1-1-2013

Climate Change Effects on Vibrio Bacteria in the Winyah Bay Estuary and the Projected Spread of Vibrio under Future Climatic Scenarios.

Reem Deeb
University of South Carolina

CLIMATE CHANGE EFFECTS ON *VIBRIO* BACTERIA IN THE WINYAH BAY ESTUARY AND THE PROJECTED SPREAD OF *VIBRIO* UNDER FUTURE CLIMATIC SCENARIOS

by

Reem Deeb

Bachelor of Science
Lebanese University, 2011

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of

Earth and Environmental Resource Management

College of Arts and Sciences

University of South Carolina

2013

Accepted by:

Daniel Tufford, Major Professor

Kirstin Dow, Committee Member

Janet Moore, Committee Member

Geoffrey Scott, Committee Member

Lacy Ford, Vice Provost and Dean of Graduate Studies

DEDICATION

I dedicate this thesis to my dearest family and friends. I am thankful to all the support I received particularly from my sister Sally, thank you for putting up with me throughout those two years. I am just grateful to have such a great family that always had faith in me, you were my inspiration and without you Mom, Dad, Wael, Sally, and Ramee I would not be able to do it. Every one of you supported me in his own way and I needed it all. Finally special regards to my dear friends Azza, Gowtham, Georges and Martin.

ACKNOWLEDGEMENTS

I take this opportunity to thank Professor Daniel Tufford for all his efforts. I am extremely grateful and indebted to him for his expert, sincere, and valuable guidance and encouragement extended to me. Without his guidance and consistent help this thesis would not have been possible.

I would like to express my deepest appreciation to Professor Geoffrey I. Scott, who continuously conveyed a great enthusiasm and excitement about the project. I truly appreciate your help, concern, and engagement.

I also thank my committee members, Professor Kirstin Dow, Professor Janet Moore, and Mr. Paul Conrads. I am very grateful for Professor Janet Moore's help, who also gave me the opportunity to intern with her. It was an enriching experience from which I learned a lot. I am indebted to Mr. Paul Conrads whose help and expertise allowed this thesis to come to light.

Finally, I also place on record, my sense of gratitude to one and all, who directly or indirectly have lent their helping hands in this venture.

ABSTRACT

While there are several studies on the distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in estuarine waters around the world, there is little information on the distribution of both organisms in South Carolina waters. Monthly sampling of surface and bottom water from 9 sites in Winyah Bay was conducted over the period April-October 2012. Both organisms were enumerated on CHROMagar *Vibrio* media. The *Vibrio* counts obtained were mainly less than 20 Colony Forming Units (CFU)/ml which is typical for what was found elsewhere along the coast of the Carolinas. The *Vibrio vulnificus* counts were the highest when salinity ranged between 5 ppt and 20 ppt. *Vibrio parahaemolyticus* did not show a clear pattern with salinity, indicating the possibility of other factors that interact to control its occurrence and abundance. Turbidity on the other hand showed a positive association with both *Vibrio vulnificus* and *Vibrio parahaemolyticus.* Temperature values were within *Vibrio*'s optimal range for growth and seemed to have a lesser effect. In this study we are particularly interested in the relation between *Vibrio* and conductivity in order to couple this relation with the estimated climatic scenarios calculated by the Pee Dee River and Atlantic Intracoastal Waterway Salinity Intrusion Model 2 (PRISM2). PRISM2 integrates predictions of future streamflow and sea level in an artificial neural network model that predicts specific conductance at several locations in the Winyah Bay estuary. The specific conductance projections anticipated a higher number of spikes of higher specific conductance periods with longer duration in almost all of the sea-level rise scenarios (current condition, 1.0ft,

2.0ft, and 3.0ft sea-level rise). The estimated future conductivity upper levels did not show any substantial increase in the maximum specific conductance than the measured in the current historical records. The model derived was a conservative model which showed a projected increase in *Vibrio*'s occurrence in the future. Climate change effects potentially increasing sea level rise will consequently raise specific conductance to *Vibrio vulnificus* optimal range in Winyah Bay waters. The model was tested by predicting for post hurricane Sandy sampling date (29OCT2012). The *Vibrio vulnificus* counts fell within the predictive interval of the model. Thus, the conservative model is able to predict for *Vibrio vulnificus* under normal and post low impact storm events. In the future the increased relative risks of optimum *Vibrio* growth based on specific conductance will increase up to 36X based upon location and range of sea level rise. These increased periods of optimal growth conditions for *Vibrio*s may result in increased risk for swimmers and shellfish consumers, if Virulent forms occur with more regularity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

INTRODUCTION

Estuaries are ecological zones of transition between rivers and oceans. They are susceptible to a variety of changes driven by a myriad of climatic factors. Increased global temperature and sea level rise are expected to alter the geographic range of *Vibrio* spp and extend their growing season (Baker-Austin et al., 2010). These increases would have an adverse economic impact on the shellfish harvesters because it is expected to reduce the number of locations and shorten the seasons for shellfish harvesting. In South Carolina, for instance, recreational and commercial harvesting is prohibited from May 15 and September 1 due to the potential of high bacterial concentrations (Keith et al., 2010). The harvest season is determined depending upon the spawning season of the oysters and by environmental factors, such as the amount of rainfall within a certain time period. Salt water intrusion is expected to cause future pressure on the fresh water supplies and may alter the salinity regime in estuarine and brackish water habitats. The coastal regions in the southeastern U.S. are facing stresses from the continuous increase of anthropogenic impacts from coastal development and associated rapid population growth demands, which is challenging the delivery of fresh water. The pressures on those coastal ecosystem resources are also driven by changes in inland environments that provide a continuous supply of nutrients to the estuaries along with the urban developmental pressures along the coast per se. These activities increase the potential for

eutrophication and hypoxia conditions as well as harmful algae blooms that will ultimately threaten water quality.

The southeastern climate showed an increase in annual average temperature of 2ºF since the 1970s, with the greatest increases in the winter months (USGCRP, 2009) which is of significance since this is when shellfish are harvested and shellfish consumption is greatest. Since the mid 1970's, climate records show that the number of freezing days in the Southeast decreased by four to seven days per year. The warming rates that the climate models projected for the Southeast are expected to be more than double the rates experienced during 1975 (USGCRP, 2009). By 2080 the average temperatures in the region are projected to increase by 4.5°F or 9°F under low emission or high emission scenarios, respectively (USGCRP, 2009). The increase in sea surface temperatures in the Southeast U.S. is positively correlated with the anticipated destructive hurricanes in the Atlantic (Emanuel, 2005; Knutson et al., 2010). A number of observational studies have documented a substantial increase in Atlantic tropical cyclone frequency modeled by the increase of sea surface temperatures. Increase in surface temperature and variation in tropical atmosphere thermodynamic state are projected to increase the upper limit of the distribution of tropical cyclones (Emanuel, 1987; Knutson and Tuleya, 2004). An increase in both mean intensity and frequency of tropical cyclones are projected by high resolution models (Oouchi et al., 2006; Bender at al., 2010). These storms will create significant differences in the distribution of salt water in estuaries through storm water runoffs and wind induced flushing. Even in the absence of hurricanes coastal inundation and shoreline retreat would increase with the sea-level rise, which represents one of the most certain consequences of climate change (Field et al.,

2007). This will increase the likelihood of salt-water intrusions into the fresh water of coastal rivers in the Southeast U.S.

Outbreaks of *Vibrio* associated illnesses were commonly observed after storms in the region. Following Hurricane Ophelia, *Vibrio*s concentrations increased significantly (Fries et al., 2008). *Vibrio* outbreaks driven by hurricanes represent a potential health threat to the public. Storms were able to transport pathogens like *Vibrio* and others upstream and into fresh water resources (Fries et al. 2007). Similar trends may be expected to be observed after the Super Storm Sandy and other major hurricanes. After the upward shift sediments may represent a reservoir of storm-driven *Vibrio*, which introduces those pathogens into more inland locations which used to be considered "*Vibrio* free" (Baker-Austin et al., 2010).

The two most important environmental factors that control *Vibrio* dynamics in estuaries are temperature and salinity (Randa et al., 2004). *Vibrio* abundance is typically higher during the summer than in the winter when *Vibrio* levels are generally below detectable concentrations. For example, the optimum temperature range for *Vibrio vulnificus* is between 15 to 30°C (Baker-Austin et al., 2010); (Table 1.1) and the optimum salinity range is between 5 and 25 ppt (Wetz et al., 2008).Whereas for *Vibrio parahaemolyticus* the optimal temperature is 37°C and its optimum salinity range is between 17 and 23 ppt (FDA, 2005; Colwell, 2006).

Vibrio species are heterotrophic bacteria that occur naturally in estuaries worldwide. *Vibrio* has been a focus of study because it can be a virulent human pathogen. The average annual incidence of all *Vibrio* infections has increased by 41 % between 1996 and 2005 (CDC, 2005). The main species of *Vibrio* that are being studied and

monitored are: *Vibrio cholera*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus*. They are commonly known as foodborne pathogens that cause illness through seafood consumptions. *V. vulnificus* can also cause serious and life threating injuries through open wounds in contaminated water (Oliver, 2005a). The Centers for Disease Control and Prevention (CDC) estimates 8,028 *Vibrio* infections and 57 deaths occur annually in the U.S. (Mead et al., 1999). *V. vulnificus* is the leading cause of death from raw shellfish consumption. It accounts for 50 % of all deaths in the U.S. due to seafood consumption.

V. parahaemolyticus is the leading cause of seafood-associated bacterial gastroenteritis, with an estimated 4,500 cases occurring annually in the United States according to the CDC (Johnson et al., 2010). In 2009, 825 cases of Vibriosis were reported (excluding *Vibrio cholerae*) in the U.S. Out of these 825 cases, 256 were from the Atlantic coast states, representing 31 % of U.S. all cases. Vibriosis cases along the U.S. coasts showed distinct pattern of seasonality, with a definite peak during the summer months (CDC, 2009). Most cases of infection were between May and September, while August was the month with the greatest number of cases (CDC, 2009). Among the 825 cases 30 % were wound-associated and 59 % were seafood-associated (CDC, 2009).

Out of the 4,754 *Vibrio* infections during the period 1997-2006 in the United States, 1210 (25 %) of the infections were NFVI's (Dechet et al., 2008). The NFVIs infections were dominated by *V. vulnificus* that accounted for 35 % of all NFVIs cases in the U.S. (Dechet et al., 2008). NFVI's accounted for 70 % of patient exposures from recreational activities since *V. vulnificus* causes infections through open wounds (Dechet et al., 2008). The Atlantic region accounted for the second largest number of NFVIs in

the nation accounting for 24 % (285 of all the cases from 1997-2006); (Dechet et al., 2008).

The increase and spread of *Vibrio* worldwide and domestically is thought to be correlated with climate change variations and new oceanic patterns that introduce warmer waters into colder regions, and altering the balance between fresh and salt water (Baker-Austin et al., 2012). A significant proportion of *Vibrio* species in estuaries exist in the sediments and more bacteria migrate to sediments when environmental conditions are unfavorable; (i.e. low temperatures and/or salinities) making the sediments a reservoir of *Vibrio* bacteria that can re-suspend in the water upon any physical exposure like high speed wind for example (Wetz et al., 2008). In addition, *Vibrio* is capable of entering a viable but nonculturable state (VBNC) when environmental conditions are not in its favor. This state does not grow on conventional media; however the gram negative bacteria can regain viability (Colwell et al., 1985). The poleward movement of *Vibrio* was evident in both the Northern and Southern hemispheres; *Vibrios* have been recently detected in areas where it was previously absent or rarely reported (Baker-Austin et al., 2010). The spread of *Vibrios* will increase the exposure of shellfish consumers throughout the coastal regions in the U.S. including the Southeast.

In order to assess the potential for increased incidence of *Vibrios* in estuaries in the Southeast U.S., Winyah Bay estuary was chosen as a representative site estuary to monitor the occurrence and concentrations of *Vibrio* with respect to the trends of salinity variation, now and into the future. The study aims to identify niche-based differences in the abundance of *Vibrio* in the Winyah Bay site, and to predict *Vibrio* future trends based on correlations between environmental factors and *Vibrio* concentrations.

1.1 Literature Review

1.1.1 *Vibrio vulnificus* and *Vibrio parahaemolyticus* Pathogenicity

The three main diseases caused by *V. vulnificus* are wound infections, gastrointestinal infections, and primary septicemia (Daniels, 2011). There are three major biotypes of *V. vulnificus* strains that have been categorized based on their biochemical and pathogenic characteristics (Baker-Austin et al., 2010). Biotype 1 strains are human pathogens and cause most of the *V. vulnificus* human infections reported around the world (Baker-Austin et al., 2010).

Severe systemic infections are caused through consumption of contaminated seafood. The symptoms include fever, chills, nausea, hypotensive shock, and the formation of secondary lesion on patients' extremities (Klontz et al., 1988). The most lethal infection due to *V. vulnificus* is the primary septicemia with an average mortality rate over 50 % (Feldhusen, 2000). Septicemia is most common in patients with suppressed immune systems, especially males. Patients with Cirrhosis of the liver, alcoholic liver disease, or chronic hepatitis B or C are at high risk for septicemia and death (Daniels, 2011). High levels of iron, documented by high transferrin saturation, are also common in patients with liver disease and other diseases that cause a compromised immune system; these are the people who develop *V. vulnificus* infections (Shapiro et al., 1998). Human and animal studies show that *V. vulnificus* growth and lethality increase with high levels of free iron in the blood of patients (Hor et al., 1999, 2000). *V. vulnificus* releases extracellular toxins, which destroy the basement membrane and the tissue of blood vessels, and possesses a capsule that makes it harder on the immune system to

respond (Haq and Dayal, 2005). These properties cause hemorrhage and edematous skin that are commonly found on patients' extremities.

Similar to the systemic disease, *V. vulnificus* wound infections also cause severe infections such as cellulitis, ecchymoses, and bullae, which can develop into necrotizing fasciitis (Bowdre et al., 1981). Fever, chills, diarrhea, nausea, vomiting, septic shock, and skin lesions are typical symptoms (Daniels, 2011). *V. vulnificus* rapid ability to cross the intestinal mucosa results in skin lesions formation within 24 hours of an infection. Although infections frequently require amputations, the mortality rates for wound infections are lower than those of systemic disease (CDC, 1993).

Gastrointestinal illness caused by *V. vulnificus* is generally self-limited with low recorded death rates (Daniels, 2011). It is characterized by vomiting, diarrhea, and abdominal pain following the consumption of contaminated sea food (Neupane, 2012).

V. vulnificus possesses several virulent factors. Hemolysin/cytolysin is an enzyme that lyses mammalian erythrocytes and is extremely cytotoxic to a variety of tissue culture cell lines (Gray and Kreger, 1985). The hemolysin gene sequence is highly conservative and has been used to identify *V. vulnificus* (Quiñones-Ramirez, 2010), however numerous other virulence-associated factors have been identified including the polysaccharide capsule, type IV pili, acid neutralization pathways (Rhee et al., 2002) and other enzymes which cause tissue damage such as proteases, elastase, hialuronidase, lecithinase, phospholipases, mucinase and metalloprotease (Miyoshi and Shinoda 1992; Garcia and Landgraf 1998; Shao and Hor 2000; Wu et al., 2001; Miyoshi 2006).

V. parahaemolyticus is less virulent than *V. vulnificus*, and rarely causes death (Baker-Austin et al., 2010). However, *V. parahaemolyticus* infections are much more common than other *Vibrio*s in the U.S. and count for the highest percentage of illnesses associated with sea-food consumption (Nordstrom et al., 2007). Freezing and refrigeration do not completely destroy *V. parahaemolyticus* (CDC, 1999). *V. parahaemolyticus* infections are characterized with abdominal cramps, diarrhea, nausea, headaches, fever and chills (Honda and Lida, 1993). Symptoms typically last for 72 hours, but may last for 10 days in patients that are immunocompromised (Baker-Austin et al., 2010).

During pathogenesis *V. parahaemolyticus* strains produce a variety of virulence factors. The most consistent factor is the thermostable direct haemolysin (*tdh*) (Nishibuchi et al., 1992; Bej et al., 1999). It is responsible for the Kanagawa haemolysis and the *tdh*-related haemolysin (*trh*) (Honda et al., 1988). The biological activity of (*tdh*) and (*trh*) genes include haemolytic, enterotoxic and cytotoxic activities (Park et al., 2004). Those two genes are used as universal markers for testing the virulence potential of strains (Kaysner and DePaola, 2001).

V. parahaemolyticus is responsible for acute diarrheal illness and acute gastroenteritis in humans (Pal and Das, 2010). Occasionally it causes bloody diarrhea and, rarely, sudden cardiac arythmia (Honda et al., 1976). The virulence gene *tdh* causes intestinal fluid secretion; this secretion is induced by tdh encoding genes. Those encoding genes raise the cytosolic free calcium concentration as well (Raimondi et al., 2000).

In general *V. parahaemolyticus* infections are confined to mild gastroenteritis; however 8% of those infections progress into primary septicemia (Hlady and Klotzy, 1996). High concentration of *tdh* is capable of disrupting the epithelial barrier and permit *Vibrios* to enter the bloodstream and invade the host (Raimondi et al., 2000). However, there are other explanations for *V. parahaemolyticus* invasiveness. For example, Akeda et al. (1997) showed that it possesses a cytotoxic factor that can act on the cell cytoskeleton in a calcium-independent fashion.

Before 1996, *V. parahaemolyticus* cases were disperse and caused by multiple, diverse serotypes (Baker-Austin et al., 2010). In 1996, the epidemic expansion of the O3:K6 started when a sudden emergence of infections occurred in Calcutta area in India, within two years the clone spread out to most South-East Asia (Okuda et al., 1997). In 1997, the first infection by this clone out of Asia was detected in South America, launching the first pandemic expansion of the clone (GonzalezEscalona et al., 2005; Martinez-Urtaza et al., 2008). The O3:K6 serotype that caused the wide spread illness associated with sea-food consumption is suggested to possess an unusual ability to be transmitted in the environment and cause infections in humans (Yeung et al., 2002). Non-pandemic strains of *V. parahaemolyticus* have also been recorded large outbreaks in different parts of the world like the Pacific Coast, Northwest of Spain, and Alaska (Baker-Austin et al., 2010).

1.1.2 *VIBRIO VULNIFICUS* AND *VIBRIO PARAHAEMOLYTICUS* ASSOCIATION WITH ENVIRONMENTAL FACTORS.

Worldwide researchers are voicing concerns over the potential increase of Vibriosis infections under the current and future climate change. The correlations between the sea surface temperature, salinity gradients and *Vibrio* are being studied internationally in several marine systems. The Baltic Sea, for instance, is one of the largest low salinity marine ecosystems and it is witnessing a rapid increase in temperature (Mackenzie, and Schiedek, 2007). The Baltic Sea salinity level, <25 ppt, is one of the major factors contributing to the prominent emergence of *Vibrio* (Motes et al., 1998) because it falls in the optimum range for *Vibrio* growth. Thus, the studies were able to show that *Vibrio* abundance was highly correlated to temperature variations (Baker-Austin et al., 2012). Using generalized linear models (GLMs), a strong association was shown between the maximum annual sea surface temperature (SST) and *Vibrio* cases (Baker-Austin et al., 2012). Randa et al. (2004) used a risk model for the Baltic Sea, where the SST was acting as the risk predictor, which was based on both the SST and salinity levels. The salinity levels determined the regions of interest in Barnegat Bay where historically high concentrations of *Vibrio* were found in intermediate and low salinity levels (Randa et al., 2004).

In the North Sea, levels of *Vibrio* infections from bathing waters were associated with temperature and salinity; the samples contained *Vibrio* from late May till October 2009 (Schetsa et al., 2011). The distribution of *Vibrio* species among the sampling sites reflected their salinity tolerance. Species that tolerate high salt concentrations, like *V. alginolyticus*, *V. parahaemolyticus* and *V. fluvialis*, were found in sites with high salinity

(Oliver and Kaper, 1997). *V. cholerae* and *V. vulnificus* which have lower salinity tolerance were isolated in sites with low salinity (Oliver and Kaper, 1997; Motes et al., 1998). The total aquatic bacteria isolated including *Vibrio* spp. depended on the dominant species found in the samples, which had a strong association with salinity levels (Schetsa et al., 2011). The common occurrence of *Vibrio* species in the Netherlands, with relatively low water temperatures, indicates the need for further analysis about other environmental factors like salinity (Schetsa et al., 2011).

Vibrio presence coincided with warm temperatures in several studies. For instance, the Gulf Coast oysters had the highest concentrations of *V. vulnificus* during warm months (DePaola et al., 1994). In addition, high levels of *Vibrio* in the Chesapeake Bay were reported during summer months while the concentrations of *Vibrio* during winter months were below detection levels (Wright et al., 1996).

Motes et al. (1998) developed a linear regression model that relates *V. vulnificus* occurrence in Gulf coast oysters to temperature and salinity. "The role of salinity in determining *V. vulnificus* levels becomes clear when the full model is compared with one lacking the salinity terms." (Motes et al., 1998). The salinity factor was able to explain 10% more of the total variability in the log-transformed most probable number (MPN) model; it also explained the difference among the sampling sites (Motes et al., 1998). Hsieh et al. (2007) found that salinity was the strongest predictor for *Vibrio* and *Escherichia coli* in surface water in the Neuse River Estuary in North Carolina.

Motes et al. (1998) also sampled oysters in Atlantic coastal waters during summer months and used their linear regression model to predict *V. vulnificus* occurrence. The

field results showed that high numbers of *Vibrio* occurred where salinity was below 22 ppt. The model predictions of *V. vulnificus* were lower than the measured counts. They then compared the residuals with salinity concentrations and found a positive correlation in the low-salinity sites and a negative correlation in high salinity sites (Motes et al., 1998).

Salinity can play a critical role in *Vibrio* distribution. When salinity ranges are too high or too low, *Vibrio* numbers were low. A study of oysters in the Apalachicola Bay in August and September 1995 found lower counts of *V. vulnificus* than in the same months in 1994. During 1995, abnormally high salinity concentrations coincided with the low counts of *V. vulnificus* (Jackson et al., 1997). Between Alabama, Florida, and Louisiana sites; the Louisiana site had different salinity readings than Alabama and Florida and did not depress the *V. vulnificus* growth. Both the low salinity conditions in Alabama and the high salinity levels in Florida reduced the counts of *V. vulnificus.* The results suggest that salinity extremes may play a critical role in the abundance of *V. vulnificus* (Motes et al., 1998). They concluded that salinities below 25 ppt favor high *V. vulnificus* densities, where salinities higher than 25 ppt appear to lower their numbers, even in warm waters.

The relationship between *V. parahaemolyticus*, salinity, and turbidity also has been documented. For instance, Zimmerman et al. (2007) found significant associations ($P < 0.001$) between *V. parahaemolyticus* distribution, salinity, and turbidity in both water and oysters samples at sites in Mississippi site. *V. parahaemolyticus* prefers temperatures between 5 and 43°C and salinities between 10 and 30 ppt (Lake et al., 2003; FDA, 2005) with an optimal salinity of 22 ppt (DePaola et al., 1990). Thus, at the limit of viability for both parameters the density of *V. parahaemolyticus* is expected to be low.

The marked spread of *Vibrio* during the last few decades is being observed in different parts of the world. In 1991, the seventh pandemic of *Vibrio* cholera reached Peru and then spread out through the American continents. Then in 1997 another *Vibrio* outbreak occurred in South America, when *V. parahaemolyticus* was isolated outside of Asia for the first time. The emergence and distribution pattern of *V. parahaemolyticus* infections were explained by coastal patterns. The infections were closely related to the arrival and propagation of the 1997 El Niño along the coast of South America. The *V. parahaemolyticus* consistently showed a southward spread (Martinez-Urtaza et al., 2008). This result confirms the projected poleward movement of *Vibrio* around the world (Baker-Austin et al., 2012; Ansede-Bermejo et al., 2010).

Changes in temperature and salinity of sea water consistently have shown a direct association with the occurrence of *Vibrio* and outbreaks of aquatic pathogens (Martinez-Urtaza et al., 2008; Randa et al., 2004). Salinity is a critical factor influencing the distribution of different *Vibrio* species according to their salt tolerance (Martinez-Urtaza et al., 2008). *Vibrio* epidemics have shown strong relationships with the environmental dynamics; these pathogens are expected to be one of the most sensitive to the global warming. Large coastal areas are witnessing patterns of warming and alterations of salinity due to drastic changes in climate patterns (i.e. rainfall, river flows, sea-level rise) (Gavilan and Martinez-Urtaza, 2011). In South America, for example, the increase in water temperatures and decrease in salinity due to increased rainfall will provide better conditions for the spread of *Vibrio* along the coast (Gavilan and Martinez-Urtaza, 2011). This phenomenon is also predicted to expand the geographic distribution of *Vibrio* into

areas that used to be considered too cold for them to survive and grow (Baker-Austin et al., 2010).

1.2 Research Objective

The objective of the current study is to quantify the distribution of *Vibrio* species in the Waccamaw River/Winyah Bay estuary under current conditions and to predict potential changes in distribution under future climate conditions. There are no previous studies in South Carolina that address this issue, although there have been baseline *Vibrio* studies conducted in North Inlet, Murrels Inlet and Charleston (Vernberg et al., 1997; and Bass, 2004). *Vibrios* are not only a major economic threat to marine shellfish growing but also represent a public health threat as they are readily transmitted to humans through seafood consumption and open wounds. *V. vulnificus* and *V. parahaemolyticus* are naturally occurring pathogens in aquatic environments which contribute to the cycling of carbon and other nutrients (Riemann et al., 2002). The range of environmental conditions in which these species can thrive suggest that human exposure to *Vibrio* cannot be eliminated. However, monitoring of environmental factors that indicate high risk of *Vibrio* spp. can be used to forecast high risk *Vibrio* conditions which may support management to reduce the incidence of illness. This research study aims to provide information that may help decision makers (regulatory agencies, the shellfish industry, public health officials, and the public) manage both shellfish harvesting and recreational activities to reduce the public exposure to *Vibrio* species. This will be done by combining field data on *Vibrio* species concentrations collected during 2012 with the Pee Dee River and Atlantic Intracoastal Waterway Salinity Intrusion Model (PRISM2) decision support system (DSS) (Conrads and Roehl, 2007). PRISM2 predicts specific conductance in the

Waccamaw River under future climate scenarios. The field data collected during 2012 was analyzed for empirical relationships among *Vibrio*, salinity, conductivity, turbidity and temperature. Salinity and conductivity are both measures of the dissolved salt in the water. Salinity compares the dissolved salt in a water sample to the salt concentration of the ocean (CWT, 2004). Conductivity measures a water sample ability to conduct electricity, which is directly proportional to the amount of ions present due to the dissociation of salt into positive and negative ions (CWT, 2004). The estimates of conductivity values were used to predict where and when *Vibrio* species may be present and represent a potential threat to human health.

1.3 SIGNIFICANCE

V. parahaemolyticus and *V. vulnificus* are ubiquitous and native to estuaries globally. Similar to other *Vibrio* species, *V. parahaemolyticus* and *V. vulnificus* are autochthonous to coastal areas and their distribution is determined by changes in environmental parameters. Seasonal and geographical variations are based on water temperature, salinity, turbidity and other environmental factors. The influence of temperature on *Vibrio* distribution in water and sediment has been reported in several studies for both *V. parahaemolyticus* and *V. vulnificus*. (Kelly and Dan Stroh, 1988; Oliver, 1989; O'Neill et al., 1992; Kasper and Tamplin, 1993; Motes et al., 1998). *Vibrio* species are halophilic species that occur most frequently in waters with moderate salinities. Ecological studies have shown that *Vibrios* can thrive in fresh and very low salinities coupled with high water temperatures and organic matter concentration (Singleton et al., 1982; and Miller et al., 1984). Thus, the combined effect of environmental parameters enables *Vibrio* to persist and overcome the deleterious effect of

low salinity. Models that predict the abundance of *Vibrio* populations rely on monitoring the different environmental factors that allow optimum growth conditions. However, the developed models across the various geographical regions have resulted in different predictive relationships due to geographical ecological variations. For instance, models developed for the Gulf of Mexico may not be applicable to the Atlantic coast or to the Pacific coast (Tamplin et al., 1982; Jiang and Fu, 2001; Hsieh et al., 2008; Johnson et al., 2010).

Winyah Bay, similar to the rest of the East Coast, is likely to be impacted by climate change. Sea-level rise, increased inundation, and increased inter-annual climate variability will potentially cause an increase in saltwater intrusions into estuaries along the East Coast. Several studies have reported on saltwater intrusion occurrence in the United States (Barlow and Reichard, 2010) and along the Atlantic coast, and even more specifically in Carolina's coastal areas (Barlow and Wild, 2002; Conrads et al., 2012). Saltwater intrusion into freshwater coastal estuaries and rivers affects fresh water quantity and quality. During 2002 and later in 2011 the Waccamaw River experienced saltwater intrusions that threatened drinking water due to elevated salinity levels. In 2002, the high salinity levels were caused by the over 50% decrease in Pee Dee River normal flow (Hicks, 2002). Summer of 2011 was dry and heavy rains were very confined, causing an increase in the upriver extent of the saltwater wedge. This restricted local utilities from utilizing the Waccamaw River waters during mid-July at high tide due to elevated salinity (Fuller, 2011). During 2012, high tides were encountered due to Hurricane Sandy. Climate change effects, including sea level rise and changes in stream flow, suggest that such intrusion events are expected to be more common and/or longer

duration in the future. Based on what is known about *Vibro* occurrence from other locations it is likely that Winyah Bay estuary will experience changes in *Vibrio* distribution and density due to changes in the salinity patterns as a result of climate change.

The aim of preventing and mitigating *Vibrio*-associated infections has been explored by developing predictive models for *Vibrio* spp Numerous studies have quantitatively compared the distribution of *V. vulnificus* and *V. parahaemolyticus* to environmental parameters. The most common adapted variables that have been effective in predicting the occurrence and abundance of *Vibrio* are water temperature and salinity (Randa et al., 2004; Zimmerman et al., 2007; Hsieh et al., 2008; Constantin de Magny et al., 2009; Johnson et al., 2010). While there are extensive studies on the distribution of *V. vulnificus* and *V. parahaemolyticus* in estuarine waters globally, there is little information on the abundance of both organisms in South Carolina waters (Vernberg et al., 1997; and Bass, 2004). Assessing *Vibrios* presence and correlating occurrence based on environmental predictors has been done along other regions of the Atlantic Coast, including North Carolina and Florida (Yamazaki and Esiobu, 2007; Banakar et al., 2012; and Jacobs et al., 2012). Thus, *Vibrio* analysis in South Carolina will help complete the picture of the geographic distribution and variation of *Vibrio* spp. along the Southeast coast.

In addition to helping complete the understanding of the geographic distribution of *Vibrio* abundance along the Atlantic Coast, this project developed a prediction model of *Vibrio* distribution that, similar to other studies, was based on its relation to environmental parameters. We then coupled the model with the PRISM2 model

prediction of salinity under future climate and sea level conditions. This enabled us to predict potential counts of *Vibrio* for the years 2055-2069 under future different sea-level and streamflow conditions. Without accurate predictions of *Vibrio* populations, recreational users and shellfish consumers may be exposed to life-threatening situations during times and in locations currently considered free of *Vibrio* exposure. The potential of *Vibrio* transport upstream increases the chances of contact with the public using the river for recreation or a source for shellfish. Thus, it is important to be able to predict the expansion of *Vibrio* populations based on future climate scenarios.

1.4 Study Site Description

Winyah Bay is the study site estuary in South Carolina to monitor and assess the potential increase of *Vibrio* exposure depending on the varying trends of salinity intrusions. Winyah Bay is an estuary formed at the confluence of the Waccamaw River, the Pee Dee, the Sampit, and the Black Rivers in Georgetown County (Figure 1). Winyah Bay is part of the North Inlet-Winyah Bay National Estuarine Research Reserve system. The Reserve covers 4988.56 hectares (12,327 acres) of natural area and is located around 80.5 kilometers north of Charleston (NERRS reserves, 1992). Winyah Bay is the estuary with the third largest watershed on the east coast, where more than 90 % of North Inlet's watershed is in its natural forested state (NERRS reserves, 1992). Fresh water inputs ranges from 56.63 to 28 316.84 cubic meter per second, while the runoff average is around 424.75 cubic meter per second (NERRS, 2009). The river flow is strong enough to limit the ocean intrusions into the bay during the winter and spring. Winyah Bay experiences salt wedge effects with the occurrence of the flood tide, when saline water moves up the river despite the freshwater that is flowing to the ocean (NERRS, 2009).

Periods of low river flow permit the flood tide to move salt water up to 24 km upstream of the Highway 17 Bridge over the Waccamaw River. The salinity differences between surface and bottom water can be more than 20 ppt. During normal river flow salt water penetration is within 1.6 km of the bridge (NERRS, 2009).

CHAPTER 1 TABLES AND FIGURES

Table 1.1: *Vibrio vulnificus* and *Vibrio parahaemolyticu*s optimal and range of tolerance for temperature and salinity.

	Vibrio Vulnificus	*Vibrio parahaemolyticus*	References
Optimal Salinity (ppt)	5-25 ppt	17-23 ppt	Wetz et al., 2008; Colwell, 2006.
Salinity tolerance range (ppt)	5-38 ppt	3-35 ppt	Kaspar et al., 1993; Johnson et al., 2010.
Optimal Temperature (°C)	15-30°C	37°C	Baker-Austin et al., 2010; FDA, 2005
Temperature tolerance range (°C)	7-36°C	5-43°C	Motes et al., 1998; FDA, 2005.

CHAPTER 2:

MATERIALS AND METHODS

2.1 BACTERIAL IDENTIFICATION

The study examined nine sampling sites in the Winyah Bay/Waccamaw River area. Surface and bottom water samples were collected in pre-sterilized 2 liter polypropylene containers every month between April and October 2012. A subsequent sampling occurred October 29 after Hurricane Sandy passed off the coast of South Carolina. Smaller containers of surface and bottom waters were collected during each sampling event for turbidity measurement, which was done using a Hach 2100P turbidimeter. This was used to represent the total suspended solids (Fries et al., 2007). The sampling was performed from a boat, which collected surface water, bottom water, and sediment samples along with physicochemical water parameters including water temperature, salinity, pH, and other field parameters were recorded at each site. Water and sediment samples were transported in coolers with frozen/cold gel packs to the microbiology lab at the National Oceanic and Atmospheric Administration's Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, S.C. and analyzed within 6-8 hours of collection. Water samples were tested for the presence of *Vibrio vulnificus* and *parahaemolyticus*. Various volumes of surface and bottom water samples and dilutions of water samples in phosphate buffered saline (PBS) were filtered through sterile nitrocellulose 0.45 micron, 47 mm filters on a sterile vacuum

manifold to determine bacterial plate counts on CHROMagar *Vibrio* media. Plates were incubated overnight (16-24 hours) at 37°C and colonies were counted as presumptive *V. vulnificus* and *V. parahaemolyticus* colony forming units (CFU/mL). Turquoise colonies were identified as *V. vulnificus* and mauve colonies were identified as *V. parahaemolyticus* (Williams et al., 2011). After counting, seven individual CFUs of presumptive *V. vulnificus* and *V. parahaemolyticus* were picked from the CAV plates using sterile loops, and were put into tubes containing 700 µl of tryptic soy broth with 2% sodium chloride (TSB + NaCl). Tubes with presumptive *Vibrio* spp. colonies were labeled, vortexed, and incubated at 37°C for another twenty four hours.

2.2 DNA EXTRACTION FOR ISOLATE CONFIRMATION

A volume of 300 µl was removed from each tube and put into 1.5 ml micro centrifuge tubes to undergo DNA extraction. The 300 µl of the bacterial solution was centrifuged at 5,000 x g for five minutes to form a cell pellet. The TSB + NaCl supernatant was removed and the pellet underwent one wash using AE buffer (reagent in the Qiagen DNeasy Tissue Kit). Then, 200 µl of AE buffer was added to each tube and centrifuged again at 5,000 x g for five minutes. The supernatant was removed, and 200 more µl of AE buffer was added to each tube. The tubes were then boiled for 10 minutes and then centrifuged at 5,000 x g for five minutes. From each of the tubes 100 µl of the supernatant was put into new micro centrifuge tubes. With the 100 µl of supernatant, 200 µl of 100% molecular grade ethanol and 10 µl of 3 molar sodium acetate, with pH of 5.2, were added to each tube. The solution was mixed by inverting the tubes and the tubes were then incubated for at least 30 minutes at -20 °C. After 30 minutes, the tubes were centrifuged at 14,000 x g for 10 minutes. The supernatant was then removed and 1 ml of

100% ethanol was then added. The tubes were then vortexed and centrifuged again at 14,000 x g for five minutes. The supernatant was then removed, and the pellets air dried before being re-suspended in 100 µl of AE buffer. The extracted DNA was then stored at -80°C until further analysis was conducted.

The TaqMan based real-time PCR assay of Paniker & Bej (2005) was used for *V. vulnificus* identification, using the *hemolysin A* gene (*vvhA*) as the marker. For *V. parahaemolyticus,* the *thermolabile hemolysin* (*tlh*) gene was used as the target (Nordstrom et al., 2007). The Nordstrom et al., 2007 *V. parahaemolyticus* qPCR method has a detection limit equivalent to 20 CFU for the species specific *tlh* gene in V. parahaemolyticus, and most of the CHROMagar plate counts were <20 CFU. When qPCR results using purified DNA extracts were compared to the plate counts there was a general agreement with the plates that had levels above 20 CFU, thus we can be reasonably confident that the CHROMagar plate technique for identification is accurate, except at low bacterial numbers at the lower limits of detection.

2.3 Statistical Analysis

2.3.1 Previous studies statistical analysis

Vibrio association with environmental parameters has been assessed in several ways. Generally, cell abundance is $\log_{10}$ transformed to achieve a normal distribution prior to any further statistical analysis. Correlations between *Vibrio* species abundance and the environmental parameters measured were determined by using Spearman's coefficient or Pearson's coefficient of correlation (Randa et al., 2004; Wetz et al., 2008). Randa et al. (2004) performed linear regressions for each of the environmental

parameters, while Wetz et al. (2008) conducted a multiple regression analyses using Pearson's correlations between *Vibrio,* temperature and salinity, and with salinity and temperature combined. A number of studies generated simple and multiple linear regressions to determine the environmental factors contributing to the variability in the concentrations of *Vibrio* species (Wright et al., 1996; Motes et al., 1998; Pfeffer et al., 2003; Ramirez et al., 2009). For instance, Hseih et al. (2008) developed a multi-linear regression model to predict *Vibrio* concentration using six environmental parameters: temperature, salinity, chlorophyll a, dissolved organic carbon, pH, and total suspended solids. Banakar et al. (2012) used the logit model for *V. vulnificus* based on the data quantified by qPCR. The logistic regression was based on temperature, salinity and their interaction. Banakar et al. (2012) adapted Jacobs et al. (2010) model, which does not require the data to be normally distributed, and replaced the salinity median by the actual value of salinity that was determined. Martinez-Urtaza et al. (2007) analyzed *V. parahaemolyticus* association with environmental parameters using Pearson correlation coefficients, multiple logistic regression, and multiple linear regression. The logistic regression model was used to generate the predicted probabilities and odds ratios. The multiple linear regression analysis was developed to identify the environmental conditions that affected *V. parahaemolyticus* abundance in Rias of Galicia, Spain. Johnson et al. (2010) used the generalized linear mixed model (GLMM) to assess *Vibrio* relationship to environmental parameters in oyster, sediment, and water. The GLMM regression analysis, is a continuous latent distribution underlying the discrete observations of abundance, which was assumed to follow a log-normal distribution, with the mean $\log_{10}$ densities generally considered to be linearly related. Due to the salinity

ranged observed in Johnson et al. (2010) study, a quadratic polynomial was utilized to model the effect of salinity on *Vibrio*.

2.3.2 Statistical Analysis on the field data gathered

The data set in this study was compiled to analyze temperature, turbidity, and conductivity effect on the occurrence of *V. vulnificus* and *V. parahaemolyticus*. *Vibrio* abundance was plotted as a function of temperature, salinity, conductivity, and turbidity. Prior to any statistical tests the bacterial counts were transformed to their $\log_{10}$. Pearson's correlations between *Vibrio* and the different environmental parameters were computed. The sampling for *Vibrio* spp. in Winyah Bay was conducted at 9 sites, collecting surface and bottom water samples from each site. Thus, correlations were done for each species for surface and bottom water separately and combined. The computation of Pearson's correlation coefficient was used to evaluate the degree to which the two variable movements were associated (De Souza Costa Sobrinho et al., 2010; Yamazaki and Esiobu, 2012) and to be able to compare our results with previous studies. Initial scatter plots of *V. vulnificus* and *V. parahaemolyticus* plate counts against temperature, salinity, and turbidity helped clarified which environmental variable represented a log-linear relationship with *Vibrio* species. The linear regression equation is as following $Y = a + bX$. Motes et al. (1998) used the linear relationship to assess the relationship between *Vibrio* concentrations and selected environmental parameters. A multi-linear regression was conducted to evaluate the relation of the environmental variables on *V. vulnificus* and *V. parahaemolyticus.* The scatter plot revealed that the relation between *Vibrio* and conductivity was not linear and followed a quadratic polynomial relation; *Vibrio* counts showed an increasing trend with conductivity until conductivity falls out of *Vibrio*'s

optimal range and *Vibrio* counts start to decrease. This pattern is what is known as the quadratic trend, which is why we used the quadratic value for conductivity, which is conductivity^2, to assess its effect, similar to Johnson et al. (2010). We started with a full model that contained all the following variables: temperature, salinity, conductivity, and turbidity. We then used a backward selection to select our final model based on a cutoff point of a P-value 0.05. The coefficient for each of the variable was reported and the model was used to predict future trends of *V. vulnificus* based on conductivity from PRISM2 model. For that we assumed fixed values for all the other variables in the full model which was equal to the average calculated from the field data. The model does not reflect the seasonal variation of *Vibrio* abundance with respect to temperature. The *Vibrio* counts obtained are the whole bacterial population without any determination of the percentage of virulent strains. Thus, the model gives an estimate of the total population abundance in the water according to its association with conductivity. All the analysis was done using SAS software 9.3.

2.4 Predictive Tool

Results from the above analyses were coupled with projected scenarios of conductivity estimates calculated by the updated Pee Dee River and Atlantic Intracoastal Waterway Salinity Intrusion Model Decision Support System (PRISM2 DSS) (Conrads et al., 2012). The model integrates a historical streamflow database, artificial neural network models, projections of future streamflow and sea level, and model controls. The historical data set is composed of hourly samples of freshwater flows, tidal forces, and specific conductance between 7/11/1995 - 8/20/2009 (Conrads et al., 2012). Then the model calculates conductivity dynamics as sea-level, tidal ranges, and freshwater inflows

vary. *Vibrio*'s relationship with conductivity derived from the field data, as described in the statistical analysis, was used to generate current and predictive models for *Vibrio* species in Winyah Bay/ Waccamaw River. PRISM2 provided us with historical specific conductance values for three of our sampling sites stations in the Waccamaw River, which showed that the number of higher specific conductance spikes has been increasing over the years. PRISM2 also integrates sea-level rise and reduced streamflow scenarios. Future specific conductance estimates following the current conditions and 1.0ft, 2.0ft, and 3.0ft sea-level rise scenarios were run for WR-5, WR-4, and WR-3 stations which are USGS gages 02110809, 021108125, and 02110815 used for PRISM2 (stations 809, 8125, and 815 hereafter). These projections at three stations represent an example of future prediction approaches for *Vibrio* utilizing the conductivity estimates generated by PRIMS2 combined with the relationship developed by the current field data for *Vibrios*.

CHAPTER 2 TABLES AND FIGURES

Figure 2.1: The Winyah Bay estuary and sampling sites in the Bay and the Waccamaw River.

CHAPTER 3:

RESULTS

3.1 FIELD DATA RESULTS AND DESCRIPTION

The maximum culturable *Vibrio* count in bottom water samples was 75 CFU/mL; while in surface water samples it was 59 CFU/mL. *V. vulnificus* counts ranged between 0 and 58 CFU/mL, with an average of 10.66 CFU/mL. *V. parahaemolyticus* counts at our study sites ranged between 0 and 34 CFU/mL, with an average of 5.27 CFU/mL. The total number of counts of *V. vulnificus* from all the collected samples both surface and bottom water was more than twice the count of *V. parahaemolyticus*. Overall, sampling detected both *V. vulnificus* and *V. parahaemolyticus* in all water samples and study sites throughout the nine months of sampling. Temperatures measured at our study sites were always between 18 and 30ºC. The total counts of *Vibrio* species were higher in bottom water samples; in addition the total *V. vunlificus* and *V. parahaemolyticus* bottom water counts were higher than the total *V. vunlificus* and *V. parahaemolyticus* surface water counts.

V. vulnificus was retrieved from a wide range of salinity conditions, ranging from 0.05 ppt and 27.6 ppt. Counts were the highest when salinity was between 5 and 20 ppt. Lower counts were observed when salinity was lower than 5 ppt and higher than 20 ppt. In terms of conductivity the highest counts were between 10mS/cm and 30mS/cm and counts decreased at both the lower and upper range of values (Figure 3.1). The scatter

plot of *V. vulnificus* logcount versus temperature did not show a clear effect although the highest counts were at the upper end of the temperature range (Figure 3.2). Turbidity ranged between 0 and 262 NTU; *V. vulnificus* tended to increase with increased turbidity (Figure 3.3). Correlation analysis of all samples showed that the abundance of *V. vulnificus* in Waccamaw River/Winyah Bay was positively correlated to the water temperature (r =0.18754; P <.0355), conductivity (r =0.37351; P <.0001), salinity (r=0.18754; P <0.034661) and turbidity (r=0.52370; P <.0001). *V. vulnificus* from surface water samples showed different associations to temperature, salinity, and turbidity from those in bottom water samples. A summary of the Pearson's correlation for surface and bottom water samples is provided in Table 3.1. Salinity and turbidity recorded a higher correlation and significance with *V. vulnificus* abundance in bottom water samples. Temperature showed a stronger association with *Vibrio*'s abundance in the surface water samples. Bottom turbidity was highest of all.

V. parahaemolyticus range counts, average, and total counts were lower than *V. vulnificus*. *V. parahaemolyticus* bacteria were collected over the whole range of salinity gradient (0.05-27.63ppt) with no clear dominance within a certain salinity range. The scatter plot of *V .parahaemolyticus* versus conductivity showed an unclear pattern (Figure 3.4). Similarly, temperature did not show a strong association with *V. parahemolyticus* in general, but an inhibition of growth was observed when the temperature was >25°C (Figure 3.5). Turbidity ranged between 0 and 262 NTU, the log count of *V. parahaemolyticus* and turbidity were associated in a positive manner (Figure 3.6). Correlation analysis of all samples showed that the abundance of *V. parahaemolyticus* in Waccamaw River/ Winyah Bay was negatively correlated to water temperature (r = -

0.14236; P < 0.1274) and positively correlated to conductivity (r = 0.18292; P < 0.0494), salinity (r = 0.17727; P < 0.0570), and turbidity (r =0.51898; P < 0.0001). The negative correlation with water temperature was insignificant. Surface and bottom water temperatures did not exhibit large variation (Table 3.2). Salinity, conductivity, and turbidity values were higher in bottom water than surface water samples. Unlike surface water samples, bottom water samples showed significant Pearson's correlation coefficient with conductivity and turbidity, and salinity was on the marginal border of significance with a P= 0.0577 (Table 3.2).

V. vulnificus and *V. parahaemolyticus* exhibited a different response to temperature in both surface and bottom waters (Figure 3.7). In general, *V. vulnificus* had higher counts than *V. parahaemolyticus* in surface and bottom samples during every month except April. *V. vulnificus* counts were higher when the temperature was higher in surface and bottom waters, while *V. parahaemolyticus* was not affected by temperature.

Figure 3.8 and 3.9, show that salinity and conductivity ranges and averages did not vary a lot between surface and bottom water. Stations WR-5, WR-4, WR-3, WR-2, WR-1, and BR-1 were more sensitive to salinity and conductivity increases especially *V. vulnificus* densities. Shellfish Station 05-20, Shellfish Station 05-21, and Shellfish Station 05-25 recorded higher salinities and conductivities values which are expected due to them being located in marine waters. However, *Vibrios* average densities were higher at the upriver stations WR-1, WR-2, and BR-1 where salinity and conductivity recorded lower values than those of the shellfish stations. This indicates the protective effects of high salinity in reducing *Vibrio* abundances.

Figure 3.10 shows that *Vibrio* spp are directly proportional to turbidity. Turbidity measurements and total *Vibrio* counts were higher in bottom waters in comparison to the surface water turbidity and *Vibrio* spp counts.

3.2 Prediction Model Results

The multi-linear regression model beta values for the environmental variables in relation to *V. vulnificus* are as follows: Conductivity parameter estimate= 0.06455, P<.0001; Conductivity^2 parameter estimate= -0.00163, P<.0001; Turbidity parameter estimate= 0.00357, P<.0001; Temperature parameter estimate= 0.01748, P=0.0654. This multiple linear model explained 62.47 % of *V. vulnificus* variability (Table 3.3). Turbidity and conductivity were both significant with a p<0.0001, while temperature was on the borderline with a P<0.0654. The residuals by regression for logcount _Vv with temperature, turbidity, conductivity and conductivity^2 are depicted in Figure 3.11.

The multi-linear regression model beta values for the environmental variables in relation to *V. parahaemolyticus* are the following: Temperature parameter estimate= -0.03115, P= 0.0096; Turbidity parameter estimate= 0.00414, P<.0001; Conductivity parameter estimate= 0.01223, P= 0.1925; Conductivity^2 parameter estimate= -0.00024169, P= 0.3586. The model explained 34.25 % of *V. parahaemolyticus* variability, which was expected since *V. parahaemolyticus* did not show any clear relationship with temperature, salinity, and conductivity (Table 3.4). Turbidity was the most correlated variable to the bacteria with a P<0.0001. The residuals by regression for logcount _Vp with temperature, turbidity, conductivity and conductivity2 are depicted in Figure 3.12. The model also showed that temperature was significant, but conductivity

did not show a strong correlation. The reason for this negative relation between *V. parahaemolyticus* and temperature could be explained in part by the limited temperature range recorded during the sampling, (18-30°C). The temperature range is within *Vibrio parahaemolyticus* tolerance range however it is still below its optimum temperature. Thus, the relatively low temperatures and the other environmental factors interaction could be behind this negative association we obtained.

The model enabled us to predict for *V. vulnificus* in gages 809, 815, 8125. However, the insignificant relation between *V. parahaemolyticus* and conductivity hindered us from providing estimates of its future counts according to the predicted conductivity levels by PRISM2. Our study showed that conductivity and turbidity are strong predictors of the occurrence and abundance of *V. vulnificus*. In contrast, conductivity was a very weak predictor of *V. parahaemolyticus.* Turbidity again was a strong predictor, as was temperature, although the reason for the temperature relationship is unclear from these data, as mentioned above.

3.3 *VIBRIO VULNIFICUS* LINEAR REGRESSION MODEL WITHOUT THE SAMPLING DATE AFTER HURRICANE SANDY (29/OCT/2012)

We reran *V. vulnificus* linear regression model without considering the samples on 29/10/2012, which are post hurricane Sandy. The model beta values for the environmental variables in relation to *V. vulnificus* are found in Table 3.5. The model this time explained 61.94% of *V. vulnificus* distribution. Then we predicted for 29/10/2012 sampling date, by entering the real environmental variables values measured on that date. The observed *V. vulnificus* logcounts obtained from the sampling were within the model

predictive interval. Figure 3.13 shows the observed logcounts obtained from the sampling, the model prediction counts, and the predictive interval. Thus, the conservative model generated was able to predict for a post hurricane event. The hurricane Sandy actual counts did not differ greatly than the other sampling counts, which is why the model was able to predict for it. However, if a future event caused *Vibrio* counts to be above the average obtained then the model will not be able to predict it. Thus, the model is capable of predicting for *V. vulnificus* under normal conditions and post low impact storm events with a reasonable level of confidence.

CHAPTER 3 TABLES AND FIGURES

Table 3.1: A summary of the Pearson's correlation coefficients of *Vibrio vulnificus* for surface and bottom water samples.

Surface Water **Pearson Correlation Coefficients** **Prob > |r| under H0: Rho=0** **Number of Observations**					
	logcount_VV	**temperature**	**salinity**	**conductivity**	**turbidity**
logcount_VV logcount_VV	1.00000 61	0.21930 0.0895 61	0.25367 0.0485 61	0.28132 0.0281 61	0.28735 0.0556 45
Bottom Water **Pearson Correlation Coefficients** **Prob > |r| under H0: Rho=0** **Number of Observations**					
	logcount_VV	**temperature**	**salinity**	**conductivity**	**turbidity**
logcount_VV logcount_VV	1.00000 65	0.18150 0.1479 65	0.39889 0.0010 65	0.43027 0.0003 65	0.60259 <.0001 48

Table 3.2: A Summary of the Pearson's Correlation Coefficients of *Vibrio parahaemolyticus* for Surface and Bottom Water Samples.

Surface Water **Pearson Correlation Coefficients** **Prob > |r| under H0: Rho=0** **Number of Observations**					
	logcount_VP	**temperature**	**salinity**	**conductivity**	**turbidity**
logcount_VP logcount_VP	1.00000 55	-0.13728 0.3176 55	0.05160 0.7083 55	0.05696 0.6796 55	0.07160 0.6693 38
Bottom Water **Pearson Correlation Coefficients** **Prob > |r| under H0: Rho=0** **Number of Observations**					
	logcount_VP	**temperature**	**salinity**	**conductivity**	**turbidity**
logcount_VP logcount_VP	1.00000 61	-0.14367 0.2693 61	0.24441 0.0577 61	0.25605 0.0464 61	0.60564 <.0001 44

Table 3.3: *Vibrio vulnificus* Linear Regression Model.

Root MSE	0.27819	**R-Square**	0.6247
Dependent Mean	0.79661	**Adj R-Sq**	0.6076
Coeff Var	34.92165		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	-0.03719	0.21618	-0.17	0.8638
temperature	temperature	1	0.01748	0.00937	1.87	0.0654
turbidity	turbidity	1	0.00357	0.00065721	5.43	<.0001
conductivity	conductivity	1	0.06455	0.00764	8.45	<.0001
conductivity2	conductivity2	1	-0.00163	0.00022105	-7.39	<.0001

Table 3.4: *Vibrio parahaemolyticus* Linear Regression Model.

Root MSE	0.32089	**R-Square**	0. 3425
Dependent Mean	0.64209	**Adj R-Sq**	0.3084
Coeff Var	49.97505		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	1.06756	0.26179	4.08	0.0001
temperature	temperature	1	-0.03115	0.01173	-2.65	0.0096
turbidity	turbidity	1	0.00414	0.00076539	5.40	<.0001
conductivity	conductivity	1	0.01223	0.00930	1.31	0.1925
conductivity2	conductivity2	1	-0.00024169	0.00026170	-0.92	0.3586

Table 3.5: *Vibrio vulnificus* Linear Regression Model without Oct29 Sampling Date.

Root MSE	0.28218	**R-Square**	0.6194
Dependent Mean	0.79436	**Adj R-Sq**	0.6006
Coeff Var	35.52338		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	0.05251	0.23506	0.22	0.8238
temperature	temperature	1	0.01425	0.01015	1.40	0.1639
turbidity	turbidity	1	0.00341	0.0006797 4	5.01	<.0001
conductivity	conductivity	1	0.06527	0.00830	7.87	<.0001
conductivity2	conductivity2	1	-0.00167	0.0002372 5	-7.02	<.0001

Table 3.6: *Vibrio vulnificus* linear regression model without October/29/2012 sampling date.

Root MSE	0.28218	**R-Square**	0.6194
Dependent Mean	0.79436	**Adj R-Sq**	0.6006
Coeff Var	35.52338		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	0.05251	0.23506	0.22	0.8238
temperature	temperature	1	0.01425	0.01015	1.40	0.1639
turbidity	turbidity	1	0.00341	0.0006797 4	5.01	<.0001
conductivity	conductivity	1	0.06527	0.00830	7.87	<.0001
conductivity2	conductivity2	1	-0.00167	0.0002372 5	-7.02	<.0001

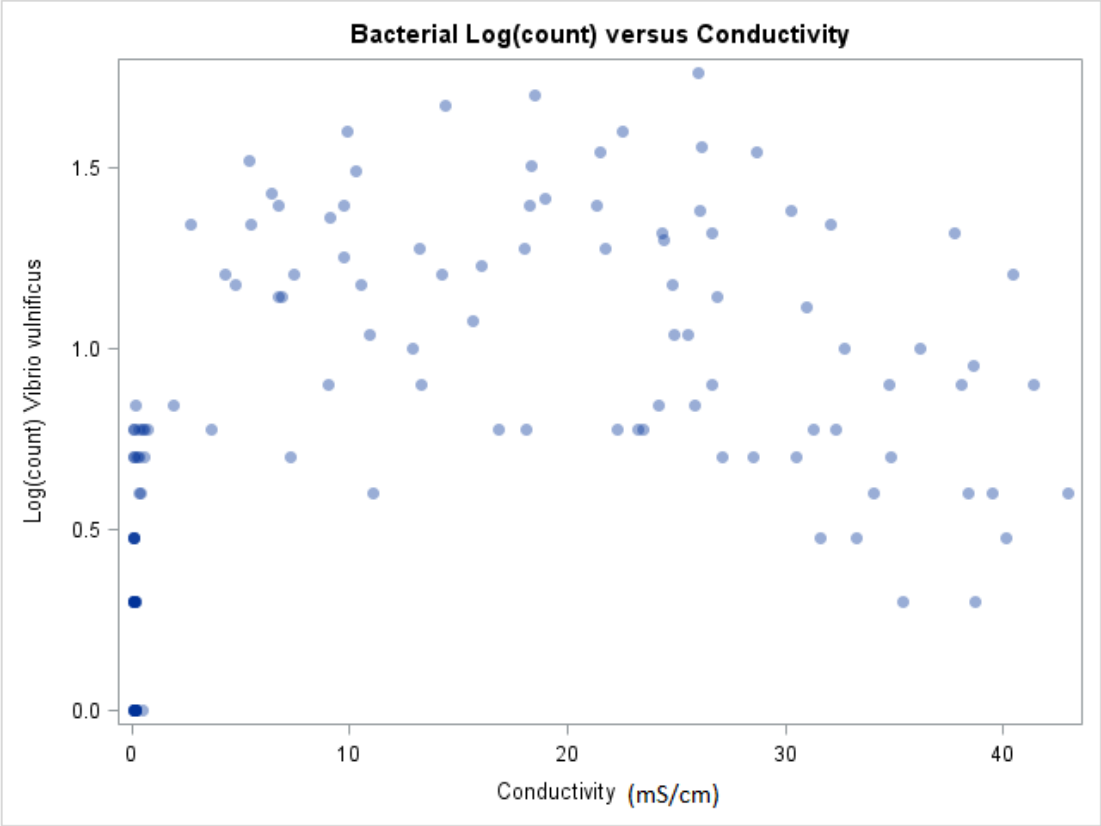

Figure 3.1: Scatter plot of *Vibrio vulnificus* logcount versus Conductivity (mS/cm).

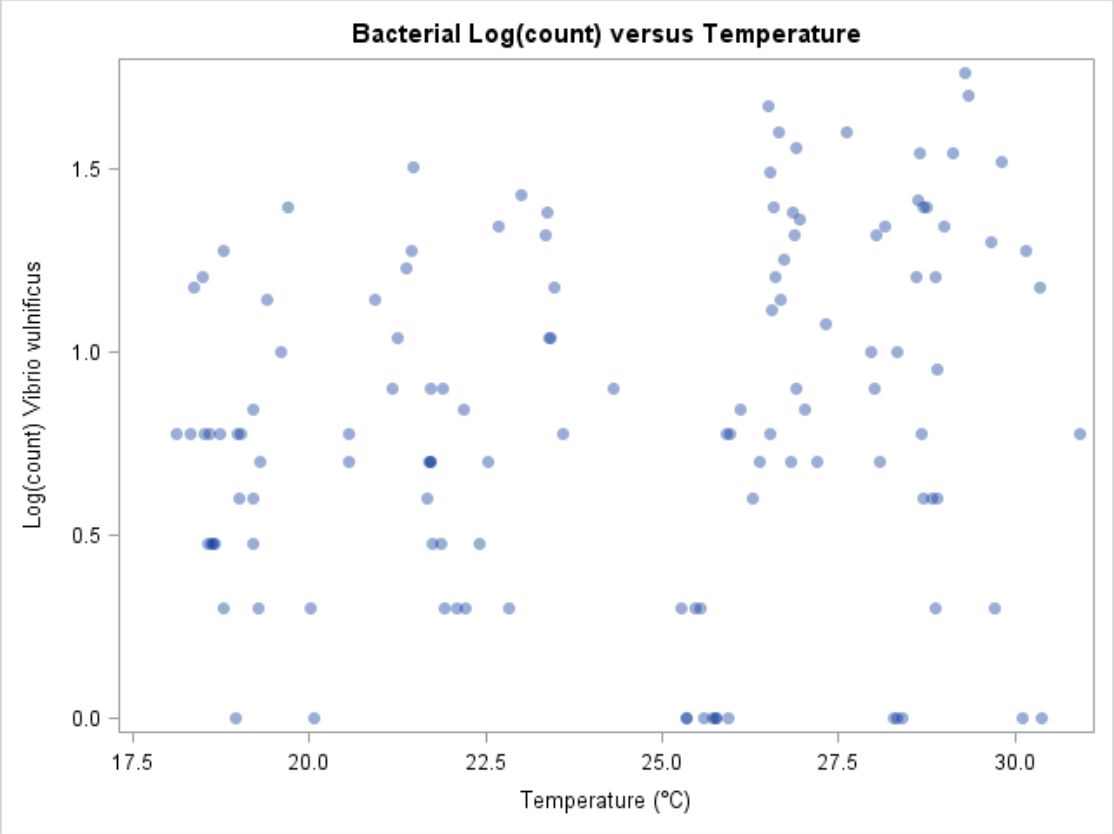

Figure 3.2: Scatter plot of *Vibrio vulnificus* logcount versus Temperature (°C).

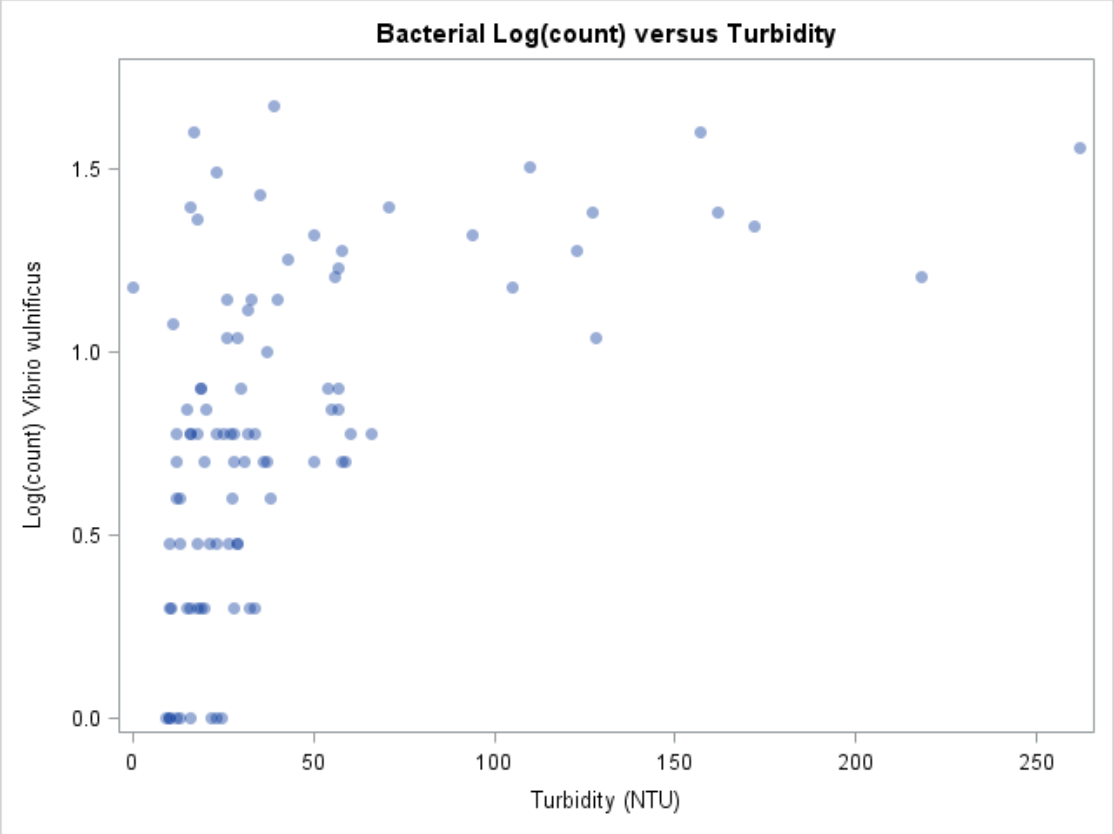

Figure 3.3: Scatter plot of *Vibrio vulnificus* logcount versus Turbidity (NTU).

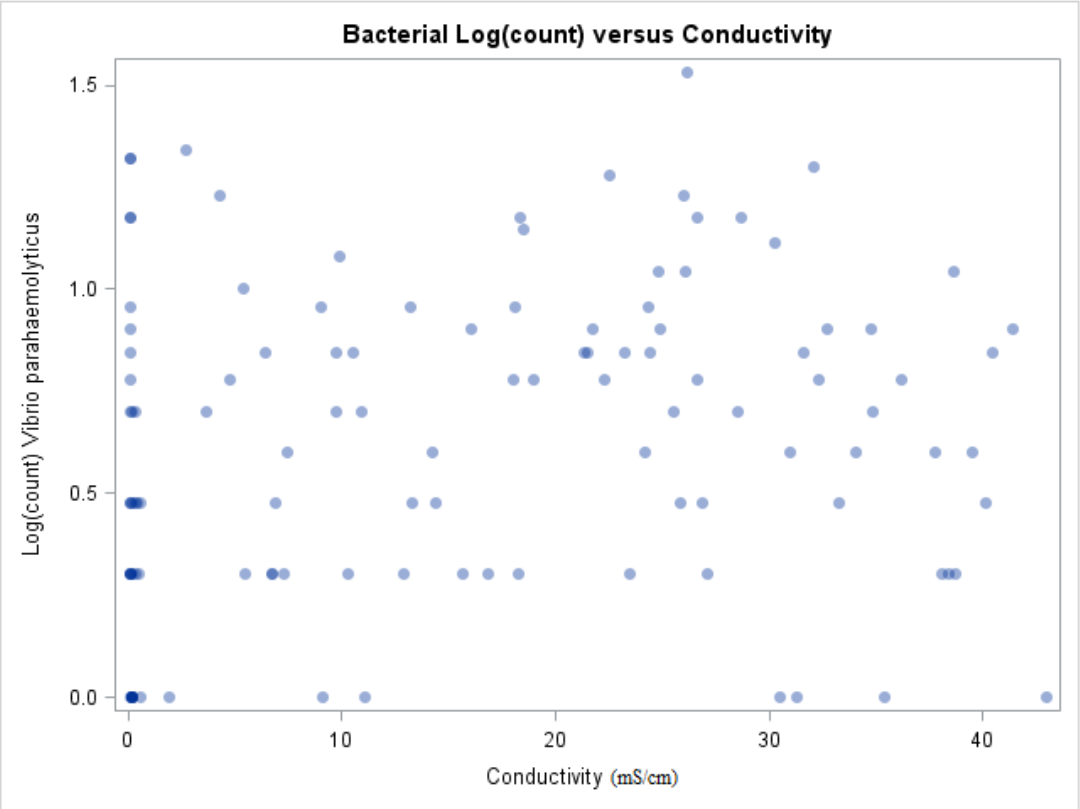

Figure 3.4: Scatter plot of *Vibrio parahaemolyticus* logcount versus Conductivity (mS/cm).

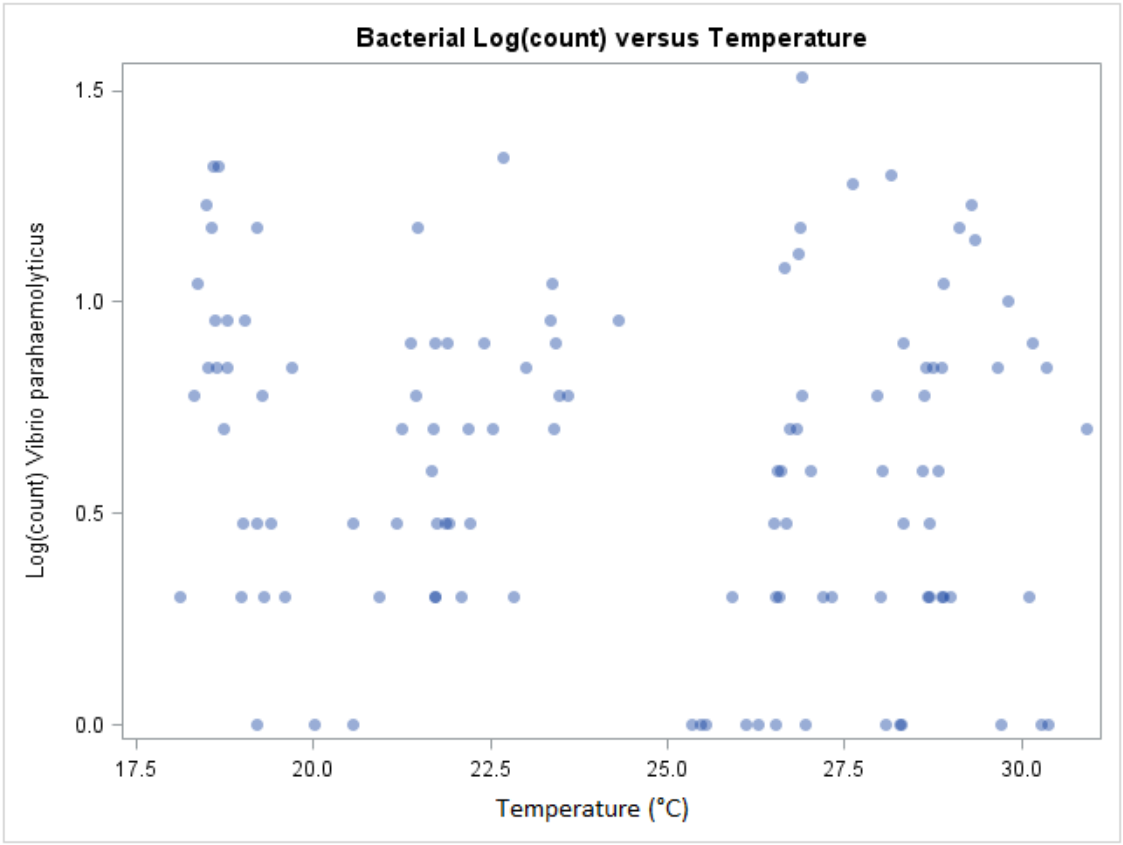

Figure 3.5: Scatter plot of *Vibrio parahaemolyticus* logcount versus Temperature (°C).

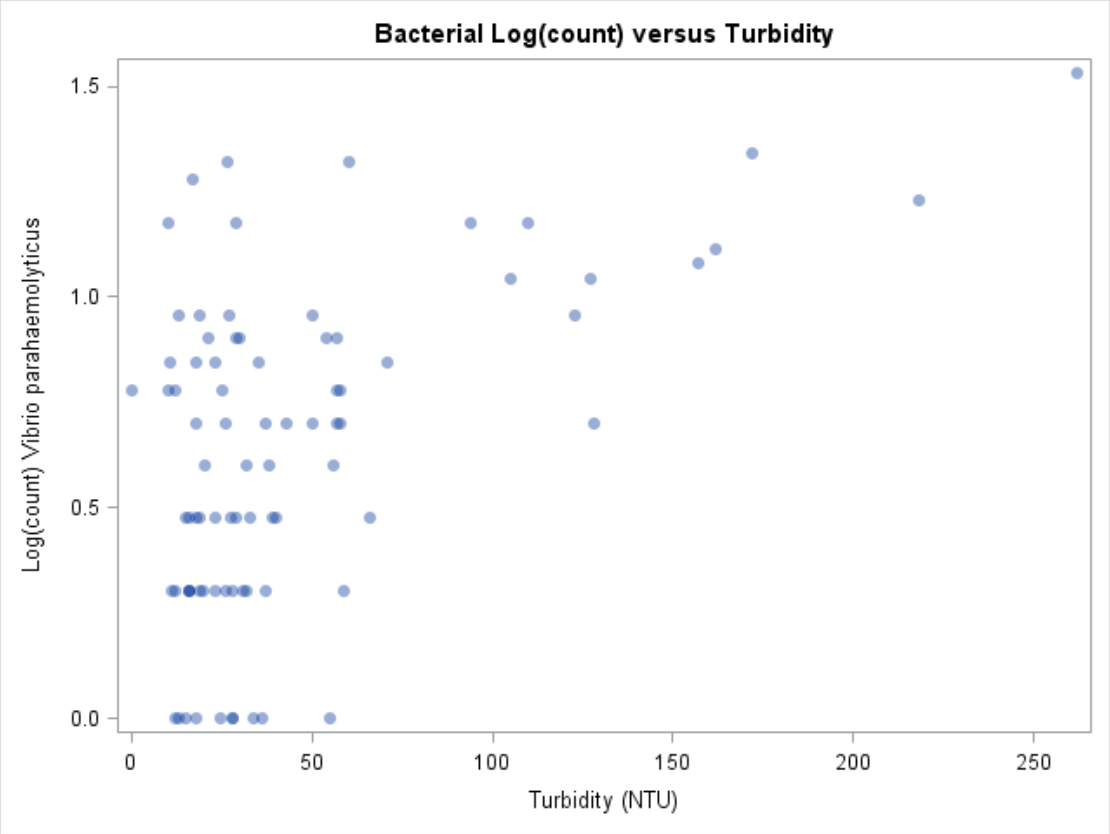

Figure 3.6: Scatter plot of *Vibrio parahaemolyticus* logcount versus Turbidity (NTU).

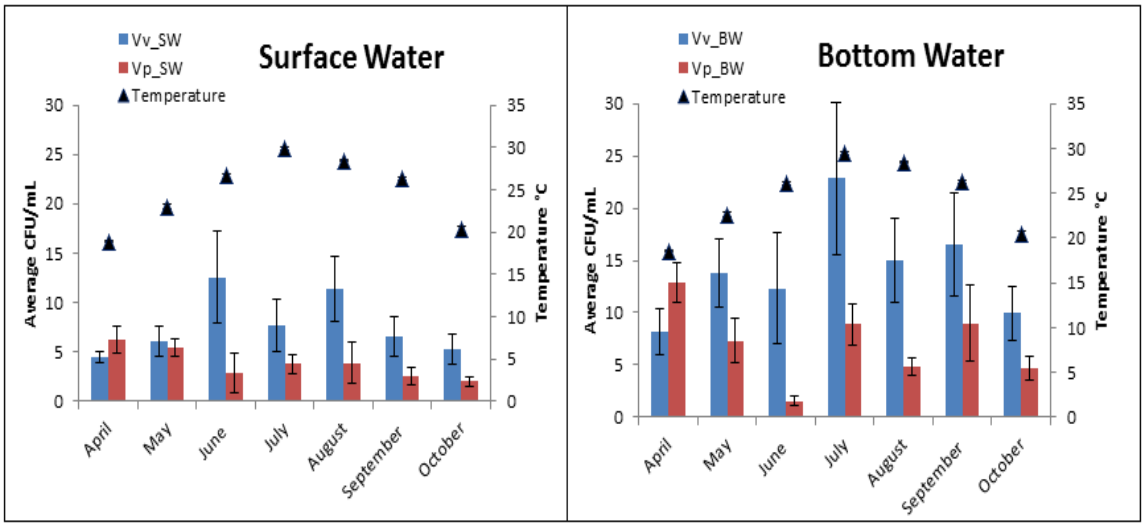

Figure 3.7: *Vibrio vulnificus* and *Vibrio parahaemolyticus* surface and bottom water average counts in relation to the average temperature with the standard error.

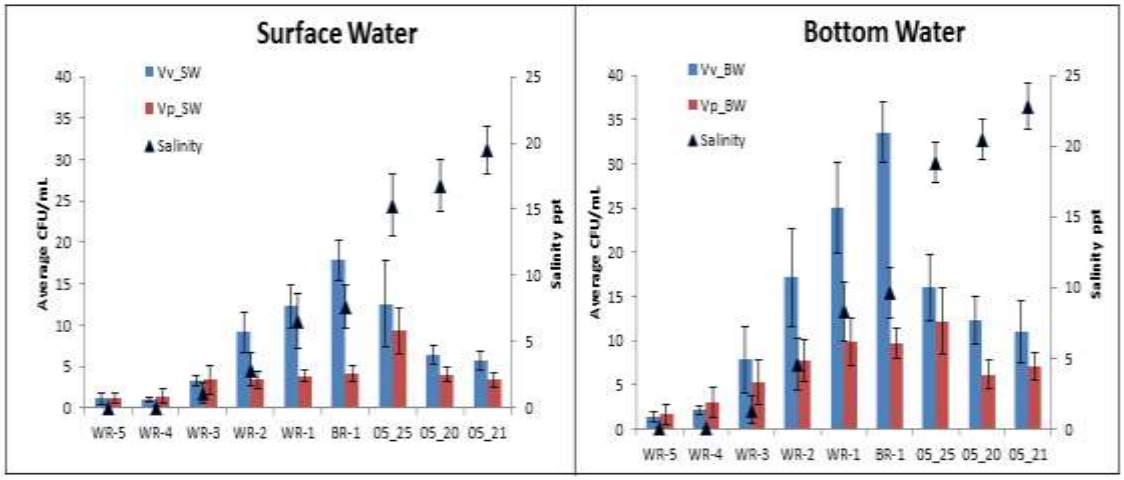

Figure 3.8: *Vibrio vulnificus* and *Vibrio parahaemolyticus* surface and bottom water average counts in relation to the average salinity with the standard error.

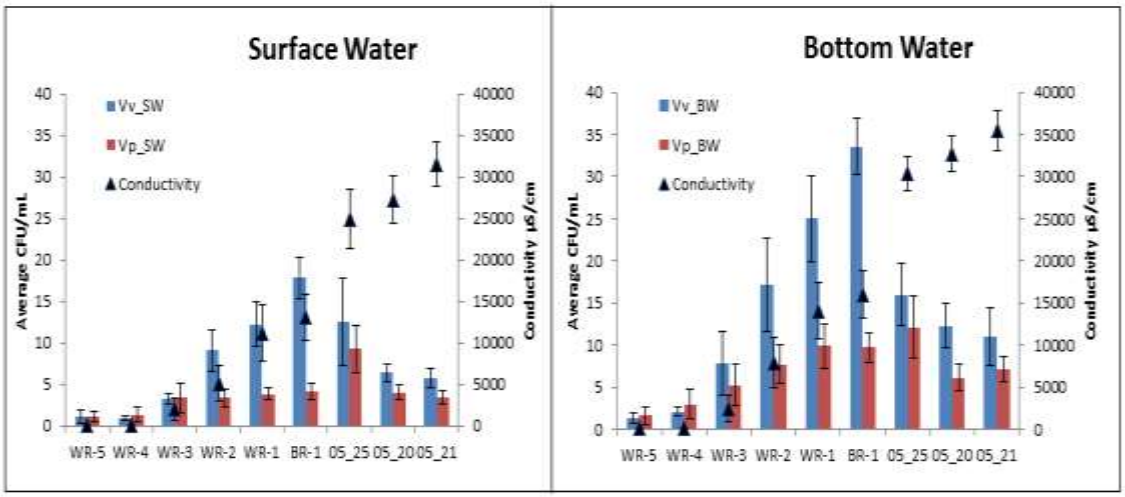

Figure 3.9: *Vibrio vulnificus* and *Vibrio parahaemolyticus* surface and bottom water average counts in relation to the average conductivity with the standard error.

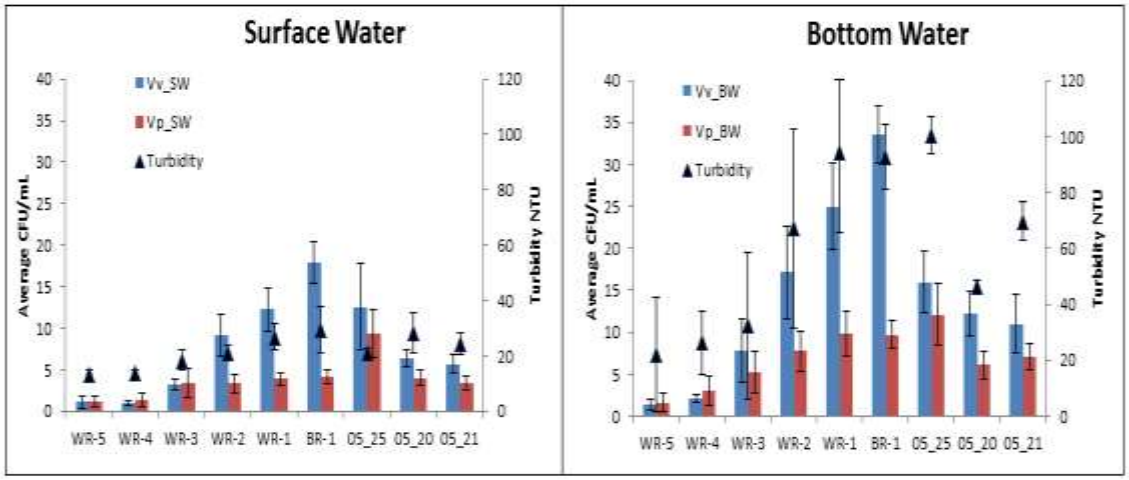

Figure 3.10: *Vibrio vulnificus* and *Vibrio parahaemolyticus* surface and bottom water average counts in relation to the average turbidity with the standard error.

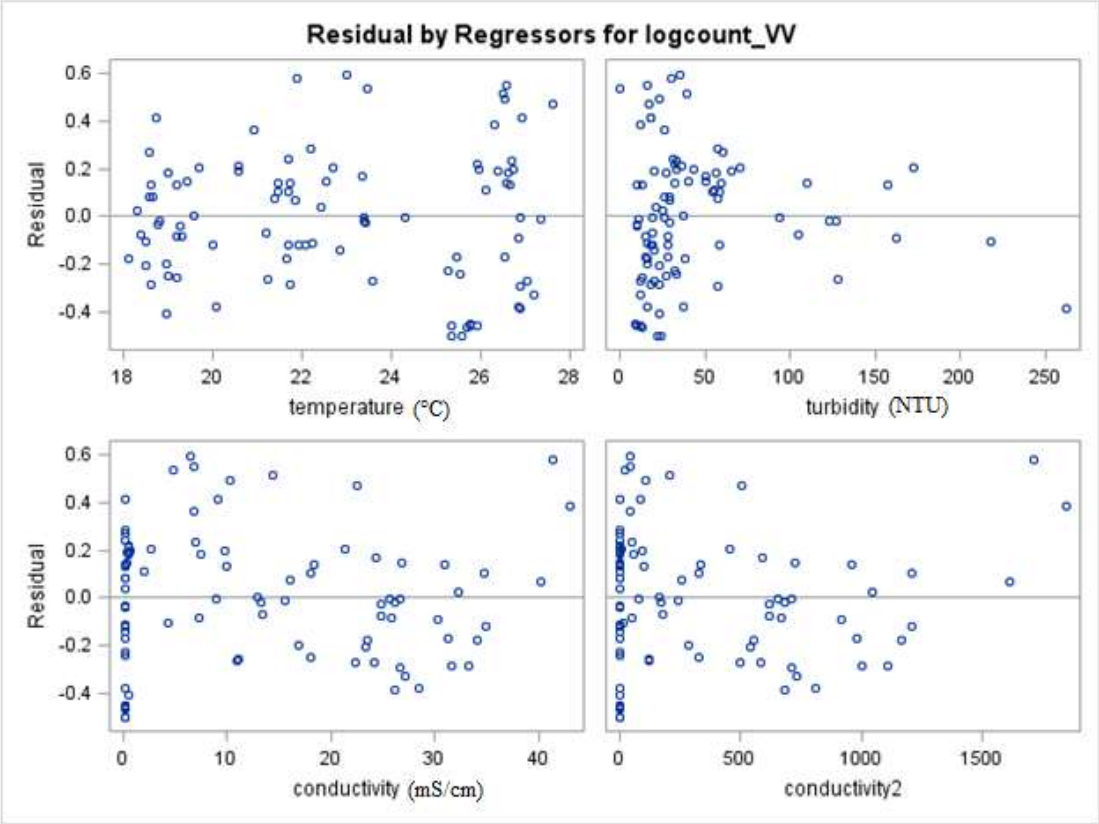

Figure 3.11: Temperature, Turbidity, Conductivity, and Conductivity2 Residuals by Regressors for logcount_Vv.

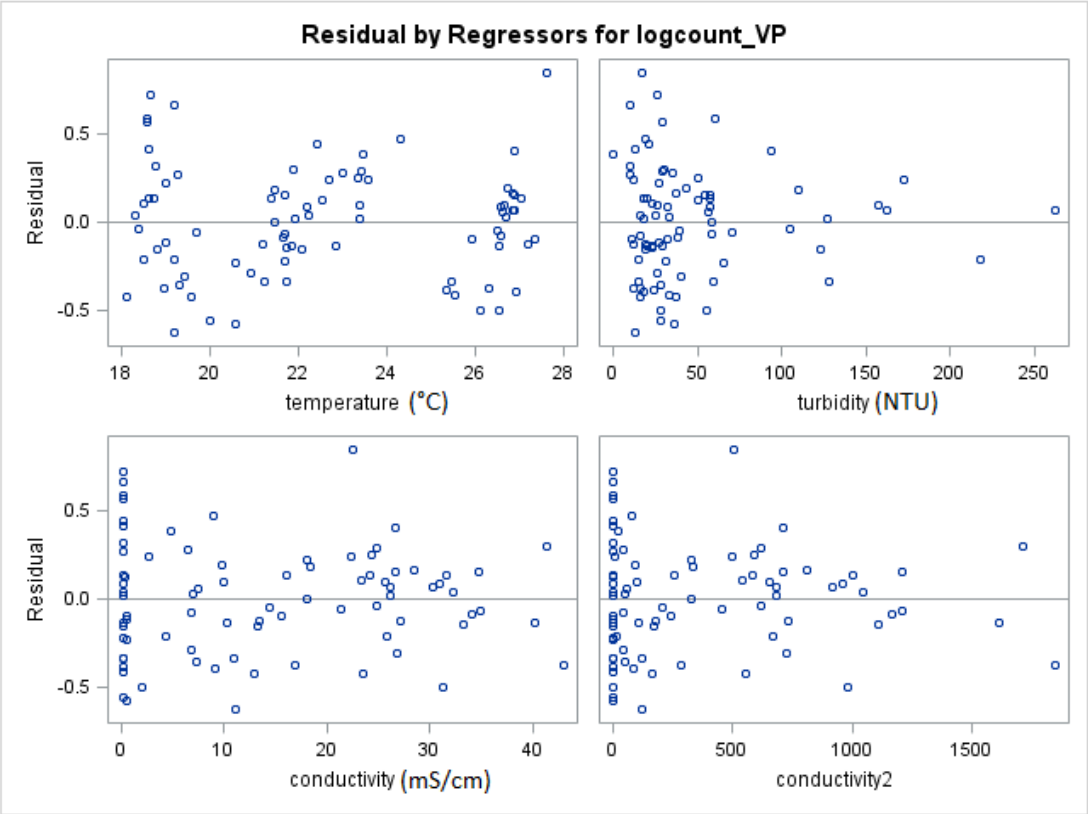

Figure 3.12: Temperature, Turbidity, Conductivity, and Conductivity2 Residuals by Regressors for logcount_Vp.

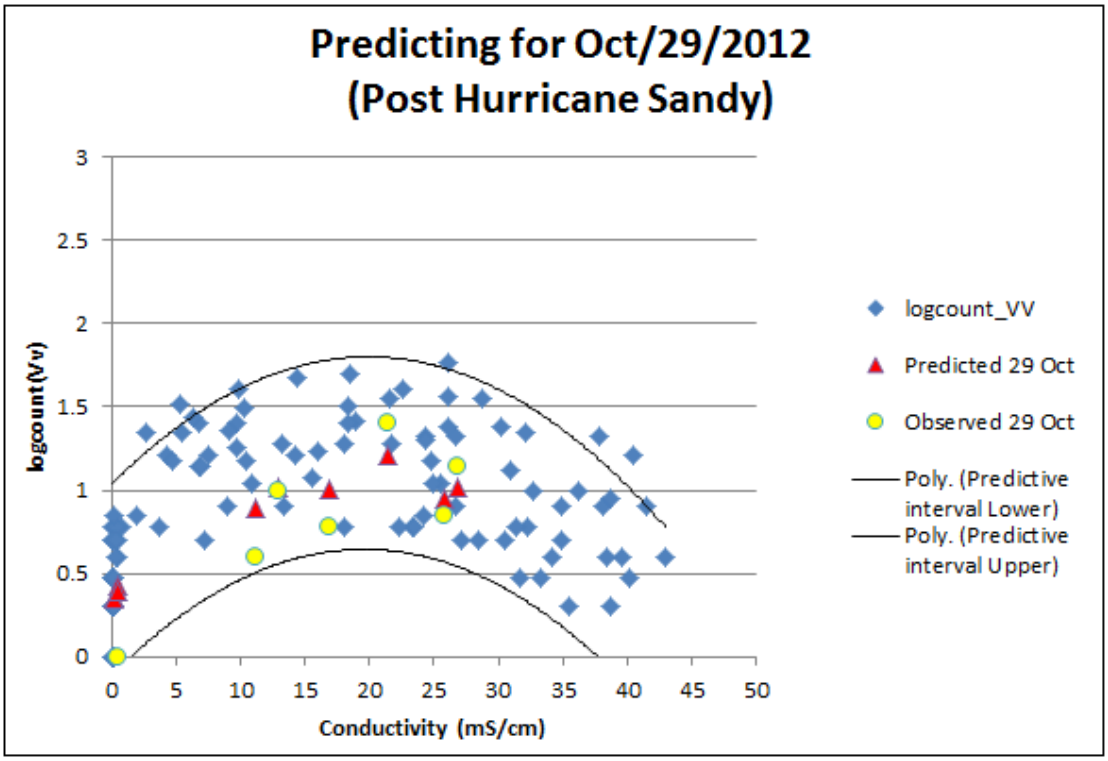

Figure 3.13: Model prediction for October/29/2012

CHAPTER 4:

SPECIFIC CONDUCTANCE LEVELS IN WACCAMAW RIVER: HISTORIC AND PROJECTED (1995-2069)

The Pee Dee River and Atlantic Intracoastal Waterway Salinity Model intrusion (PRISM2) (Conrads et al., 2013) was used to evaluate the impact of climate change on salinity intrusion. PRISM2 is a "decision support system" (DSS) designed for use by water managers as an aid for adaptation planning related to salinity intrusion along the Waccamaw River and Intracoastal Waterway. The field portion of this study collected samples at three of the gages in the Waccamaw River that are included in the PRISM2 model. The PRISM2 model predicts specific conductance at those three gage locations under user-defined scenarios of sea level, tide stage, and freshwater inflow. The existence of the PRISM2 model provided the opportunity to couple its specific conductance prediction capability with the relationships observed with the field data. The specific-conductance models for the three gages of our interest (809, 8125 and, 815) in Waccamaw River/Winyah Bay had R^2 between 0.87 and 0.96 (coefficient of determination) and PME (percent model error) between 2.1 and 7.0 % (Conrads and Roehl, 2007). For a complex tidal system like the Waccamaw River, the statistical accuracy and predictive capability of the model are satisfactory.

The predicted specific conductance was generated by PRISM2 using streamflow predictions for the period 2055-2069 and sea level rise estimates of 0.0ft (current conditions projection), 1.0ft, 2.0ft, and 3.0ft. The streamflow predictions were made

using precipitation and temperature predictions made by the ECHO coupled ocean-atmosphere general circulation model (GCM). The GCM does not incorporate tropical storms and hurricanes in its stimulation, which limits the ability to predict the effect of super storms and hurricanes on salinity intrusions. The predicted conductivity levels made by PRISM2 coupled with the field data enabled us to predict *Vibrio* occurrence according to the significant water quality relationships derived for Waccamaw River/Winyah Bay, discussed earlier.

In order to evaluate the conductivity level in the Waccamaw River, the specific conductance of the three gages 809, 8125, and 815 with respect to the different sea-level rise scenarios were plotted. The historic and the predicted specific conductance levels allowed us to project the possible occurrence of *Vibrio* bacteria in such environments using the relationship between specific conductance and *Vibrio* concentration observed in the field data collected during 2012.

4.1 Specific Conductance Historic Records (1995-2009)

Gage 809 specific conductance records cover 1995 until 2009, at 0.0ft- 1.0ft- 2.0ft- 3.0ft sea-level rise scenarios are illustrated in Figure 4.1. The specific conductance did not exceed 4000µS/cm in all of the scenarios. However, the graphs show a noticeable increase in the number and the duration of specific conductivity spikes is observed as sea-level rise increase.

The exceedance graph (Figure 4.2) of the specific conductance of gage 809 clearly shows the increase in the specific conductance in coherence with the increase in mean sea-level among the different scenarios. The difference in the specific conductance

among the sea-level rise scenarios is higher when the specific conductance is greater than 500µS/cm. Thus, the impact of sea level rise is more evident when specific conductance is higher than 500µS/cm. The 2ft, 2.5ft, and 3ft sea-level rise scenarios show no variation at all for conductivity levels above 3700µS/cm. At 0.0ft (current conditions), 1% of the time the specific conductance was 500µS/cm or more, while at the 3.0ft sea-level rise scenario, 1% of the time the specific conductance was 3750µS/cm or more. The other scenarios fall between those two values, increasing as the mean sea-level increase. The conductivity level of this gage is below the typical range for *Vibrio*s occurrence [9000-39000µS/cm (Kaspar and Tamplin, 1993)]; however, in the summer months when temperatures were warm enough for *Vibrio* to grow with measurable *Vibrio* colonies during our sampling, even at extremely low conductivity levels (110µS/cm). In addition, *Vibrio* can enter a viable but nonculturable state (VBNC) when environmental conditions are not favorable. When gram-negative bacteria enter a VBNC state, they no longer grow on conventional media like CHROMagar *Vibrio*, but remain intact and retain viability (Colwell et al., 1985). Thus, relying on direct viable counts obtained from CHROMagar media may underestimate actual *Vibrio* spp densities (Oliver, 2005b). However, VBNC and sediments populations were beyond the scope of our study and thus this issue was not addressed.

Gage 8125 specific conductance records from 1995 to 2009, at 0.0ft- 1.0ft- 2.0ft- 3.0ft sea-level rise scenarios are illustrated in Figure 4.3. At this gage, specific conductance range is higher than gage 809. A similar trend is observed as sea-level rise increases, specific conductance increases and reaches a maximum value of 16000µS/cm.

Specific conductance increase was proportional to the sea-level rise increase (Figure 4.4). The variation in conductivity among the different scenarios is greater when conductance is above 500µS/cm. 2.0ft, 2.5ft, 3.0ft sea-level rise scenarios did not show a great impact on conductivity when conductivity was higher than 12000µS/cm and lower than 500µS/cm. At 0.0ft, 1 % of the time the specific conductance was 8000µS/cm or more. At 3.0ft sea-level rise scenario, 1 % of the time the specific conductance was 12000µS/cm or more. The difference between the current condition and the highest sea-level rise scenario was not as significant as the other gages. In general, the increase in specific conductance was slow but steady along the sea-level rise scenarios. Despite the moderate increase in conductivity with sea-level rise scenarios, this gage still represents a potential threat for the growth of *Vibrio* since at 0.0ft 1 % of the time the specific conductance falls in *Vibrio*'s viability range. The sea-level rise scenarios strengthen *Vibrio*'s occurrence and abundance by a moderate increase in conductivity. In addition, in our sampling *V. vulnificus* and *V. parahaemolyticus* were collected at gage 8125 (WR-4) at conductivity levels ranging between 110 and 473µS/cm.

Gage 815 specific conductance records from 1995 till 2009 at 0.0ft- 1.0ft- 2.0ft- 3.0ft sea-level rise scenarios are illustrated in Figure 4.5. Gage 815 had the highest range of specific conductance among the three gages. This reflects its geographical location in the river that is more susceptible toward tidal forces effects. At the current conditions the gage 815 recorded the highest specific conductance values in comparison to the other gages. The period 1995-2009 includes a wide range of flows, including the high flows of the El Niño in 1998 to the low flows of the extended drought from 1998 to 2002 (Conrads and Roehl, 2007). Examining the graph, on the upper left of figure 4.5, which

represents the historical data, it shows that during periods of high flows (1998) the specific conductance was generally low. While periods of low flow (streamflow less than 5,000 ft3/s) (Conrads and Roehl, 2007), specific-conductance recorded high values manifesting periods of salinity intrusions. Sea-level rise once again caused an increase in the specific conductance, recording 35000µS/cm as the highest value.

Gage 815 represents the gage with the highest conductivity in all scenarios (Figure 4.6). At 0.0ft, 1% of the time the specific conductance is 15000µS/cm or more, while at 3.0ft scenario 1% of the time specific conductance is 31000µS/cm or more. The chart shows a gradual increase in conductivity from the 0.0ft scenario to the 3.0ft scenario. By recording the highest conductivity levels gage 815 manifests the highest potential threat for *Vibrio*s abundance. For instance at 3.0ft sea-level rise scenario 10% of the times specific conductance was 23000µS/cm or more, which is within the optimal conductivity level for *Vibrio* to grow as mentioned before.

PRISM2 estimates for specific conductance at gages 809, 8125, 815 were run for 0.0ft, 1.0ft, 2.0ft, and 3.0ft sea-level rise scenarios plus streamflow reduction by 5, 10, 15, 20, and 25 % from the Pee Dee River. Streamflow reductions could be caused by environmental factors (e.g. climate change, droughts, and precipitation trends) and/or human activities (e.g. irrigation, ground water pumping, and dams).

The reduced flow scenarios for the recorded conditions in all of the three gages did not cause a great variation in the specific conductance (Figure 4.7). However, consistent slight increase in conductivity was observed as the percentage of reduction in stream flow increased. The three gages did not show a significant variation in

conductivity in relation to streamflow reduction which indicates that at these sites the tide is more dominant in determining the specific conductance. This may underscore the importance of drought which would reduce stream flows and allow enhancement of this tidal effect.

The combined effect of sea-level rise and streamflow reductions also can be viewed (Figures 4.8 and 4.9). The magnitude of effect on specific conductance of sea-level rise is larger than for streamflow reductions at all of the gages under all scenarios. This is the expected outcome based on the results of the individual effects analysis presented earlier. Streamflow reductions and sea-level rise are both potential impacts of climate change that can affect the frequency and longevity of saltwater intrusion events. However, referring to the historical data for Waccamaw River shows that sea-level rise has a more substantial effect on conductivity, which can potentially affect *Vibrio*s abundance, than streamflow reduction.

4.2 Projected Specific Conductance over 2055-2069

Gage 809 projected specific conductance from 2055 till 2068 at 0.0ft- 1.0ft- 2.0ft- 3.0ft sea-level rise scenarios are illustrated in Figure 4.10. In comparison to Figure 4.1, the future trends show an increase in the number of peaks in all the scenarios. The specific conductance upper limit of the gage 809 is still under 4000µS/cm but the fact that *Vibrio* spp were found at lower conductivities (Figure 3.1 and 3.4) during favorable temperature indicates that such levels present a potential threat from *Vibrio* exposure.

The exceedance graph for the predicted specific conductance for gage 809 (Figure 4.11); shows once again an increase in conductivity with the increase in sea-level rise.

For current conditions, 1 % of the time conductivity is 500µS/cm or more while a 1.0ft sea-level rise makes the conductivity 1 % of the time to be 2000µS/cm or more. At 2.0ft and 3.0ft sea-level rise levels, 1% of the time conductivity 3700µS/cm or more.

The predicted future specific conductance at gage 8125 (Figure 4.12) shows that even with no sea-level rise, the number of peaks of specific conductance shows an increase over the historic period (Figure 4.3). The number of spikes and their duration increases as the sea-level rise increases.

The estimated specific conductance for current conditions shows a decrease in specific conductance level, where 1 % of the time conductivity is 6000µS/cm or more, while it was 8000µS/cm or more between 1995 and 2009 (Figure 4.13) this decrease does not indicate that specific conductance will not record high values of conductivity. This decrease would not result in a decrease of *Vibrio* spp in the area, since *Vibrios* were collected at even lower conductivities. However, it might cause *Vibrio* spp to enter the VBNC state. Under 1.0ft sea-level rise, specific conductance is expected to be 11,000µS/cm or more 1 % of the time. As the sea-level rise scenarios increase conductivity continues to increase into the optimal range of *Vibrio*'s growth and viability.

The predicted future specific conductance at Gage 815 (Figure 4.14) shows a higher number of spikes in all of the scenarios in comparison to the historical records and scenarios in Figure 4.5. As mentioned before Gage 815 is within *Vibrio*'s optimal range of conductivity, thus an increase in the number of spikes in such levels indicates an increase in the probability of *Vibrio*s occurrence and abundance, especially during the warm months.

Under current conditions, conductivity 1 % of the time is around 13000µS/cm or more (Figure 4.15). In relation to the field data the highest counts of *V. vulnificus* were observed when conductivity was between 10000µS/cm and 30000µS/cm. Thus, gage 815 without any sea-level rise assumptions represents a favorable niche for *V. vulnificus*. The estimated sea-level rise scenarios increased conductivity levels to *Vibrio*'s optimal range 1 % of the time. The estimated specific conductivity at 3.0ft sea-level rise scenario was 10 % of the time around 11500µS/cm or more. Thus, 10 % of the time conductivity will be in *Vibrio*'s range of viability. This represents a 10 fold increase in optimal *Virbio* growth conditions under this future sea level rise scenario.

Gage 809 is not expected to have any peaks within *V. vulnificus* optimal range, under any of the future scenarios. While gage 8125 showed an increase in the number of peaks upon the increase in mean sea level rise, the sea level rise scenarios showed that the peaks are commonly occurring between June and November. At 3.0ft sea level rise a peak is anticipated to occur in March 2065, which is out of *Vibrio's* optimum occurrence in the site. In addition, gage 815 specific conductivity future estimates showed that the longest peaks (up to 29days) within *V. vulnificus* optimal range are in October and November. Once again sea level rise was able to induce peaks of high specific in months that are typically out of *V. vulnificus* optimum abundance, at 3.0ft sea level rise two peaks are anticipated in January 2056 and March 2065.

The fact that *V. vulnificus* and *V. parahaemolyticus* were found at stations WR-5, WR-4, and WR-3 or gages 809, 8125, 815, respectively, shows the upriver movement of this organism. The distance between the shellfish stations which are at the closest proximity to the ocean and the furthest station (WR-5) is around 37 Km. According to the

field data conductivity levels were lower in stations WR-5, WR-4, and WR-3 in comparison to the other stations. However, *Vibrio* spp were still found at those conditions (Figure 3.9). This indicates that an increase in conductivity in the upper stations, where conductivities are typically lower than the growth range of *Vibrio*, may induce growth of *Vibrio* populations. In the shellfish stations conductivity was within *Vibrio*'s optimal range, thus it is not a limiting factor for its growth and the lower numbers observed are probably caused by other factors.

4.3 *VIBRIO VULNIFICUS* LOGCOUNT PREDICTIONS ACCORDING TO THE ESTIMATED PREDICTED SPECIFIC CONDUCTIVITY

The linear model developed to predict *Vibrio* spp., replaces conductivity values by a quadratic polynomial to model the effect of conductivity on *Vibrio* distribution. The model explained 62.47% of *V. vulnificus* and 34.25% of *V. parahaemolyticus* distribution. Due to the low percentage of explanatory for *V. parahaemolyticus,* we only predicted for *V. vulnificus* based on the future conductivity calculated by PRISM2. The predicted counts are computed based on the relation found between *V. vulnificus*, conductivity, temperature, and turbidity from the field data. The predicted *V. vulnificus* concentration at WR-5, WR-4, and WR-3 was based on the estimated predicted conductivity with temperature and turbidity values fixed on their average computed from the field data. Thus, our projection has a number of limitations: the models' errors, the inconsideration of temporal changes, the weak association with temperature due to the narrow water temperature range obtained during the sampling, and finally the lack of identification of virulent *Vibrio* species. However, the model shows how *V. vulnificus*

reacts to the variation of conductivity. Those predicted counts represent the expected trends of *V. vulnificus* concentrations according to the different sea-level scenarios.

Under the current conditions, station WR-5 predictions for log *V. vulnificus* show that years 2056, 2059, and 2066 are expecting higher concentrations of *V. vulnificus* in comparison to the other years (Figure 4.16) as the frequency of optimum growth conditions increased significantly based on model predictions. However, in terms of maximum densities or counts (anti-log), the predicted counts of *V. vulnificus* for the years 2055 till 2066 are similar to the counts quantified in our study and no substantial increase overall *Vibrio* abundances are expected. The sea-level rise scenarios show an increase in the number of peaks of *Vibrio* occurrence but not an increase in the maximum *V. vulnificus* densities per se. This indicates that *V. vulnificus* population presence is going to be more persistent in case of sea-level rise.

Station WR-4 in comparison to station WR-5 shows a higher number of peaks and longer durations in all scenarios (Figure 4.17). The expected (anti-log) counts are also relatively higher than station WR-5 and the recorded counts in our data. As sea-level rise scenarios increase the persistence of *V. vulnificus* in the water increases. The rise in sea-level by 3.0ft shows that *V. vulnificus* may occur over all the 14 years (2055-2068).

The WR-3 trends for *V. vulnificus* show an increase in persistence along with the increase of sea-level rise (Figure 4.18). Station WR-3 shows that the predicted *V. vulnificus* counts are the highest among the three stations. The duration of *Vibrio* occurrence is expected to be longer. As sea-level rise scenarios increase the number of peaks increases and the model projects that the bacteria will persist longer.

The model predictions provided us with the expected *V. vulnificus* trends at WR-5, WR-4, and WR-3 according to the specific conductivity variations that are anticipated to occur. It also showed the potential climate change effects on conductivity and consequently on *V. vulnificus* trends. If we compare the future estimated conductivity trends with *V. vulnificus* predicted trends, there is a noticeable increase in conductivity peaks due to sea-level rise scenarios that tracks the increase in *V. vulnificus* logcount with the sea-level rise. Thus, the prediction represents climate change potential effect on specific conductivity and consequently on *V. vulnificus*. It is anticipated that climate change will enable *V. vulnificus* to further thrive at those sites. However, those predictions are not intended for public health planning because our predictions were based solely on projections of specific conductivity without having information about future temperature and turbidity as well as lacking information on *Vibrio* virulence.. Thus, in order to assess *V. vulnificus* abundance the model needs inputs about conductivity, temperature, and turbidity. However the model does provide environmental managers information on a microbe of significant public health concern and predictions of whether or not this microbe will continue to be a health concern in the future.

CHAPTER 4 TABLES AND FIGURES

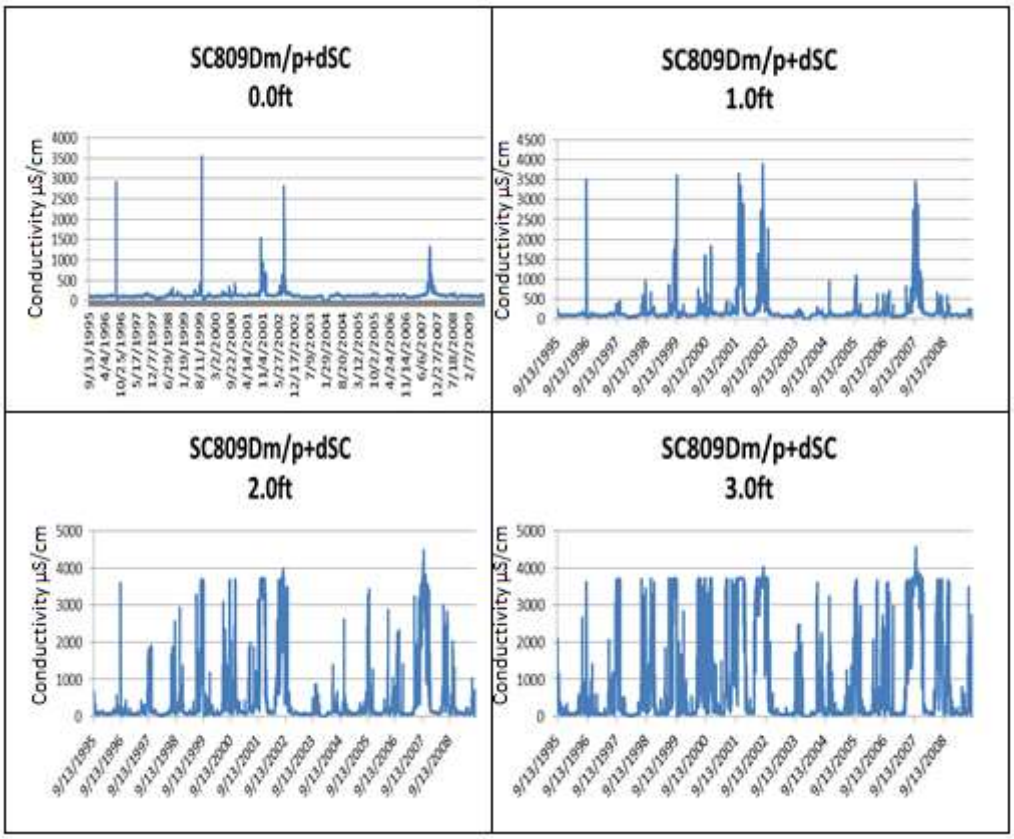

Figure 4.1: Gage 809 Specific Conductance (µS/cm) records from 1995 till 2009, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea-level Rise Scenarios. The 0.0 ft panel represents current mean sea level.

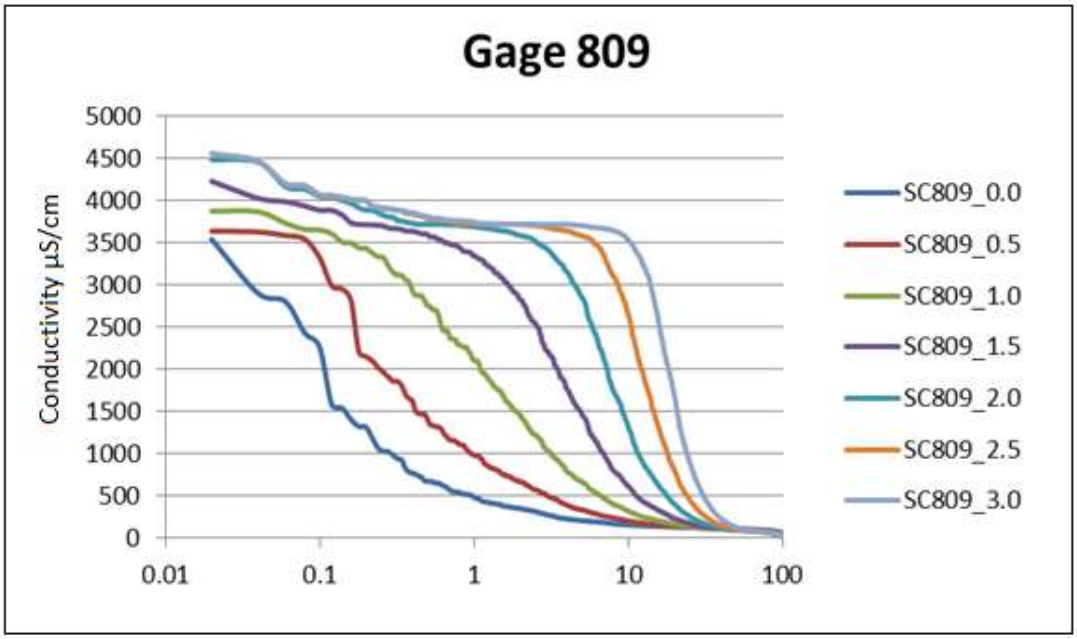

Figure 4.2: Exceedance graph of the Specific Conductance (µS/cm) of Gage 809 as mean sea varies from 0-ft (current condtions) to plus 3-ft.

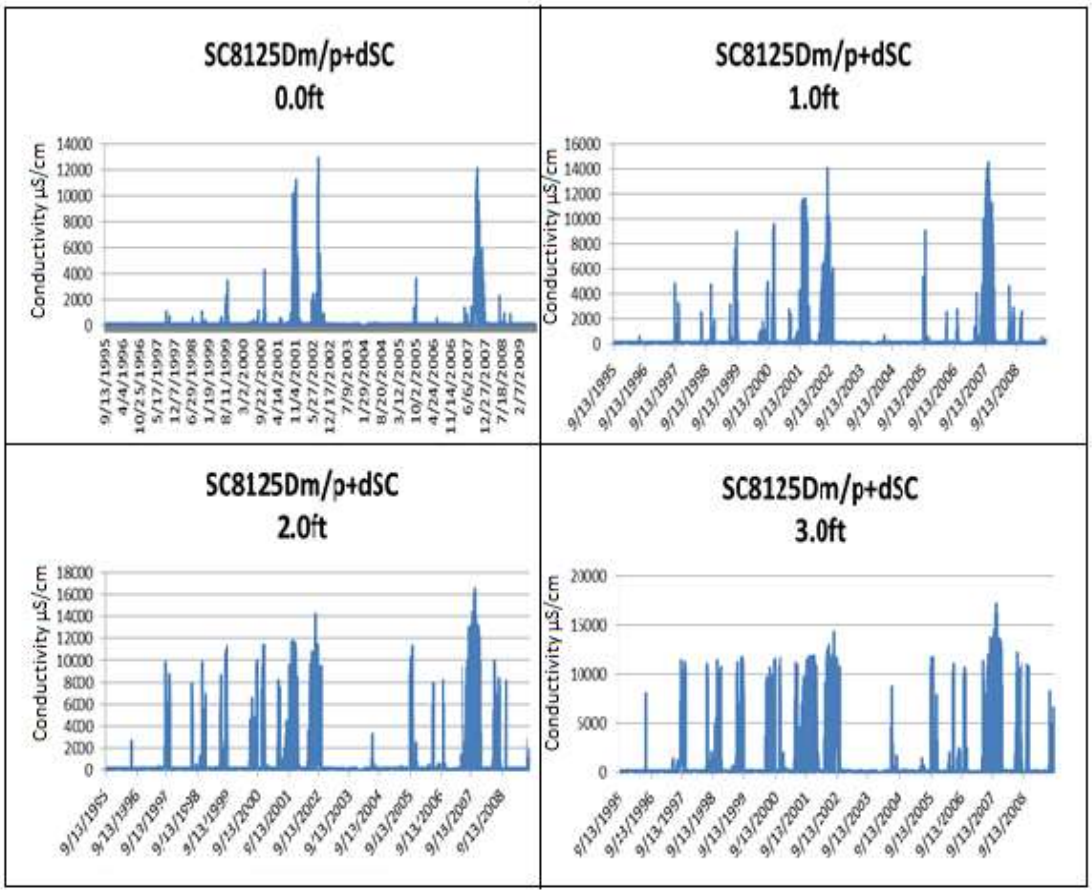

Figure 4.3: Gage 8125 Specific Conductance (µS/cm) records from 1995 till 2009, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea-level Rise Scenarios.

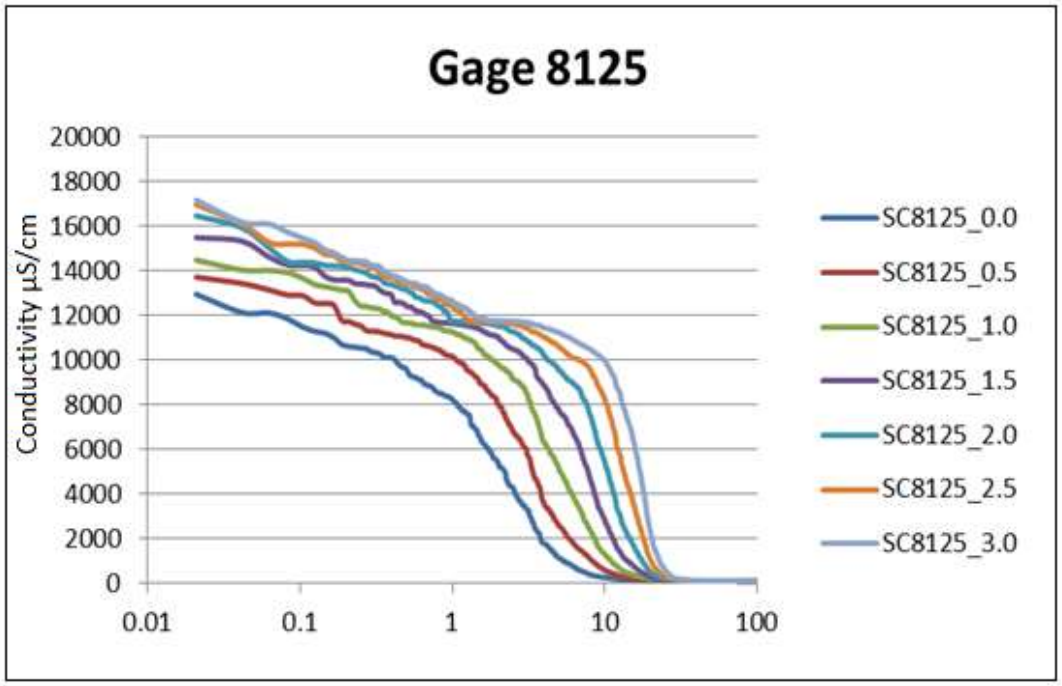

Figure 4.4: Exceedance graph of the Specific Conductance (µS/cm) of Gage 8125, as mean sea varies from 0-ft (current conditions) to plus 3-ft.

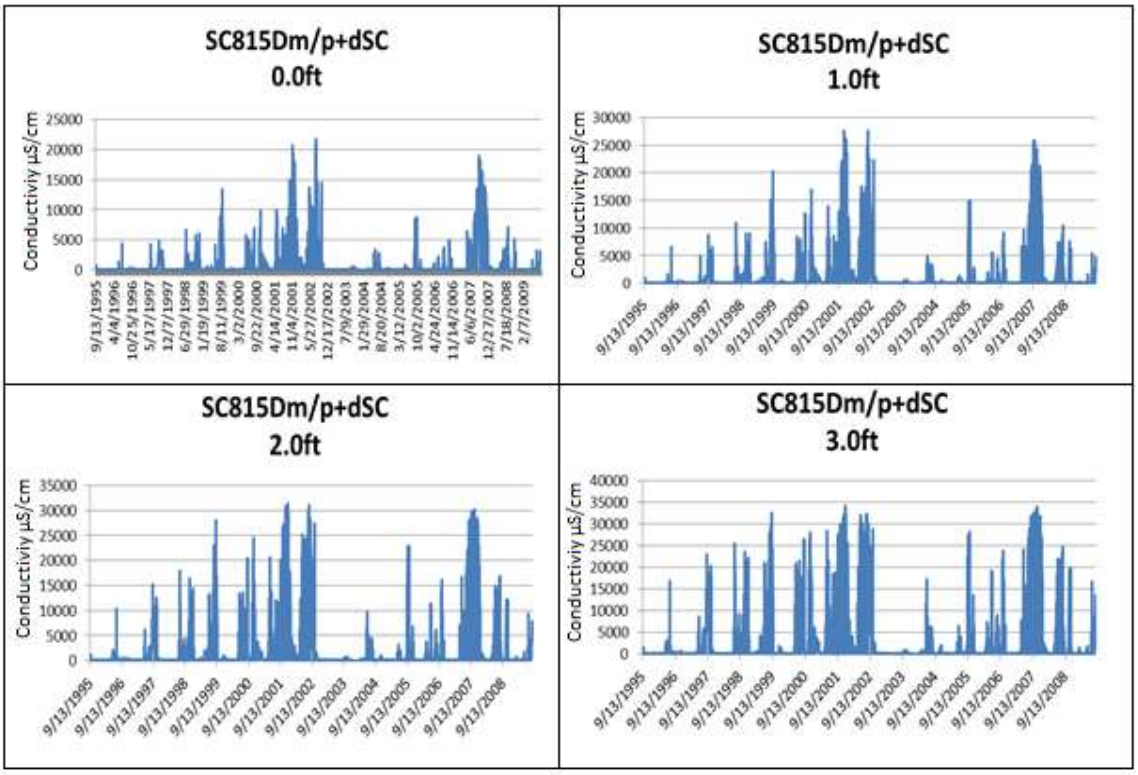

Figure 4.5: Gage 815 Specific Conductance (µS/cm) records from 1995 till 2009, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea-level Rise Scenarios.

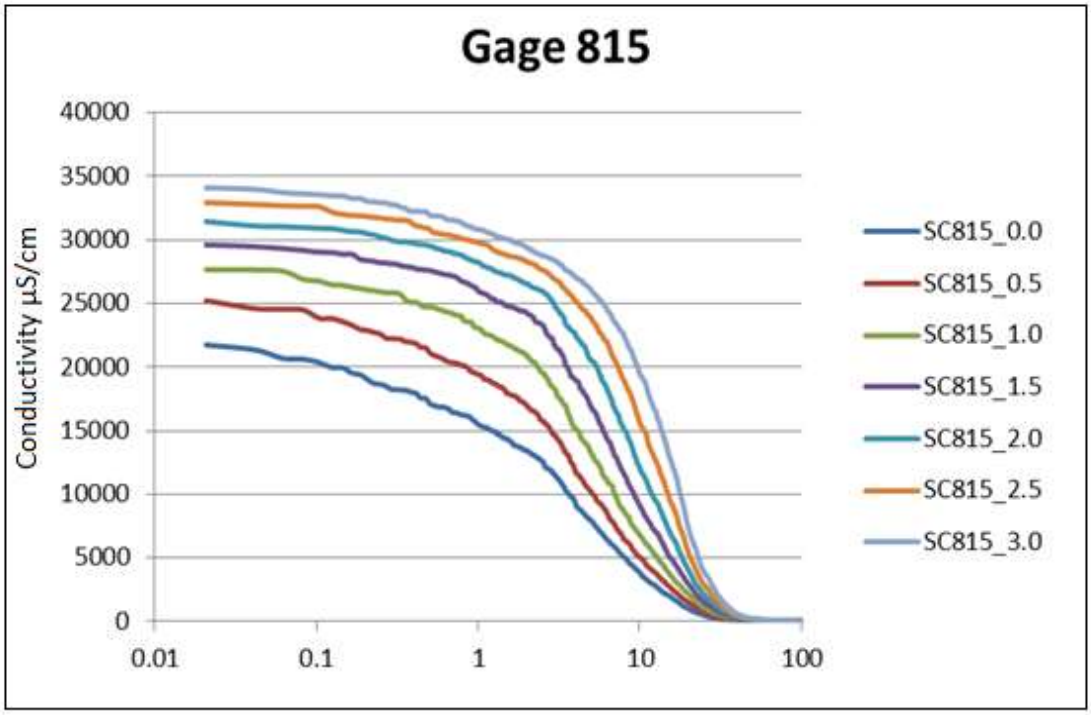

Figure 4.6: Exceedance graph of the Specific Conductance (µS/cm) of Gage 815, as mean sea varies from 0-ft (current conditions) to plus 3-ft.

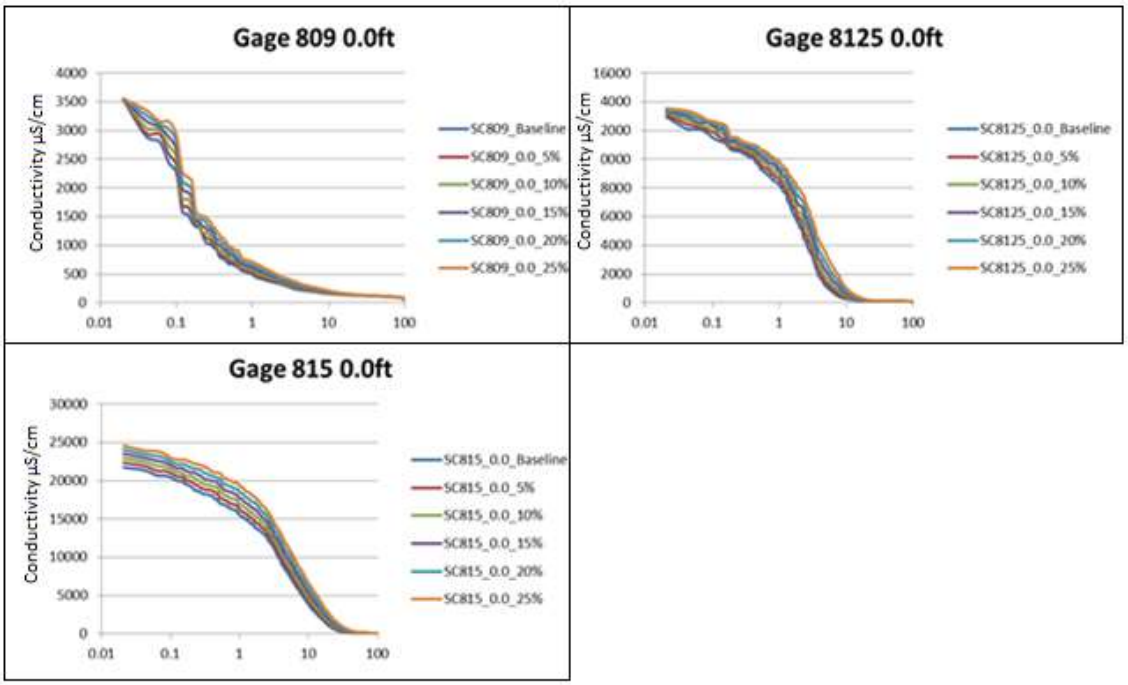

Figure 4.7: Exceedance graphs for the Reduced Flow Scenarios from 1995 till 2009 in Gages 809, 8125, and 815 (µS/cm).

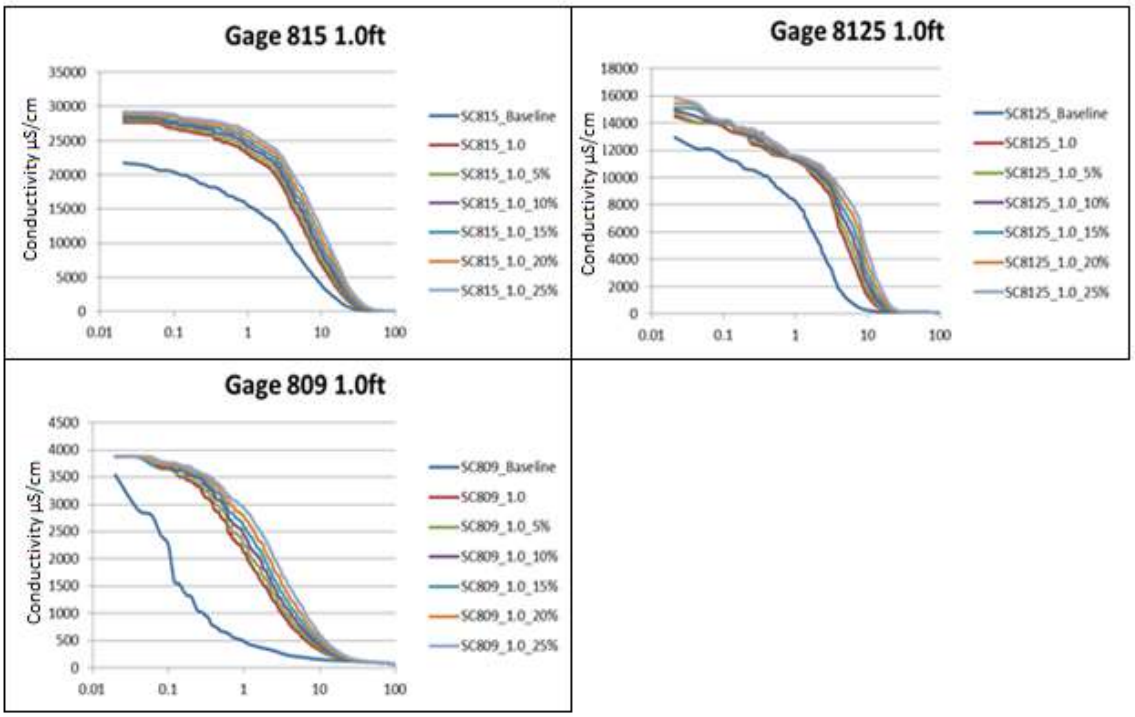

Figure 4.8: Exceedance graphs for the Reduced Flow Scenarios (µS/cm) from 1995 till 2009 at 1.0ft Sea-level Rise Scenario in Gages 809, 8125, and 815. Baseline is current conditions.

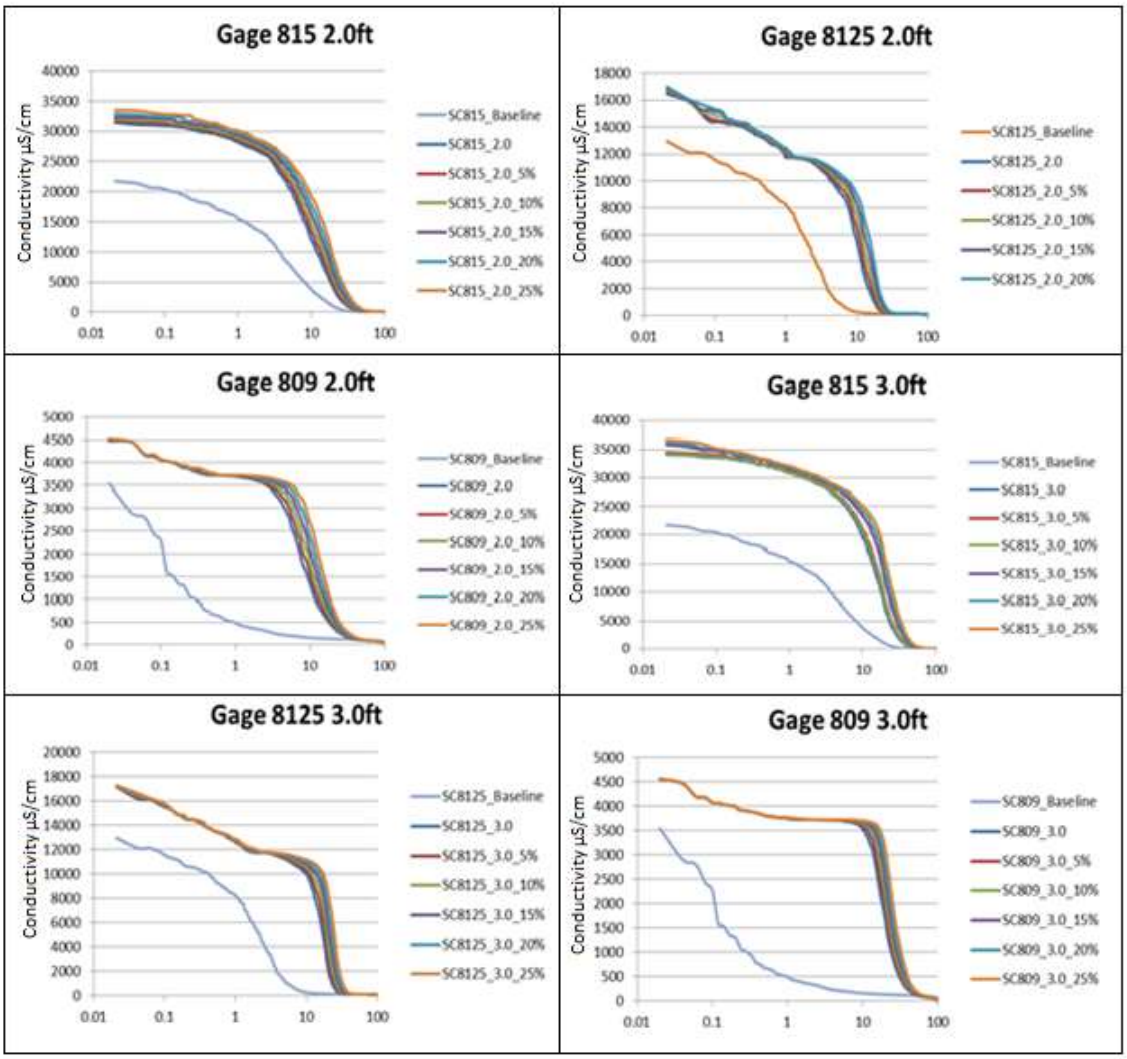

Figure 4.9: Exceedance graphs for the Reduced Flow Scenarios (µS/cm) from 1995 till 2009 at 2.0ft and 3.0ft Sea-level Rise Scenarios in Gages 809, 8125, and 815. Baseline is current conditions.

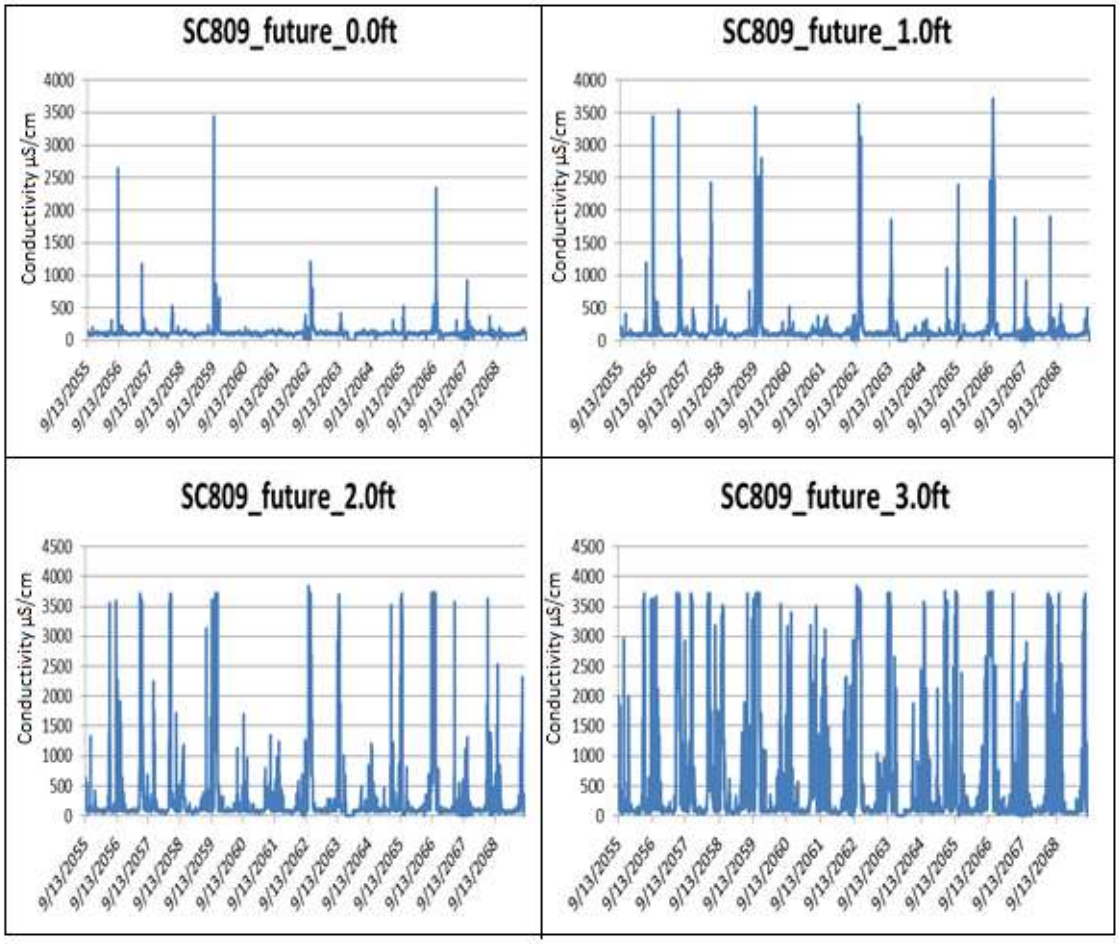

Figure 4.10: Gage 809 predicted Specific Conductance (µS/cm) for years 2055-2068, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea- level Rise Scenarios.

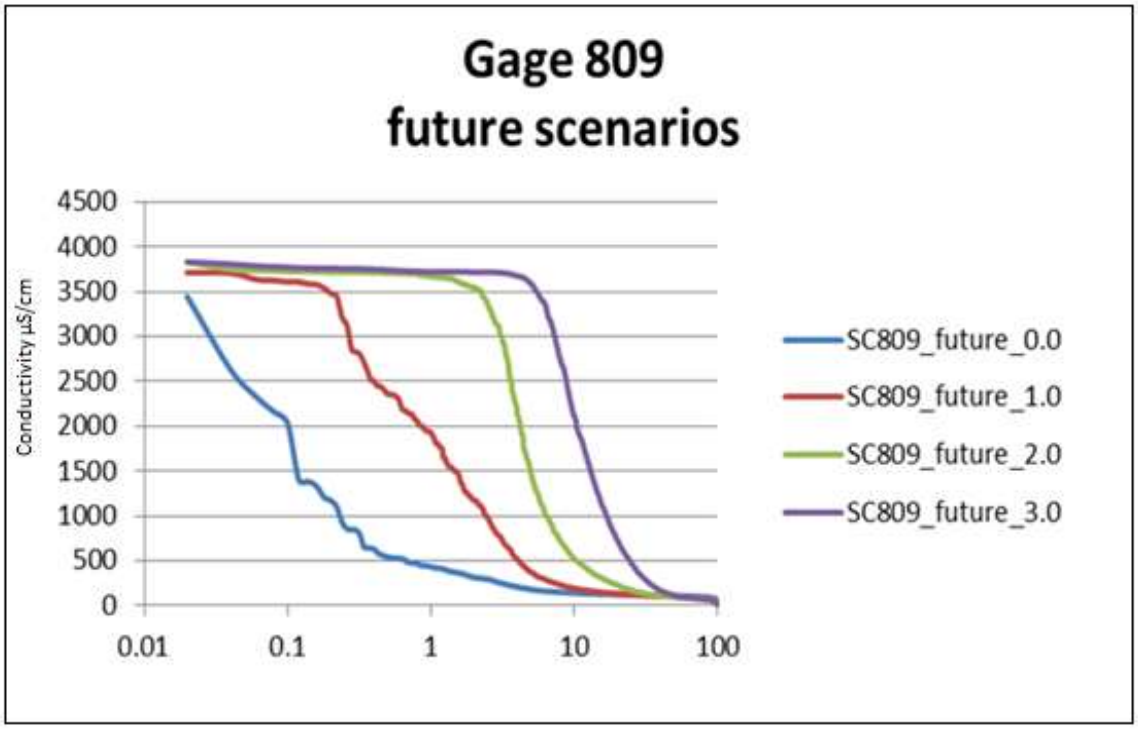

Figure 4.11: Exceedance graph of the predicted specific conductance (µS/cm) for Gage 809.

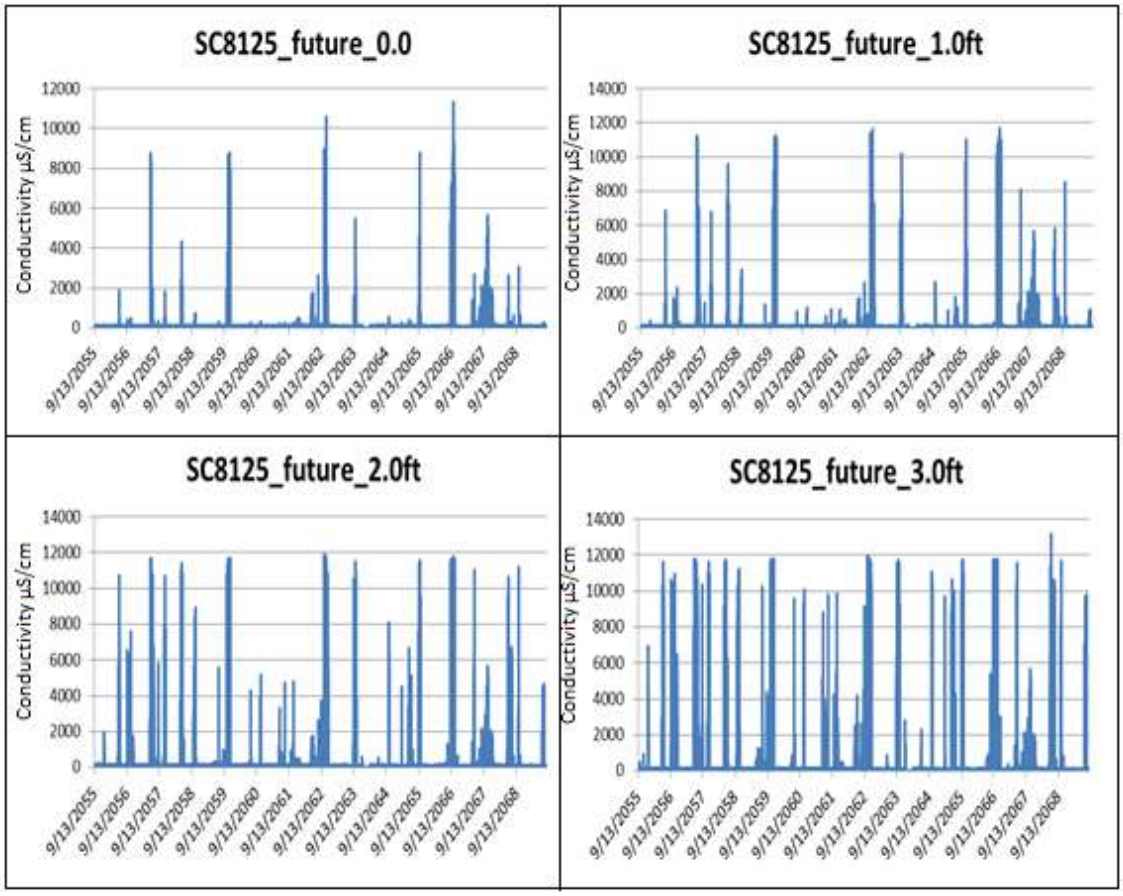

Figure 4.12: Gage 8125 predicted Specific Conductance (µS/cm) for years 2055-2068, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea-level Rise Scenarios.

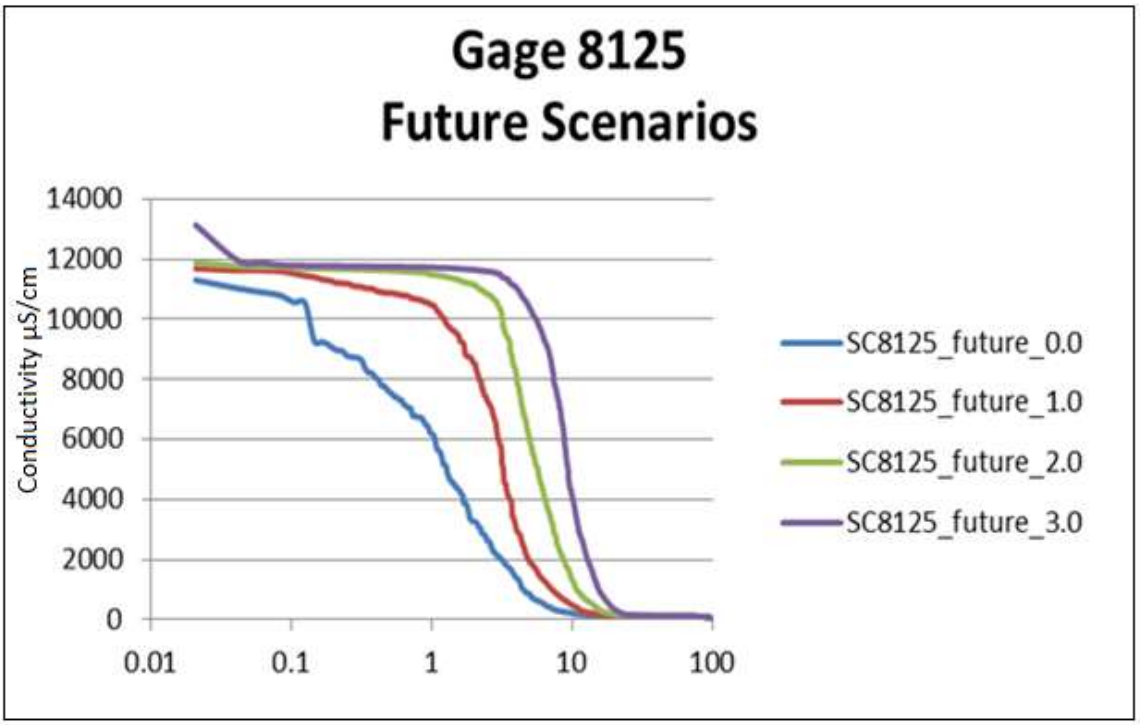

Figure 4.13: Exceedance graph of the predicted specific conductance (µS/cm) for Gage 8125.

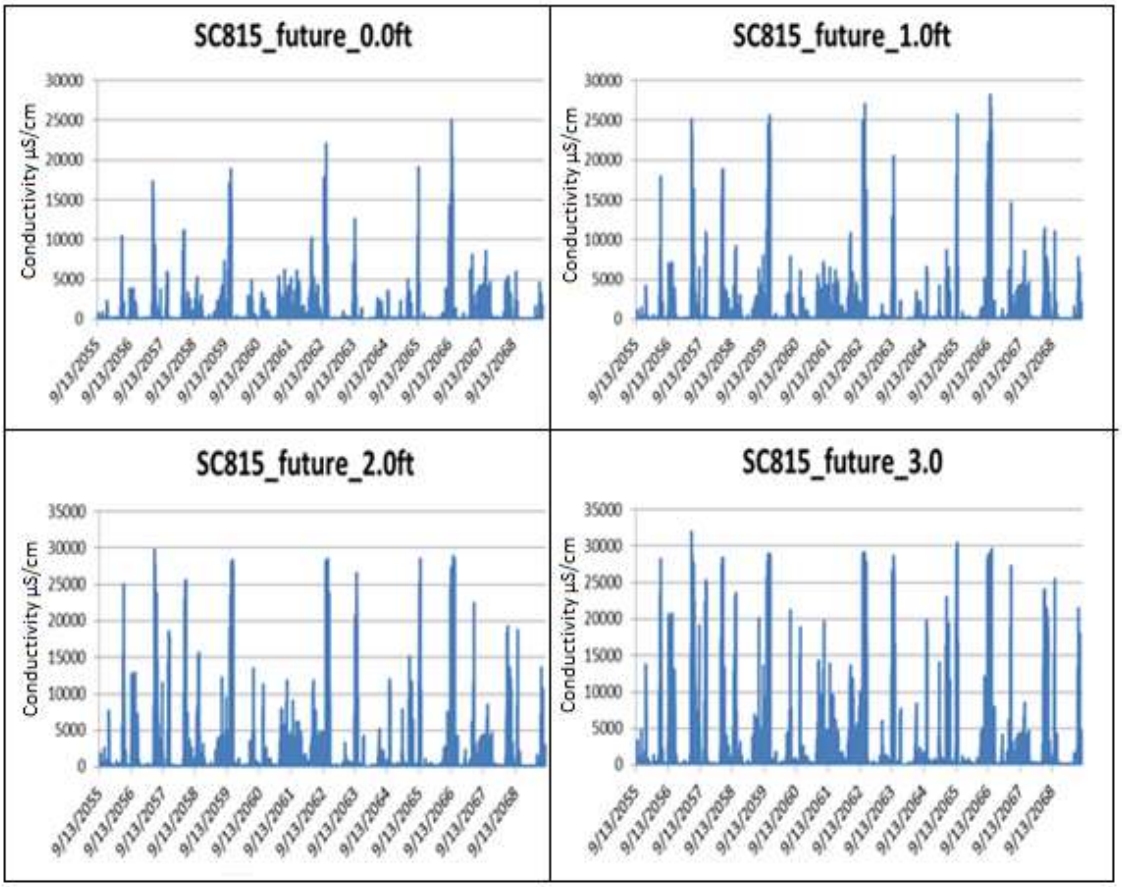

Figure 4.14: Gage 815 predicted Specific Conductance (µS/cm) for years 2055-2068, at 0.0ft- 1.0ft- 2.0ft- 3.0ft Sea-level Rise Scenarios.

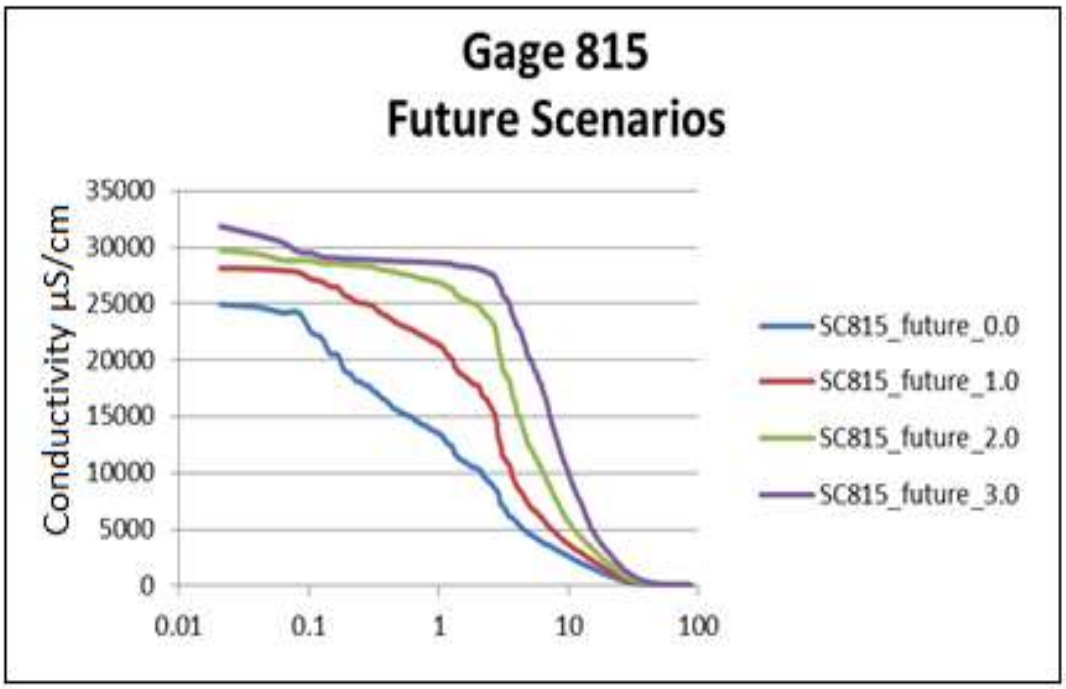

Figure 4.15: Exceedance graph of the predicted specific conductance (µS/cm) for Gage 815.

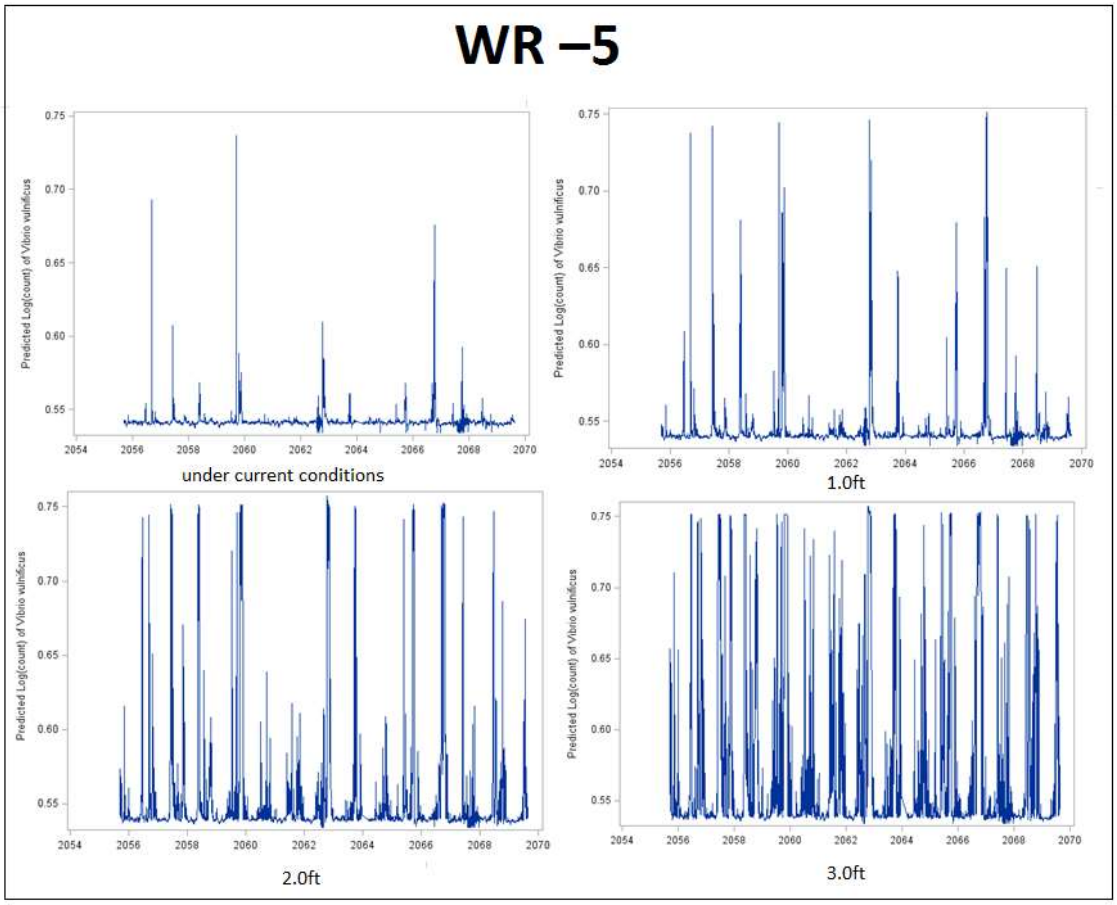

Figure 4.16: Logcount prediction of *Vibrio vulnificus* at WR-5 under 0.0ft, 1.0ft, 2.0ft, and 3.0ft scenarios.

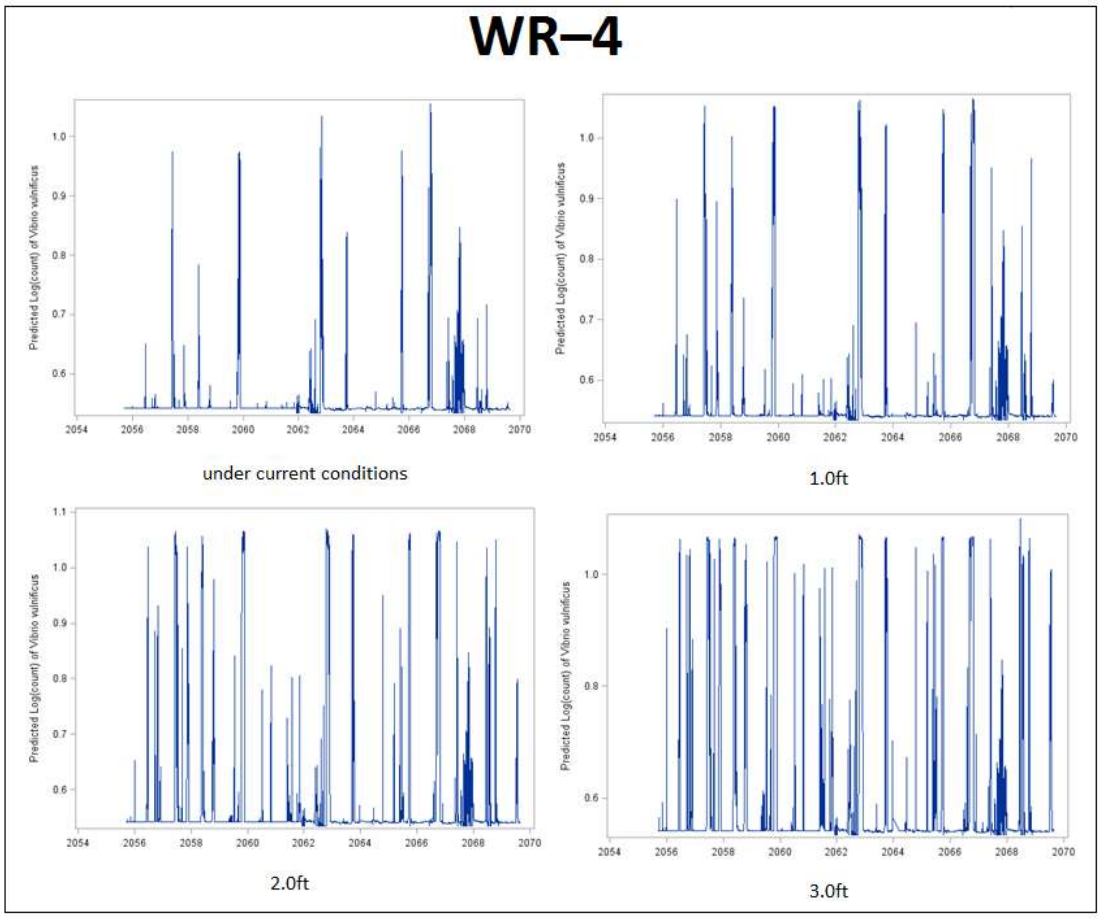

Figure 4.17: Logcount prediction of *Vibrio vulnificus* at WR-4 under 0.0ft, 1.0ft, 2.0ft, and 3.0ft scenarios.

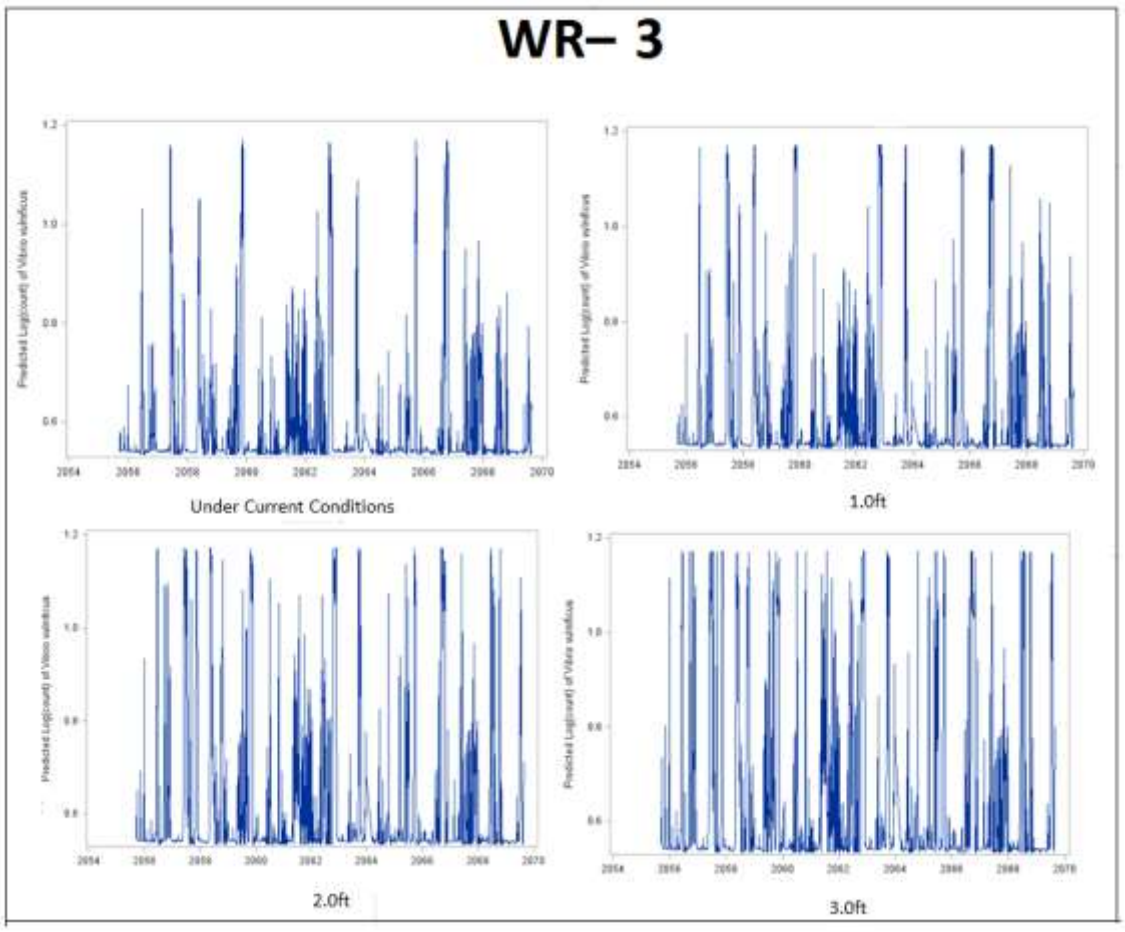

Figure 4.18: Logcount prediction of *Vibrio vulnificus* at WR-3 under 0.0ft, 1.0ft, 2.0ft, and 3.0ft scenarios.

CHAPTER 5:

DISCUSSION

V. vulnificus total counts were relatively low in comparison to the counts retrieved from studies in the Gulf coast where counts reached to more than 100 CFU/ml (Wetz et al., 2008). However, the ecological study of *V. vulnificus* in eastern North Carolina observed a monthly average range of 0.01 to 23 CFU ml−1, with the highest concentrations detected during the warm-weather months (Pfeffer et al., 2003). Thus, the counts observed in our study are common for Carolina waters. On the other hand, *V. vulnificus* virulence is due to its possession of an antiphagocytic polysaccharide capsule (Simpson et al., 1987). The virulence of encapsulated single-celled organisms is extremely high with a 50% lethal dose of less than 10 CFU (Starks et al., 2010; Wright et al., 1981).

The dynamic that *V. vulnificus* showed with salinity corresponds to its optimal salinity range of between 5 ppt and 20 ppt (Randa et al., 2004). Interestingly turbidity, not conductivity, showed the highest Pearson's correlation with *V. vulnificus*. Turbidity in this study ranged between 0 and 262 NTU. Correlation with turbidity was found in several other studies (Pfeffer et al., 2003; Johnson et al., 2010). Oliver et al. (1983) and Pfeffer et al. (2003) studies identified a positive correlation among *Vibrio* spp., other estuarine bacteria, and turbidity. The field data obtained in this study indicated that *V. vulnificus* and turbidity are associated in a positive manner, where the log count of *V.*

vulnificus tended to increase with the increase of turbidity. The positive relationship between turbidity and *Vibrio* spp was expected, since *Vibrio* like many aquatic bacteria are frequently attached to the sediments (Cooksey et al., 1995). Thus, in general, the particulate matter suspended in the water column creates a favorable habitat for a greater density of *Vibrio* spp. This correlation between *Vibrio*s and turbidity may be of great importance as other water quality indicator bacteria, such as *Eneterococcus*, have been highly correlated with turbidity (Fries et al., 2006; 2008). In the Great Lakes, real time turbidity measurements are used to regulate contact recreation on swimming beaches there, as *Enterococcus* levels are highly correlated with turbidity (Byappanahalli et al., 2010). Since contact recreational water quality is currently regulated with Enterococcus, which is also highly correlated with turbidity, it may be possible to correlate further *Enterococcus*, *Vibrio*s and Turbidity associations and use the current *Enterococcus* warning levels to protect swimmers and bathers from *Vibrio* bacterial risk. Further study if this issue will better define this potential.

Evidence on the relationship between salinity and *V. vulnificus* abundance has shown to be contradictory. Kaspar and Tamplin (1993) study shows that the occurrence of *V. vulnificus* is highly affected by salinity levels. Other studies, like Hoi et al. (1998), did not reveal significant correlations between *V. vulnificus* abundance and salinity. Wright et al. (1996) showed that salinity was inversely correlated with *V. vulnificus* in their field survey in the Chesapeake Bay. While studies conducted in the Gulf of Mexico by Motes et al. (1998) and Lipp et al. (2001) showed that when salinity is below 15ppt, *V. vulnificus* was positively correlated to salinity, and was negatively correlated to salinity when salinity levels were elevated above 15 ppt. The inconsistency among the various

studies may be explained with the fact that the different studies have been executed in locations with various salinity gradients and the possibility that temperature and salinity effect may be interdependent (Kaspar and Tamplin, 1993). In addition, there could be other environmental factors, like turbidity, causing those variations without being detected. Based on the analysis of the field data compiled in this study, we found that *V. vulnificus* was positively correlated with salinity. These various findings at different locations with different salinity ranges underscore the importance of salinity as a controlling variable in the growth of *Vibrio*s but also the importance of other environmental variables. The highest counts of *V. vulnificus* were obtained when the salinity ranged between 5 and 19 ppt. This range falls in the optimal range of *Vibrio* tolerance to salinity. Notably, at salinities as low as 2 and 3 ppt *V. vulnificus* counts in some cases recorded high counts. Such salinity concentrations fall out of the range of *Vibrio* tolerance. The presence of *V. vulnificus* and *V. parahaemolyticus* at gage 809, that recorded low conductivity levels, is surprising and implies the influence of other factors, like sediments conditions or the presence of *Vibrio*s vectors and hosts. Those factors are beyond the scope of this study, we only examined the abiotic factors in surface and bottom water. Previous studies have not reached a mechanistic explanation for these *V. vulnificus* dynamics; the inconsistency in the results among the studies reflects the complexity of physiochemical effects on *V. vulnificus* occurrence and abundance in estuaries.

Typically *V. vulnificus* population dynamics have been strongly correlated with temperature. Studies have shown that water temperature is a major variable influencing *V. vulnificus* seasonal fluctuation in estuarine waters (Randa et al., 2004; Thomson et al.,

2004). In comparison with other studies, temperature has shown a higher correlation to *Vibrio* abundance than in this study case. Randa et al. (2004) examined the temperature effect on *Vibrio* population in Barnegat Bay and found *Vibrio* abundance was highly correlated with temperature ($r_s = 0.775$; $P < 0.001$). However, Randa et al. (2004) sampling conducted between June 2001 and February 2003. Such time interval covers winter and summer months which helps manifest the full seasonal fluctuation that *Vibrio* typically shows with temperature. Water column and shellfish *Vibrio* counts were the highest when water temperatures were generally exceeding 20°C (Wright, 1996) and when salinities were moderate (ranging from 5 to 25 ppt) (Kasper and Tamplin, 1993). Not only the abundance of *V. vulnificus* but also the incidences of *V. vulnificus*-associated illness decrease with temperature (Randa et al., 2004). *Vibrio* in Barnegat Bay, N.J., between the summer and the winter months demonstrated a concentration variation of two orders of magnitude in total *Vibrio* (Thompson et al., 2004). Significant growth of *V. vulnificus* has been repeatedly documented to be associated to elevated sea surface temperatures along the Atlantic, Gulf, and Pacific Coasts of the United States (Kelly, 1982; Kaysner et al., 1987; O'Neill et al., 1992; Kaspar and Tamplin1993; Lipp et al., 2001; Pfeffer et al., 2003; Randa et al., 2004).

Contrary to the findings of most environmental studies of this bacterium, temperature was not highly correlated with *V. vulnificus* isolation frequency in our study. However, no winter samplings were conducted due to the known undetectable limits of *Vibrio* in the area of study from previous samplings during the winter. The temperatures measured at our study sites were always between 18 and 30ºC, values within the growth range for *V. vulnificus* which is between 7-36°C (Motes et al., 1998); while its optimal

range is between 13 and 22ºC (Kaspar and Tamplin, 1993). Thus, it is likely that no significant relationship was found between temperature and *V. vulnificus* because temperature records at our study sites were not limiting the growth of this bacterium. Therefore, the narrow temperature variation explains the relatively low correlation of *Vibrio*s abundance to temperature. Despite the general relationship between *V. vulnificus* and temperature, the cause of the decrease of *V. vulnificus* populations in the winter is still not verified. It may be directly due to temperature-mediated mortality or to the availability of its hosts and vectors (e.g. Oysters, clams, mussels; (Hoi et al., 1998)) or the result of the organism entering the viable but nonculturable (VBNC) state.

Interestingly, both salinity and turbidity recorded a higher correlation and significance with *V. vulnificus* abundance in bottom water samples. Despite *V. vulnificus* wide range of tolerance of salinity and temperature, it is observed in higher abundance in water when temperatures are above 20°C and salinities are between 15 and 25 ppt (Kelly et al., 1982; Kaspar and Tamplin, 1993). The fact that salinity and turbidity values were higher in bottom water than in surface water samples may have contributed to the observed results. Venkateswaran et al. (1988) study in the Sea of Japan showed that the bottom water samples had the highest population of total *Vibrios* among the different sampling locations. This observation was consistent with our study sites.

V. parahaemolyticus range counts, average, and total count were lower than *V. vulnificus*. Cook et al. (2002) found that shellfish harvested from the Gulf Coast typically contained higher densities of *V. parahaemolyticus* than the shellfish harvested from the North Atlantic or mid-Atlantic coast. However, *V. parahaemolyticus* infections are very

common worldwide, and account for most of bacterial illnesses due to seafood consumption in the U.S. (Nordstrom et al., 2007).

V. parahaemolyticus has a wide salinity range which is between 3-35 ppt (Johnson et al., 2010). Therefore, recovering *V. parahaemolyticus* bacterium in our study site when salinities were between 5 and 25 ppt was not surprising; however at the lower end of the salinity gradient *V. parahaemolyticus* counts were as high as the rest of the gradient. This indicates *V. parahaemolyticus* euryhaline ability to thrive in the environment even at very low salinities (0.05 ppt). Martinez-Urtaza et al. (2008) found that the highest incidence of *V. parahaemolyticus* in the Rias of Galicia, Spain occurred during the phases of decreasing salinity. On the other hand, the Ria of Pontevedra that was significantly more saline with higher temperatures, showed a significantly lower incidence of *V. parahaemolyticus*.

Turbidity recorded the highest correlation with *V. parahaemolyticus*. This finding is consistent with the significant relationships identified in previous studies between turbidity and *V. parahaemolyticus* occurrence (Parveen et al., 2008; Julie et al., 2010; Nigro et al., 2011). As mentioned earlier, *Vibrio* spp have shown a positive association with turbidity due to their habit of attaching onto sediments (Cooksey et al., 1995). A major contributor to turbidity is sediment resuspension in the water column. Therefore, any activity causing a resuspension of sediment is potentially redistributing reservoirs of *Vibrio* into the water column. This could be the reason behind the strong association, especially with *V. parahaemolyticus* bottom water samples.

V. parahaemolyticus has shown a strong correlation with warm temperatures and tropical areas. Several outbreaks of *V. parahaemolyticus* have been correlated to the water temperatures increases potentially caused by climate change, in a number of studies. (e.g. Gonzalez-Escalona et al., 2005; McLaughlin et al., 2005; Martinez-Urtaza et al., 2008). The weak Pearson's correlation between *V. parahaemolyticus* and water temperature probably is due to the low temperature variation among the period of sampling (min: 18 °C – max: 30°C). Studies that also had low temperature variations **(**Deepanjali et al., 2005) similarly observed no statistically significant correlation with seawater temperature. In contrast to *V. parahaemolyticus* relatively consistent positive correlation with sea surface temperature, its relation to salinity is mainly inconsistent. Deepanjali et al., 2005; Martinez-Utraza et al., 2008; and de Souza Costa Sobrinho et al., 2010 studies have shown either no relation between the two parameters or a negative correlation between *V. parahaemolyticus* and salinity. Similar to our study DePaola et al., (2003) reported a positive correlation between the occurrence of *V. parahaemolyticus* and salinity.

V. parahaemolyticus studies have used multiple linear regressions to assess the relationship between the bacteria and the environmental parameters. De Souza Costa Sobrinho et al. (2010) study results show that salinity unlike temperature was not significant for linear effects. Even though the study shows a correlation between seawater temperature and *V. parahaemolyticus* abundance, the bacterial mean densities formed a plateau at temperatures above 24°C and below 20°C. In these plateaus temperature was not significantly affecting the density of *V. parahaemolyticus*. DePaola et al. (2003) has also observed the same effect of temperature on *V. parahaemolyticus*.

Although it has been consistently shown that temperature is a strong predictor of abundance of *Vibrio* spp, *V. parahaemolyticus* pathogenic subspecies that contain *tdh* and/or *trh* genes association to seawater temperatures was inconsistent and inversely related (DePaola et al, 2003; Zimmerman et al, 2007). Thus, environmental factors like salinity and turbidity can differentially affect the distribution of *V. parahaemolyticus*.

As for climate results, in general, the future estimated specific conductance at the three gages showed a higher number of spikes in almost all of the scenarios (current condition, 1.0ft, 2.0ft, and 3.0ft sea-level rise) in comparison with the historic specific conductance records. Despite the increase in the number of spikes, the upper limit or the maximum value of conductivity did not show any substantial variation in the gages. Table 5.1 shows how the specific conductance limits did not vary a lot between the historic records and the future estimates. Thus, the upper range of specific conductance values are not expected to increase in the years 2055-2068, but the number of spikes and the duration of higher conductivity are expected to increase. This projection of future specific conductivity was also reflected in the prediction of *V. vulnificus*. *V. vulnificus* is expected to occur in the estuarine waters whenever the specific conductance is high enough for its viability. The predicted *V. vulnificus* counts are not expected to increase in comparison to the counts from our samplings in the year 2012.

These results suggest that optimum *Vibrio* density is not expected to increase but the frequency of occurrence and duration of the occurrence of *Vibrio* growth conditions are anticipated to be longer. This indicates a potential increase in the opportunity for exposure to *Vibrio* in the Waccamaw River. Our study reconfirmed the association between *Vibrio* and specific conductivity, and that was depicted in the future estimated

specific conductivity and *V. vulnificus* logcounts. *V. vulnificus* at WR-5, WR-4, and WR-3 number of logcount peaks increased with sea-level rise, which was also accompanied by an increased number of days of high specific conductivity at those sites. Table 5.2 depicts the *V. vulnificus* risk in the future with respect to the sea level rise scenarios. The *V. vulnificus* abundance risk is not expected to increase in WR-5, WR-4, and WR-3 stations. However, stations WR-4 and WR-3 are anticipating an increase in the number of days of optimum *V. vulnificus* specific conductance between 2055 and 2069 with the increase of mean sea level. WR-3 under current conditions is expected to record 16 peaks and 124 days above 9000uS/cm. This indicates that for more than 4 months *Vibrio* bacteria are anticipated to occur in high abundance. WR-3 under a 3.0ft sea level rise scenario, more than 17 months are expected to be in *V. vulnificus* optimal range in this location. Thus, climate change and consequently sea level rise can potentially increase specific conductance in Winyah Bay and by that increase the occurrence of *Vibrio* bacteria in the water.

CHAPTER 5 TABLES AND FIGURES

Table 5.1: Comparison of the Specific Conductivity levels between the historic records and the estimate future values.

	Gage 809 Historic conductivity	Gage 809 Future Conductivity	Gage 8125 Historic Conductivity	Gage 8125 Future Conductivity	Gage 815 Historic Conductivity	Gage 815 Future conductivity
Current condition: 1% of the time	500µS/cm or more	500µS/cm or more	8000µS/cm or more	6000µS/cm or more	15000µS/cm or more	15000µS/cm or more
3.0ft sea-level rise: 1% of the time	3750µS/cm or more	3700µS/cm or more	12000µS/cm or more	12000µS/cm or more	31000µS/cm or more	27000µS/cm or more

Table 5.2 Summary of predicted *Vibrio vulnificus* risks in Winyah Bay with future sea level rise scenarios.

Maximum *V. vulnificus* Abundance (cfu/ml)					Duration (days) Optimum V. v. Growth Conditions*			
Station	Current Condition	1ft	2ft	3ft	Current Condition	1ft	2ft	3ft
WR5	5.45	5.63	5.71	5.72	0	0	0	0
WR4	11.34	11.62	11.75	12.59	9	82	173	324
WR3	14.81	14.83	14.83	14.83	124	188	339	514
Maximum *V. vulnificus* Abundance Risk**					Optimum V. v. Growth Conditions Risk***			
Station	Current Condition	1ft	2ft	3ft	Current Condition	1ft	2ft	3ft
WR5	Baseline	1.03	1.04	1.049	Baseline	1	1	1
WR4	Baseline	1.024	1.036	1.11	Baseline	9.11	19.22	36
WR3	Baseline	1.001	1.001	1.001	Baseline	1.51	2.73	4.14

* = Optimum Conductivity for growth for V.v. is between 9000 uS/cm and 39000 uS/cm.

** = Risk = Future Maximum Abundance/Current Condition Maximum Abundance for 1, 2 and 3 Ft Scenarios at each station.

*** = Risk= Future Optimum Growth Period/Current Optimum Growth Period for 1, 2 and 3 Ft Scenarios at each station.

CHAPTER 6:

CONCLUSION

The ability to predict *V. vulnificus* and *V. parahaemolyticus* is invaluable and offers many potential benefits, given the public health significance of these bacterial in shellfish illnesses and wound infections. The reported relationship between *Vibrio* spp and environmental parameters has been inconsistent among the different geographic locations (Atlantic, Pacific, Gulf of Mexico, and Black Sea). This represents the complexity of *Vibrio* spp distribution, and indicates the possibility that there are interacting factors that control *Vibrio* occurrence and abundance in a given location.

The multi-linear prediction model would be more robust by incorporating qPCR quantifications instead of the raw plate counts. The qPCR records are more accurate and specific (Tarr et al., 2007). Relevance for public health planning could be strengthened by determining the potentially pathogenic subpopulations of environmental *Vibrios* out of the total *Vibrio* population. This could be done by conducting qPCR targeting the thermostable direct hemolysin (*tdh*) and tdh-related hemolysin genes (*trh*) that are markers for potentially pathogenic *V. parahaemolyticus* (Shirai et al., 1990; Nishibuchi et al., 1992) and hemolysin gene (*vvhA*) for the identification of *V. vulnificus* species (Morris et al., 1987; Wright et al., 1993). Calculating the percentage of pathogenic

subpopulation would be more helpful for public health decision makers. The current model does not include *Vibrio* virulence. Future models should include these understanding associations between these predictive variables and virulence which is more correlated with illness.

An annual sampling of *Vibrio* spp in Waccamaw River/Winyah Bay would result in a better association with temperature that can potentially project *Vibrio* more accurately. The ability to develop a model that can predict sea surface temperature in Waccamaw River/Winyah Bay and integrate it with PRISM2 would enable us to give more accurate projections of *Vibrio* spp distribution.

Studying the interaction between the environmental parameters, especially temperature and salinity (the two dominant predictors of *Vibrio*) will enhance the ability to project *Vibrio* spp. Thus, it would be interesting to see if there is a combinatorial effect between temperature and salinity and perhaps other unidentified parameters. Determining interactions between temperature and salinity may explain some of *Vibrio* distribution variability, since optimal salinity was influenced by temperature under experimental conditions (Chase and Harwood, 2011). Soto et al. (2009) noted that significant differences in growth rates of *Vibrio* due to salinity existed only when temperatures were low.

It would also be interesting to incorporate biotic factors, such as the presence and abundance of *Vibrio* hosts and vectors (i.e. oysters, clams, mussels, crabs, shrimps, lobsters; (Twedt, 1989; Oliver and Kaper, 1997; and Hoi et al., 1998). Those hosts are

mobile species (in some life stages) that may help in the introduction and distribution of *Vibrio* bacteria into new regions.

Evaluating the effect of storms and hurricanes on *Vibrio* distributions in an estuary would require sampling of water and sediment, and measuring environmental parameters before and after the event to observe the difference. Such studies will enable us to understand the effect of large storms when they occur and estimate their potential threat. Forecast of *Vibrio* pathogens will help reduce the risk of infections and provide critical information to public health and safety managers. Short and long-term projections can enable policy makers and public health officials to take preventive measures which are very important for the shellfish harvest stations in order to decrease the exposure possibility to *Vibrio* when it is prevalent.

REFERENCES:

Akeda, Y., Nagayama, K., Yamamoto, K., and Honda, T. 1997. "Invasive phenotype of *Vibrio parahaemolyticus*." J Infect Dis.; 176:822–824.

Ansede-Bermejo, J., Gavilan, R. G., Trinanes, J., Espejo, R. T., and Martinez-Urtaza, J. 2010. "Origins and colonization history of pandemic *Vibrio parahaemolyticus* in South America." Molecular Ecology; 3924–3937.

Baker-Austin, C., Stockley, L., Rangdale, R., and Martinez-Urtaza, J. 2010. "Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective." Environ. Microbiol. Rep. 2:7–18.

Baker-Austin, C., Trinanes, J. A., Taylor, N. G. H., Hartnell, R., Siitonen, A., and Martinez-Urtaza, J. 2012. "Emerging *Vibrio* risk at high latitudes in response to ocean warming." Nature Climate Change 3, 73–77.

Banakar, V., Constantin de Magny, G., Jacobs, J., Murtugudde, R., Huq, A., Wood, R. J., and Colwell, R. R. 2012. "Temporal and Spatial Variability in the Distribution of *Vibrio vulnificus* in the Chesapeake Bay: A Hindcast Study." EcoHealth; 10.1007/s10393-011-0736-4.

Barlow, P., and Wild, E. 2002. "Bibliography on the occurrence and intrusion of saltwater in aquifers along the Atlantic Coast of the United States, 1-30." Northborough, Massachusetts: US Geological Survey.

Barlow, P., and Reichard, E. 2010. "Saltwater intrusion in coastal regions of North America." Hydrogeology Journal, 18(1), 247-260.

Bass, Kellie Ballenger. 2004. "Establishing a Baseline for *Vibrio Vulnificus* Densities in Oyster and Water Samples from Charleston, South Carolina." Graduate School -- College of Charleston, American oyster.

Bej, A. K., Patterson, D. P., Brasher, C. W., Vickery, M. C. L., Jones, D. D., and Kaysner, C. 1999. "Detection of total and hemolysin producing *Vibrio parahaemolyticus* in shell-fish using multiplex PCR amplification of *tl*, *tdh*, and *trh*." J Microbiol Methods 36: 215–225.

Bender, M. A., Knutson T. R., Tuleya, R. E., Sirutis, J. J., Vecchi, G. A., Garner, S. T., and Held, I. M. 2010. "Modeled impact of anthropogenic warming of the frequency of intense Atlantic hurricanes." Science 327, 454–458.

Bowdre, J. H., Hull, J. H., and Cocchetto, D. M. 1983. "Antibiotic efficacy against *Vibrio vulnificus* in the mouse: superiority of tetracycline." J. Pharmacol. Exp. Ther. 22595-598.

Byappanahalli, M. N., Whitman, R. L., Shively, D. A., Nevers, M. B. 2010. "Linking non-culturable (qPCR) and culturable enterococci densities with hydrometeorological conditions." Science of the Total Environment; 408(16):3096-101.

Centers for Disease Control and Prevention. 1993. "*Vibrio vulnificus* infections associated with raw oyster consumption." Morb. Mortal. Wkly. Rep. 42**:**405-407.Centers for Disease Control and Prevention. 1999. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound—Connecticut, New Jersey, and New York. MMWR Morb. Mort Wkly. Rep. 48:48–51.

Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. MMWR Morb Mortal Wkly Rep. 2006;55:392–5.

Centers for Disease Control and Prevention (CDC). COVIS Annual Summary, 2009. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2011. http://www.cdc.gov/nationalsurveillance/PDFs/CSTEVibrio2009.pdf

Chase, E., and Harwood, V. J. 2011. "Comparison of the effects of environmental parameters on growth rates of *Vibrio vulnificus* biotypes I, II, and III by culture and quantitative PCR analysis." Appl. Environ. Microbiol. 77:4200–4207.

Clean Water Team (CWT). 2004. Electrical conductivity/salinity Fact Sheet, FS-3.1.3.0 (EC). In: The Clean Water Team Guidance Compendium for Watershed Monitoring and Assessment, Version 2.0. Division of Water Quality, California State Water Resources Control Board (SWRCB), Sacramento, CA. http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3130en.pdf

Colwell, R. R., Brayton, P. R., Grimes, D. J., Roszak, D. R., Huq, S. A., and Palmer, L. M. 1985. "Viable, but non-culturable, Vibrio cholerae and related pathogens in the environment: implications for release of genetically engineered microorganisms." Biotechnol. 3:817-820.

Colwell, R. R. 2006. "A global and historical perspective of the genus Vibrio. In: Thompson FL. Austin B., Swing (eds) The Biology of Vibrios. ASM press, Washington, DC pp 3-11.

Conrads, P. A., and Roehl, E. A. 2007. "Analysis of salinity intrusion in the Waccamaw River and Atlantic Intracoastal Waterway near Myrtle Beach, South Carolina, 1995–2002: U.S." Geological Survey Scientific Investigations Report 2007–5110, 41 p., 2 apps.

Conrads, P. A., Roehl, E. A., and Cook, J. 2012. “Development of a Decision support system for Estimating Salinity Intrusion Effects Due to Climate Change on the South Carolina and Georgia Coast.” Found at http://acwi.gov/monitoring/conference/2012/D4/D4Conrads.pdf.

Constantin de Magny, G., Long, W., Brown, C. W., Hood, R. R., Huq, A., Murtugudde, R., and Colwell, R. R. 2009. “Predicting the Distribution of *Vibrio* spp. in the Chesapeake Bay: A *Vibrio* cholerae Case Study.” EcoHealth; 10.1007/s10393-009-0273-6.

Cook, D. W., Bowers, J. D., and DePaola, A. 2002. “Density of total and pathogenic (*tdh*$^{+}$) *Vibrio parahaemolyticus* in Atlantic and Gulf Coast molluscan shellfish at harvest.” J. Food Prot. 65**:**1873-1880.

Cooksey, K., and Wigglesworth-Cooksey, B. 1995. “Adhesion of bacteria and diatoms to surfaces in the sea: a review.” Aquatic Microbial Ecology. 9:87-96.

Courtney, S. P., Hite, M. F., and Oliver, J. D. 2003. “Ecology of *Vibrio vulnificus* in Estuarine Waters of Eastern North Carolina.” Appl. Environ. Microbiol; 69(6):3526.

Daniels, N. A. 2011. “*Vibrio vulnificus* Oysters: Pearls and Perils.” Clinical Infectious Diseases; 52(6):788–792.

Dechet, A. M., Yu, P. A., Koram, N., and Painter, J. 2008. "Nonfoodborne *Vibrio* infections: an important cause of morbidity and mortality in the United States, 1997-2006." Clin Infect Dis 46(7): 970-976.

Deepanjali, A., Kumar, H. S., Karunasagar, I., and Karunasagar, I. 2005. “Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India.” Appl. Environ. Microbiol. 71:3575-3580.

DePaola, A., Capers, G. M., and Alexander, D. 1994. "Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast." Appl Environ Microbiol 60(3): 984-988.

DePaola, A., Nordstrom, J. L., Bowers, J. C., Wells, J. G., and Cook, D. W. 2003. “Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters.” Appl. Environ. Microbiol. 69:1521-1526.

De Souza Costa Sobrinho, P., Destro, M. T., Franco, B., and Landgraf, M. 2010. “Correlation between Environmental Factors and Prevalence of *Vibrio parahaemolyticus* in Oysters Harvested in the Southern Coastal Area of Sao Paulo State, Brazil.” Appl. Environ. Microbiol. 4: 1290-1293.

Emanuel, K. 1987. “The dependence of hurricane intensity on climate.” Nature326, 483–485.

Emanuel, K. 2005. "Increasing destructiveness of tropical cyclones over the past 30 years." Nature 436(7051): 686-688.

Feldhusen, F. 2000. “The role of seafood in bacterial foodbourne diseases. Microbes Infect. 21651-1660. CDC.” 1993. *Vibrio vulnificus* infections associated with raw oyster consumption. Morb. Mortal. Wkly. Rep. 42405-407.

Field, C. B., Mortsch, L. D., Brklacich, M., Forbes, D. L., Kovacs, P., Patz, J. A., Running, S. W., and Scott, M. J. 2007. North America. In: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Parry, M.L., O.F. Canziani, J.P. Palutikof, P.J. van der Linden, and C.E. Hanson (eds.)]. Cambridge University Press, Cambridge, UK, and New York, pp. 617-652.

Fries, J. S., Characklis, G. W., and Noble, R. T. 2006. "Attachment of fecal indicator bacteria to particle suspensions in the Neuse River Estuary." Environ Eng 132: 1338–1345.

Fries, J. S., Characklis, G. W., and Noble, R. T. 2008. "Sediment-water exchange of *Vibrio* spp. and fecal indicator bacteria: implications for persistence and transport in the Neuse River Estuary, North Carolina, USA." Water Res 42(4-5): 941-950.

Fries, J. S., Noble, R. T., Kelly, G. M., and Hsieh, J. L. 2007. "Storm Impacts on Potential Pathogens in Estuaries." EOS, Vol. 99, No.8.

Fuller, K. M. 2011. “Waccamaw River water impacted by more salt water creeping in.” Waccamaw Times: Georgetown, SC, July 21, 2011.

Garcia, M., and Landgraf, M. 1998. “Virulence factors and pathogenicity of *Vibrio vulnificus* strains isolated from seafood.” J Appl Environ Microbiol. 84:747–751.

Gavilan, R. G., and Martinez-Urtaza, J. 2011. "Environmental drivers of emergence and spreading of *Vibrio* epidemics in South America." Rev Peru Med Exp Salud Publica 28(1): 109-115.

Gonzalez-Escalona, N., Cachicas, V., Acevedo, C., Rioseco, M. L., Vergara, J. A., Cabello, F., Romero, J. 2005. “Vibrio parahaemolyticus diarrhea, Chile, 1998 and 2004.” Emerg Infect Dis 11: 129–131.

Gray, L. D., Kreger, A. S. 1985. “Purification and characterization of an extracellular cytolysin produced by *Vibrio vulnificus*.” Infect Immun. 48:62–72.

Haq, S. M., and Dayal, H. H. 2005. “Chronic liver disease and consumption of raw oysters: a potentially lethal combination—a review of *Vibrio vulnificus* septicemia.” Am J Gastroenterol; 100:1195–9.

Hicks, B. 2002. Hopes Sink As River's Pulse Ebbs. The Post and Courier, Charleston.SC. Available online at http://www.accessmylibrary.com/article-1G1-92036123/hopessink-rivers-pulse.html.

Hlady, W. G., and Klontz, K. C. 1996. "The epidemiology of *Vibrio* infections in Florida, 1981–1993." J Infect Dis; 173:1176–1183.

Hoi, L., Larsen, J. L., Dalsgaard, I., and Dalsgaard, A. 1998. "Occurrence of *Vibrio vulnificus* in Danish marine environments." Appl. Environ. Microbiol. 64:7-13.

Honda, T., Goshima, K., Takeda, Y., Sugino, Y., Miwatani, T. 1976. "Demonstration of the cardiotoxicity of the thermostable direct hemolysin (lethal toxin) produced by *Vibrio parahaemolyticus*." Infect Immun; 13:163–171.

Honda, T., Ni, Y., and Miwatani, T. 1988. "Purification and characterization of a hemolysin produced by a clinical isolate of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin." Infect Immun 56: 961–965.

Honda, T., and Lida, T. 1993. "The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct haemolysin and related haemolysins." Rev Med Microbiol 4: 106–113.

Hor, L. I., Chang, T. T., and Wang, S. T. 1999. "Survival of *Vibrio* vulnificus in whole blood from patients with chronic liver diseases: association with phagocytosis by neutrophils and serum ferritin levels." J Infect Dis;179:275–8.

Hor, L. I., Chang, Y. K., Chang, C. C., Lei, H.Y., and Ou, J. T. 2000. "Mechanism of high susceptibility of iron-overloaded mouse to *Vibrio vulnificus* infection." Microbiol Immunol; 44:871–8.

Hsieh, J., Fries, J., and Noble, R. 2007. "*Vibrio* and phytoplankton dynamics during the summer of 2004 in a eutrophying estuary." Ecol Appl 17:S102–S109.

Hsieh, J., Fries, J., and Noble, R. 2008. "Dynamics and predictive modeling of *Vibrio* spp. in the Neuse River estuary, North Carolina, USA." Environmental Microbiology, 10 (1), 57 – 64. doi: 10.1111/j.1462-2920.2007.01429.

Jackson, J. K., Murphree, R. L., and Tamplin, M. L. 1997. "Evidence that mortality from *Vibrio vulnificus* infections results from single strains among heterogeneous populations in shellfish." Clin Microbiol 35:2098–2101.

Jacobs, J. M., Rhodes, M., Brown, C. W., Hood, R. R., Leigh, A., Long W. 2010. "Predicting the distribution of *Vibrio vulnificus* in the Chesapeake Bay." Cooperative Oxford Laboratory, Oxford, MD.

Jiang, S. C., and Fu, W. 2001. “Seasonal abundance and distribution of *Vibrio cholera* in coastal waters quantified by a 16S-23S intergenic spacer probe.” Microb. Ecol. 42:540-548.

Johnson, C. N., Flowers, A. R., Noriea, N. F., Zimmerman, A. M., Bowers, J. C., DePaola, A., and Grimes, D. J. 2010. “Relationships between Environmental Factors and Pathogenic *Vibrios* in the Northern Gulf of Mexico.” Appl Environ Microbiol; 76(21): 7076–7084.

Julie, D., Solen, L., Antoine, V., Jaufrey, C., Annick, D., and Dominique, H. 2010. “Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll a.” Environ. Microbiol. 12:929–937.

Kaspar, C. W., and Tamplin, M. L. 1993. “Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish.” Appl. Environ. Microbiol. 59:2425-2429.

Kaysner, C., Abeyta, C., Wekell, M., DePaola, A., Stott, R., and Leitch, J. 1987. “Virulent strains of *Vibrio vulnificus* isolated from estuaries of the United States West Coast. Applied and Environmental Microbiology 53:1349–1351.

Kaysner, C., and DePaola, A. 2001. *Vibrio*. In Downes, F.P., and Ito, K. (eds). Microbiological Examination of Foods. Washington, DC, USA: American Public Health Association, pp. 405–428.

Keith, W. J., and Anderson, W.D. 2010. “Oysters and Clams.” South Carolina Department of Natural Resources.
http://www.dnr.sc.gov/marine/pub/seascience/oyster.html

Kelly, M. 1982. “Effect of temperature and salinity on *Vibrio* (Beneckea) *vulnificus* occurrence in a Gulf Coast environment.” Applied and Environmental Microbiology 44:820–824.

Kelly, M., and Dan Stroh, E. M. 1982. “Occurrence of Vibrionaceae in natural and cultivated oyster populations in the Pacific Northwest.”Diagn. Microbiol. Infect. Dis. 9:1-5.

Klontz, K. C., Lieb, S., Schreiber, M., Janowski, H. T., Baldy, L. M., and Gunn, R. A. 1988. “Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiological features in Florida cases, 1981-1987.” Ann. Intern. Med. 109318-323.

Knutson, T. R., and Tuleya, R. E. 2004. “Impact of CO_2-induced warming on simulated hurricane intensity and precipitation: sensitivity to the choice of climate model and convective parameterization.” J. Clim. 17, 3477–3495.

Knutson, T. R., McBride, J. L., Chan, J., Emanuel, K., Holland, G., Landsea, C., Held, I., Kossin, J. P., Srivastava, A. k., and Sugi, M. 2010. "Tropical cyclones and climate change." Nature Geoscience 3, 157 – 163.

Lake, R., Hudson, A., and Cressey, P. 2003. "Risk profile: *Vibrio parahaemolyticus* in seafood." Retrieved from: http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Vibrio-Science_Research.pdf.

Lipp, E. K., Rodriquez-Palacios, C., and Rose, J. B. 2001. "Occurrence and distribution of the human pathogen *Vibrio vulnificus* in a subtropical Gulf of Mexico estuary." Hydrobiologia 460:165-173.

Mackenzie, B. R., and Schiedek, D. 2007. Daily Ocean monitoring since the 1860s shows record warming of northern European seas. Glob. Change Biol. 13, 13351347.

Martinez-Urtaza, J., Lozano-Leon, A., Varela-Pet, A., Trinanes, J., Pazos, Y., and Garcia-Martin, O. 2007. "Environmental Determinants of the Occurrence and Distribution of *Vibrio parahaemolyticus* in the Rias of Galicia, Spain." Appl Environ Microbiol; 74(1): 265–274.

Martinez-Urtaza, J., Huapaya, B., Gavilan, R. G., Blanco-Abad, V., Ansede-Bermejo, J., Cadarso-Suarez, C., Figueiras, A., and Trinanes, J. 2008. "Emergence of Asiatic *Vibrio* diseases in South America in phase with El Niño." Epidemiology 19(6): 829-837.

McLaughlin, J. B., DePaola, A., Bopp, C. A., Martinek, K. A., Napolilli, N. P., Allison, C. G., Murray, S. L., Thompson, E. C., Bird, M. M. and Middaugh, J. P. 2005. "Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters." N Engl J Med 353, 1463–1470.

Mead, P. S., Slutsker, L., Griffin, P. M., Tauxe, R. V. 1999. Food-related illness and death in the United States. Emerg Infect Dis. 5(6):841-842.

Miller, C., Drasar, B., and Feachem, R. 1984. "Response of toxigenic *Vibrio cholerae* 01 to physico-chemical stress in aquatic environments." J Hyg (Lond) 93:475–495.

Miyoshi, A. I., and Shinoda, S. 1992. "Activation mechanism of human Hageman factor-plasma kallikrein-kinin system by *Vibrio vulnificus* metalloprotease." FEBS Lett. 308:315–319.

Miyoshi, S. I. 2006. "*Vibrio vulnificus* infection and metalloprotease." J Dermatol. 33:589–595.

Morris, J. G., Wright, A. C., Roberts, D. M., Wood, P. K., Simpson, L. M., and Oliver, J. D. 1987. "Identification of environmental *Vibrio vulnificus* isolates with a DNA probe for the cytotoxin-hemolysin gene." Appl Environ Microbiol 53:193-5.

Motes, M. L., DePaola, A., Cook, D. W., Veazey, J. E., Hunsucker, J. C., Garthright, W. E., Blodgett, R. J., and Chirtel, S. J. 1998. "Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*)." Appl Environ Microbiol 64(4): 1459-1465.

National Estuarine Research Reserve System, 2009. "Tidal Range and River Flow." http://nerrs.noaa.gov/NERRSReserve.aspx?ID=194&ResID=NIW.

National Estuarine Research Reserve System, 1992. "North-Inlet Winyah, SC." http://nerrs.noaa.gov/Reserve.aspx?ResID=NIW.

Neupane, G. P., Kim, D. M., Yun, N. R., Shin, S. H., Lim, S. C., and Choi, C. H. 2012. "Quantitative PCR and in vivo efficacy of antibiotics in the treatment of *Vibrio vulnificus* infection in a mouse model." Eur J Clin Microbiol Infect Dis 31:2461–2467.

Nigro, O. D., Hou, A., Vithanage, G., Fujioka, R., and Steward, G. 2011. "Temporal and spatial variability in culturable pathogenic *Vibrio* spp. in Lake Pontchartrain, Louisiana, following hurricanes Katrina and Rita." Appl. Environ. Microbiol. 77:5384–5393.

Nishibuchi, M., Fasano, A., Russell, R. G., and Kaper, J. B. 1992. "Enterotoxigenicity of *Vibrio parahaemolyticus* with and without genes encoding thermostable direct hemolysin." Infect Immun 60: 3539–3545.

Nordstrom, J. L., Vickery, M. C. L., Blackstone, G. M., Murray, S. L., and DePaola, A. 2007. Development of a multiplex real-time PCR assay with an internal amplification control for detection of total and pathogenic *Vibrio parahaemolyticus* in oysters. Appl. Envir. Microbiol. 73:5840-5847.

Okuda, J., Ishibashi, M., Hayakawa, E., Nishino, T., Takeda, Y., Mukhopadhyay, A. K. 1997. "Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travellers arriving in Japan." J Clin Microbiol 35: 3150–3155.

Oliver, J. D., Warner, R. A., and Cleland, D. R. 1982. "Distribution and Ecology of *Vibrio vulnificus* and other Lactose-fermenting Marine *Vibrio*s in Coastal Waters of the Southeastern United States." Appl. Environ. Microbiol; 44:1404–1414.

Oliver, J. D., Warner, R. A., and Cleland, D. R. 1983. "Distribution of *Vibrio vulnificus* and other Lactose-Fermenting *Vibrios* in the Marine Environment."Appl. Environ. Microbiol. 45:985–998.

Oliver, J. D. 1989. "*Vibrio vulnificus* Foodborne bacterial pathogens." Marcel Dekker, Inc, New York, NY.

Oliver, J. D., and Kaper, J. B. 1997. *Vibrio* species. In: Doyle, M.P., Beuchat, L.R., Montvilee, T.J. (Eds.), Food Microbiology—Fundamentals and Frontiers. ASM Press, Washington,DC.

Oliver, J. D. 2005a. "Wound infections caused by *Vibrio vulnificus* and other marine bacteria." Epidemiology and Infection; 133(3):383–91.

Oliver, J. D. 2005b. "The Viable but Nonculturable State in Bacteria." J Microbiol. Spec No:93-100.

O'Neill, K., Jones, S., and Grimes, D. 1992. "Seasonal incidence of *Vibrio vulnificus* in the Great Bay estuary of New Hampshire and Maine." Applied and Environmental Microbiology 58:3257–3262.

Oouchi, K., Yoshimura, J., Yoshimura, H., Mizuta, R., Kusunoki, S., and Noda, A. 2006. "Tropical cyclone climatology in a global-warming climate as simulated in a 20km-mesh global atmospheric model: frequency and wind intensity analysis." *J. Meteorol. Soc. Jpn* 84, 259–276.

Pal, D., and Das, N. 2010. "Isolation, identification and molecular characterization of *Vibrio parahaemolyticus* from fish samples in Kolkata." Eur. Rev. Med. Pharmacol. Sci. 14:545–549.

Panicker, G. and Bej, A. K. 2005. "Real-time PCR detection of *Vibrio vulnificus* in oysters: comparison of oligonucleotide primers and probes targeting *vvha*." Appl. Envir. Microbiol. 71: 5702-5709.

Park, K. S., Ono, T., Rokuda, M., Jang, M. H., Okada, K., Iida, T., and Honda, T. 2004. "Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus*." Infect Immun 72: 6659–6665.

Parveen, S., Hettiarachchi, K. A., Bowers, J. C., Jones, J. L., Tamplin, M. L., and McKay, R. 2008. "Seasonal distribution of total and pathogenic *Vibrio parahaemolyticus* in Chesapeake 13 Bay oysters and waters." J Food Microbiol 128: 354-361.

Pfeffer, C. S., Hite, M. F., and Oliver, J. D. 2003. "Ecology of *Vibrio vulnificus* in estuarine waters of eastern North Carolina." Appl Environ Microbiol;69(6):3526-31.

Quiñones-Ramireza, E. I., Bonifaciob, I. N., Betancourt-Ruleb, M., Ramirez-Vivesb, F., and Vazquez-Salinas, C. 2010. "Putative virulence factors identified in *Vibrio vulnificus* strains isolated from oysters and seawater in Mexico." International Journal of Environmental Health Research, 395–405.

Raimondi, F., Kao, J. P., Fiorentini, C., Fabbri, A., Donelli, G., Gasparini, N., Rubino, A., and Fasano, A. 2000. "Enterotoxicity and cytotoxicity of *Vibrio parahaemolyticus* thermostable direct hemolysin in in-vitro systems." Infect. Immun; 68:3180–3185.

Ramirez, G. D., Buck, G. W., Smith, A. K., Gordon, K. V., and Mott, J. B. 2009. "Incidence of *Vibrio vulnificus* in estuarine waters of the south Texas Coastal Bend region." J Appl Microbiol 107, 2047-2053.

Randa, M. A., Polz, M. F., and Lim, E. 2004. "Effects of temperature and salinity on *Vibrio vulnificus* population dynamics as assessed by quantitative PCR." Appl. Environ. Microbiol. 70:5469–5476.

Riemann, L., and Azam, F. 2002. "Widespread N-acetyl-D-glucosamine uptake 673 among pelagic marine bacteria and its ecological implications." Appl Environ 674 Microbiol 68:5554-62.

Rhee, J. E., Rhee, J. H., Ryu, P. Y., and Choi, S. H. 2002. "Identification of the cadBA operon from *Vibrio vulnificus* and its influence on survival to acid stress." FEMS Microbiol Lett 208: 245–251.

Ruple, A. D., and Cook, D. W. 1992. "*Vibrio vulnificus* and indicator bacteria in shellstock and commercially processed oysters from the Gulf coast." J Food Prot. 55:667–671.

Schetsa, F.M., van den Berga, H. H. J. L., Marcheseb, A., Garbomc, S., de Roda Husmana, A. M. 2011. "Potentially human pathogenic *Vibrios* in marine and fresh bathing waters related to environmental conditions and disease outcome." International Journal of Hygiene and Environmental Health 214(5):399-406.

Shao, C. P., and Hor, L. I. 2000. "Metalloprotease is not essential for *Vibrio vulnificus* virulence in mice." Infect Immun. 68:3569–3573.

Shapiro, R. L., Altekruse, S., Hutwagner, L., Bishop, R., Hammond, R., Wilson, S. 1998. "The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996." J Infect Dis;178:752–9.

Shirai, H., Ito, H., Hirayama, T., Nakamoto, Y., Nakabayashi, N., Kumagai, K., Takeda, Y., and Nishibuchi, M. 1990. "Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of *Vibrio parahaemolyticus* with gastroenteritis." Infect Immun 58:3568-73.

Simpson, L. M., White, V. K., Zane, S. F., and Oliver, J. D. 1987. "Correlation between virulence and colony morphology in *Vibrio vulnificus*." Infect. Immun. 55:269-272.

Singleton, F., Attwell, R., Jangi, M., and Colwell, R. 1982. "Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms." Appl Environ Microbiol; 43:1080–1085.

Soto, W., Gutierrez, J., Remmenga, M. D., Nishiguchi, M. K. 2009. “Salinity and temperature effects on physiological responses of *Vibrio fischeri* from diverse ecological niches.” Microb Ecol. 57:140–150.

Starks, A. M., Schoeb, T. R., Tamplin, M. L., Parveen, S., Doyle, T. J., Bomeisl, P. E., Escudero, G. M., and Gulig, P. A. 2000. “Pathogenesis of infection by clinical and environmental strains of *Vibrio vulnificus* in iron-dextran-treated mice.” Infect. Immun. 68:5785-5793.

Tamplin, M., Rodrick, E., Blake, N. J., and Cuba, T. 1982. “Isolation and characterization of *Vibrio vulnificus* from two Florida estuaries.” Appl. Environ. Microbiol. 44:1466-1470.

Tarr, C. L., Patel, J. S., Puhr, N. D., Sowers, E. G., Bopp, C. A., and Strockbine, N. A. 2007. “Identification of *Vibrio* isolates by a multiplex PCR assay and rpoB sequence determination.” J. Clin. Microbiol. 45:134-140.

Thompson, J. R., Randa, M. A., Marcelino, L. A., Tomita-Mitchell, A., Lim, E., and Polz, M. F. 2004. “Diversity and dynamics of a North Atlantic coastal *Vibrio* community.” Appl. Environ. Microbiol. 70:4103-4110.

Twedt, R. M. 1989. “*Vibrio parahaemolyticus*.” In M. P. Doyle, ed. Foodborne Bacterial Pathogens, p543-568. New York, Marcel Decker, Inc.

United States Food and Drug Administration. 2005. *Vibrio parahaemolyticus* risk assessment – appendix 5: details of the data analysis for exposure assessment. Retrieved fromhttp://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm185746.htm.

U.S. Global Change Research Program. 2009 “Southeast Region.” http://globalchange.gov/publications/reports/scientific-assessments/us-impacts/full-report/regional-climate-change-impacts/southeast.

Valasek, M A., and Repa, J. J. 2005. “The power of real-time PCR.” Advan in Physiol Edu 29:151-159, doi: 10.1152/advan.00019.2005.

Venkateswaran, K., Nakano, H., Okabe, T., Takayama, K., Matsuda, O., and Hashimoto, H. 1989. “Occurrence and distribution of *Vibrio* spp., Listonella spp., and Clostridium botulinum in the Seto Inland Sea of Japan.” Applied and Environmental Microbiology; 0099-2240.

Vernberg, W. B., Scott, G. I., Strozier, S. H., Bemiss, J., and Daugomah, J. W. 1997. “The effects of urbanization on human and ecosystem health.” In: F. J. Vernberg, W. B., and Siewicki, T. editor. Sustainable development in the Southeastern Coastal Zone, vol. 20. Belle W. Baruch Library in Marine Science.

Wetz, J. J., Blackwood, A. D., Fries, J. S., Williams, Z. F., Noble, R. T. 2008. "Trends in total *Vibrio* spp. and *Vibrio vulnificus* concentrations in the eutrophic Neuse River Estuary, North Carolina, during storm events." Aquat Microb Ecol Vol. 53: 141–149, doi: 10.3354/ame01223.

Williams, T. C., Froelich, B. A., and Oliver, J. D. 2011. "Comparison of two selective and differential media for the isolation of *Vibrio vulnificus* from the environment." Retrieved from http://www.chromagar.com/fichiers/1310459233VB_WILLIAMS.pdf?PHPSESSID=168f8ebd8a36a09c1e56e1bba032028a.

Wright, A. C., Simpson, L. M., and Oliver, J. D. 1981. "Role of iron in the pathogenesis of *Vibrio vulnificus* infections." Infect. Immun. 34:503-507.

Wright, A. C., Miceli, G. A., Landry, W. L., Christy, J. B., Watkins, W. D., and Morris, J. G. 1993. "Rapid identification of *Vibrio vulnificus* on nonselective media with an alkaline phosphatase-labeled oligonucleotide probe." Appl Environ Microbiol 59:541-6.

Wright, A. C., Hill, R. T., Johnson, J. A., Roghman, M. C., Colwell R. R., and Morris, J.G. 1996. "Distribution of *Vibrio vulnificus* in the Chesapeake Bay." Appl Environ Microbiol 62(2): 717-724.

Wu, S. I., Lo, S. K., Shao, C. P., Tsai, H. W., and Hor, L. I. 2001. "Cloning and characterization of a periplasmic nuclease of *Vibrio vulnificus* and its role in preventing uptake of foreign DNA." Appl Environ Microbiol. 67:82–88.

Yamazaki, K., and Esiobu, N. 2012. "Environmental Predictors of Pathogenic *Vibrios* in South Florida Coastal Waters." The Open Epidemiology Journal; 5, 1-4.

Yeung, P. S., Hayes, M. C., DePaola, A., Kaysner, C. A., Kornstein, L., and Boor, K. J. 2002. "Comparative phenotypic, molecular, and virulence characterization of *Vibrio* parahaemolyticus O3:K6 isolates." Appl Environ Microbiol 68: 2901–2909.

Zimmerman, A. M., DePaola, A., Bowers, J. C., Krantz, J. A., Nordstrom, J. L., Johnson, C. N., and Grimes, D. J. 2007. "Variability of total and pathogenic *Vibrio parahaemolyticus* densities in northern Gulf of Mexico water and oysters." Appl. Environ. Microbiol. 73:7589-75.