same time, salinity values and stratification are highest in the southeastern region, an area that encompasses the long-term monitoring Station D (Applebaum et al., 2005; Montagna and Ritter, 2006). The benthic macrofaunal community structure and metrics between Station D and its most comparable Station E have been well differentiated in multiple comparison studies over time—the most recent study covering the time period of 1988 to 2007 (Montagna and Froeschke, 2009). Because of time constraints, to date, only station D samples have been processed past the year 2009. Given the above, only samples collected at Station D were used for the current analysis (Figure 1).

Benthic Macrofaunal Sampling

To assess the relationship among benthic community diversity (a measure that accounts for both species richness and evenness) and water quality dynamics in southeastern Corpus Christi Bay, benthic macrofaunal community abundance, biomass, species richness, and species evenness data from station D was used in analysis (Figure 1). During the period of October 1987 to October 2017, sediment core samples at Station D were collected on a quarterly basis (January, April, July, and October) with a period of missing data beginning October 1988 and continuing until July 1990.

During a quarterly sampling trip, three replicates of a 6.7 cm diameter core were collected, sectioned into depth intervals of 0-3 and 3-10 cm, preserved using 5% buffered formalin, and stained with Rose Bengal. Macrofauna inside the cores were sorted out using 0.5 mm sieves, identified to the lowest taxonomic level possible, and counted for abundance measures. Identified macrofauna were then grouped into higher tax categories (Polychaeta, Bivalvia, Gastropoda, and Crustacea), dried at 50 ^{0}C for a period of 24-48 hours, and individually weighed for biomass measures. For macrofauna with carbonate shells, the shells were dissolved in 1 N hydrochloric acid and washed before being dried and weighed. Species richness was represented by an index of the number of present species in a core (Hill 1973). Species evenness was calculated using Pielou's evenness index represented by Equation 1 below (Ludwig and Reynolds, 1988 Montagna and Ritter, 2006).

Equation 1: $$\mathrm{J}' = \frac{-\Sigma(p_i \ln p_i)}{\ln(S)}$$

Where: p_i = abundance of species i divided by the total abundance
S = Species richness (number of present species in a core)

All resulting data collected over time was archived. Benthic macrofaunal sampling and identification techniques used in this study followed that of Montagna and Kalke (1992) with one exception: benthic macrofaunal data from the sectioned depth intervals (0-3 cm and 3-10 cm) were combined (after being archived) and treated as a complete 0-10 cm core for analysis. In previous studies, section depth has been treated as a main effect and hypoxia has been found to have a relationship with the vertical distribution of infauna in the bay (Montagna and Ritter, 2006). This study, however, was only interested in looking at the benthic community as an entire whole during analysis.

Water Quality Sampling

To describe southeastern Corpus Christi Bay's water quality dynamics as well as to assess its relation to benthic community diversity, hydrographic profiles and water samples were taken alongside each quarterly benthic sample. A Hydrolab Sonde (1988 to 1999) and a YSI 6 sonde (2000 to present) measured parameters of water temperature (˚C), salinity (PSU), dissolved oxygen (mg L^{-1}), pH, and depth (m) while wind speed, wind direction, cloud cover, and wave height were observed during collection and documented. A top (0.1 m from the surface) and bottom (0.1 m from the bay bottom) sonde measurement were always recorded. Because the study was interested in multiple stressors (including hypoxia) acting on benthic macrofauna, only bottom sonde measurements were used in analysis. This is due to the close location of the bottom sonde measurement (0.1 m from the bay bottom) to the benthic macrofauna being measured as well as the fact that previous field observations (unpublished data from the Montagna lab) have found hypoxic measurements near the bay bottom which were not recognized higher in the water column.

For nutrient analysis, water samples were collected 0.1 m from the bay bottom using a horizontally mounted Van Dorn sampler. Samples (of at least 24 mL of filtrate) were filtered into a collection tube out in the field using a 60 mL syringe and a 0.7 µm Whatman Glass microfiber filter 25 mm in diameter. To keep the integrity of the sample as well as not to damage the filter, the filtering rate was set to about 6 drops per second (GF/F filter rate = 325 sec/100 mL). Collected samples were stored on ice and covered until returning from the field where they were placed immediately into a freezer. Nutrient samples were later filtered using 0.45 µm polycarbonate filters and analyzed using an O.I. Analytical Flow Solution IV autoanalyzer combining segmented flow and flow injection analysis techniques with computer controlled sample selection and peak processing. Nutrients measurements (µmol/L) were acquired for phosphate (PO_4), ammonium (NH_4), nitrite+nitrate (NO_x), and Silicate (SiO_4).

Statistical Analyses

Univariate metrics such as abundance of indicator species, indicator ratios and diversity metrics are frequently used in monitoring ecosystem health (Ellis et al., 2015). However, multivariate metrics, using the same information with a focus at the community level, are also available for use. Though ecological systems are complex, relationships among and between variables can still be explained in a simple and easy to understand manner. Principal Component Analysis, Spearman rank-order correlations, stepwise regression, and nonparametric multidimensional scaling analysis were all utilized and are further described below.

PCA and Spearman Rank-Order Correlation

Principal Component Analysis (PCA) was used to evaluate the underlying structure of the water quality dataset. Spearman rank correlation was used to assess the relationships between hydrographic variables and benthic metrics. PCA is a variable reduction technique that reduces a data matrix by using linear combinations and variance to extract variables or components that are mutually orthogonal to each other. The motive behind using PCA was to reduce the large set of variables into a smaller uncorrelated (orthogonal relationship) set that still contained most of the original data's information. The underlying structure of the variables would then be understood and the dataset visualized in a meaningful way.

The final products of a PCA are principal component (PC) variable loads and PC sample scores. Bivariate plots for both the PC loads and the sample scores were plotted. PC loads were plotted to visualize correlations or contributions between original variables and new variables or components. Sample scores were plotted to visualize each sample's contribution among different PC variable loads across sampling dates as well as differing salinity ranges. Plots of PC variable loads and their subsequent scores were then compared.

Spearman rank-order correlation is a nonparametric measure of association based on the ranks of the data values. Results from the correlation will identify significant relationships that exist between PC indexes and a specific benthic metric. Therefore, a Spearman rank-order correlation analysis was conducted among new PC variables and benthic community metric response variables (abundance, biomass, richness, and evenness) to measure the strength of association and direction of relationships among the two types of variables.

Prior to PCA, all data was log transformed using ln (x +1) and standardized to a normal distribution with a mean value of zero and variance of one (N 0,1). The pH measurements were excluded from log transformation due to their already being log transformed. The PROC FACTOR procedure (SAS version 9.4) was used using PC methods on a correlation matrix to obtain a data set that consisted of both vector loads and sample scores. A VARIMAX rotation method was used on the PC axes for better visualization and interpretation. Spearman Rank-Order Correlation was calculated using PROC CORR (SAS version 9.4).

Stepwise Regression

A multiple adaptive regression spline analysis was performed using water quality data as independent variables in order to determine if water quality variables could predict benthic responses. Adaptive regression was calculated using PROC ADAPTIVEREG (SAS version 9.4). PROC ADAPTIVEREG uses a nonparametric regression technique that combines an adaptive regression spline model using knot values, with model reduction using model selection techniques (Kuhfeld and Cai, 2013). Knots or partitions in the data were created automatically, basis functions were constructed, and forward then backward selection techniques were applied to generate a final model that was parsimonious and did not overfit the data. The importance of each variable was calculated by taking the square root value of the generalized cross validation (GCV) statistic from the submodel (with all that variable's basis functions removed), subtracting from it the selected model's square root GCV value, and finally scaling the result so that the largest importance value was 100. Calculated values of variable importance were used to define the leading variables of prediction regarding benthic abundance, biomass, and richness. Per SAS (2015) and Kuhfield and Cai (2013) examples, only variables with an importance value of 50 or greater were selected as variables of importance.

Prior to regression analysis, data was log transformed using ln (x +1) and standardized to a normal distribution with a mean value of zero and variance of one (N 0,1). The pH measurements were excluded from log transformation due to its already being log transformed. For each of the dependent variables: benthic abundance, biomass, and species richness, separate regressions were run with the independent variables DO (mgL^{-1}), temperature (˚C), salinity (PSU), pH, phosphate (PO_4), ammonium (NH_4), nitrite+nitrate NO_x, and silicate (SiO_4).

Nonparametric Multidimensional Scaling

The community structure of benthic macrofauna was analyzed by nonparametric multivariate methods using Primer-e software (Clarke and Gorley 2015). First, a Bray-Curtis similarity index between sample pairs was calculated, and then the similarities were visualized in a nonparametric multidimensional scaling (nMDS) bivariate plot. In addition, a hierarchical cluster analysis, using a group average technique, was performed on the similarity index and a resulting similarity rank overlaid on the nMDs plot.

RESULTS

Status and Trends

Using a 30-year record of quarterly samples taken from the fixed Station D, the averages and standard deviations of both water quality (temperature, salinity, DO, pH, phosphate, ammonium, nitrite+nitrate, silicate) and benthic macrofaunal (abundance, biomass, species richness, species evenness) variable measurements were determined (Table 1). Water quality variables: temperature, salinity, phosphate, ammonium, and silicate measured highest on average for the July samples. DO concentrations averaged lowest among the July samples while the highest averages occurred among January samples (the opposite was true for temperature). Average salinity values were highest among July sampling months but appeared relatively high year-round.

Excluding silicate, nutrient averages from Station D for the study period were low (Table 1). In general, nutrient averages tended to be highest during the sampling month of July. Small differences, however, in nutrient averages existed among sampling months. The variable pH was a log based variable and thus had a small range of average values across months sampled.

Average values for abundance, biomass, and species richness were lowest for the sampling months of July and October. Species evenness averaged lowest during the sampling month of October, however, in general the averages for species evenness appeared similar amongst different sampling months.

Table 1. Means and Standard Deviations (SD) of water quality (Temperature, Salinity, DO, pH, Phosphate, Ammonium, Nitrite+Nitrate, Silicate) and benthic macrofaunal (Abundance, Biomass, Richness, Evenness) variables sampled October 1987 to October 2017 at Station D

Variable	**Month Sampled**									
	All Months		**January**		**April**		**July**		**October**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Temperature (˚C)	22.3	6.32	12.9	2.72	21.2	2.21	29.4	0.661	25.3	1.93
Salinity (PSU)	33.4	5.40	31.3	3.14	30.6	3.68	36.7	5.89	35.0	6.01
DO (mg L^{-1})	6.06	2.48	8.76	0.763	6.90	1.33	2.95	2.01	5.96	0.931
pH	8.15	0.244	8.16	0.190	8.05	0.311	8.17	0.189	8.20	0.254
Phosphate **(**µM L^{-1})	0.646	0.853	0.374	0.301	0.362	0.453	1.01	1.14	0.589	0.543
Ammonium **(**µM L^{-1})	1.86	2.91	0.766	0.872	1.00	0.946	3.37	4.07	1.67	2.78
Nitrite+Nitrate **(**µM L^{-1})	0.778	1.46	0.615	1.68	0.850	1.75	0.674	1.04	0.753	1.28
Silicate **(**µM L^{-1})	46.6	34.7	29.9	19.7	31.8	26.5	71.2	41.6	56.0	30.4
Abundance (n/m2)	39525	39783	46223	34103	44947	29262	22740	27794	32325	38417
Biomass (g/m2)	11.02	14.82	13.00	22.7	15.5	11.6	7.08	9.41	7.14	11.1
R Richness (S/sample)	27.3	15.0	31.7	11.5	37.3	13.5	19.7	11.7	18.0	11.2
J' Evenness (J'/samples)	2.05	0.663	2.11	0.439	2.06	0.470	2.08	0.856	1.99	0.811

Long-term trends at Station D for surface and bottom DO concentrations, temperatures, and salinities over the 30-year period were analyzed with linear regression using an alpha significance level of 0.05 (Figure 2). Separate linear regressions were run over time (independent variable) for each water quality variable: DO, temperature, and salinity (dependent variables). Resulting plots are displayed in Figure 2 and include a corresponding regression equation, regression line, r squared value, and p value.

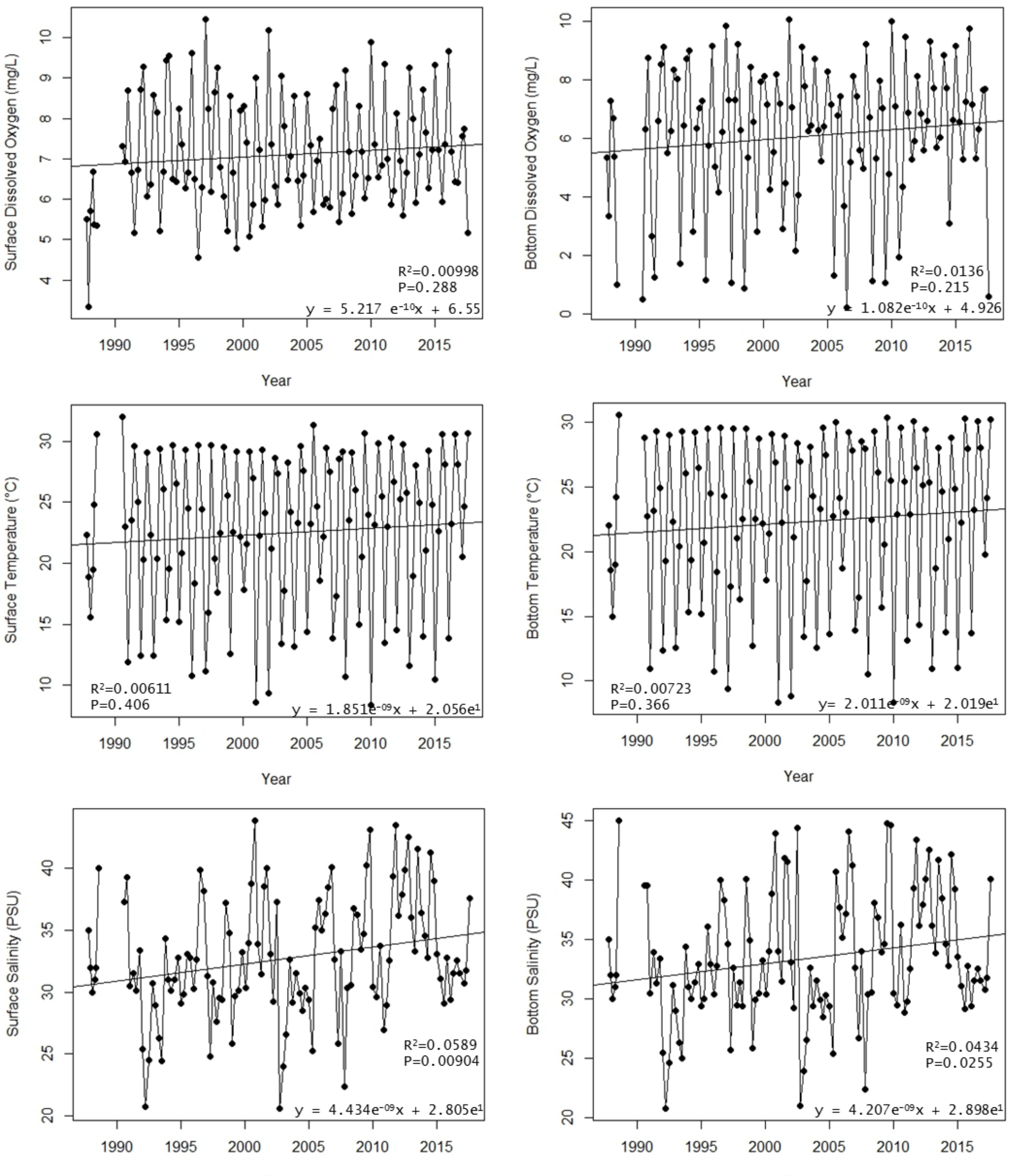
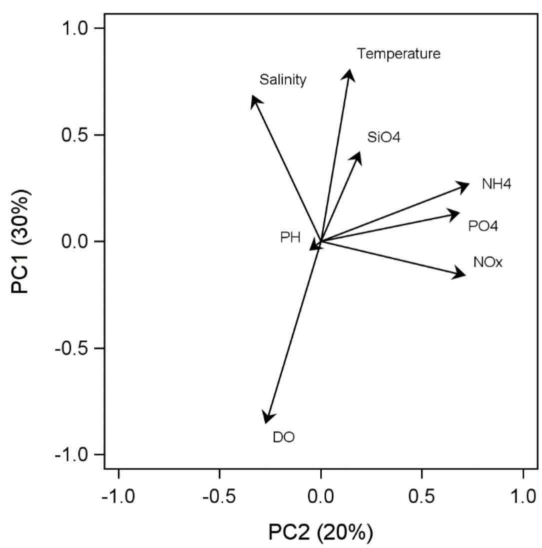


Figure 2. Water quality time series plots with linear regression equation overlay Top: surface and bottom DO concentrations. Middle: surface and bottom temperatures. Bottom: surface and bottom salinities. All contain: R squared (R^2) values, P values (P), linear regression equation

Only salinity values (both surface and bottom values) appeared to significantly change over the 30-year period of the study at Station D (Figure 2). Surface salinity values have significantly increased at a rate of $4.207e^{-9}$ PSU (p=0.00904), while bottom salinity values have significantly increased at a rate of $4.434e^{-9}$ PSU (p=0.0255). DO concentrations and temperatures (surface and bottom), however, were more variable and did not change significantly over time.

PCA and Spearman Rank-Order Correlation

PCA was used to assess relationships between hydrographic variables and benthic community metrics. Three PCs were selected to explain variance in the dataset using eigenvalue criteria (eigenvalue > 1). Resulting bivariate variable loading plots from the PCA are displayed in Figure 3. The first second and third PCs explained 30%, 20%, and 13% (63% total) of the variation within the data set respectively. The first principal component (PC1) axis explained 30% of the variability in the data set and contained strong associations between the negative PC variable load DO and the positive PC variable loads salinity and temperature. All PC1 variable loads had an absolute PC coefficient of 0.69 or higher, meaning all were relatively important variables contributing to variance of the new PC1 variable. The second principal component (PC2) axis explained 20% of the variability in the data set. The PC2 variable loads PO_4 (phosphate), NH_4 (ammonium), and NO_x (nitrate+nitrite) were relatively important to the new PC2 variable having a PC coefficient of 0.69 and higher. All PC2 variable loads had strong positive associations among each other. The third principal component (PC3) axis explained 13% of the variability in the data set. There was only one large PC variable load contributor, pH, that showed any relative importance to the new PC3 variable by having a PC coefficient value of 0.93. Silicate (SiO_4) had a PC coefficient of about .42 for both the PC1 and PC3 axes but did not contribute as a large PC variable load for any component.



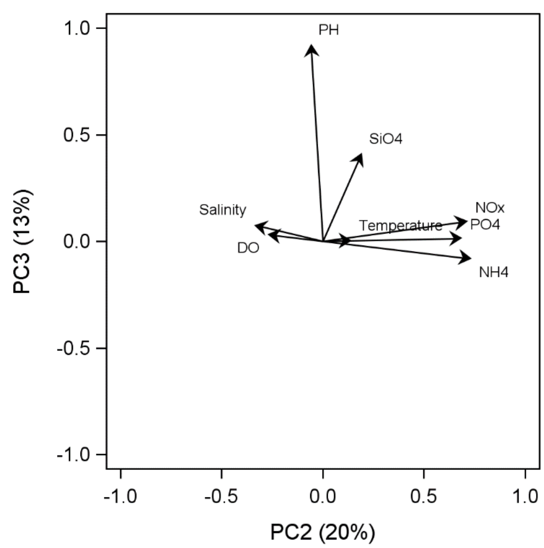


Figure 3. Principal components analysis of water quality variables; vector loads. Left plot: PC1 and PC2 vector loads for dissolved oxygen (DO), temperature, salinity, pH, phosphate (PO_4), ammonium (NH_4), nitrate+nitrite (NO_x), and silicate (SiO_4), Right plot: PC2 and PC3 vector loads

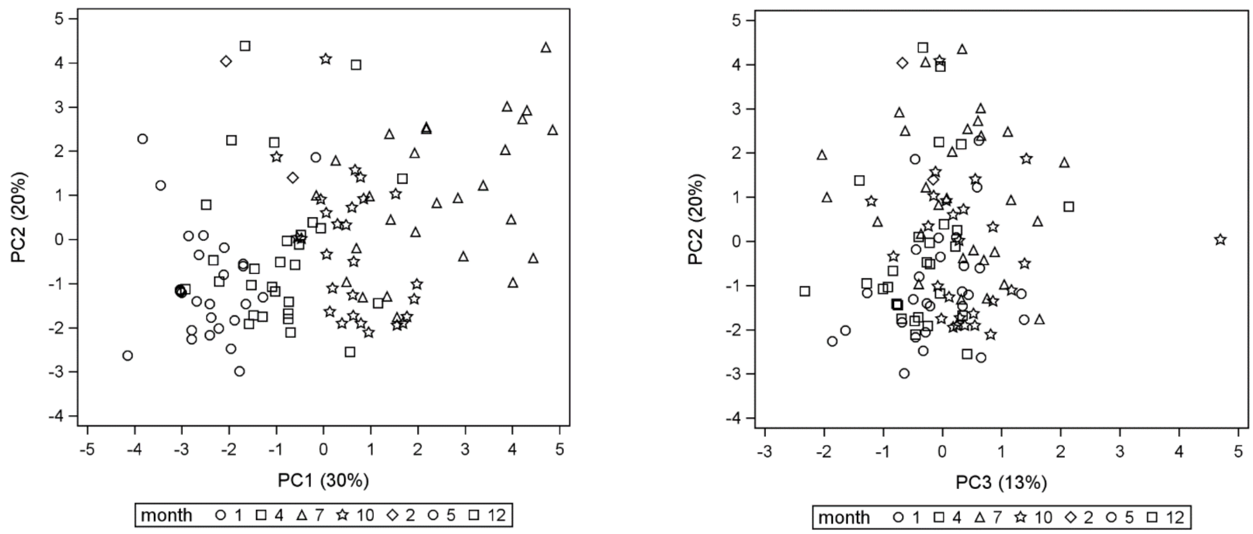


Figure 4. Principal components analysis of hydrological variables; sample scores symbolized by month sampled. Left plot: PC1 and PC2 sample scores, where each sample is labeled by its sampling month. Right plot: PC2 and PC3 sample scores.

Bivariate sample score plots from the PCA, using month of sampling as symbols, are displayed in Figure 4. Sample scores followed a spatial pattern along the PC1 axis with the month of July having the highest scores which decreased over the represented months of October, April, and January respectively. In contrast to PC1, there were no spatial distribution patterns of the sample scores along the PC2 and PC3 axis.

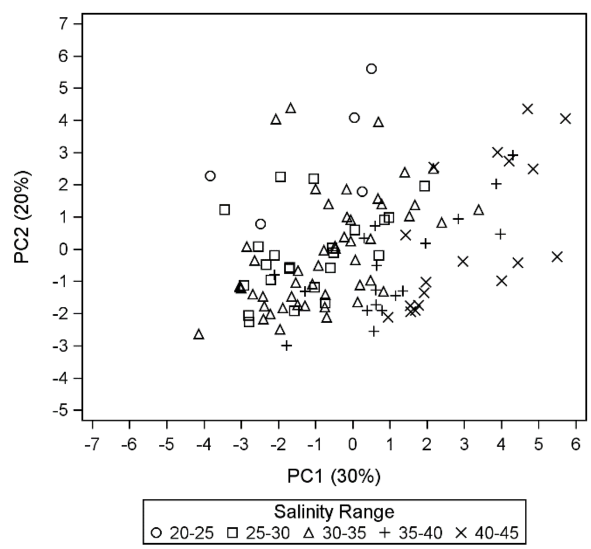


Figure 5. Principal components analysis of water quality variables; sample scores symbolized by salinity range. Left plot: PC1 and PC2 sample scores, where each sample is labeled by its salinity range value. Right plot: PC2 and PC3 sample scores.

A bivariate sample score plot from the PCA using salinity range value as symbols is displayed in Figure 5. Sample scores did not follow a strong spatial pattern along the PC2 axis. Sample scores of the salinity ranges 25 PSU to 45 PSU were, in general, evenly spread across the PC2 axis and showed no patterns. Sample scores of the lowest valued salinity range, 20-25 PSU, were concentrated completely in the positive section of the PC2 axis.

Spearman rank-order correlations among new PC variables and benthic metric response variables resulted in more than one significant inverse relationship. Spearman correlation coefficients and their corresponding probability values are shown in Table 2.

Table 2. Spearman Rank correlations (and probability values) for principal components (PCs) and benthic metrics with respect to benthic community abundance, biomass, species richness, and species evenness.

Benthic Metrics	**Spearman Rank Correlation (Probability)**		
	PC1 (Multiple Stressor Index)	**PC2 (Nutrient Index)**	**PC3 (Acidification Index)**
nm2 Abundance(n/m²)	-0.241 (0.0096)	0.0760 (0.4197)	-0.0924 (0.3261)
lnm2 Abundance(Ln(n+1)/m²)	-0.321 (0.0005)	-0.0229 (0.8081)	-0.187 (0.0453)
gm2 Biomass(g/m²)	-0.142 (0.1303)	-0.105 (0.2620)	-0.182 (0.0511)
lgm2 Biomass(Ln(g+1)/m²)	-0.213 (0.0226)	-0.115 (0.2213)	-0.254 (0.0062)
R Richness(S/sample)	-0.360 (<.0001)	-0.0212 (0.8217)	-0.187 (0.0460)
J' Evenness(J'/samples)	-0.00143 (0.9879)	0.0313 (0.7400)	-0.0360 (0.7027)

There were significant negative relationships between the PC1 axis and benthic community abundance, biomass (natural logged only), and species richness. There were also significant negative relationships between the PC3 axis and benthic community abundance (natural logged only), biomass, and species richness. In contrast, there were no significant relationships represented between the PC2 axis and any of the benthic responses. All significant relationships found had a negative correlation coefficient between PC1 or PC3 indexes and benthic response.

Stepwise Regression

A stepwise multiple linear regression analysis using spline models was used to determine if water quality variables could predict benthic response. Fit statistics along with a listing of variables of importance for the resulting regressions are shown in Table 3. The resulting regression models explained 55% of the variation in abundance, 31% of the variation in biomass, and 41% of the variation in species richness. Variables of importance were represented on a scale of 0-100 and variables with an importance value greater than 50 were selected. DO was the only leading explanatory variable for predicting benthic abundance in southeastern Corpus Christi Bay having an importance value of 100. Temperature could also be considered an explanatory variable because the importance value was 48.63. Temperature, pH, and NO_x (nitrate+nitrite) were leading explanatory variables for predicting benthic biomass having the importance values 100, 79.01, and 65.45 respectively. Temperature was the only leading explanatory variable for predicting species richness having an importance value of 100.

Table 3. Stepwise regression analysis fit statistics and variables of relative importance in respect to predicting benthic community abundance, biomass, and richness.

Benthic Metrics	**Fit Statistics**				
	R-Square	**Adjusted R - Square**	**Mean Square Error**	**Average Square Error**	**Variables of Importance (Importance value > 50)**
Abundance($Ln(n+1)/m^2$)	0.548	0.505	1.41	1.28	Dissolved Oxygen
Biomass($Ln(g+1)/m^2$)	0.314	0.282	0.811	0.769	Temperature, pH, NO_x
Richness (S/sample)	0.406	0.367	142	132	Temperature

Nonparametric Multidimensional Scaling

A total of 202 benthic macrofaunal species were found over the 30-year study. Average abundances as well as their total and cumulative percentages for the top 15 species represented are shown in Table 4. Based on percentages, community structure was characterized by high abundances of *Mediomastus ambiseta*, *Polydora caulleryi*, *Streblospio benedicti*, *Tharyx setigera*, Oligochaetes, and *Paraprionospio pinnata*.

Table 4. Average abundances, total percentages, and cumulative percentages for Station D over the period October 1987 through October 2017

Obs	**SpName**	**Abundance (n/m^2)**	**Total %**	**Cum %**
1	*Mediomastus ambiseta*	4166	31.70%	31.70%
2	*Dipolydora caulleryi*	2820	21.46%	53.15%
3	*Streblospio benedicti*	1162	8.84%	62.00%
4	*Tharyx setigera*	776	5.91%	67.90%
5	Oligochaeta (unidentified)	714	5.43%	73.33%
6	*Paraprionospio pinnata*	256	1.95%	75.28%
7	*Nemertea (unidentified)*	210	1.60%	76.87%
8	*Schizocardium sp.*	195	1.48%	78.36%
9	*Paleanotus heteroseta*	179	1.36%	79.72%
10	*Gyptis brevipalpa*	162	1.23%	80.95%
11	*Haploscoloplos foliosus*	142	1.08%	82.04%
12	*Glycinde solitaria*	142	1.08%	83.11%
13	*Mulinia lateralis*	136	1.03%	84.14%
14	*Cossura delta*	118	0.90%	85.05%
15	*Schistomeringos sp. A*	111	0.84%	85.89%
	187 Other Species	1854	14.11%	100.00%
	Total	13143	100.00%	

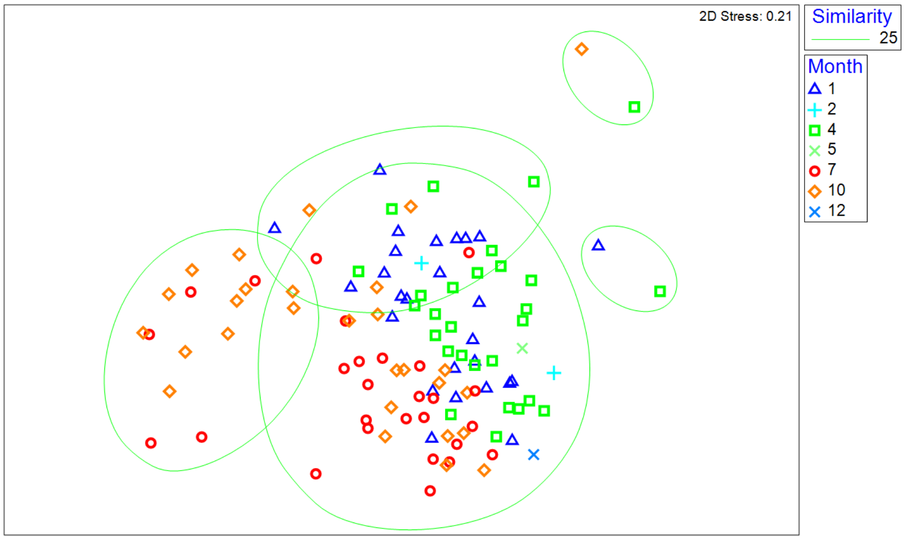


Figure 6. Nonparametric Multidimensional Scaling plot of benthic macrofaunal community structure at Station D. Symbol indicate the month samples were acquired. Macrofaunal abundances are grouped by 25% similarity

In the resulting nMDS plot, five major groups (25% similarity) were determined through cluster analysis (Figure 6). Benthic community assemblages of the same month appeared to cluster together. Overlap, however, occurred among samples of different months and community assemblages of differing months also clustered together. The January and April samples appeared more clustered; likewise, July samples clustered more with October samples. Variability over time for the months of July and October was greater or more spread in comparison with the months of January and April. Though differences between samples were slight, there was some separation from the main cluster within the months July and October.

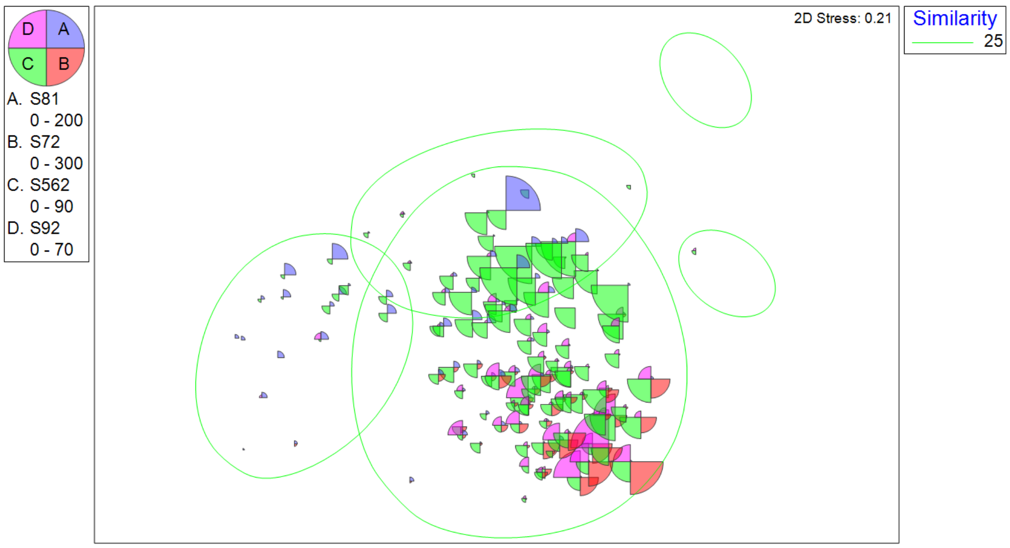


Figure 7. Nonparametric Multidimensional Scaling bubble plot of benthic macrofaunal community structure Station D. Symbol labels indicate abundant species: A. *Streblospio benedicti*, B. *Polydora caulleryi* C. *Mediomastus ambiseta* D. *Tharyx Setigera*. Bubble size represents abundance of dominant species. Macrofaunal abundances are grouped by 25% similarity

The nMDS plot in Figure 6 was overlaid with a bubble plot representing dominance for the four most abundant species for Station D: *Mediomastus ambiseta*, *Polydora caulleryi*, *Streblospio benedicti*, and *Tharyx setigera* (Figure 7). In the plot, there was some overlap regarding the dominant species of the sampled communities. Samples with dominance in species *Tharyx setigera* and *Polydora caulleryi*, however, appeared strongly clustered amongst each other. Samples with dominance in species *Mediomastus ambiseta* appeared more variable, or spread, over the period of the study, though a cluster of *Mediomastus ambiseta* dominance alone was apparent. Samples with dominance in the species *Streblospio benedicti* did not overlap greatly with samples of the dominant species *Tharyx setigera* and or *Polydora calleryi*.

DISCUSSION

Status and Trends

The Means and standard deviations of both water quality and benthic macrofaunal variable measurements were summarized in Table 1. DO concentrations averaged their lowest for the July samples and their highest for the January samples while temperature acted in opposite. This agrees with previous studies that associate hypoxia in Corpus Christi Bay to the summertime season (Ritter and Montagna, 1999). Average salinity values were highest during the July sampling months but appeared to be relatively high year-round. Excluding silicate, nutrient averages from Station D for the study period were low (Table 1). In general, nutrient averages tended to be highest during the sampling month of July. Nutrient averages, however, appeared to have small variability across sampling months and thus seasonality is not clear.

Average Station D values for abundance, biomass, and species richness over the study period were lowest for the sampling months of July and October (Table 1). Species evenness averaged its lowest during the sampling month of October, however, in general, the averages for species evenness appeared similar among sampling months. Therefore, abundance, biomass, and species richness would be expected to be in a period of decline when temperature and salinity were high and DO concentrations low.

Long-term trend analysis indicated only salinity values (surface and bottom values) to have significantly changed over the 30-year study period (Figure 2). Both surface and bottom salinity values increased over the study period while DO and temperature did not significantly change (though both had small positive slopes to their corresponding regression lines).

These results contrast with results found by Appelbaum et al. (2005). Appelbaum et al. (2005) conducted a linear regression using surface water data from the Texas Parks and Wildlife Department over a 20-year period (1982 to 2002). Appelbaum et al. (2005) found salinity to be variable and not significantly changing, while temperature and DO were significantly changing over time (temperatures increasing, DO decreasing). The Appelbaum study differed from this study in the amount of data points used (6,319 compared to this study's 115), the time period involved, and spatial extent covered (all of Corpus Christi Bay compared to one station).

Water Quality Dynamic

The underlying structure of the water quality dataset was summarized by the three principal components representing a multiple stressor index, a nutrient index, and an acidification index, together contributing to 63% of the variability in the dataset (Figure 3).

Multiple Stressor Index

In the first principal component (PC1), accounting for 30% of the dataset's variability, DO was inversely related to both salinity and temperature. Temperature and salinity both have an indirect relationship with gas solubility in the water column (Ritter et al., 2002; Rabalais et al., 2010). Given the presence of high temperature and salinity values in the water column, oxygen solubility would be lowered and thus one would expect to observe lower DO concentrations. In addition, salinity may also act as a stratification barrier among water masses preventing or slowing DO diffusion downwards to bottom depths (Rabalais et al., 2010; Ritter and Montagna, 1999). Both temperature and salinity influence stratification strength, and therefore they influence the degree of DO diffusion (Rabalais et al., 2002). Extended periods of stratification can allow DO concentrations in the bottom layers of the water column to become and remain low. Thus, PC1 likely represents a multiple stressor index where increased temperature and salinity are associated with a decrease in bottom DO concentrations.

Sample scores plotted along the PC1 axis (multiple stressor index), using month sampled as a symbol for each point, were distributed in a definite seasonal pattern (Figure 4). The most positive sample scores (higher temperatures and salinities) were represented by samples collected during the July and October sampling months respectively. The most negative sample scores (higher dissolved oxygen concentrations) were represented by samples collected during the January and April sampling months respectively. Thus, sample scores plotted along the multiple stressor index appear to show a seasonality pattern in which minimum DO concentrations are reached near the July sampling month when temperature and salinity values are at their sampled maximum.

In the southeastern region of Corpus Christi Bay, hypoxia is recognized to be a chronic summertime occurrence over bottom waters when water column temperatures and salinities are generally at their highest values (Montagna and Kalke, 1996; Ritter and Montagna, 1999; Applebaum et al., 2005; Coopersmith et al., 2011). At the same time, low water circulation, low wind speeds, and high evaporation rates during the summertime allow for the occurrence of salinity stratification in the southeastern region of the bay (Applebaum et al., 2005). Hypoxia in the bay is inversely correlated to water column stratification where water mass differences are influenced by salinity values (Ritter and Montagna 1999). Therefore, the first principal component and multiple stressor index of the PCA appears to indicate that DO concentrations in southeastern Corpus Christi Bay are influenced by a combination of water column temperatures, salinity levels, and salinity driven stratification; the combination of which follows a seasonal cycle where minimum DO concentration is likely to occur during the summertime season.

Nutrient Index

In the second principal component (PC2) accounting for 20% of the variability in the dataset, nutrients: phosphate (PO_4 µmol L^{-1}), ammonium (NH_4 µmol L^{-1}), and nitrate+nitrite (NO_x µmol L^{-1}) were positively correlated among each other. Freshwater inflow introduces nutrients into an estuary (Alber, 2002). Nutrients, therefore, are generally positively correlated to inflow (Alber, 2002; Drinkwater and Frank, 1994). Salinity is also affected by inflow with the two having an inverse relationship (Montagna et al., 2013). Many studies have interpreted principal components composed of high nutrient loads that are inversely related to a high salinity loads to represent some sort of inflow affect (Russell et al., 2006; Arismendez et al., 2009; Pollack et al., 2009; Shank et al., 2009; Palmer et al., 2011; Paudel and Montagna, 2014; Palmer and Montagna, 2015). Salinity, though not a large loading contributor to PC2 (Figure 3), was negatively correlated to nutrients. Thus, PC2 likely represents an inflow affect or nutrient index, where increased freshwater inflow is associated with an increase in nutrient concentrations.

Sample scores plotted along the PC2 axis (nutrient index), using month sampled as a symbol for each point, did not appear to be distributed in any definite pattern (Figure 4). Determining a seasonality pattern for the nutrient component is complicated by the fact that nutrients do not all follow the same seasonal patterns in Corpus Christi Bay. In addition, nutrient baseline studies in Corpus Christi Bay have observed slightly different trends over different parts of time. In a recent study by Turner et al., (2015), the baseline of nutrients phosphate, ammonium, and nitrite+nitrate in Corpus Christi Bay were described during a dry year. Phosphate maximums were observed during the fall season, while ammonium maximums were observed during the fall and spring seasons. In contrast, nitrite + nitrate concentrations, though much more variable, reached an observed general maximum during the winter season. In contrast, Gardner et al. (2006) found no seasonality trends in nitrite and nitrate flux in Corpus Christi Bay. Moreover, ammonium fluxes were observed in the study to be higher in the summer season (August) versus the spring season (April). Inflow balance for Texas estuaries is generally lowest in the summertime months reaching a minimum around August (Montagna et al., 2007). One would then expect nutrient concentrations introduced by inflow to be relatively lower in the summertime. Sample scores along the nutrient index, however, does not reflect a clear minimum in nutrients for the July month. Instead, sample scores for all months were evenly spread along the nutrient axis. Seasonality, therefore, does not appear to be confounded with the nutrient component in southeastern Corpus Christi Bay over the course of the study.

Sample scores plotted along the PC2 axis (nutrient index), using salinity range as a symbol for each point, did not appear to be distributed in any strong pattern (Figure 5). Positive sample scores along the nutrient index correlated with higher rates of inflow or nutrient input while negative sample scores correlated with lower rates of inflow or nutrient input. Sample scores of the salinity ranges 25 PSU to 45 PSU were, in general, evenly spread across the PC2 axis and showed no patterns. Sample scores of the lowest valued salinity range 20-25 PSU, however, were concentrated completely in the positive section (higher inflow and nutrient input rates) of the nutrient index. Associations between a large range of salinity values and nutrient concentration were weak which perhaps explains why salinity was not a large loading contributor to PC2. Salinity values, therefore, do not appear to be confounded with the nutrient component in southeastern Corpus Christi Bay.

DO concentrations in southeastern Corpus Christi Bay are strongly correlated to salinity and temperature but not nutrients. Due to the orthogonal relationship between components, we assume that the nutrient component PC2 does not correlated to the multiple stressor component PC1. This is different from an eutrophication-type model where hypoxia occurs as a consequence of increased nutrient concentrations or loadings (Rabalais et al., 2002; Rabalais et al., 2010). In this scenario eutrophication, resulting from increased nutrient loading, stimulates phytoplankton productivity, which heavily contributes to a larger quantity of organic matter settling onto the seabed. The organic matter is decomposed by aerobic microbes which require the consumption of oxygen. At the same time, stratification is acting to isolate the diffusion of oxygen from the surface to the bottom so that oxygen replenishment is low. Corpus Christi Bay is not considered to be eutrophic based on chlorophyll-a and nutrient values (Turner et al., 2015). Moreover, chlorophyll-a values in Corpus Christi Bay are not significantly changing over time (Applebaum et al., 2005). Nitrogen loading into the Nueces estuary is comparatively low to other estuaries which experience hypoxia (Applebaum et al., 2005). Both nitrogen and phosphorus in the bay may be limited and low in average concentration (Brock, 2001; Applebaum et al., 2005; Turner et al., 2015). Because nutrients in Corpus Christi Bay are relatively low and surface chlorophyll-a, phosphorus, and DIN concentrations in the southeastern region are not significantly changing over time, it is not likely that eutrophication resulting from high nutrient loading is causing hypoxia in Southeastern Corpus Christi Bay (Applebaum et al., 2005). This argument is strengthened by the orthogonal relationship between the multiple stressor and nutrient components as well as the strong inverse relationship between DO concentration and temperature within the multiple stressor component.

Acidification Index

In the third principal component (PC3), accounting for 13% of the variability in the dataset, pH is the only large loading contributor (Figure 3). Th pH values are expressed on a logarithmic scale where $pH = -\log_{10}[H^+]$. Thus, PC3 likely represents an acidification index. Sample scores, symbolized based on month, did not appear to be distributed in any definite spatial pattern along the acidification index (Figure 4). Positive sample scores along the index correlated with higher pH measures while negative sample scores correlated with lower pH measures. Because sample scores were evenly spread along the acidification index, there appears to be no definite seasonality trend to the dataset's pH measurements. Therefore, the third principal component of the PCA likely represents an acidification index for pH values which do not relate to season.

The acidification component, PC3, was orthogonal to the multiple stressor (PC1) and nutrient (PC2) components of the dataset and therefore did not correlate to either of them. While eutrophication has been shown to lead to deoxygenation as a result of organic matter decomposition by aerobic microbes in a stratified environment, the same decomposition process releases CO_2 and increases bottom water acidity (Cai et al., 2011). Assuming that the remineralization of organic matter follows the Redfield stoichiometry, it would follow that increased oxygen consumption would be coupled with a larger release of CO_2 and minor alkalinity reduction (Hu and Cai, 2013). Significant positive correlations between bottom DO concentrations and pH levels have been observed and attributed to eutrophication remineralization (Cai et al., 2011; Zhai et al., 2012). Cai et al. (2011) also attributed the decrease in DO concentrations and pH levels to reduced buffer capacity as a consequence of CO_2 enrichment. As discussed before, Corpus Christi Bay is not considered to be eutrophic. In addition, quarterly nutrient averages calculated over the time period of this study were found to be low year-round ($< 2\ \mu M\ L^{-1}$) (Tablel 1). Furthermore, alkalinity, or the ability of water to resist changes in pH, increases down the Texas coast and has been significantly correlated to pH levels (Hu et al., 2015). Organic matter production and its subsequent decomposition in Corpus Christi Bay may be lower than the levels represented in the studies that attributed eutrophication and remineralization to coupled hypoxia and acidification. In addition, the alkalinity levels or buffering capacity may be higher for Corpus Christi Bay and therefore more able to resist changes in pH during CO_2 enrichment.

Benthic Response

Two different approaches were used to understand relationships between southeastern Corpus Christi Bay water quality dynamics and benthic macrofaunal diversity. In one approach, benthic community metrics were correlated among principal components from the study's resulting PCA described above. In the second approach, benthic community abundance, biomass, and species richness were used as individual dependent variables in a stepwise (adaptive) regressions using water quality data as independent variables in order to determine if water quality variables could predict benthic responses.

Principal Component Correlation

Benthic community abundance, biomass, species richness and species evenness, were correlated with principal components representing the multiple stressor index, nutrient index, and acidification index. Both the multiple stressor and acidification indexes had significant inverse relationships to benthic abundance, biomass, and species richness. No significant correlations existed between any of the benthic community metrics and the nutrient component. Species evenness did not correlate significantly to any of the principal components.

Temperature, salinity, and DO combined to make up the multiple stressor index, which negatively correlates to benthic abundance, biomass, and species richness. Thus, a reduction in DO concentrations (or related increase in temperature and salinity) in the bay would lead to reduced benthic diversity (abundance, biomass, and species richness declines). Hypoxia influenced reductions in benthic community abundance, biomass, and or species richness in Corpus Christi Bay is supported by the findings of previous bay studies (Ritter and Montagna, 1999; Montagna and Ritter, 2006; Montagna and Froeschke, 2009). Ritter and Montagna 1999 compared normoxic stations to hypoxic stations in Corpus Christi Bay and observed a fivefold reduction in abundance and a twelvefold reduction in biomass. The study determined that the degree of decline in abundance and biomass was a function of hypoxia intensity. Observed species richness in Corpus Christi Bay was also shown to be reduced at hypoxic stations in comparison to normoxic stations (Montagna and Ritter, 2006; Montagna and Froeschke, 2009). Again, the observed declines were attributed to hypoxia due to the fact that species intolerant to low DO concentrations were missing from hypoxic site samples but were observed in the normoxic samples (Montagna and Froeschke, 2009).

Temperature influences both oxygen solubility and respiration rates (via metabolic processes) and could therefore worsen the negative effects of hypoxia on macrofauna (Brown et al, 2004; Rabalias et al., 2010). In the metabolic theory (Brown et al., 2004), under warming temperatures respiration rates should increase faster than rates of photosynthesis due to differences in activation energies. Increased temperatures could therefore lead to an overall decrease in DO concentrations and a possible increase in the occurrence or severity of hypoxic events. Diaz and Rosenberg (1995) noted a trend in increased cases of hypoxia as well as increased duration, severity, extent, and intensity in areas where hypoxia is already historically established. Increased respiration rates would also mean an increase in oxygen demand possibly affecting the threshold value for which negative hypoxic effects would begin to occur. Vaquer-Sunyer and Duarte (2011) showed through meta-analysis that increasing temperatures significantly affect the threshold values for hypoxic effects and benthic macrofaunal metabolic oxygen demand; both of which associated with each other. It was also observed, that under hypoxic conditions, increased temperatures reduced benthic macrofaunal survival times and increased the oxygen requirements for benthic survival. Though not directly affecting benthic macrofauna, temperature may play an indirect part in benthic community structuring due to its influence on hypoxia and DO concentrations.

Temperature may also play a role in the benthic macrofaunal community structuring of southeastern Corpus Christi Bay due to its ties to seasonality. Evidence of benthic abundance, species richness, recruitment, distribution, and mortality following a seasonality trend has been found across time (Boesch et al., 1977; Crisp, 1964; Barry et al., 1995; Southward et al., 1995; Hiscock et al., 2004). Negative effects of benthic macrofauna, in a nearby Lavaca-Colorado estuary, have been linked to temperature influenced seasonality where the seasonal signal was observed to be strong (Pollack et al., 2011). Though not a primary driver, temperature was found to play a part in the community structuring of Lavaca-Colorado estuarine benthic macrofauna (Pollack et al., 2011). The multiple stressor index of the study follows a clear seasonality pattern and includes temperature as a strong PC variable load. It is therefore possible that temperature could be influencing the benthic community structure of southeastern Corpus Christi Bay in a similar way to what has been shown to in the nearby Lavaca-Colorado estuary.

Salinity gradients affect macrofaunal abundance, distribution, composition, structure, and function (Montagna and Kalke, 1992, Pollack et al., 2009; Kim Montagna, 2012). Macrofaunal diversity in Corpus Christi Bay has been shown to increase along an increasing salinity gradient (Montagna and Kalke, 1996). To a lesser nonsignificant extent, a benthic macrofaunal biomass density gradient also exists along this salinity gradient where biomass increases moving towards eastern Corpus Christi Bay. Kim and Montagna (2012) determined biomass in Texas estuaries to be driven by a percent change in mean salinity. Salinity, therefore can influence benthic macrofaunal communities.

The pH variable was the only large loading contributor to the acidification index which was negatively correlated to benthic abundance and biomass. Thus, a reduction in pH levels (or increase in acidity) in the bay would lead to reduced benthic diversity (abundance, biomass, and species richness declines). Organisms that make calcium carbonate shells are believed to be more sensitive to acidification because of their sensitivity to carbonate mineral saturation states which have positive correlations with pH (Shirayama and Thornton, 2005; Gazeau et al., 2007; Zeebel et al., 2008). Negative effects of acidification, however, have also been found for a variety of aquatic organisms including non-calcifying ones (Zeebel et al., 2008; Zhai et al., 2012).

Acidification influenced reductions in benthic biomass is supported by previous studies which have observed declines in the calcification rates of specific gastropod and bivalve species following an increase in acidity (Berge et al., 2006; Shirayama and Thornton, 2005; Gazeau et al., 2007; Hall-Spencer et al., 2008; Zeebel et al., 2008; Zhai et al., 2012). In addition to decreased calcification rates, some calcifying bivalves have been observed to maintain their internal pH by either decreasing their metabolic rates or by dissolving their shells (Bamber, 1990; Michaelidis et al., 2005; Berge et al., 2006; Gazeau et al., 2007). It is therefore possible for acidification to influence the calcification, dissolution, and metabolic rates of organisms and subsequently affect the production, maintenance, and stability of the shells and skeletons of calcifying organisms.

Acidification influenced reductions in benthic species abundance and richness is supported in studies that observed declines in the survivorship of bivalve species following an increase in acidity (Green et al., 2004; Shirayama and Thornton, 2005). Shirayama and Thornton (2005) found significant negative effects on the longtime survival of shallow water benthos. Green et al. (2004) studied the short-term effects of calcium carbonate saturation state on the survivorship of different sized *Mercenaria mercenaria* juveniles transitioning from a pelagic larval stage. Evidence was found of calcium carbonate based dissolution as well as significant mortality related to low carbonate saturation. Thus, it is possible for acidification to influence benthic survivorship which could further lead to a selection of more tolerant species. Moreover, it is possible for acidification to influence bivalve recruitment, distribution, and population size which subsequently affects the greater benthic community structure.

Explaining the negative correlation between acidification and benthic abundance, biomass, and species richness however, is complex due to pH tolerance level variability in benthos as well as the natural evolutionary history of marine and estuarine organisms. Significant variability exists in the pH tolerances among benthic organisms where many specific species' tolerances are not yet known (Gazeau et al., 2007; Zeebel et al., 2008; Widdicombe et al., 2009). In addition, marine and estuarine organisms have evolved naturally under a pH range of 7.6 to 8.4 with certain cases having a pH range beginning as low as 7.0 (Bamber, 1990). A large majority (~92%) of the measured pH values taken during the study were within the pH range for natural marine and estuarine organism evolution. To clearly understand why benthic abundance, biomass and species richness in Corpus Christi Bay were correlated to the acidification index, more work beyond the scope of this study is required.

Adaptive Regression

In a stepwise multiple linear regression analysis, DO was the only leading explanatory variable for predicting benthic abundance in southeastern Corpus Christi Bay. Temperature could also be considered an explanatory variable for abundance because the importance value (48.63) was very close to our cut off value of 50. Temperature, pH, and NO_x (nitrite+nitrate) were leading explanatory variables for predicting benthic biomass. Temperature was the only leading explanatory variable for predicting species richness. With the exception of NO_x as a variable of importance for biomass prediction, the results from the adaptive regression were in agreement with the results from the spearman correlations.

Nitrite+Nitrate may be good predictors for benthic macrofaunal biomass due to their relations with primary productivity and subsequently secondary productivity. Kim and Montagna (2012) suggested a theoretical link between inflow derived nutrient or organic matter input and both primary production as well as secondary production. The 2012 study was able to demonstrate that nutrient input from inflow was important in maintaining secondary productivity as well as subsequent ecosystem health and stability in Texas estuaries. In addition, the Kim and Montagna (2012) study showed that decreasing nutrient concentrations were related to increased deposit feeder biomass and decreased suspension feeder biomass.

Benthic Community Structure

The benthic community structure analysis indicates a stressed community which is in part influenced by low DO events that over time have resulted in a community made up of species more adapted to low DO conditions.

A total of 202 macrofaunal species were found over the 30-year study (Table 4). Community structure was characterized by high abundance based dominances of *Mediomastus ambiseta*, *Polydora caulleryi*, *Streblospio benedicti*, *Tharyx setigera*, Oligochaetes, and *Paraprionospio pinnata*. Higher dominances in the abundance of opportunistic species has been observed in hypoxic regions of Chesapeake Bay (Dauer et al., 1992). *Mediomastus ambiseta*, *Streblospio benedicti*, oligochaetes, and *Paraprionospio pinnata* are considered opportunist species because they are a relatively smaller, short-lived species that often dominates disturbed or stressed habitats (Dauer et al., 1992; Diaz and Rosenberg, 1995; Ritter and Montagna, 1999). Moreover, *Streblospio benedicti* and oligochaetes may be more tolerant to low oxygen conditions as they often dominate low oxygen environments in Corpus Christi Bay but not normoxic regions (Ritter and Montagna, 1999; Montagna and Froeschke, 2009). *Glycinde solitaria*, and *Mulinia* lateralis are also opportunistic species (according to Dauer et al. 1992) and appear in the table of top 15 abundant species (Table 4). The community structure of the study, therefore, appears to indicate a stressed environment, possibly due to low DO concentrations.

In the nMDS plot, benthic community assemblages of the same month appeared to cluster together and thus showed similarity in community structure over time (Fig 6). Overlap occurred among samples of different months indicating community similarity existed between differing months as well. The January and April samples clustered together and likely had very similar community structure. Likewise, but to a lesser degree, July samples clustered more with October samples. Variability over time for the months of July and October was greater or more spread in comparison with the months of January and April. Though differences between samples were slight, there was some clear separation from the main cluster within the months July and October.

During the study, July sampling occurred during the summer hypoxia season when species more tolerant to low oxygen conditions were being selected for. As the year and seasons progressed, hypoxia stress would no longer be actively occurring and less tolerant species would be able to recover. This could explain why the July and October samples were more separated as the July samples were acquired during the hypoxic season and the October samples only a few months after. July would then appear most separated from the April and January sampling months with longer recovery times.

The original nMDS plot to this study was overlaid with a bubble plot accounting for the dominance of the study's four most abundant species *Mediomastus ambiseta*, *Polydora caulleryi*, *Streblospio benedicti*, and *Tharyx setigera* (Figure 7). Samples that dominated in species *Tharyx setigera* and *Polydora caulleryi* appeared strongly clustered amongst each other and thus showed similarity in community structure amongst samples. Samples that dominated in the species *Mediomastus ambiseta* appeared more variable, or spread over the period of the study, though a cluster of highly dominating *Mediomastus ambiseta* samples was clearly apparent. This likely indicates that many samples had high abundances of *Mediomastus ambiseta* and specifically a number of samples had a clear dominance of *Mediomastus ambiseta*. Samples that dominated in the species *Streblospio benedicti* did not overlap greatly with samples of the dominant species *Tharyx setigera* and or *Polydora calleryi* and thus community structure amongst the two groupings were not similar.

Ritter and Montagna (1999) found community dominance in the hypoxic region of Corpus Christi Bay follows a trend based on category of DO intensity. In the first intensity level they defined (a DO concentration > 5 mg L^{-1}), *Tharyx setigera* and *Polydora caulleryi* co-dominated. Intensity level 2 (4-5 mg L^{-1}) was dominated by *Mediomastus ambiseta* and intensity level 4 (2-3 mg L^{-1}) was dominated by *Streblospio benedicti* (Ritter and Montagna, 1999). Results from this study's nMDS bubble plot appears to show some similarities to the Ritter Montagna (1999) trend. *Streblospio benedicti* only dominated in the July and October months, a period during and immediately after the hypoxia season. High abundances of *Mediomastus ambiseta* appeared well spread throughout the samples, however, there was little overlap between the samples that dominated in *Streblospio benedicti* and samples that dominated in the species *Tharyx setigera* and *Polydora caulleryi*. Moreover, samples that dominated in *Tharyx setigera* and or *Polydora caulleryi* were clustered amongst each other which would agree with Ritter and Montagna's (1999) finding of codominance.

Therefore, based on nMDS plots, it appears that low DO events are affecting benthic community structure. It has been proposed that the macrofaunal community for the area of study is a result of remainder species that are more adapted to low oxygen conditions (Ritter and Montagna, 1999; Montagna and Ritter, 2006). This would explain why community similarity existed between differing months in the nMDS plot.

CONCLUSION

Results demonstrate that multiple stressors, including low DO concentrations, high temperature, and high salinity are collectively acting on benthic communities in southeastern Corpus Christi Bay. PCA performed in the study demonstrated close associations among the water quality variables DO, temperature, and salinity, which were confounded with season. Together, DO, temperature, and salinity significantly related to the benthic macrofaunal community metrics of abundance, biomass, and richness where a decrease in DO (or increase in temperature and salinity) would result in observed benthic macrofaunal declines. The water quality variable pH exhibited a significant negative effect on benthic macrofauna. Nutrient concentration averages were low over the course of the 30-year study and did not correlate to the other water quality variables such as DO, temperature, salinity, or pH. Moreover, nutrients were not significantly correlated with benthic community structure. The dominance of opportunistic species is evidence the benthic community over the study period is a stressed community, which is in part influenced by low DO events that have resulted in a community made up of species more adapted to low DO conditions. Multiple predictors, however, were found for benthic macrofaunal metrics in the bay. Moving forward, the close associations among the water quality variables (DO, temperature, and salinity) in the southeastern region of Corpus Christi Bay should be observed and monitored together in management practices or environmental assessments.

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