Vibrio vulnificus Monitoring in Recreational Waters
Final Report

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Vibrio vulnificus Monitoring in Recreational Waters

Joanna Mott, Ph.D. and Gregory Buck, Ph.D.

EXECUTIVE SUMMARY

Vibrio vulnificus is a common halophilic organism in coastal waters of some parts of the U.S. It is a well documented pathogen and is the leading cause of death in the U.S. related to seafood consumption. This species is also responsible for wound infections in persons who have had contact with marine waters. In the Coastal Bend region there have been recent cases of this pathogen infecting fishermen and causing death or severe illness and loss of limbs. There is no ongoing bacteria monitoring for this species in Texas coastal waters. In order to determine the distribution of the pathogen, for future education of the public, this study examined levels of *V. vulnificus* in Coastal Bend estuarine waters in relation to other environmental parameters such as temperature, salinity/conductivity, and dissolved oxygen.

The objective of this study was to determine the occurrence and distribution of the pathogen, *V. vulnificus* in south Texas coastal waters by establishing baseline data on *V. vulnificus* occurrence and abundance at six sites in the CBBEP area, monitoring seasonal variation in *V. vulnificus* at each site over a one year period and evaluating certain factors that may be associated with elevated levels of *V. vulnificus*. e.g. temperature, salinity, enterococci.

Due to the lack of data on *V. vulnificus* populations in Coastal Bend area waters, site selection was made with a goal of including several bays with different characteristics. The staff and management at CBBEP selected six public-use beaches, fishing areas, and/or recreational waters for the project. These included sites in the Laguna Madre (Bird Island Basin), Corpus Christi Bay (Cole Park), Nueces Bay, Copano Bay and Redfish Bay. Overall consideration was given to accessibility and safety. Water sampling was conducted at each site monthly for one year from August 2006 to July 2007 to provide seasonal data. *V. vulnificus* is known to enter a viable but non-culturable (VBNC) state during colder months in cooler waters. For South Texas waters where temperatures remain high, seasonal variation may differ.

Field sampling procedures documented in the TCEQ *Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003) were followed for collection of water samples and measurement of field parameters. Two water samples were collected from each site using sterile, one liter screw-cap polypropylene bottles. Field parameters were measured with an YSI water quality multiprobe instrument. Field data sheets were used to document field parameters. *V. vulnificus* and enterococci were isolated and enumerated following the U.S. FDA Bacteriological Analytical Manual Chapter 9. *Vibrio*. Online May, 2004, (http://www.cfsan.fda.gov/~ebam/bam-9.html), and U.S. EPA Method

1600 for enterococci, respectively. There is no one-step method to identify and enumerate *V. vulnificus.* Presumptive isolates were obtained by filtration and plating on VVA (*V. vulnificus* agar) and mCPC (modified cellobiose-polymyxin B-colistin) agar. Isolates from VVA were confirmed using a molecular technique described in the U.S. FDA Bacteriological Analytical Manual and FDA/Gulf Coast Seafood Laboratory Protocol (Vv-ISSC) 11/02 in which plating for colony isolation is followed by colony hybridization with a specific alkaline phosphatase-labeled gene probe that targets the cytolysin gene, *vvhA*.

Baseline data on the occurrence and abundance of *V. vulnificus* in the CBBEP area was collected. *V. vulnificus* was ubiquitous in the marine waters sampled and was found throughout the year, being isolated from each site each sampling event at levels as high as 3.97 X 10^4 CFU/100mL. Numbers of *V. vulnificus* varied seasonally over the 12 month period with highest levels generally found from August through October/November. However, the bacteria were isolated in low numbers throughout the winter months at all six sites. The highest numbers of *V. vulnificus* were found at Nueces Bay in June 2007 with the second highest water temperature (27.4°C) and second lowest salinity (23.48 ppt) at this site. Numbers of *V. vulnificus* were positively correlated with water temperature and negatively correlated with dissolved oxygen levels. Multiple regression analyses indicated that levels of *V. vulnificus* were primarily influenced by water temperature and salinity, with these two parameters accounting for 48.2% of the variability in the concentration of *V. vulnificus.* The other 51.8% of the variability in the levels of *V. vulnificus* was caused by unknown factors. Thus, it appears that no single parameter is primarily responsible for the levels of *V. vulnificus* in south Texas coastal waters.

Enterococci levels ranged from <1 to 1.06 X 10^3 CFU/100mL at the six sites. The highest numbers of *Enterococcus* sp. were found at Cole Park. There was a significant correlation between *V. vulnificus* and enterococci levels, suggesting that the fecal indicator, enterococci, might be a possible indicator of elevated levels of *V. vulnificus* in Coastal Bend waters.

Future studies are recommended to identify other parameters influencing levels of *V. vulnificus* in south Texas coastal waters such as nutrient levels and to further elucidate the relationship between *V. vulnificus* and enterococci. Relationships between environmental factors and incidence of *V. vulnificus* could be used to develop a predictive model for elevated levels of the organism which could then form a basis to advise the public of increased risk under certain conditions. To more fully understand the ecology of the organism, populations in sediment and oysters should also be enumerated to determine distribution and levels of *V. vulnificus* in these habitats. Finally additional studies to assess diversity and characteristics of environmental strains compared to clinical strains could provide a greater understanding of the level of risk to the public from exposure to *V. vulnificus* in coastal waters.

INTRODUCTION

Vibrio vulnificus is a gram-negative halophilic bacterium which naturally occurs in marine environments. It is perhaps the most invasive of vibrio species and humans can be infected by it through direct contact of skin lesions with seawater or consumption of raw seafood (Wright et al., 1981). Septicemic infection of humans by *V. vulnificus* can result in a mortality rate of over 50% and *V. vulnificus* is responsible for 95% of all seafood related deaths (Levin, 2005). Individuals who consume seafood contaminated with this organism can experience vomiting, diarrhea, and abdominal pain. *V. vulnificus* typically causes a severe and life-threatening illness characterized by fever and chills, septic shock, and blood-tinged blistering skin lesions. Humans with liver problems or who are immuno-compromised are particularly at risk. Infections with *V. vulnificus* resulting in septicemia and high mortality have been correlated with pre-existing liver disease and hemochromatosis (Wright et al., 1981). *V. vulnificus* wound infections often present with large red edematous lesions on the extremities (bullae), which may coalesce and become necrotic, leading to gangrene within the surrounding tissues, resulting in debridement or amputation (Blake et al., 1979).

There have been several cases of wound infections caused by *V. vulnificus* in the Coastal Bend region of the Texas coast in the last few years, as reported by the Corpus Christi Caller Times newspaper. Examples include: a 63-year-old man in Port Aransas, Texas who was shucking oysters without gloves when he was cut before developing a *V. vulnificus* infection (Corpus Christi Caller-Times, April 1, 1996); a 41-year-old man who cut his finger while fishing south of Freeport, Texas and lost a finger due to *V. vulnificus* (Corpus Christi Caller-Times, August 28, 2003); an 80-year-old fisherman who suffered a leg wound that was infected with *V. vulnificus* while fishing in Corpus Christi, Texas (Corpus Christi Caller-Times, August 28, 2003). *V. vulnificus* has also been reported as the etiologic agent responsible for infecting multiple fishermen in south Texas and causing death or severe illness and loss of limbs (Corpus Christi Caller-Times, June 23, 2005; http://www.caller.com/news/2006/sep/03/outdoors-calendar-090306/). Table 1 summarizes data from the Texas Department of State Health Services on reported *V. vulnificus* infections in Texas. Additional data is available on the website http://www.dshs.state.tx.us/idcu/. A total of 126 *V. vulnificus* infections were reported in Texas between 2000 and 2006 with the highest number reported in 2004 (32 reported infections) (Table 1, Texas Department of State Health Services 2008). In the years 1997, 1998 and 1999 there were totals of 17, 8 and 15 cases reported, respectively. Most reported infections occurred between May and October (Table 1), while only two *V. vulnificus* infections were reported during the winter months (December – February) (Table 1).

Table 1: Reported *V. vulnificus* infections in Texas for the years 2000-2006 by type of exposure (Texas Department of State Health Services, 2008 customized report).

	2000	2001	2002	2003	2004	2005	2006
January Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**
shellfish consumption	-	-	-	-	-	-	-
water contact	-	-	-	-	-	-	-
Other/ unknown	-	-	-	-	-	-	-
February Total	**0**	**0**	**0**	**0**	**0**	**0**	**1**
shellfish consumption	-	-	-	-	-	-	1
water contact	-	-	-	-	-	-	-
Other/ unknown	-	-	-	-	-	-	-
March Total	**0**	**0**	**0**	**0**	**1**	**0**	**0**
shellfish consumption	-	-	-	-	1	-	-
water contact	-	-	-	-	-	-	-
Other/ unknown	-	-	-	-	-	-	-
April Total	**0**	**1**	**0**	**1**	**2**	**0**	**0**
shellfish consumption	-	1	-	-	1	-	-
water contact	-	-	-	-	-	-	-
Other/ unknown	-	-	-	1	1	-	-
May Total	**2**	**3**	**0**	**2**	**0**	**1**	**2**
shellfish consumption	2	-	-	1	-	-	1
water contact	-	2	-	1	-	1	1
Other/ unknown	-	1	-	-	-	-	-
June Total	**1**	**2**	**2**	**1**	**5**	**3**	**1**
shellfish consumption	1	2	1	-	1	1	-
water contact	-	-	1	1	3	2	1
Other/ unknown	-	-	-	-	-	-	-
July Total	**4**	**1**	**5**	**3**	**13**	**3**	**6**
shellfish consumption	1	1	1	-	2	1	3
water contact	2	-	2	1	10	2	3
Other/ unknown	1	-	2	2	1	-	-
August Total	**1**	**2**	**4**	**2**	**3**	**4**	**6**
shellfish consumption	1	1	1	1	1	-	4
water contact	-	-	2	-	2	3	1
Other/ unknown	-	1	1	1	-	1	1
September Total	**3**	**2**	**2**	**1**	**3**	**3**	**4**
shellfish consumption	3	1	-	-	-	-	4
water contact	-	1	1	1	1	1	-
Other/ unknown	-	-	1	-	2	2	-
October Total	**0**	**3**	**2**	**2**	**2**	**3**	**1**
shellfish consumption	-	3	2	2	-	-	1
water contact	-	-	-	-	2	1	-
Other/ unknown	-	-	-	-	-	2	-
November Total	**0**	**0**	**0**	**2**	**3**	**0**	**1**
shellfish consumption	-	-	-	2	1	-	1

water contact	-	-	-	-	2	-	-
Other/ unknown	-	-	-	-	-	-	-
December Total	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Shellfish consumption	-	-	-	-	-	-	-
water contact	-	-	-	-	-	-	-
Other/ unknown	1	-	-	-	-	-	-
Total	**12**	**14**	**15**	**14**	**32**	**17**	**22**

V. vulnificus has been isolated from seawater at many locations along the Gulf of Mexico, including Galveston Island, Tampa Bay, Apalachicola Bay, Mobile Bay, Mississippi Sound, Cedar Point, Black Bay, Charlotte Harbor, and Galveston Bay (DePaola et al., 1994; Kelly, 1982; Lin et al., 2003; Lipp et al., 2001; Motes et al., 1998; Tamplin et al., 1982). It has also been isolated from the Atlantic (Oliver et al., 1982; Oliver et al., 1983; O'Neill et al., 1992; Pfeiffer et al., 2003; Randa et al., 2004; Wright et al., 1996), and U.S. Pacific coastal waters (Kaysner et al., 1987). This organism has also been found globally from waters of Denmark (Høi et al., 1998a), Japan (Fukushima and Seki, 2004), Israel (Bisharat et al., 1999) and Italy (Montanari et al., 1999).

There have been several studies conducted on the prevalence of *V. vulnificus* in the Gulf of Mexico. In a study conducted by Kelly (1982), the occurrence of *V. vulnificus* in the Gulf of Mexico was found to be dependent upon presence of warm water temperatures and low salinity. *V. vulnificus* was isolated from all but one of 21 sample sites around Galveston Island, Texas during the 12-month study. Sample sites were characterized as having salinities that ranged from 7 - 16 ppt and temperatures often exceeding 20°C. Prevalence of *V. vulnificus* was highest during summer months and it was rarely isolated at temperatures below 20°C. A study conducted by Tamplin et al. (1982) examined the prevalence of *V. vulnificus* in seawater and shellfish from two Florida estuaries, Tampa Bay and Apalachicola Bay. *V. vulnificus* was isolated only at water temperatures greater than 17°C and it was found in a larger proportion of samples with temperatures above 29°C. Isolation of *V. vulnificus* was more frequent when salinity exceeded 17 ppt and it was found in an even higher proportion of samples with salinities in excess of 23 ppt. It was found more often in waters having a fecal coliform MPN of less than 3 per 100 ml, but it was also isolated when there were high fecal coliform levels. Occurrence of *V. vulnificus* between sampling events at the two bays seemed to be dependent on water temperature and salinity. Similarly, *V. vulnificus* was isolated from Mobile Bay and Mississippi Sound waters more commonly during the summer than the winter months (DePaola et al., 1994).

The effects of water temperature and salinity on the occurrence of *V. vulnificus* in the northern Gulf coast and Atlantic coast were also studied by Motes et al. (1998) who found a common seasonal distribution of *V. vulnificus* at all Gulf coast sites during a 15-month study. Levels of *V. vulnificus* were high from May - October and then decreased in November and December, remaining low from January - mid-March. There was an increase in *V. vulnificus* in March and April to levels typically found in summer. Ability to isolate *V. vulnificus* increased with water temperatures up to 26°C and remained steady at higher temperatures. Intermediate salinities between 5 and 25 ppt at Gulf coast sites were optimal for *V. vulnificus* isolation. Lower numbers of *V. vulnificus* were isolated from salinities above 28 ppt at Atlantic Coast sites. In a subtropical Gulf of Mexico estuary, Charlotte Harbor, Florida, Lipp et al. (2001) were able to isolate *V. vulnificus* throughout the year. Levels ranged from 0.058 - 1.21 X 10^3 CFU/100 ml with warm temperatures and moderate salinities favoring detection. Optimal isolation was salinity dependent and occurred at 15 ppt. Salinity was the main factor controlling the seasonal distribution of *V. vulnificus* in this subtropical estuary, but concentrations of *V. vulnificus* were also positively correlated with water temperature. Recently, incidence of *V. vulnificus* at five sites in Galveston Bay was examined in a one-year study by Lin et al. (2003). Detection was positively correlated with temperature, and *V. vulnificus* was not isolated from water between October and March. Salinity levels ranged from 5 to 25 ppt when *V. vulnificus* was detected, and there was a slight negative correlation between salinity and *V. vulnificus* concentrations.

Several studies have been conducted to examine the occurrence of *V. vulnificus* along the Atlantic coast. The distribution of *V. vulnificus* among five Atlantic coast sites from North Carolina to Georgia was described by Oliver et al. (1982). Their research showed that distribution was correlated with high hydrocarbon levels in water and salinity levels in plankton samples. In a subsequent study, Oliver et al. (1983) examined the distribution of *V. vulnificus* at 80 environmental locations along the Atlantic coast and showed that it could be isolated from southern Florida to as far north as Cape Cod, Massachusetts. Seasonal incidence of *V. vulnificus* was also examined in the Great Bay Estuary of New Hampshire and Maine by O'Neill et al., 1992. Six estuarine sites along with three upstream freshwater sites were investigated. *V. vulnificus* was isolated between July and October and temperature and salinity were the primary abiotic factors correlated with the presence of the bacteria, but other unknown factors may control the occurrence of *V. vulnificus* in the estuary studied. Isolation occurred under wide ranges of temperature and salinity (11.1°C – 29.5°C and 5-27.0 ppt, respectively). In a 21-month study of Chesapeake Bay, Wright et al. (1996) detected *V. vulnificus* in 80% of samples when water temperature exceeded 8°C, but it was not isolated at lower temperatures. Presence of *V. vulnificus* was correlated with lower salinities. The ecology of *V. vulnificus* in estuarine waters of eastern North Carolina was examined by Pfeffer et al. (2003). Water temperature was the factor most highly correlated with presence of *Vibrio* spp., which was most prevalent when mean water temperature ranged from 15 to 27°C and at salinity levels between 8 and 14 ppt. It was not detected when water temperature was below 14°C. In addition, total vibrio levels were positively correlated with levels of *V. vulnificus*, while dissolved oxygen was negatively correlated with *V. vulnificus* in this study. The effects of temperature and salinity on the presence of *V. vulnificus* were also examined by Randa et al. (2004) in Barnegat Bay, New Jersey. Again there was a strong positive correlation between levels of *V. vulnificus* and water temperature. High concentrations were found at salinity levels of 5 - 10 ppt, with a positive correlation between 20 - 25 ppt. *V. vulnificus* remained undetectable during winter months with the exception of one winter water sample which had a temperature of 6°C.

Occurrence of *V. vulnificus* along the Pacific coastline of the U.S. was examined by Kaysner et al. (1987). For estuaries located in Washington, Oregon, and California, *V. vulnificus* was detected in only 5.9% of the samples and 10 of the 24 estuaries. The organism was Isolated most frequently when water temperature exceeded 15°C and salinity remained between 15 and 30 ppt.

Prevalence of *V. vulnificus* in coastal waters has also been examined in other countries. Hoi et al. (1998a) collected water samples along the coast of Denmark to evaluate the occurrence of *V. vulnificus* in Danish marine environments. Between June and mid – September, there were low numbers of *V. vulnificus* (0.08 – 1.9 CFU/100mL) and it was isolated in 42% of water samples collected. Ranges of salinity and water temperature throughout the study were 5 to 17 ppt and 14 to 22°C. Incidence of *V. vulnificus* correlated strongly with water temperature, but *V. vulnificus* was isolated from water with a temperature as low as 7°C. A multi-year study on the ecology of *V. vulnificus* in brackish environments of the Sada River in Shimane Prefecture, Japan was conducted by Fukushima et al. (2004). *V. vulnificus* was most prevalent from water samples with salinities between 19 and 29 ppt but was also isolated at salinities between 2 and 8 ppt. Presence of *V. vulnificus* correlated positively with water temperature and it was isolated at water temperatures between 7 and 30°C. Cavallo et al. (2002) examined occurrence of *V. vulnificus* in seawater and mussels from the Ionian Sea, Italy but *V. vulnificus* was only isolated from mussels and not seawater.

Although there have been numerous studies investigating the occurrence and distribution of *V. vulnificus* in various estuaries around the U.S., there is a lack of information on its incidence and distribution in south Texas coastal waters, where water temperatures remain above 20°C for most of the year and wound infections are reported.

Induction of Viable but Non-Culturable State

V. vulnificus is known to enter into a viable but nonculturable (VBNC) state during colder months (i.e. in cooler waters below 10° C) (Oliver and Bockian, 1995; Oliver et al., 1995; Oliver, 1999; Weichart, 1999; Whitesides and Oliver, 1997). The VBNC state can be induced by a reduction of ambient temperature to 5°C, with cells becoming nonculturable within 7 days (Oliver and Bockian, 1995). In this state, cells do not develop into colonies on regular media in the laboratory setting; however, they are still metabolically active and remain virulent. However, there is a decrease in virulence over time in the VBNC state. The VBNC state appears to be an important survival mechanism in most bacteria which can frequently be found outside known environmental optima (e.g. as with *V. vulnificus* and cold water). Resuscitation of *V. vulnificus* cells to a fully culturable state can occur within 24 h when returned in warmer months (August – November) to the same waters from which they were isolated (Oliver et al., 1995).

Nutrients also appear to have a negative effect on resuscitation of *V. vulnificus* cells in the VBNC state and adding nutrient only allows for detection of culturable cells that are present. In a study by Whitesides et al. (1997), it was shown that resuscitation in the presence of nutrients required an increase in ambient temperature.

Some researchers also believe that VBNC cells are simply not capable of being revived and sometimes referred to as *active but nonculturable* (ABNC). Weichart (1999) proposed to apply the term "viable" only to recoverable cells, and the term "active but nonculturable" (ABNC) to non-recoverable (non-viable) cells such as those which have been lethally injured or senescent cells.

Culture Media

A recent review by Harwood et al. (2004), describes various media used for the enumeration of *V. vulnificus.* Two different media found to be particularly selective are modified cellobiose-polymyxin B-colistin (mCPC) agar and *Vibrio vulnificus* agar (VVA). The former was created for obtaining environmental isolates and is a modification of CPC agar. Cellobiose polymyxin B-colistin agar inhibits growth of other types of bacteria and allows *V. vulnificus* to proliferate (Massad and Oliver, 1987). Differentiation of *V. vulnificus* from other *Vibrio* spp. is accomplished by cellobiose fermentation, resulting in yellow colonies surrounded by a yellow halo. The difference between mCPC agar and CPC agar is that the concentration of colistin is reduced to 400,000 U/l (versus 1,400,000 U/l in CPC). However, the two polymyxins (B and E) in the medium can inhibit gram-negative bacteria, including some strains of *V. vulnificus* by binding to the cell membrane and altering its structure allowing it to become more permeable, resulting in cell death (Murray et al., 2005). Numerous studies have used mCPC agar to isolate *V. vulnificus* (for example: DePaola et al., 1994; Tamplin et al., 1991). Tamplin et al. (1991) used mCPC agar in conjunction with immunoassay to confirm 99.7% of isolates. DePaola et al. (1994) utilized mCPC to identify *V. vulnificus* in water, sediment, oysters, and fish intestines. Again confirmation of greater than 95% was accomplished using enzyme immunoassay. In another study (Fukushima and Seki, 2004), pre-enrichment of samples in alkaline peptone water (APWP) followed by subculturing on mCPC agar enhanced the isolation of *V. vulnificus*. Colony hybridization with the cytolysin probe has been used for >95% presumptive identification of *V. vulnificus* from mCPC plates (DePaola et al., 1994).

V. vulnificus agar (Kaysner and DePaola, 2004) is considered less selective than mCPC agar (Masad and Oliver, 1987), but was designed to be used in conjunction with the VVAP oligonucleotide probe for identification (Wright et al., 1993). By this method, cellobiose fermentation generates presumptive yellow colonies surrounded by a yellow halo. Direct detection from Gulf Coast oysters has been achieved on VVA agar and then confirmed with the VVAP probe (DePaola et al., 1997).

The U.S. Food and Drug Administration (FDA) in its Bacteriological Analytical Manual (BAM) describes two molecular methods to confirm *V. vulnificus* isolates on the two aforementioned selective media following filtration (Kaysner and DePaola, 2004). The first involves colony isolation on selective media followed by confirmation using polymerase chain reaction (PCR). The second method involves plating to achieve colony isolation and then using colony hybridization with a DNA probe to confirm colonies. The DNA probe, alkaline phosphatase-labeled and referred to as VVAP, has been employed in multiple studies to confirm the identification of environmental and clinical isolates (Cerda-Cuellar et al., 2001; DePaola et al., 1997; Hoi et al., 1998a; Wright et al, 1993; Wright et al., 1996). The VVAP probe was derived from gene sequencing of the *V. vulnificus* cytolysin structural gene, *vvhA* (Wright et al., 1993). It was proven to be very selective because it hybridized with DNA from each of the strains tested and did not display any false-positives with non-*V. vulnificus* strains.

Objectives

The objective of this study was to determine the incidence and distribution of *V. vulnificus* in south Texas estuarine waters.

Specific objectives of this study were to:

1. Establish baseline data on *V. vulnificus* occurrence and abundance at six sites in the CBBEP area.

2. Monitor seasonal variation in *V. vulnificus* at each station over a one year period.

3. Compare *V. vulnificus* levels in relation to other environmental parameters measured at each station e.g. temperature and salinity.

In addition, levels of *V. vulnificus* were compared to those of the fecal indicator, *Enterococcus* sp. to assess whether levels of *Enterococcus* (routinely monitored) can provide an indication of *V. vulnificus* levels in surface waters.

The project objective was to collect data that complies with TCEQ rules for surface water quality monitoring programs and water quality assessments. The specific objective of this project was to collect the necessary water quality monitoring data to evaluate certain factors that may be associated with elevated levels of *V. vulnificus*. These data include bacteria (*V. vulnificus* and enterococci) and routine field parameters.

METHODS

All methods followed the approved Quality Assurance Project Plan (QAPP) for this study (Mott and Buck, 2006).

Sites

Due to the lack of data on *Vibrio vulnificus* populations in Coastal Bend area waters site selection was made with a goal of including several bays with different characteristics. The staff and management at CBBEP selected six public-use beaches, fishing areas, and/or recreational waters for the project. These areas included sites in the Laguna Madre (Bird Island Basin), Corpus Christi Bay (Cole Park), Nueces Bay, Copano Bay and Redfish Bay. Coordinates are shown in Table 2 and site locations in Figures 1-6. Overall consideration was given to accessibility and safety. Historical ranges in water quality parameters at the six sampling sites from 1969 to the present are shown in Table 3 (TCEQ, 2007).

Bird Island Basin is located in the upper Laguna Madre at PINS (Padre Island National Seashore) (Figure 1). It is used as a fishing area and other recreational activities include windsurfing, kayaking, and camping. TCEQ data show salinities have reached as high as 52 ppt at this site, usually in warmer months.

Nueces Bay is a shallow bay that receives freshwater from Nueces River and exchanges saline water with Corpus Christi Bay. The sampling site was located in Nueces Bay at the north end of US Highway 181 (Figure 2). Nueces Bay is primarily used as a recreational fishing area. Water temperatures have ranged from a low of 4°C in winter to 32°C in summer. Salinity levels range from a low of 9 to 42 ppt, due to the freshwater inflow from the Nueces River.

Table 2: Texas Coastal Bend study sites and their coordinates.

Site	Coordinates
Bird Island Basin	27°28'10.01" N 97°18'29.05" W
Nueces Bay	27°50'34.19" N 97°23'42.08" W
Cole Park	27°46'02.48" N 97°23'02.61" W
Redfish Bay	27°53'02.51" N 97°07'02.50" W
Copano Bay Causeway	28°07'59.66" N 97°00'29.69" W
Copano Bay near Bayside	28°03'51.17" N 97°13'12.33" W

Table 3: Historical physical water quality parameter ranges from Bird Island, Nueces Bay, Cole Park, Redfish Bay, Copano Bay, and Bayside for 1969 – 2006 (TCEQ, 2007 http://www.tceq.state.tx.us/compliance/monitoring/crp/data/samplequery.html)

Location	**Range**	**Temp (°C)**	**Conductivity (µmhos/cm)**	**Salinity (ppt)**	**DO (mg/L)**	**pH (s.u.)**	**Secchi (m)**
Bird Island	high	32	76000	52	10	~ 8	0.8
	low	13	42000	27	4	~ 8	0.4
Nueces Bay	high	32	63000	42	17	8	> 0.55
	low	4	4000	9	4	7	0.1
Cole Park	high	29	63000	42	10	~ 8	> 0.72
	low	13	40000	25	4	~ 8	0.15
Redfish Bay	high	32	60000	39	12	9	> 15
	low	10	1000	7	4	6	0.1
Copano Bay	high	30	37000	24	13	~ 8	> 1
	low	9	2000	1	5	~ 8	0.03
Bayside	high	34	56000	34	16	9	1
	low	4	1000	1	2	7	0.03

The Corpus Christi Bay sampling site was located at the south end of Cole Park at what is known as Oleander Point (Figure 3). Recreational activities at Cole Park include windsurfing and fishing. Water temperatures range from 13 to 29° C depending on season, while salinity levels are between 25 and 42 ppt.

The Redfish Bay sampling site was located at the Morris and Cummings Cut by Stedman Island (Figure 4). Redfish Bay is primarily used by fishermen. Water temperatures are between 10 and 32°C. Salinity levels range from 7 to 39 ppt.

Two sites in Copano Bay were sampled. One sampling site was located at the north end of Copano Bay at Copano Bay Causeway at SH35 (Figure 5). Water temperature ranges from 9 to 30°C. Salinity levels range from a low of 1 to 24 ppt. The second sampling site in Copano Bay was located at the south end of the bay off FM136 near Bayside and receives freshwater inflow from the Aransas River resulting in a range of salinity from 1 to 34 ppt (Figure 6).

Microbiological analyses were conducted at the Texas A&M University-Corpus Christi Environmental Microbiology Laboratory.

FIG 1. Location of sample site in upper Laguna Madre on Bird Island Basin at PINS. Google Earth 2006.

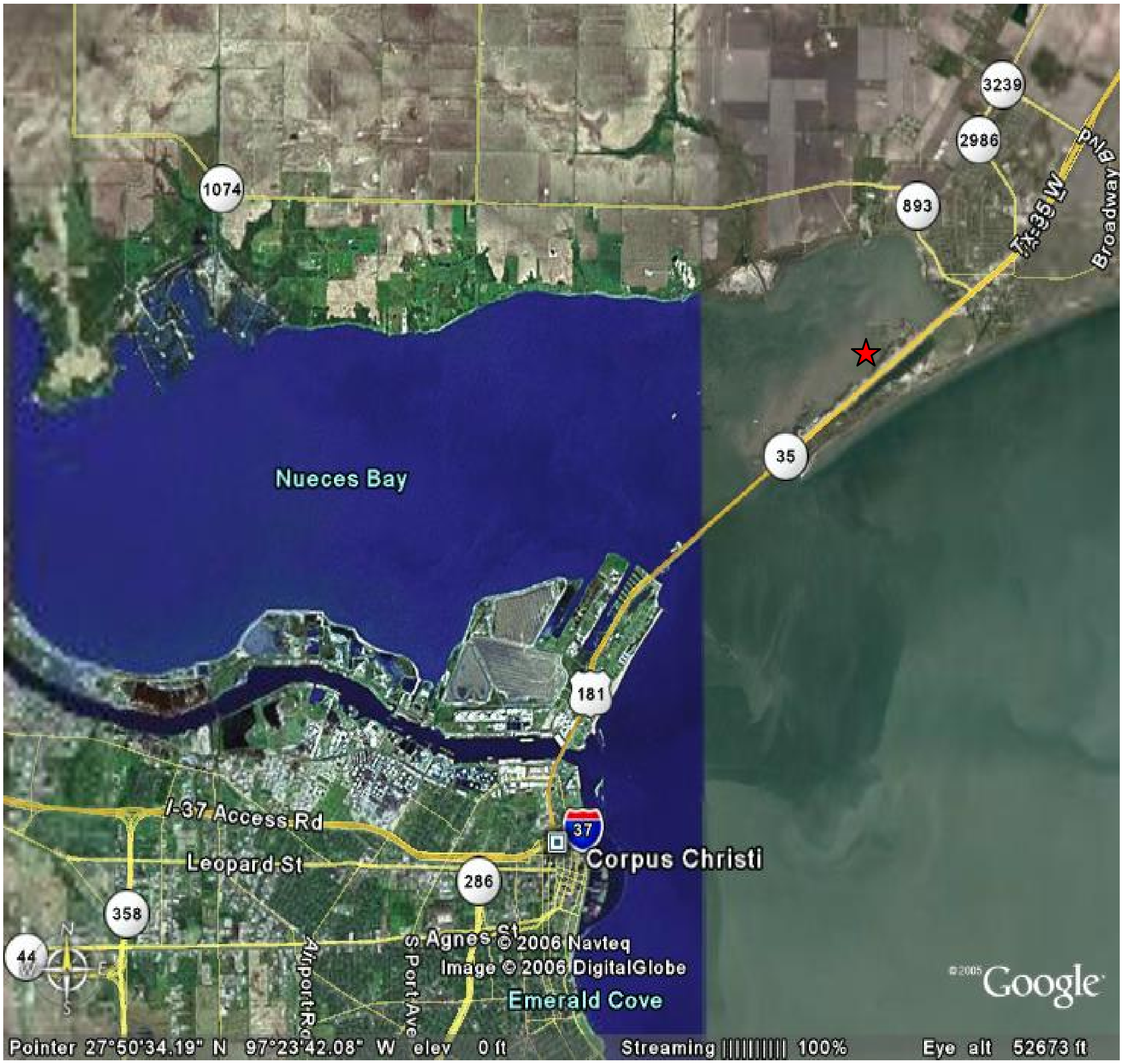

FIG 2. Location of sample site in Nueces Bay at North end of US Highway 181. Google Earth 2006.

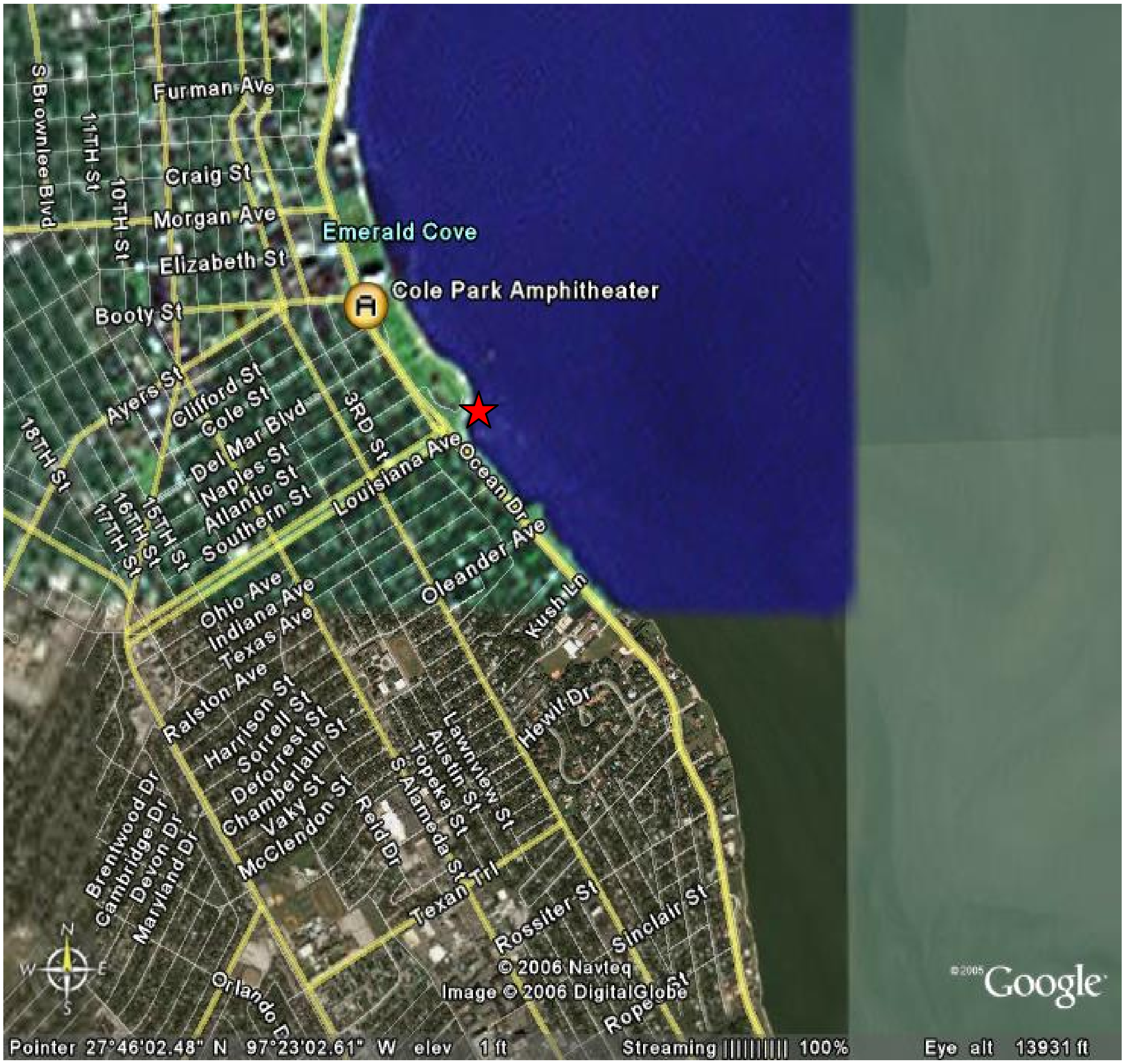

FIG 3. Location of sample site in Corpus Christi Bay at south end of Cole Park. Google Earth 2006.

FIG 4. Location of sample site in Redfish Bay at the Morris and Cummings Cut. Google Earth 2006.

FIG 5. Location of sample site at Copano Bay Causeway at SH35. Google Earth 2006.

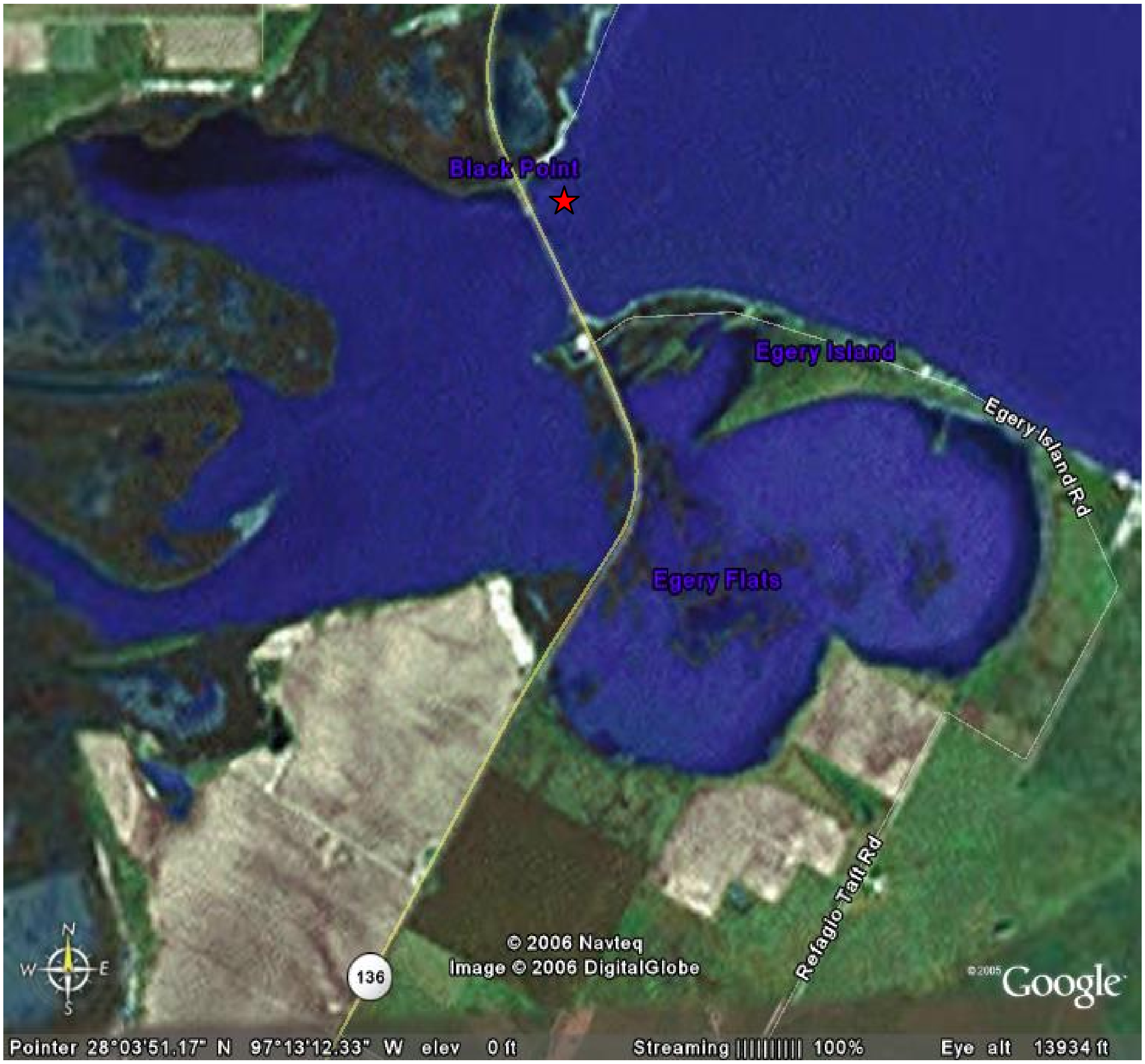

FIG 6. Location of sample site in Copano Bay off FM136 near Bayside. Google Earth 2006.

Field Collection

Sampling was conducted monthly for a one-year period from August 2006 to July 2007 to provide seasonal data. *Vibrio vulnificus* is known to enter a viable but not culturable (VBNC) state during colder months, in cooler waters (Oliver et al., 1995). For south Texas waters where temperatures remain high, seasonal variation may differ.

Field sampling procedures documented in the TCEQ *Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003) were followed for the collection of water samples and measurement of field parameters.

Standard field data sheets (as approved in the QAPP) were used to document field parameters. Abiotic factors (water temperature, salinity, conductivity, dissolved oxygen, pH, and turbidity) were measured at each station during each sampling event using a YSI water quality multiprobe instrument Model 6820 and a Secchi disk. Probe parameters were measured *in situ* when possible, but when that was not possible or unsafe, the TCEQ *Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003) protocol for field measurements from a bucket was followed. Calibration of the YSI mulitprobe instrument followed the manufacturer's instructions. Precipitation data were obtained through the National Oceanic and Atmospheric Administration (NOAA) website (www.noaa.com). Other observations (e.g. weather conditions, water appearance, odor, wind intensity, direction, etc.) were also recorded on the field data sheets (Appendix).

Two water samples were collected from six sampling stations at several public use beaches, fishing areas, and/or recreational waters within the CBBEP study area including Bird Island Basin in upper Laguna Madre, Nueces Bay, Cole Park in Corpus Christi Bay, Redfish Bay, and two stations in Copano Bay. Samples were collected using sterile, screw-cap, one liter polypropylene bottles which were cleaned and autoclaved prior to each use.

At Bird Island, Nueces Bay, Cole Park, and near Bayside in Copano Bay, water samples were collected from the shoreline by wading out to a depth of ~0.6 m. Two samples were taken using sterile, one liter polypropylene bottles by submerging one bottle at a time under water to a depth of ~0.3 m and uncapping the bottle under water until the bottle was filled to ~2.5 cm from the top; the cap was immediately replaced while the bottle was under water.

At Redfish Bay and Copano Bay Causeway, water samples were taken from a pier or bridge, respectively, using a sterile bucket. At Redfish Bay, water samples were taken at the end of the pier at the Morris and Cummings Cut. At Copano Bay Causeway, water samples were taken halfway down the bridge (~400 m). A sterile bucket was rinsed three times with ambient water before collecting the water sample. The bucket was completely submerged under water to collect the sample. The water sample was then poured from the bucket into two sterile one liter polypropylene bottles.

The one liter sampling bottles containing the water samples were transported in ice chests with ice (to ensure samples were maintained between 1^{O}C and 4^{O}C) to the TAMU-CC environmental laboratory for analysis within the required holding time (six hours plus two hours lab time). Bottles were labeled with an indelible, waterproof marker. Label information included the site identification, the date and time of sampling. A standard TCEQ approved Chain of Custody form was completed with collector signature and date/time collected. On arrival at the laboratory the TAMU-CC Laboratory Manager or a trained lab analyst checked times of collection to ensure holding times had not been exceeded. An additional bottle of water of the same specifications as the sample bottles was included in the ice chest and was used to check temperature of the samples on arrival in the laboratory.

Laboratory Analysis

Procedures for laboratory analyses were in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the TCEQ *Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003), 40 CFR 136, or other reliable procedures acceptable to TCEQ. Exceptions to this included analyses and sample matrices for which no regulated methods exist.

Enterococci were isolated and enumerated using EPA Method 1600: Membrane Filter Test Method for *Enterococcus* in Water U.S. EPA (1997, 2000, 2002). Following filtration of three volumes of water using 0.45μ membrane filters, each filter was placed onto a plate containing the selective mEI (membrane-Enterococcus Indoxyl-β-D-Glucoside agar) medium and then incubated at 41^{O}C for 24 hours. After incubation all colonies with a blue halo were counted as *Enterococcus*. Verifications of colonies were performed as outlined in the method protocol. (http://epa.gov/waterscience/methods/biological/1600enterococcus.pdf).

V. vulnificus isolations were performed by filtering multiple different volumes of each water sample (0.45μ membrane filters). Enumeration following the U.S. FDA Bacteriological Analytical Manual Chapter 9. Vibrio. Online May, 2004, http://www.cfsan.fda.gov/~ebam/bam-9.html, using the FDA/Gulf Coast Seafood Laboratory Protocol. (Vv-ISSC) 11/02 (U.S. FDA, 2002), with plating onto VVA (*Vibrio vulnificus* agar) followed by colony-blot hybridizations with a specific alkaline phosphatase-labeled gene probe (VVAP) to confirm isolates. A positive result for *V. vulnificus* was indicated by a purple colony blot, whereas a negative result was colorless, yellow, or light brown. Comparison plating was performed using mCPC (modified cellobiose-polymyxin B-colistin) agar (a more specific medium for *V. vulnificus*, but which may inhibit certain strains).

Detailed laboratory QC requirements were followed as described in the QAPP for the project. Laboratory bacteriological duplicates were performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates were evaluated by

calculating the logarithm of each result and determining the range of each pair. Performance limits were used to determine the acceptability of duplicate analyses.
The quality control for bacteriological membrane filtration methods followed *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003). For each membrane filter test, sterility of the media, petri dishes, membrane filters, dilution water and apparatus were checked using sterile water. If colonies appear on the blank then all data from samples filtered after the blank were discarded. A blank was run at the start and end of each group of samples analyzed. In cases where extremely high levels of bacteria were present in the sample, the blank run at the end of the group should have less than 1% of the colonies on the sample filter.

Additional method specific QC requirements were performed (e.g., positive controls, negative controls) as specified in Section 9020 B. *Standard Methods for the Examination of Water and Wastewater* (21st Edition, 2005), American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) 2005. Recommended positive and negative control cultures for enterococci were used as per *Standard Methods* Table 9020:V (*Enterococcus faecalis* American Type Culture Collection (ATCC) 29212 and *Streptococcus salivarius* ATCC 13419). Recommended positive and negative controls for *V. vulnificus* were used as per the U.S. FDA Online 2004 Method for *V. vulnificus* and U.S. FDA. 2002. *MPN Procedure for the enumeration of Vibrio vulnificus using gene probe for identification.* FDA/Gulf Coast Seafood Laboratory Protocol. (Vv-ISSC) 11/02. Positive controls included *V. vulnificus (*ATCC 27562 and 33817) and negative controls were *V. alginolyticus* (ATCC 17749) *V. mimicus (*ATCC 33653), and *V. parahaemolyticus* (ATCC 17802). The requirements for these samples, their acceptance criteria, and corrective action are method-specific.

Statistical Analysis

The statistical program SPSS (Statistical Package for the Social Sciences) version 12.0 for Windows was used to analyze the data. A two-way analysis of variance (ANOVA) was conducted to determine whether there was a statistically significant difference in *V. vulnificus* populations isolated using VVA versus mCPC media and between the sites sampled. The Tukey HSD test was used to determine which sites differed in *V. vulnificus* populations. *V. vulnificus* numbers were log transformed to achieve normality. A multiple regression analysis was completed, and Pearson's coefficient of correlation was also used to detect correlations between numbers of *V. vulnificus* as confirmed by gene probe, and each environmental factor at 95% and 99% confidence levels.

RESULTS

Water Quality Parameters

A total of 144 water samples were collected from the six sites. Water temperatures ranged from 9 to 31°C from August 2006 through July 2007 at all of the sites (Tables 4 and 5). Between the November and December 2006 sampling events, water temperatures dropped by 5 - 8°C from 20 - 22°C to 13 - 16°C. For Bird Island, Nueces Bay, and Cole Park water temperatures increased 8 to 10°C between the March and April 2007 sampling events from 15 - 16°C and remained > 24° C through July 2007. At Redfish Bay, Copano Bay causeway, and Copano Bay near Bayside, water temperatures increased from 9 - 12°C to 18 - 21°C between the February and March 2007 sampling events followed by a cool front, which dropped water temperatures by 4°C to 17 - 18°C during the April 2007 sampling event. Water temperatures then increased by 8 to 10°C during the May 2007 sampling event and remained greater than 26°C through July at Redfish Bay and both Copano Bay sites. Average water temperatures were between 22 and 23°C at each of the sites sampled (Table 6). The highest water temperature (31°C) occurred at Redfish Bay during the August 2006 sampling event, while the lowest water temperature (9°C) occurred at Copano Bay near Bayside in February 2007 (Table 5). Copano Bay near Bayside had the widest water temperature range of the six sites sampled (21°C) (Table 7).

There was a broad range in salinity at each of the sites sampled. Nueces Bay and Cole Park had similar average salinities of 32 – 33 ppt (Table 6). Salinity levels were between 31 and 41 ppt at Nueces Bay and Cole Park for 8 of 12 sampling events (Table 4). The highest salinities at each of these sites were 41.5 – 41.8 ppt in October 2006 (Table 4). Both sites in Copano Bay had salinity levels between 1 and 20 ppt with the exception of three sampling events that exceeded 20 ppt at the Copano Bay causeway (September 2006, 23.6 ppt; December 2006, 22.2 ppt; and January 2007, 20.6 ppt, respectively). The lowest salinities for the two sites in Copano Bay were 0.89 and 0.62 ppt in July 2007 presumably related to rainfall (see later) (Table 5). The widest range in salinity occurred at Bird Island and Redfish Bay (26.9 - 52.7 ppt and 12.4 - 37.8 ppt, respectively) (Table 7). Salinity at Redfish Bay ranged from 19.8 – 37.8 ppt with the exception of July 2007 (12.3 ppt). Bird Island had the highest mean salinity of all the sites (39.7 ppt) reaching 52.7 ppt in October 2006 (Table 4). Salinity levels changed throughout the study at each site, and even at Bayside which had the smallest range in salinity, the range was 16 ppt (range 0.62 to 16.69 ppt) (Table 7). Bayside had the lowest salinities overall of the sites sampled (Table 5). Since conductivity is strongly dependent on salinity, both parameters varied similarly (Tables 4 and 5).

Table 4: Physical water quality parameters from Bird Island, Nueces Bay, and Cole Park for August 2006 through July 2007.

Location	Date	Temp (°C)	Conductivity (μmhos/cm)	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Secchi Disk (m)
Bird Island	8/15/2006	27.76	72920	47.27	6.28	8.39	0.35
	9/6/2006	26.26	62630	42.18	4.68	8.41	0.375
	10/4/2006	27.38	76008	52.67	4.49	8.39	>0.46
	11/8/2006	21.34	63960	43.29	3.59	8.39	>0.45
	12/6/2006	13.19	63210	42.53	7.61	8.25	>0.66
	1/3/2007	13.91	52060	34.24	9.95	8.13	>0.51
	2/7/2007	15.18	46800	30.44	9.2	8.32	>0.49
	3/7/2007	15.55	54770	36.28	5.37	8.07	>0.57
	4/4/2007	25.13	61990	41.72	4.19	7.73	>0.48
	5/9/2007	24.59	60390	40.51	8.53	7.57	>0.51
	6/6/2007	26.71	58320	38.88	5.83	7.8	>0.48
	7/5/2007	26.84	41980	26.89	7.05	7.52	>0.51
Nueces Bay	8/15/2006	28.51	62800	39.24	5.88	8.04	0.175
	9/6/2006	24.86	61410	41.28	6.07	8.05	0.175
	10/4/2006	26.33	61074	41.5	4.21	8.12	0.33
	11/8/2006	21.82	51390	33.81	6.8	8.13	>0.55
	12/6/2006	14.67	53660	35.44	6.71	8.16	0.32
	1/3/2007	13.12	43600	28.09	9.48	8.2	>0.42
	2/7/2007	16.26	47070	30.65	7.48	8.37	0.31
	3/7/2007	15.68	49920	32.72	5.48	7.96	0.275
	4/4/2007	24.46	48670	31.77	5.16	7.9	0.1
	5/9/2007	24.71	45260	29.29	7.93	7.77	0.3
	6/6/2007	27.4	37180	23.48	6.75	7.78	0.225
	7/5/2007	26.06	31590	19.64	8.44	7.51	0.35
Cole Park	8/15/2006	29.45	63400	38.88	6.95	8.03	0.35
	9/6/2006	27.27	57930	38.57	6.74	8.01	0.5
	10/4/2006	27.32	62021	41.82	4.48	8.01	>0.52
	11/8/2006	22.59	52410	34.56	7.13	8.12	>0.72
	12/6/2006	15.38	53970	35.69	6.39	8.13	>0.66
	1/3/2007	14.2	51550	33.87	9.61	8.12	0.41
	2/7/2007	15.19	45730	29.67	9.65	8.01	>0.54
	3/7/2007	16.43	47870	31.23	5.58	8.11	>0.6
	4/4/2007	24.07	50110	32.83	5.93	7.96	0.15
	5/9/2007	25.42	45370	29.36	8.68	7.87	0.3
	6/6/2007	28.07	44950	28.98	6.81	7.88	0.455
	7/5/2007	27.89	39770	25.29	7.75	7.62	0.275

Table 5: Physical water quality parameters from Redfish Bay, Copano Bay, and Bayside for August 2006 through July 2007.

Location	Date	Temp (°C)	Conductivity (µmhos/cm)	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Secchi (m)
Redfish Bay	8/23/2006	31.35	57060	37.78	6.16	8.25	0.3
	9/13/2006	27.44	54400	35.92	5.99	8.08	0.72
	10/11/2006	26.21	48043	31.55	7.76	8.15	0.8
	11/15/2006	22.37	47250	30.77	5.8	8.09	0.19
	12/13/2006	16.24	47570	31.01	11.62	8.03	>1
	1/11/2007	15.6	45240	29.32	7.03	7.87	0.75
	2/14/2007	12.25	31840	19.85	10.16	8	0.59
	3/21/2007	22.09	40720	26.08	4.91	7.97	0.525
	4/11/2007	18.5	31800	19.87	9.27	8.07	0.55
	5/16/2007	26.22	45920	29.73	8.19	7.85	0.75
	6/13/2007	29.48	38440	24.31	7.01	8.09	0.95
	7/11/2007	29.15	20740	12.34	6.34	8.25	0.8
Copano Bay	8/23/2006	30.4	35500	19.95	5.88	8.02	0.3
	9/13/2006	27.09	37390	23.63	5.67	7.98	0.82
	10/11/2006	26.34	30096	19.2	6.91	7.85	1.05
	11/15/2006	21.04	29550	18.31	7.57	7.99	>1.05
	12/13/2006	14.13	35260	22.24	13.36	8.09	>0.85
	1/11/2007	14.83	32900	20.62	7.72	7.75	0.75
	2/14/2007	12.48	21720	13.08	10.75	8.17	0.73
	3/21/2007	21.61	29490	18.27	5.28	7.8	0.4
	4/11/2007	17.03	19920	11.93	10.81	8.05	0.7
	5/16/2007	27.68	17240	10.11	9.4	8.05	0.55
	6/13/2007	30.1	19750	11.68	8.7	8.01	0.55
	7/11/2007	29.46	1768	0.89	7.54	8.45	0.25
Bayside	8/23/2006	30.2	30080	16.69	5.07	7.93	0.575
	9/13/2006	26.27	26290	16.05	6.2	7.72	>0.42
	10/11/2006	24.96	15140	8.8	7.49	7.94	>0.55
	11/15/2006	20.57	23050	13.96	8.27	7.95	>0.48
	12/13/2006	15.6	27440	16.9	11.98	7.98	>0.46
	1/11/2007	15.61	22690	13.74	8.15	8.04	>0.46
	2/14/2007	9.71	16610	9.75	12.01	8.25	0.15
	3/21/2007	21.43	3740	1.98	6.9	8.02	0.03
	4/11/2007	17.26	15600	9.15	10.79	8.01	0.25
	5/16/2007	27.17	16940	9.92	9.21	7.81	0.35
	6/13/2007	30.19	15410	8.92	7.97	8.02	>0.48
	7/11/2007	29.64	1257	0.62	7.04	8.39	0.2

Table 6: Physical water quality parameter statistics from sites in south Texas coastal waters for August 2006 through July 2007.

Location	Statistic (N=12)	Temp (°C)	Conductivity (μmhos/cm)	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Secchi Disk (m)
Bird Island	Mean	21.99	59586.50	39.74	6.40	8.08	0.49
	Std Dev	5.83	9771.60	7.07	2.08	0.34	0.08
Nueces Bay	Mean	21.99	49468.67	32.24	6.70	8.00	0.29
	Std Dev	5.51	9561.62	6.70	1.48	0.23	0.12
Cole Park	Mean	22.77	51256.75	33.40	7.14	7.99	0.46
	Std Dev	5.83	7175.35	4.82	1.57	0.15	0.17
Redfish Bay	Mean	23.08	42418.58	27.38	7.52	8.06	0.66
	Std Dev	6.23	10326.47	7.28	1.98	0.13	0.24
Copano Bay	Mean	22.68	25882.00	15.83	8.30	8.02	0.67
	Std Dev	6.69	10238.11	6.48	2.42	0.18	0.26
Bayside	Mean	22.38	17853.92	10.54	8.42	8.01	0.37
	Std Dev	6.76	8833.75	5.32	2.20	0.18	0.17

Table 7: Physical water quality parameter ranges from sites in south Texas coastal waters for August 2006 through July 2007.

Location	Range	Temp (°C)	Conductivity (µmhos/cm)	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Secchi Disk (m)
Bird Island	high	27.76	76008.00	52.67	9.95	8.41	>0.66
	low	13.19	41980.00	26.89	3.59	7.52	0.35
Nueces Bay	high	28.51	62800.00	41.50	9.48	8.37	>0.55
	low	13.12	31590.00	19.64	4.21	7.51	0.10
Cole Park	high	29.45	63400.00	41.82	9.65	8.13	>0.72
	low	14.20	39770.00	25.29	4.48	7.62	0.15
Redfish Bay	high	31.35	57060.00	37.78	11.62	8.25	>1.00
	low	12.25	20740.00	12.34	4.91	7.87	0.19
Copano Bay	high	30.40	37390.00	23.63	13.36	8.45	>1.05
	low	12.48	1768.00	0.89	5.28	7.75	0.25
Bayside	high	30.20	37400.00	16.69	12.01	8.39	0.58
	low	9.71	1257.00	0.62	5.07	7.72	0.03

Dissolved oxygen (DO) levels ranged from 3.6 to 13.4 mg/L at all sites during the sampling period (Tables 4 and 5). At Bird Island, Nueces Bay, and Cole Park, DO levels ranged from 3.6 to 10 mg/L (Table 7). DO levels ranged from 4.9 to 13.4 mg/L at Redfish Bay and both sites at Copano Bay (Table 7). Average DO levels were between 6.4 and 8.4 mg/L at each of the sites sampled (Table 6). The highest DO level (13.36 mg/L) occurred at Copano Bay causeway during the December 2006 sampling event, while the lowest DO level (3.59 mg/L) occurred at Bird Island during the November 2006 sampling event (Tables 4 and 5). Overall, DO levels were highest at the two Copano Bay sites and lowest at Bird Island and Nueces Bay (Tables 4 and 5). pH means at all sites were between 7.99 and 8.08 s.u. and ranged from 7.51 – 8.45 s.u. throughout the study (Tables 6 and 7).

Measurements of turbidity using Secchi disk ranged from 0.03 to > 1.05 m (Tables 4 and 5). Secchi disk measurements ranged from 0.1 to > 0.72 m at Bird Island, Nueces Bay, and Cole Park (Table 7). At Redfish Bay and Copano Bay Causeway, Secchi disk measurements ranged from 0.19 to > 1.05 m (Table 7). At Bayside, Secchi disk measurements ranged from 0.03 to 0.58 m (Table 7). Mean Secchi disk measurements were between 0.29 and 0.67 m at each of the sites sampled (Table 6). The highest Secchi disk measurement (> 1.05 m) occurred at Copano Bay causeway during the November sampling event, while the lowest Secchi disk measurement (0.03 m) occurred at Bayside during the March sampling event (Table 5). Overall, turbidity was lower at Redfish Bay and Copano Bay causeway sites and highest at the Nueces bay site (Table 6).

Significant rainfall (over one inch, 2.5 cm) occurred the week prior to the September 2006 sampling event at Redfish Bay, Copano Bay, and Bayside, and there was also significant rainfall the week prior to July 2007 sampling event at each of the sites sampled (Tables 8 and 9). Rainfall data was gathered from the National Oceanic and Atmospheric Association. Nearly half an inch of rain (1.25 cm) occurred the day before sampling Redfish Bay, Copano Bay, and Bayside in September 2006 and nearly two and a half inches (6.25 cm) of rain occurred during the week prior to sampling (Table 9). There was three and a half inches (8.75 cm) of rain the day before sampling at Bird Island, Nueces Bay, and Cole Park in July 2007 and just over fourteen and a quarter inches (35.63 cm) of rain during the week prior to sampling (Table 8). There was over three and a half inches (8.75 cm) of rain during the week prior to sampling at Redfish Bay, Copano Bay, and Bayside in July 2007 (Table 9). The lowered salinity at each site in July 2007 was presumably related to the preceding heavy rainfall (Tables 4 and 5). Less than an inch (<2.5 cm) of rain occurred the week before each of the other sampling events. However, there was some rainfall the week before each sampling event, although most were low amounts of rain (trace or < 0.1 in.; < 0.25 cm; Tables 8 and 9).

Table 8: Rainfall: one day and one week prior to sampling at Bird Island, Nueces Bay, and Cole Park for August 2006 through July 2007 (NOAA, 2006-7, http://www.srh.noaa.gov/crp/climate/default.html).

Date	Location	Days Since Rain	Rain (inch) (1 day)	Rain (cm) (1 day)	Rain (inch) (7 days)	Rain (cm) (7 days)
8/15/2006	Bird Island	5	0.00	0.00	0.22	0.55
9/6/2006	Bird Island	1	0.01	0.03	0.04	0.10
10/4/2006	Bird Island	5	0.00	0.00	Trace	Trace
11/8/2006	Bird Island	1	0.02	0.05	0.08	0.20
12/6/2006	Bird Island	6	0.00	0.00	Trace	Trace
1/3/2007	Bird Island	4	0.00	0.00	0.03	0.08
2/7/2007	Bird Island	6	0.00	0.00	0.01	0.03
3/7/2007	Bird Island	6	0.00	0.00	Trace	Trace
4/4/2007	Bird Island	1	Trace	Trace	0.33	0.83
5/9/2007	Bird Island	1	Trace	Trace	0.01	0.03
6/6/2007	Bird Island	1	0.47	1.18	0.83	2.08
7/5/2007	Bird Island	1	3.50	8.75	14.26	35.65
8/15/2006	Nueces Bay	5	0.00	0.00	0.22	0.55
9/6/2006	Nueces Bay	1	0.01	0.03	0.04	0.10
10/4/2006	Nueces Bay	5	0.00	0.00	Trace	Trace
11/8/2006	Nueces Bay	1	0.02	0.05	0.08	0.20
12/6/2006	Nueces Bay	6	0.00	0.00	Trace	Trace
1/3/2007	Nueces Bay	4	0.00	0.00	0.03	0.08
2/7/2007	Nueces Bay	6	0.00	0.00	0.01	0.03
3/7/2007	Nueces Bay	6	0.00	0.00	Trace	Trace
4/4/2007	Nueces Bay	1	Trace	Trace	0.33	0.83
5/9/2007	Nueces Bay	1	Trace	Trace	0.01	0.03
6/6/2007	Nueces Bay	1	0.47	1.18	0.83	2.08
7/5/2007	Nueces Bay	1	3.50	8.75	14.26	35.65
8/15/2006	Cole Park	5	0.00	0.00	0.22	0.55
9/6/2006	Cole Park	1	0.01	0.03	0.04	0.10
10/4/2006	Cole Park	5	0.00	0.00	Trace	Trace
11/8/2006	Cole Park	1	0.02	0.05	0.08	0.20
12/6/2006	Cole Park	6	0.00	0.00	Trace	Trace
1/3/2007	Cole Park	4	0.00	0.00	0.03	0.08
2/7/2007	Cole Park	6	0.00	0.00	0.01	0.03
3/7/2007	Cole Park	6	0.00	0.00	Trace	Trace
4/4/2007	Cole Park	1	Trace	Trace	0.33	0.83
5/9/2007	Cole Park	1	Trace	Trace	0.01	0.03
6/6/2007	Cole Park	1	0.47	1.18	0.83	2.08
7/5/2007	Cole Park	1	3.50	8.75	14.26	35.65

Table 9: Rainfall: one day and one week prior to sampling at Redfish Bay, Copano Bay, and Bayside for August 2006 through July 2007 (NOAA, 2006-7, http://www.srh.noaa.gov/crp/climate/default.html).

Date	Location	Days Since Rain	Rain (inch) (1 day)	Rain (cm) (1 day)	Rain (inch) (7 days)	Rain (cm) (7 days)
8/23/2006	Redfish Bay	1	0.11	0.28	0.11	0.28
9/13/2006	Redfish Bay	1	0.46	1.15	2.45	6.13
10/11/2006	Redfish Bay	1	0.06	0.15	0.06	0.15
11/15/2006	Redfish Bay	4	0	0	Trace	Trace
12/13/2006	Redfish Bay	3	0	0	0.61	1.53
1/11/2007	Redfish Bay	4	0	0	0.39	0.98
2/14/2007	Redfish Bay	3	0	0	0.07	0.18
3/21/2007	Redfish Bay	6	0	0	0.02	0.05
4/11/2007	Redfish Bay	2	0	0	0.71	1.78
5/16/2007	Redfish Bay	5	0	0	0.03	0.08
6/13/2007	Redfish Bay	3	0	0	Trace	Trace
7/11/2007	Redfish Bay	4	0	0	3.55	8.88
8/23/2006	Copano Bay	1	0.11	0.28	0.11	0.28
9/13/2006	Copano Bay	1	0.46	1.15	2.45	6.13
10/11/2006	Copano Bay	1	0.06	0.15	0.06	0.15
11/15/2006	Copano Bay	4	0	0	Trace	Trace
12/13/2006	Copano Bay	3	0	0	0.61	1.53
1/11/2007	Copano Bay	4	0	0	0.39	0.98
2/14/2007	Copano Bay	3	0	0	0.07	0.18
3/21/2007	Copano Bay	6	0	0	0.02	0.05
4/11/2007	Copano Bay	2	0	0	0.71	1.78
5/16/2007	Copano Bay	5	0	0	0.03	0.08
6/13/2007	Copano Bay	3	0	0	Trace	Trace
7/11/2007	Copano Bay	4	0	0	3.55	8.88
8/23/2006	Bayside	1	0.11	0.28	0.11	0.28
9/13/2006	Bayside	1	0.46	1.15	2.45	6.13
10/11/2006	Bayside	1	0.06	0.15	0.06	0.15
11/15/2006	Bayside	4	0	0	Trace	Trace
12/13/2006	Bayside	3	0	0	0.61	1.53
1/11/2007	Bayside	4	0	0	0.39	0.98
2/14/2007	Bayside	3	0	0	0.07	0.18
3/21/2007	Bayside	6	0	0	0.02	0.05
4/11/2007	Bayside	2	0	0	0.71	1.78
5/16/2007	Bayside	5	0	0	0.03	0.08
6/13/2007	Bayside	3	0	0	Trace	Trace
7/11/2007	Bayside	4	0	0	3.55	8.88

Microbiological Analysis

i) Enterococci

Enterococci were isolated from 97 of 144 samples during the sampling period (Tables 10 and 11). Enterococci exceeded the U.S. EPA standard criterion for single samples of 104 CFU/100mL in 17 of 144 samples during this study (U.S. EPA, 1986). Rainfall of at least 0.5 in. (1.25 cm) occurred the day before seven of 17 exceedances (June and July sampling events at Nueces Bay and July sampling event at Bird Island and Cole Park), and over 0.75 in. (1.85) rain occurred the week prior to these sampling events (Tables 8 and 10). The maximum concentration of enterococci (1,055 CFU/100mL) occurred at Cole Park during the September 2006 sampling event (Table 10). Cole Park had a much higher mean enterococci concentration (245 CFU/100mL) than the other sites which all had mean enterococci concentrations of less than 48 CFU/100mL (Table 12 and Figure 7). Cole Park and Nueces Bay samples exceeded the U.S. EPA standard for enterococci for three of twelve sampling events, while Bird Island samples exceeded the standard for enterococci for two events (Table 10). However, the highest concentration at Cole Park was 1.055×10^3 CFU/100mL, whereas the highest concentration at Nueces Bay was 150 CFU/100mL. Bird Island exceeded the EPA standard by more than three-fold (350 CFU/100mL; July 2007) for one sampling event (Table 10). All three sites exceeded the standard for the July 2007 sampling event, which followed rainfall in excess of 14 in. (35.63 cm) over the previous seven days. Samples from Redfish Bay and Bayside each exceeded the U.S. EPA standard for enterococci for one sampling event, while Copano Bay samples did not exceed the standard during this study (Table 11). The exceedance at Redfish Bay was at the standard (1.04×10^2 CFU/100mL; February 2007), though the exceedance at Bayside was nearly three-fold higher than the standard (295 CFU/100mL) (Table 11). Copano Bay had consistently lower enterococci concentrations compared to the other stations, with the highest concentration of only 20 CFU/100mL (September 2006) (Table 11).

ii) *Vibrio vulnificus*

V. vulnificus was enumerated using two different media: VVA (with probe confirmation) and mCPC. The use of mCPC as a second isolation technique for *V. vulnificus* was for comparison and to add confidence to our data. However, this medium is known to inhibit some environmental strains due to the antibiotics, polymyxin B and E, included in the medium.

Table 10: Numbers of *V. vulnificus* and *Enterococcus* sp. at Bird Island, Nueces Bay, and Cole Park for August 2006 through July 2007 (CFU/100mL; average of two samples).

Location	Date	*V. vulnificus** (VVA, probe+)	*Enterococcus*** (mEI)
Bird Island	08/15/06	949	60
	09/06/06	1467	2
	10/04/06	2945	1
	11/08/06	763	5
	12/06/06	1517	1
	01/03/07	1719	125
	02/07/07	42	9
	03/07/07	132	1
	04/04/07	1761	11
	05/09/07	89	3
	06/06/07	500	1
	07/05/07	1000	350
Nueces Bay	08/15/06	3889	20
	09/06/06	7000	112
	10/04/06	7047	18
	11/08/06	1589	7
	12/06/06	1167	32
	01/03/07	1365	3
	02/07/07	467	1
	03/07/07	1219	32
	04/04/07	2750	45
	05/09/07	1875	5
	06/06/07	39711	109
	07/05/07	1667	150
Cole Park	08/15/06	14375	4
	09/06/06	13750	1055
	10/04/06	13454	24
	11/08/06	2252	14
	12/06/06	2125	3
	01/03/07	1813	5
	02/07/07	126	2
	03/07/07	1053	5
	04/04/07	4000	950
	05/09/07	1146	1
	06/06/07	1382	1
	07/05/07	8334	870

* *V. vulnificus* colonies on VVA confirmed with *vvhA* gene probe
** For values of 0, 1 was used for calculation purposes

Table 11: Numbers of *V. vulnificus* and *Enterococcus* sp. at Redfish Bay, Copano Bay, and Bayside for August 2006 through July 2007 (CFU/100mL, average of 2 samples).

Location	Date	*V. vulnificus** (VVA, Probe+)	*Enterococcus*** (mEI)
Redfish Bay	08/23/06	1389	15
	09/13/06	6125	15
	10/11/06	14000	19
	11/15/06	1886	6
	12/13/06	328	2
	01/11/07	63	1
	02/14/07	101	104
	03/21/07	198	2
	04/11/07	303	16
	05/16/07	875	1
	06/13/07	472	2
	07/11/07	1501	3
Copano Bay	08/23/06	1456	1
	09/13/06	5000	20
	10/11/06	17000	2
	11/15/06	23509	1
	12/13/06	234	1
	01/11/07	1100	1
	02/14/07	328	1
	03/21/07	594	1
	04/11/07	500	2
	05/16/07	278	1
	06/13/07	1000	1
	07/11/07	1	9
Bayside	08/23/06	3728	12
	09/13/06	2421	16
	10/11/06	7000	21
	11/15/06	8508	15
	12/13/06	4500	1
	01/11/07	1342	1
	02/14/07	3900	12
	03/21/07	29500	295
	04/11/07	2768	38
	05/16/07	2632	7
	06/13/07	385	2
	07/11/07	1875	91

* *V. vulnificus* colonies on VVA confirmed with *vvhA* gene probe
** For values of 0, 1 was used for calculation purposes

Table 12: Mean *Enterococcus* sp. and *V. vulnificus* concentrations (CFU/100mL) at sites in south Texas coastal waters for August 2006 through July 2007.

Location	Enterococci	VVA
Bird Island	47	1074
Nueces Bay	45	5812
Cole Park	245	5318
Redfish Bay	16	2270
Copano Bay	3	4636
Bayside	43	5713

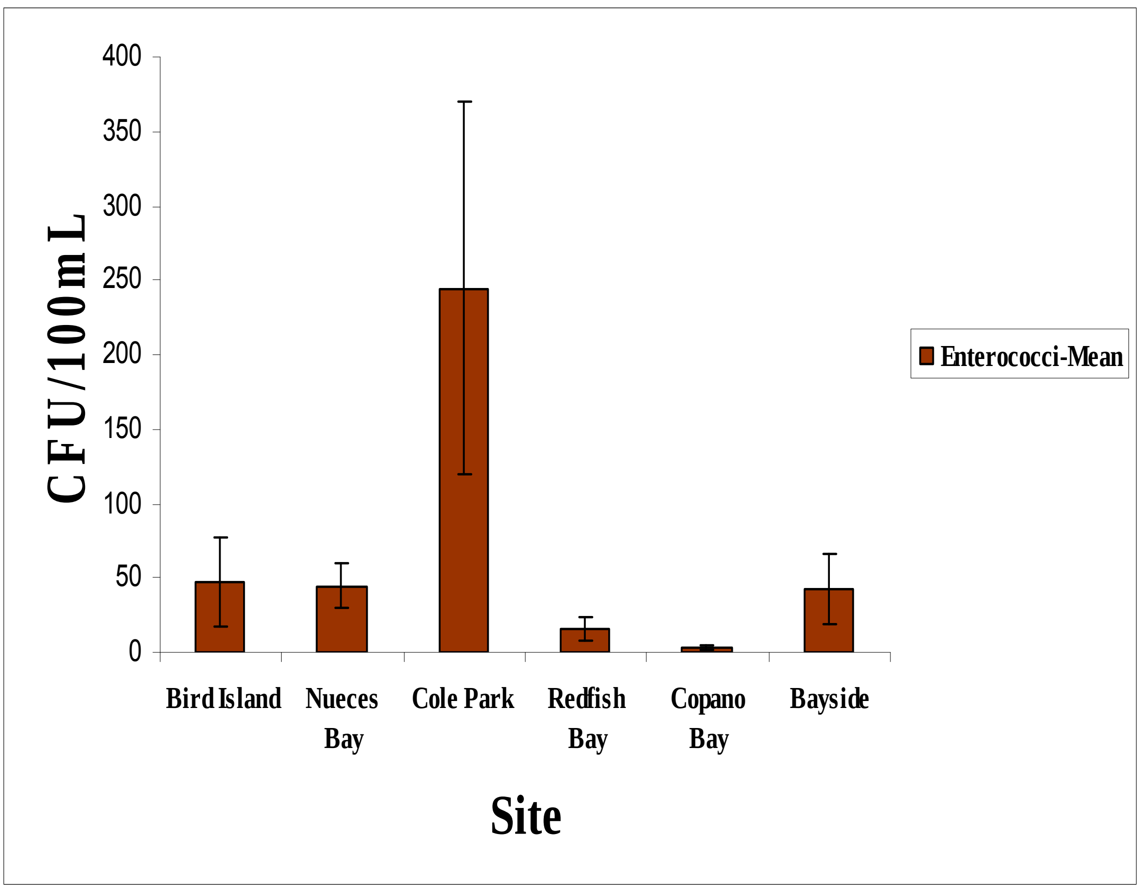

FIG 7. *Enterococcus* sp. means at sites in south Texas coastal waters from August 2006 through July 2007.

Table 13: Analysis of *V. vulnificus* data using a two-way ANOVA: Tests of Between-Subjects Effects Dependent Variable: LOG10CFU/100mL *V. vulnificus.*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34.160	11	3.105	7.015	0.000
Intercept	1171.008	1	1171.008	63.870	0.034
Media	14.961	1	14.961	100.854	0.000
Site	17.609	5	3.522	23.751	0.002
Media * Site	0.741	5	0.148	0.336	0.891
Error	57.895	131	0.442		
Total	1261.712	143			
Corrected Total	92.156	142			

* R Squared = 0.371 (Adjusted R Squared = 0.318)

A two-way analysis of variance (ANOVA) was conducted to determine whether there was a statistically significant difference in *V. vulnificus* populations enumerated using the two techniques.(Table 13). The numbers of *V. vulnificus using* VVA and confirmed with colony hybridization were significantly higher than the numbers determined using mCPC ($F = 100.854$, df = 1,131, $P < 0.05$), probably due to the inhibition of some environmental strains. The overall mean (all sites) for *V. vulnificus* isolated using VVA and probe confirmation was 4.13×10^3 CFU/100mL, while using mCPC the mean was 1.19×10^3 CFU/100mL. Pearson's coefficient of correlation showed a significant correlation between *V. vulnificus* numbers using VVA versus mCPC ($r=0.514$; $p<0.01$). Therefore the *V. vulnificus* results described for this study are those based on the data using the standard U.S. FDA method (VVA with confirmation by DNA probe). In addition, there was not a significant relationship between the two media and sites ($F=0.336$, df = 5,131, $P = 0.891$) indicating the number of isolates obtained on each media showed the same trend at each site.

Levels of *V. vulnificus* at sites

V. vulnificus was isolated from every site on every sampling date, including the winter months, with the exception of July 2007 at Copano Bay (Tables 10 – 11; Figures 8 - 9).

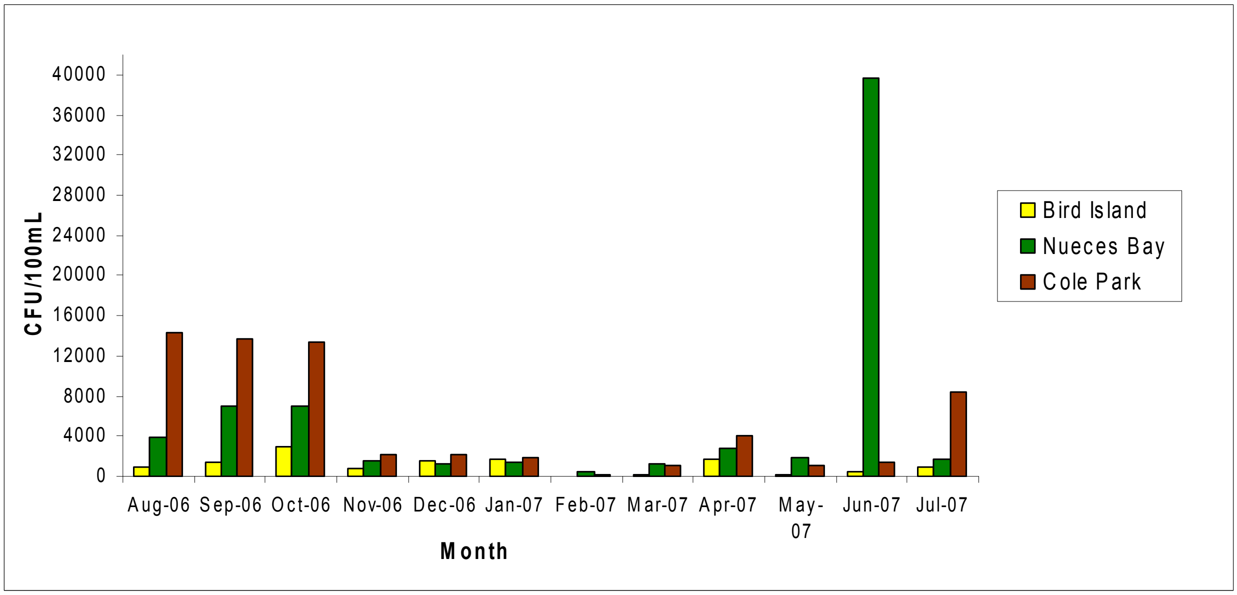

FIG 8. Numbers of *V. vulnificus* at Bird Island, Nueces Bay and Cole Park sites from August 2006 to July 2007.

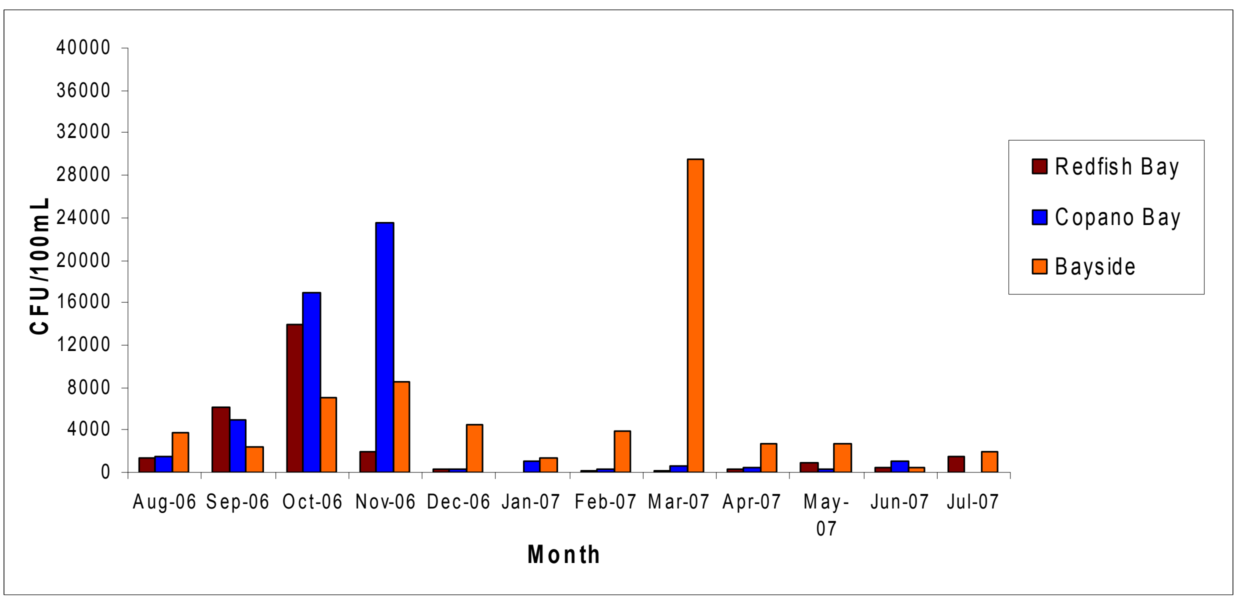

FIG 9. Numbers of *V. vulnificus* at Redfish Bay, Copano Bay and Bayside sites from August 2006 to July 2007.

Bird Island

At Bird Island, *V. vulnificus* was isolated each sampling event (Table 10) but had the lowest mean *V. vulnificus* level (1.07 X 10^3 CFU/100mL) of any of the sites sampled (Table 12). Levels of *V. vulnificus* increased from the start of the sampling period (August 2006) and peaked in October 2006 at Bird Island reaching 2.95 X 10^3 CFU/100mL (Table 10, Figures 8 and 10). At this event salinity was at its highest level of any of the sites (52 ppt), and water temperature (27° C) was its second highest for Bird Island (Table 4, Figure 10). Dissolved oxygen (DO) was 4.49 mg/L for the October 2006 sampling event. During the February, March, and May 2007 sampling events *V. vulnificus* levels using VVA were less than 1.33 X 10^2 CFU/100mL (Table 10, Figure 10). Water temperatures were 15 and 16° C during the February and March 2007 sampling events (Table 4, Figure 10). However, the water temperature in May was 25° C. DO was 9.2 mg/L during the February 2007 sampling event when levels of *V. vulnificus* using VVA were 42 CFU/100mL (negative correlation) (Tables 4 and 10; Figure 10).

Nueces Bay

At Nueces Bay, *V. vulnificus* was isolated each sampling event and had the highest mean *V. vulnificus* levels (5.81 X 10^3 CFU/100mL) of all the sites sampled (Tables 10 and 12; Figures 8 and 11). Levels of *V. vulnificus* increased from the start of the sampling period (August 2006) through October 2006 reaching 7.05 X 10^3 CFU/100mL (Table 10, Figure 11). Similarly, water temperature reached its second highest level (26.33° C) for Nueces Bay during the October 2006 sampling event (Table 4, Figure 11). In addition, DO levels were at their lowest (4.21 mg/L) and salinity was at its highest (41.5 ppt) during this sampling event (Table 4 and Figure 18). The highest number of *V. vulnificus* during this study at any of the sites (3.97 X 10^4 CFU/100mL) was found at Nueces Bay during the June 2007 sampling event (Table 10, Figure 11). Water temperature reached its second highest (27.4° C) and salinity its second lowest (23.48 ppt) for Nueces Bay at this June 2007 sampling event with a DO level of 6.75 mg/L (Table 4, Figure 11). Approximately 0.5 inches (1.25 cm) of rainfall occurred the day before sampling and 0.83 inches (2.33 cm) of rain fell the week prior to sampling in June 2007 (Table 8). The lowest levels of *V. vulnificus* at Nueces Bay (4.67 X 10^2 CFU/100mL) occurred in February 2007 when the water temperature was 16.26° C (Tables 4 and 10; Figure 11).

Cole Park

At Cole Park, *V. vulnificus* was isolated each sampling event (Table 10). Cole Park samples had the third highest mean of *V. vulnificus* of all the sites (5.32 X 10^3 CFU/100mL) (Table 12). Levels of *V. vulnificus* were highest in the first sampling event (August 2006; 1.44 X 10^4 CFU/100mL) and decreased through February 2007 to their lowest level of 1.26 X 10^2 CFU/100mL (Table 10, Figure 12). Water temperature was highest and salinity was its second highest in August 2006 (29.45° C; 38.88 ppt) (Table 4, Figure 12). Water temperature decreased to 15.38° C in December 2006 and remained ~15° C or less through the February 2007 sampling event (Table 4, Figure 12). In February 2007 when levels of *V. vulnificus* were lowest, DO was at its highest reaching 9.65 mg/L and salinity was 29.67 ppt (Tables 4 and 10; Figure 12).

Redfish Bay

At Redfish Bay, *V. vulnificus* was isolated each sampling event but had the second lowest mean (2.27 X 10^3 CFU/100mL) (Tables 11 and 12). Levels increased from the start of the sampling period (August 2006) through October 2006 when they peaked at 1.4 X 10^4 CFU/100mL (Table 11, Figure 13). Water temperature was 26.21° C and salinity was 31.55 ppt for the October 2006 sampling event (Tables 5 and 11, Figure 13). Levels of *V. vulnificus* and water temperature each decreased through the January 2007 sampling event reaching their lowest and second lowest levels of the sampling period at Redfish Bay (6.3 X 10 CFU/100mL and 15.6° C respectively) (Tables 5 and 11, Figure 13). Numbers of *V. vulnificus* remained less than 3.04 X 10^2 CFU/100mL through April 2007 (Table 11, Figure 13).

Copano Bay

V. vulnificus from Copano Bay Causeway was isolated in eleven of twelve sampling events (Table 11). In July 2007, none of the presumptive *V. vulnificus* colonies from VVA were confirmed by colony hybridization, and this was the only time this occurred during the study. During this sampling event, salinity at Copano Bay Causeway was its lowest of the sampling period (0.89 ppt), and 3.55 inches of rainfall occurred during the seven days prior to sampling (Tables 5 and 9, Figure 14). Copano Bay Causeway had a mean *V. vulnificus* level of 4.64 X 10^3 CFU/100mL (Table 12). Numbers of *V. vulnificus* increased from the start of sampling (August 2006) through November 2006 when they peaked at 2.35 X 10^4 CFU/100mL (Table 11, Figure 14). Water temperature was 21.04° C and salinity was 18.31 ppt for the November 2006 sampling event when numbers of *V. vulnificus* were highest (Table 5, Figure 14).

Bayside

At Copano Bay near Bayside, *V. vulnificus* was found at each sampling event (Table 11) and the second highest mean *V. vulnificus* level was found at this site (5.71 X 10^3 CFU/100mL) (Table 12). Levels of *V. vulnificus* were highest during the March 2007 sampling event (2.95 X 10^4 CFU/100mL) (Table 11, Figure 15) when the second lowest salinity measurement (1.98 ppt) occurred (Table 5, Figure 15). Water temperature was 21.43° C during this sampling event (Table 5, Figure 15). The lowest number of *V. vulnificus* were isolated in June 2007 (3.85 X 10^2 CFU/100mL), and this was the only time levels of *V. vulnificus* using VVA were less than 1.34 X 10^3 CFU/100mL (Table 11, Figure 15).

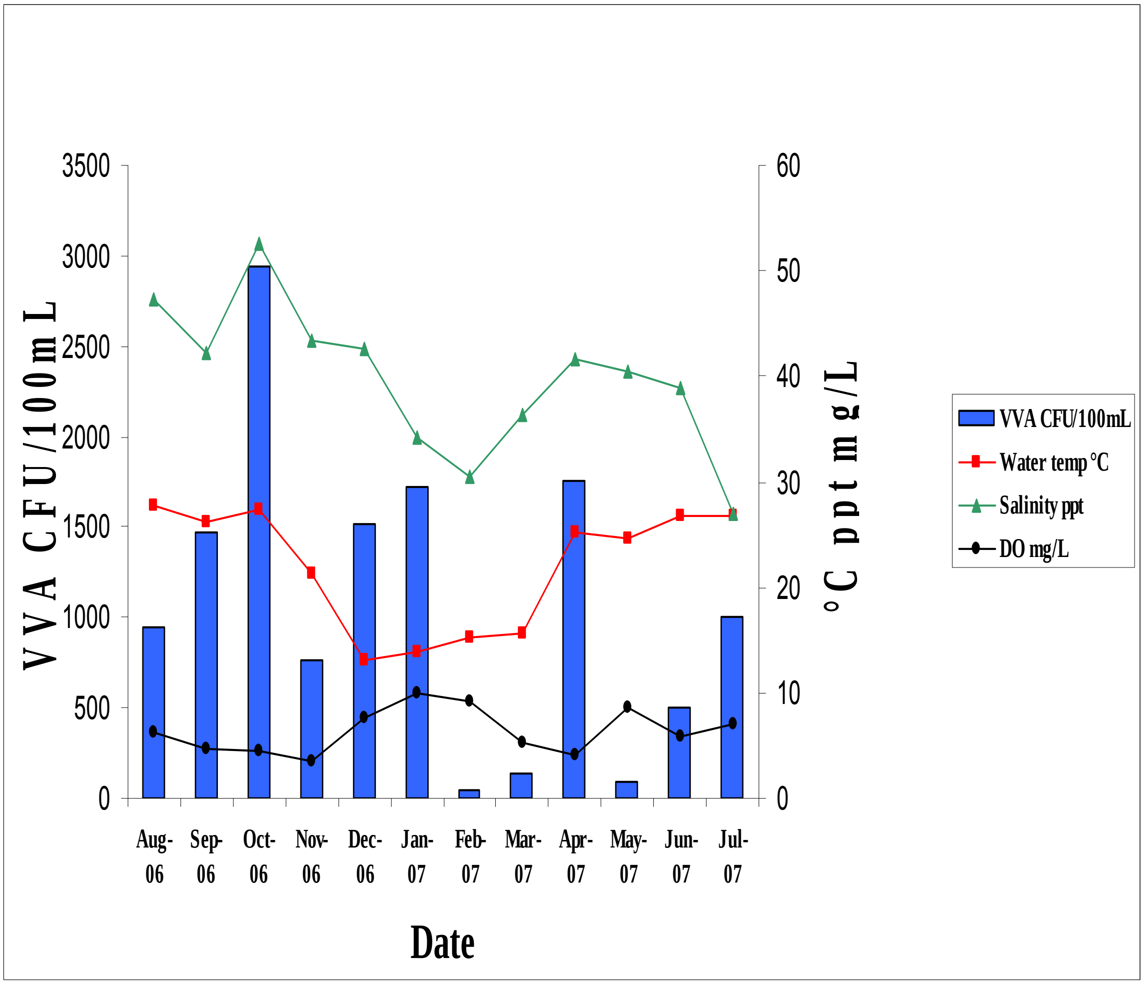

FIG 10. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Bird Island from August 2006 through July 2007.

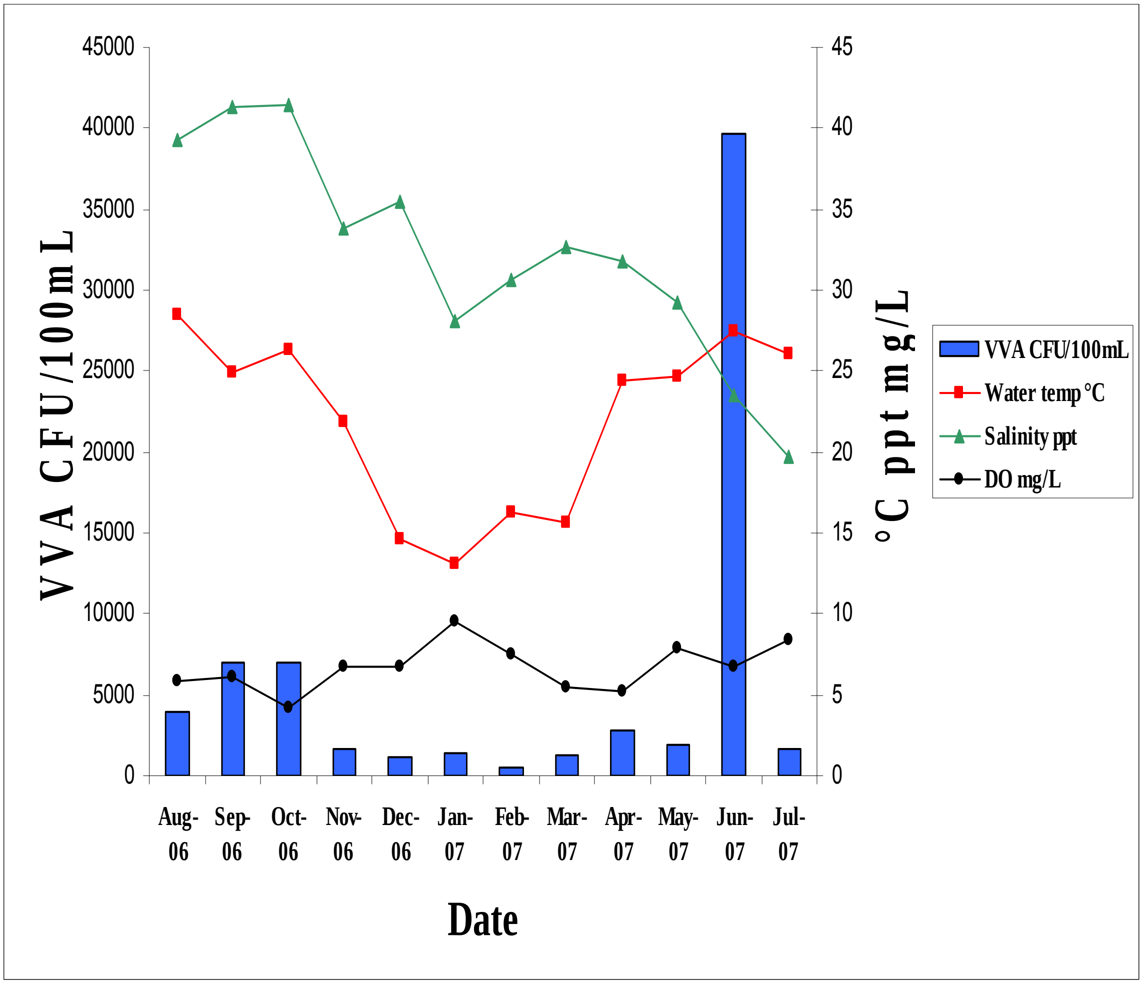

FIG 11. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Nueces Bay from August 2006 through July 2007.

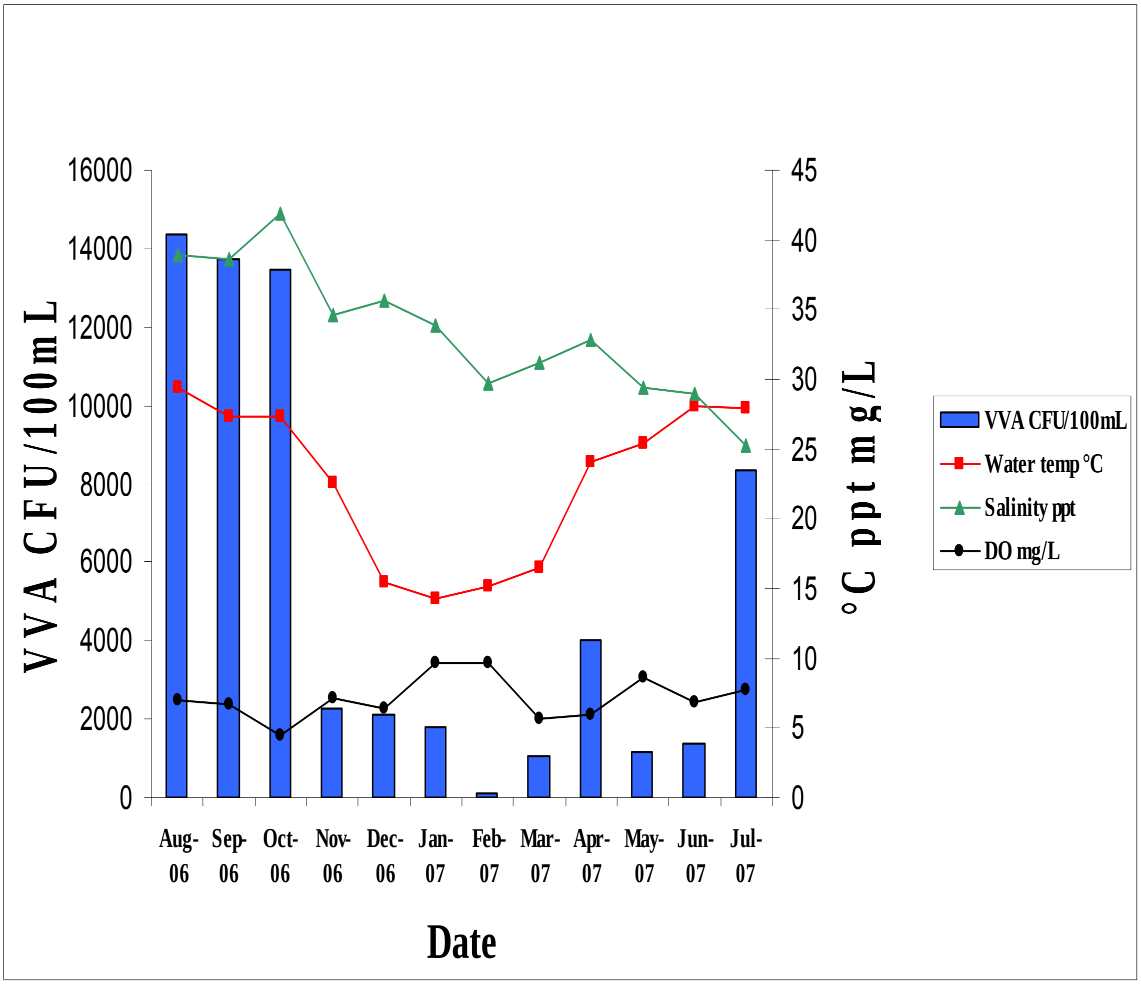

FIG 12. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Cole Park from August 2006 through July 2007.

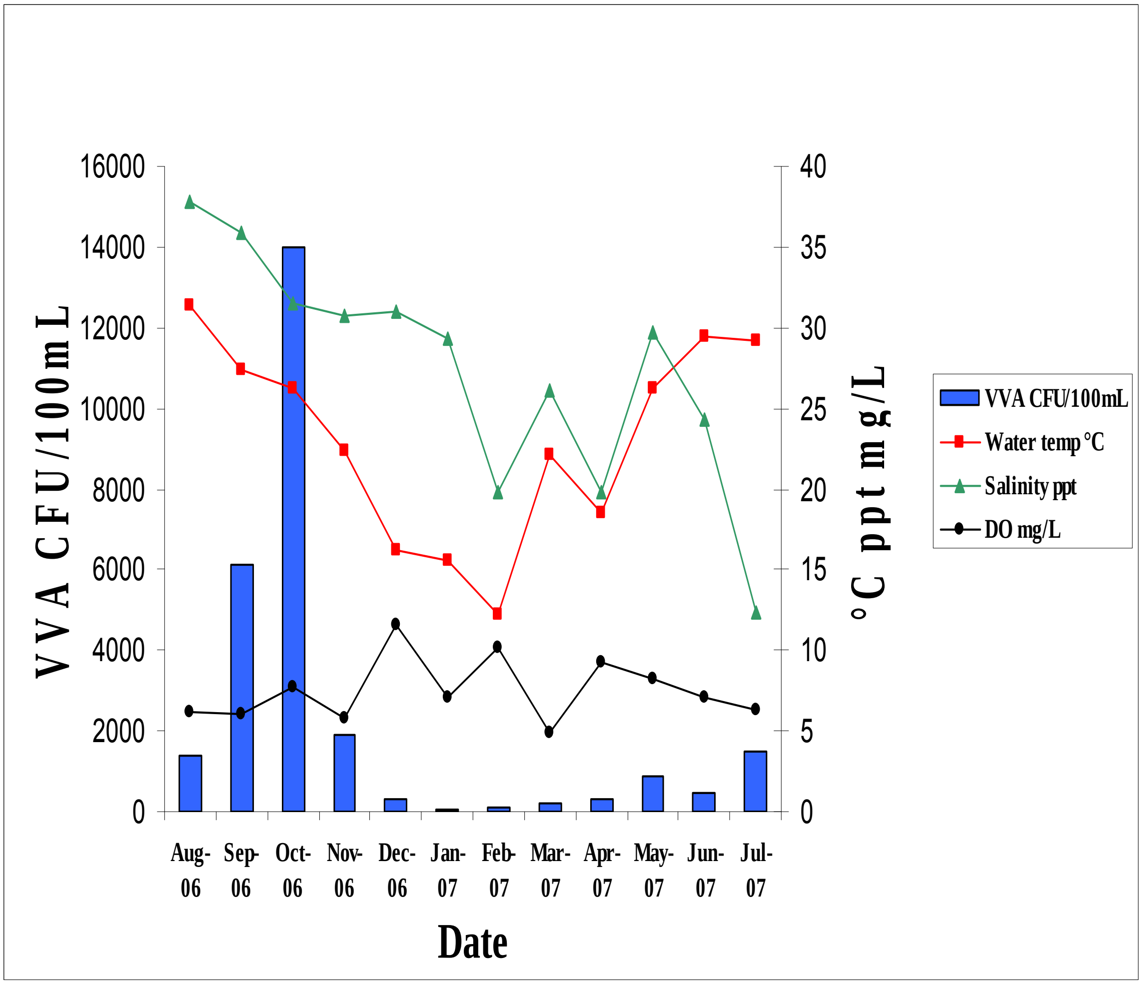

FIG 13. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Redfish Bay from August 2006 through July 2007.

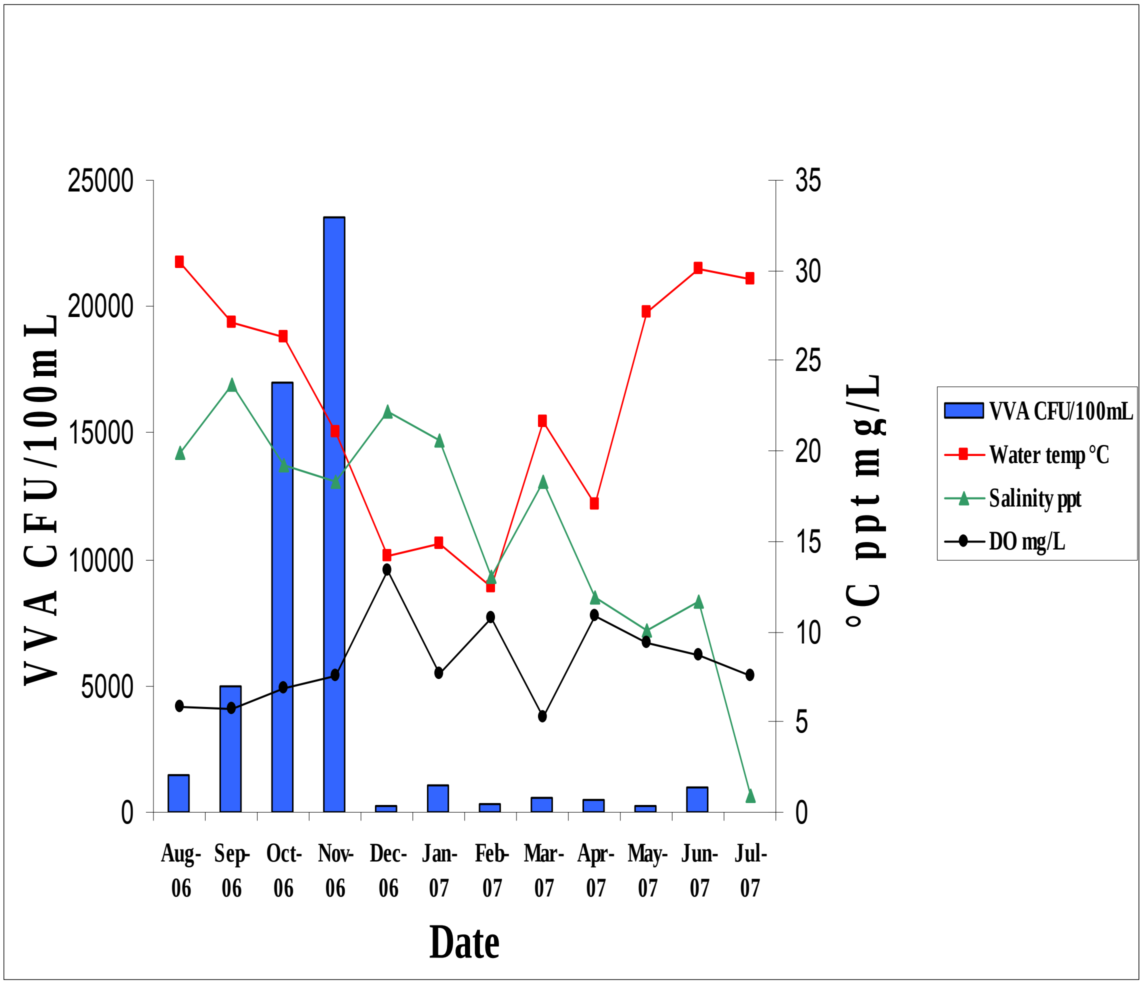

FIG 14. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Copano Bay Causeway from August 2006 through July 2007.

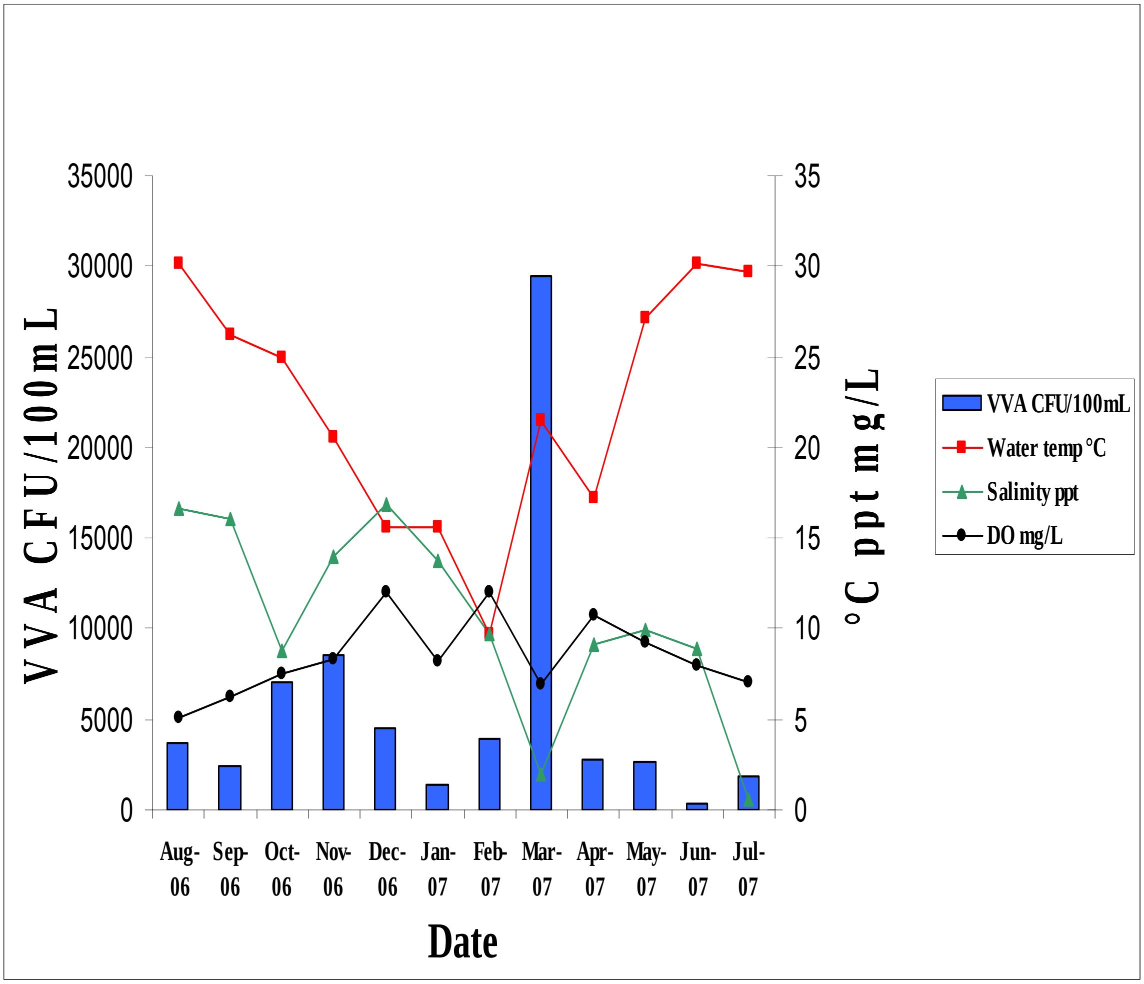

FIG 15. Water temperature, salinity, and dissolved oxygen values versus numbers of *V. vulnificus* at Copano Bay near Bayside from August 2006 through July 2007.

Statistical Analysis

Pearson's coefficient of correlation was used to detect correlations between numbers of *V. vulnificus* and each environmental factor at 95% and 99% confidence levels. Analysis with Pearson's correlation coefficient (r) revealed that levels of *V. vulnificus* were positively correlated with water temperature ($r = 0.368$; $P < 0.01$) (Table 14). Water temperatures between May and July 2007 and August and October 2006 exceeded 25° C, and this is generally when numbers of *V. vulnificus* were highest at each site (Tables 4 – 5, 10 - 11). When temperatures were 16° C or below between December 2006 and February 2007 levels of *V. vulnificus* isolated were generally their lowest (Tables 10 and 11). Numbers of *V. vulnificus were* slightly negatively correlated with both salinity and conductivity (Table 15) and were correlated with DO ($r = -0.260$; $P < 0.05$) (Table 14). There was no correlation between numbers of *V. vulnificus* and pH or between *V. vulnificus* and Secchi disk (Table 14). Enterococci concentrations were significantly correlated with *V. vulnificus* levels (r = 0.424, $P < 0.01$) (Figures 16 and 17).

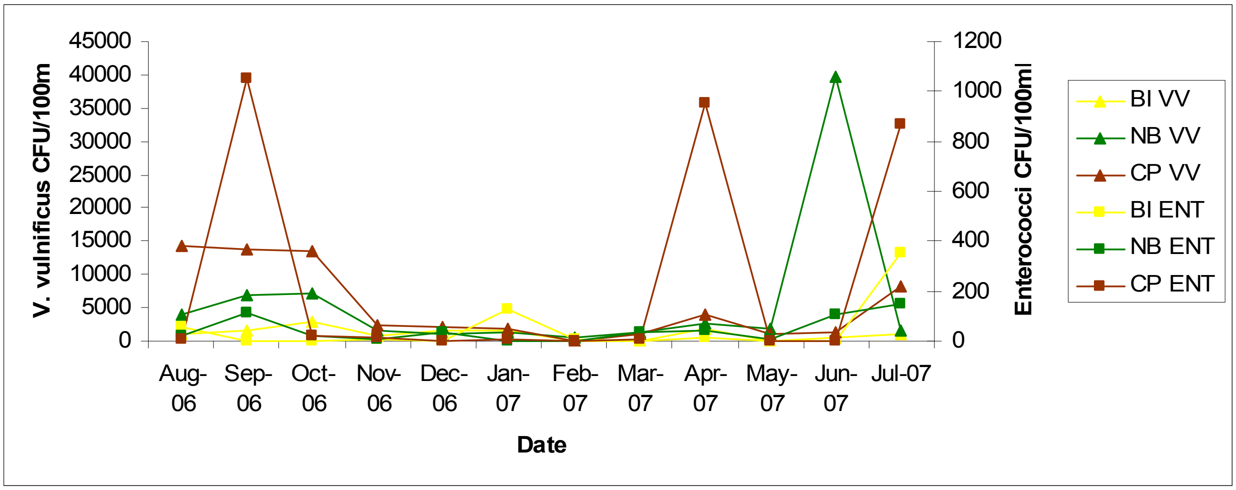

FIG 16. Numbers of *V. vulnificus* vs. enterococci at Bird Island, Nueces Bay and Cole Park sites from August 2006 to July 2007.

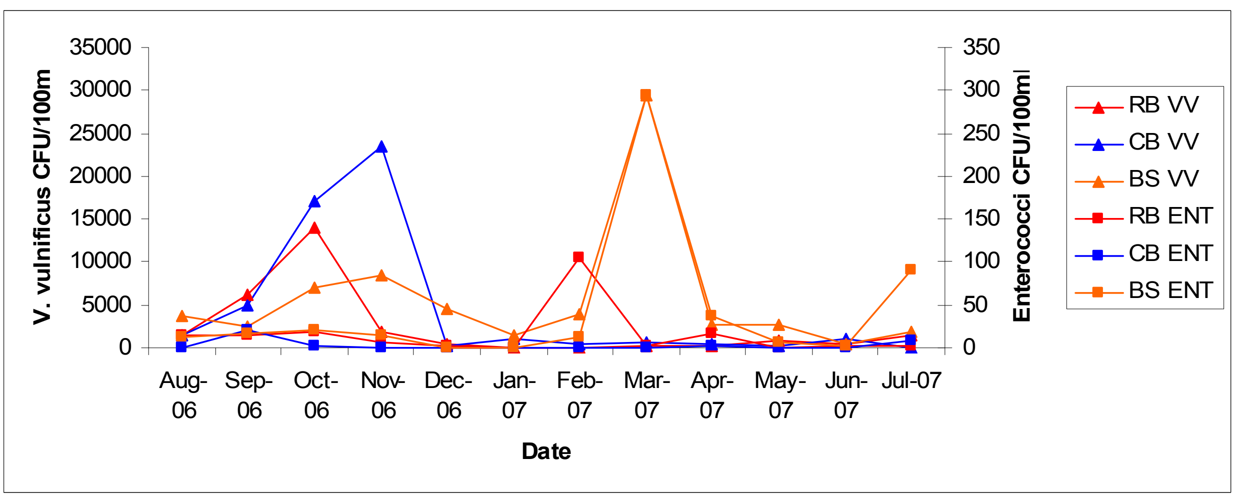

FIG 17. Numbers of *V. vulnificus* vs. enterococci at Redfish Bay, Copano Bay and Bayside sites from August 2006 to July 2007.

A two-way analysis of variance (ANOVA) was conducted to determine whether there was a statistically significant difference in *V. vulnificus* numbers between the sites sampled (Table 13). The difference in the levels of *V. vulnificus* isolated from the six sites was statistically significant ($P < 0.05$). Nueces Bay, Bayside, Cole Park, and Copano Bay each had a mean of at least 4.64 X 10^3 CFU/100mL of *V. vulnificus* with Nueces Bay having the highest mean at 5.81 X 10^3 CFU/100mL (Table 12). The lowest means for *V. vulnificus* were 1.07 X 10^3 CFU/100mL at Bird Island and 2.27 X 10^3 CFU/100mL at Redfish Bay, while highest means were at Cole Park and Bayside (Table 12). Although there was a significant difference in the mean number of *V. vulnificus* among sites, only Bird Island and Bayside had significantly different means ($p < 0.05$; Table 15).

Table 14. Correlation matrix for *V. vulnificus vs.* physical water quality parameters from sites in south Texas coastal waters for August 2006 through July 2007.

	Temp	Conductivity	Salinity	DO	pH	Secchi Disk
Pearson Correlation	0.368	-0.032	-0.032	-0.260	-0.077	-0.199
p – value	0.002**	0.792	0.789	0.028*	0.525	0.096

** Correlation is significant at 99% confidence interval (2-tailed)

* Correlation is significant at 95% confidence interval (2-tailed)

Table 15: Statistical comparison of *V. vulnificus* levels at sites, analyzed using the Tukey HSD: Multiple Comparisons Dependent Variable: LOG10CFU/100mL.

(I) Station	(J) Station	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Bird Island= 1	2	-0.619	0.243	0.126	-1.333	0.095
	3	-0.638	0.243	0.106	-1.352	0.076
	4	-0.058	0.243	1.000	-0.771	0.656
	5	-0.323	0.249	0.784	-1.053	0.407
	*6	-0.729	0.243	0.043	-1.442	-0.015
Nueces Bay= 2	1	0.619	0.243	0.126	-0.095	1.333
	3	-0.019	0.243	1.000	-0.733	0.694
	4	0.561	0.243	0.206	-0.153	1.275
	5	0.296	0.249	0.840	-0.434	1.026
	6	-0.110	0.243	0.998	-0.824	0.604
Cole Park= 3	1	0.638	0.243	0.106	-0.076	1.352
	2	0.019	0.243	1.000	-0.694	0.733
	4	0.581	0.243	0.176	-0.133	1.294
	5	0.315	0.249	0.801	-0.415	1.045
	6	-0.090	0.243	0.999	-0.804	0.623
Redfish Bay= 4	1	0.058	0.243	1.000	-0.656	0.771
	2	-0.561	0.243	0.206	-1.275	0.153
	3	-0.581	0.243	0.176	-1.294	0.133
	5	-0.265	0.249	0.892	-0.995	0.464
	6	-0.671	0.243	0.077	-1.385	0.043
Copano Bay= 5	1	0.323	0.249	0.784	-0.407	1.053
	2	-0.296	0.249	0.840	-1.026	0.434
	3	-0.315	0.249	0.801	-1.045	0.415
	4	0.265	0.249	0.892	-0.464	0.995
	6	-0.406	0.249	0.581	-1.135	0.324
Bayside= 6	*1	0.729	0.243	0.043	0.015	1.442
	2	0.110	0.243	0.998	-0.604	0.824
	3	0.090	0.243	0.999	-0.623	0.804
	4	0.671	0.243	0.077	-0.043	1.385
	5	0.406	0.249	0.581	-0.324	1.135

* The mean difference is significant at the 0.05 level.

A multiple regression analysis was completed and the model used accounted for 48.2% of the variability in the numbers of *V. vulnificus* (R^2 = 0.375) (Table 16). The environmental factors used in this model were water temperature and salinity. An ANOVA for this model displayed a significant relationship between these factors and the dependent variable, *V. vulnificus* (Table 17). Water temperature had a standardized partial regression coefficient of (b' = 0.368; $P < 0.01$), while salinity had a standardized partial regression coefficient of (b' = 0.411; $P < 0.05$) (Table 18). The positive correlation between *V. vulnificus* and water temperature was significant, but there was only a slight negative correlation between *V. vulnificus* and salinity (Table 18; Figures 18 and 19). However, salinity had a significant positive correlation with the residuals from the model including all the other variables. The other 51.8% of the variability in the isolation of *V. vulnificus* from south Texas coastal waters was caused by unknown factors. However, there was a significant negative correlation between dissolved oxygen levels and *V. vulnificus* (Figure 20).

Table 16: Multiple Regression analysis for levels of *V. vulnificus* : Model Summary

Model	R	R square	Adjusted R square	Std. Error of the Estimate
1	0.694(a)	0.482	0.375	0.509

a. Predictors: (Constant), Site, Water temp, Sal

Table 17: ANOVA from multiple regression analysis for levels of *V. vulnificus*

Model	Sum of Squares	df	Mean Square	F	Sig.
1 Regression	13.966	12	1.164	4.495	0.000(a)
Residual	15.018	58	0.259		
Total	28.984	70			

a. Predictors: (Constant), Site, Water temp, Sal

b. Dependent Variable: VVA

Table 18: Coefficients with P-values from multiple regression analysis for levels of *V. vulnificus.*

Model	Unstandardized Coefficie		Standardized Coefficier	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	3.628	0.548		6.616	0.000
BI	-2.176	0.816	-1.277	-2.667	0.010
NB	-2.292	0.842	-1.344	-2.720	0.009
CP	-2.497	0.833	-1.464	-2.998	0.004
RB	-3.043	0.794	-1.785	-3.830	0.000
CB	-1.749	0.768	-0.990	-2.275	0.027
BI WTEMP	0.020	0.027	0.269	0.751	0.456
NB WTEMP	0.061	0.028	0.816	2.197	0.032
CP WTEMP	0.068	0.026	0.938	2.576	0.013
RB WTEMP	0.071	0.025	1.004	2.881	0.006
CB WTEMP	0.038	0.024	0.504	1.591	0.117
BS WTEMP	-0.015	0.023	-0.211	-0.674	0.503
WTEMP	0.040	0.012	0.368	3.285	0.002
SAL	0.023	0.011	0.411	2.135	0.037

a. Dependent Variable: VVA

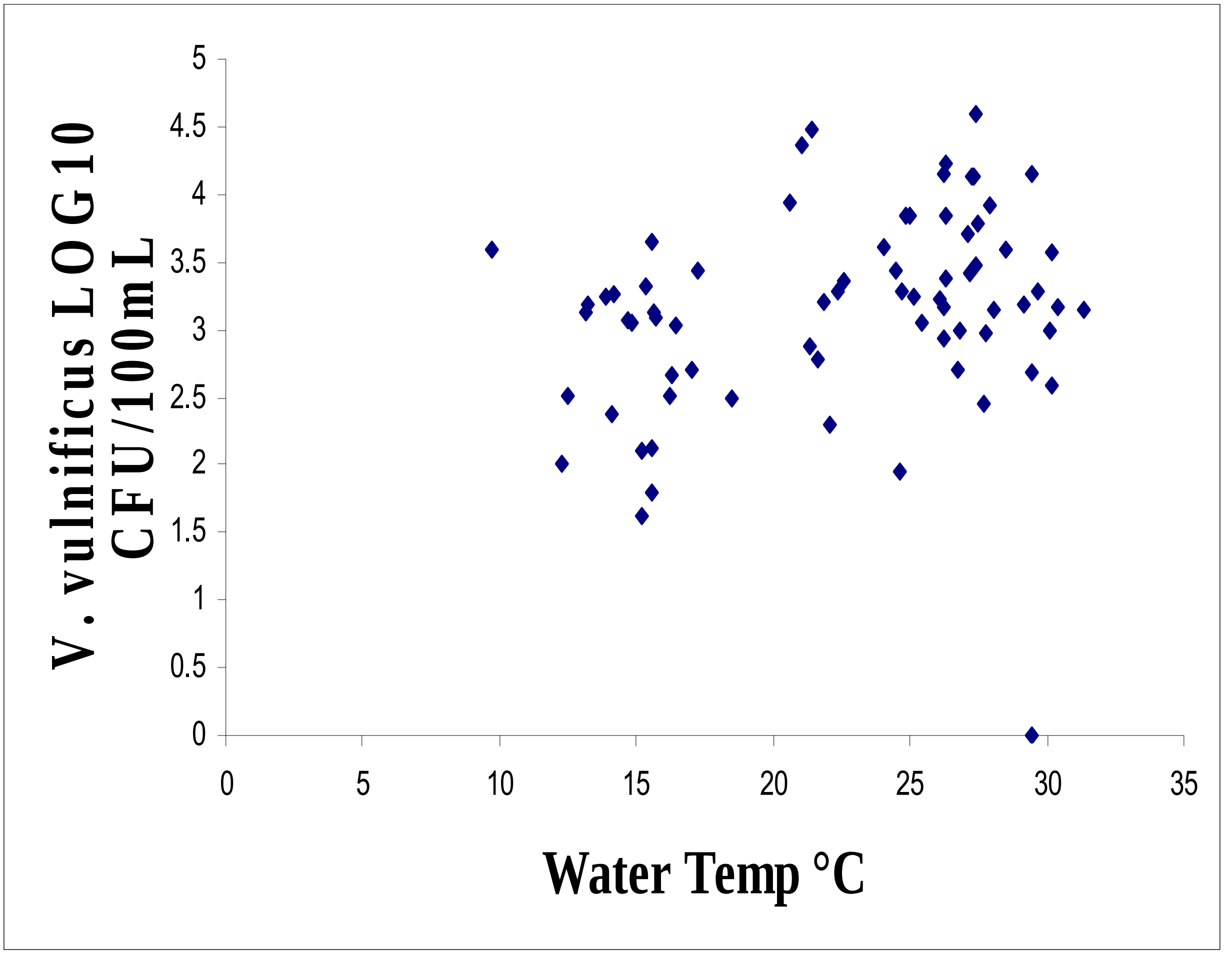

FIG 18. Water temperature versus *V. vulnificus* at sites in south Texas coastal waters from August 2006 through July 2007.

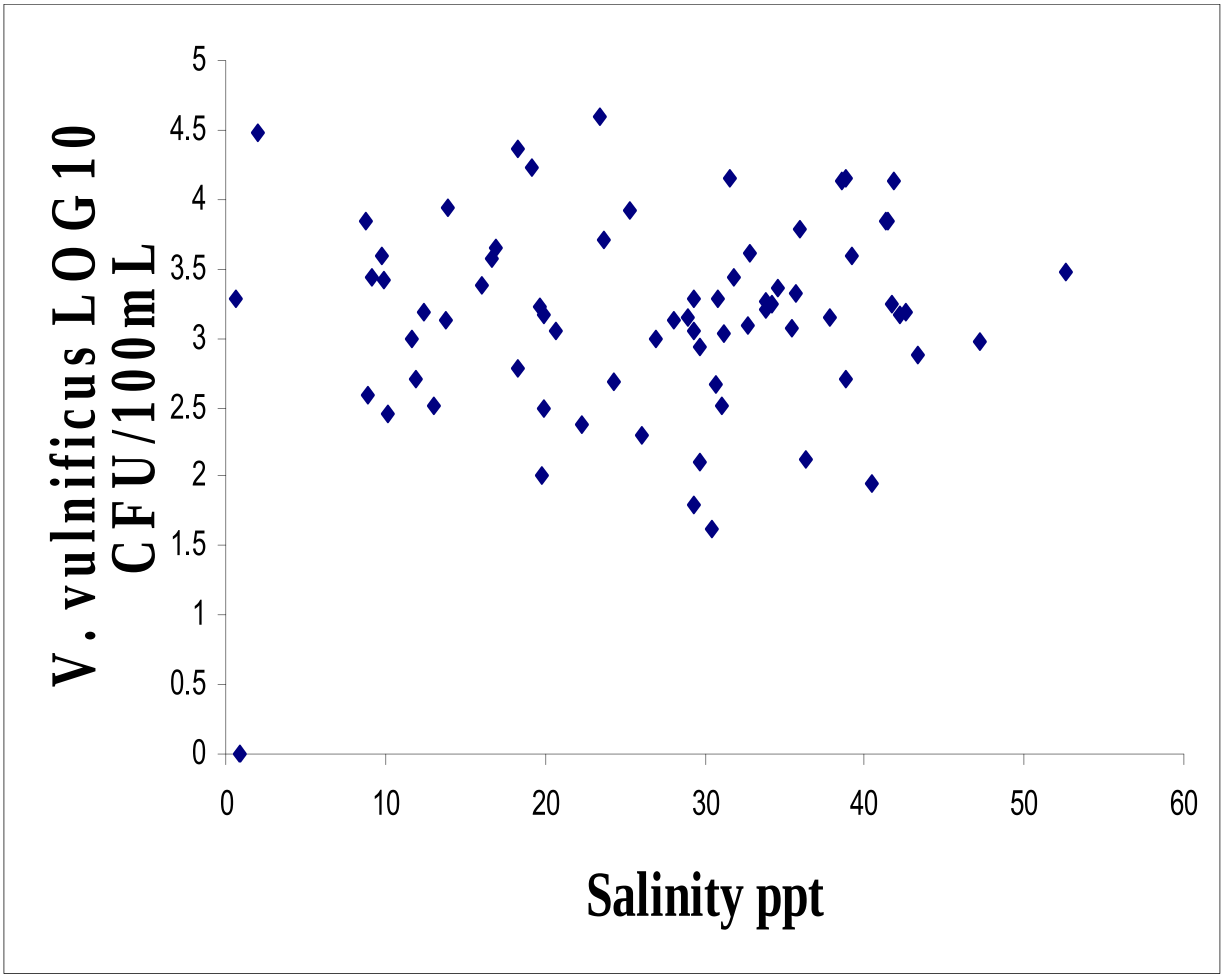

FIG 19. Salinity versus *V. vulnificus* at sites in south Texas coastal waters from August 2006 through July 2007.

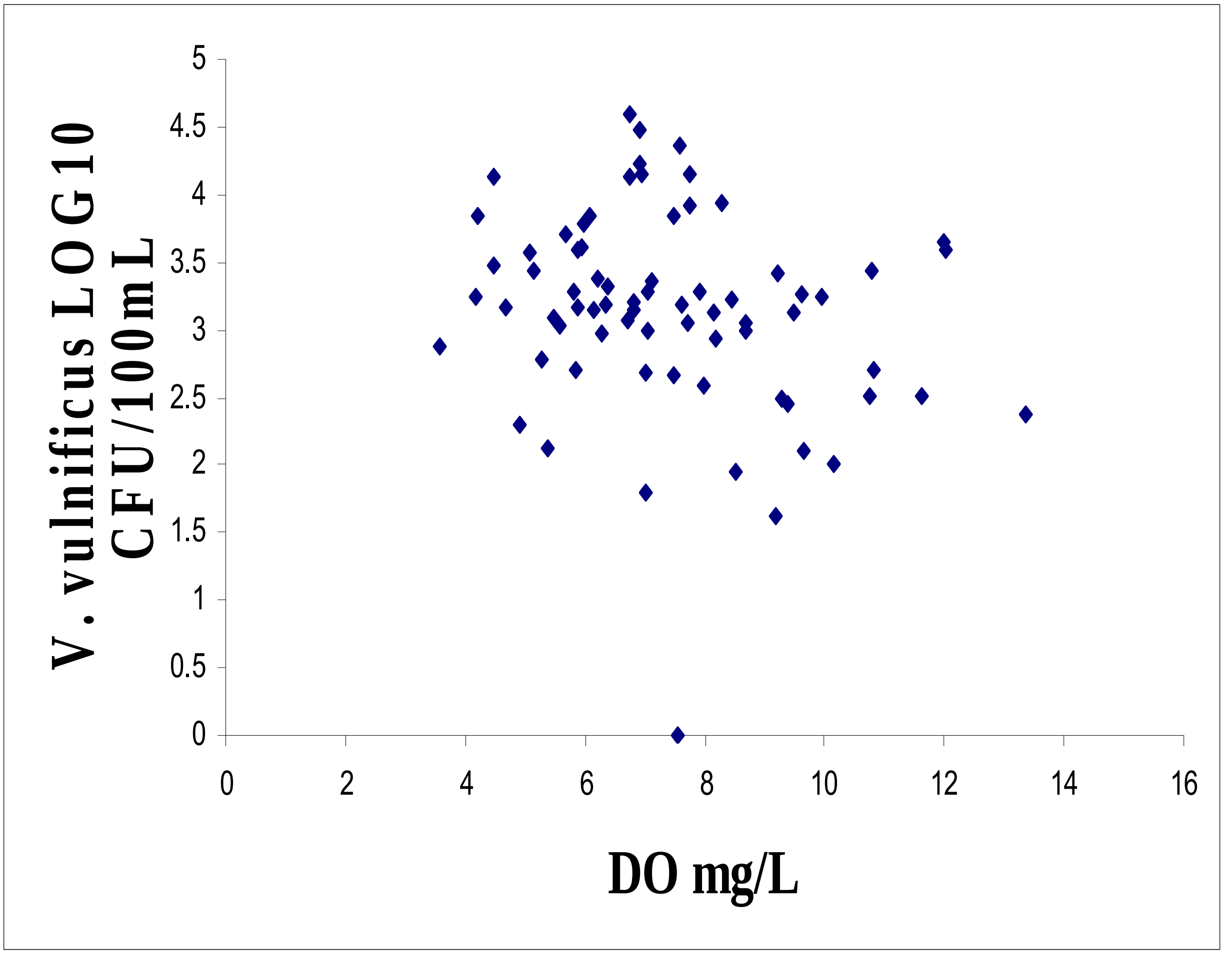

FIG 20: Dissolved oxygen versus *V. vulnificus* at sites in south Texas coastal waters from August 2006 through July 2007.

CONCLUSIONS

V. vulnificus was isolated from each site at each sampling event at levels as high as 3.97×10^4 CFU/100mL, with the exception of the July Copano Bay Causeway samples when the presumptive colonies failed to confirm as *V. vulnificus* using the DNA probe. However, *V. vulnificus* was detected this sampling event using mCPC, suggesting that an error may have occurred during analysis on this date (positive controls provided a normal response). Levels of *V. vulnificus* isolated in the present study ranged from < 1 to 3.97×10^4 CFU/100mL at the six sites, similar to those of other environmental studies in which *V. vulnificus* was isolated from estuarine waters (DePaola et al., 1994; Lipp et al., 2001; Oliver et al., 1982; O'Neill et al., 1992; Pfeiffer et al., 2003; Randa et al., 2004; Tamplin et al. 1982; Wright et al., 1996).

Numbers of *V. vulnificus* varied seasonally over the 12 month period with highest levels generally found from August through October/November. However, the bacteria were isolated in low numbers throughout the winter months at all six sites. The highest numbers of *V. vulnificus* were found at Nueces Bay in June 2007 with the second highest water temperature (27.4° C) and second lowest salinity (23.48 ppt) at this site.

Like many other studies that have examined *V. vulnificus* levels in coastal waters (Gulf of Mexico, Atlantic, and U.S. Pacific coasts) (DePaola et al., 1994; Kaysner et al., 1987; Kelly, 1982; Lipp et al., 2001; Oliver et al., 1982, 1983, 1995; O'Neill et al., 1992; Pfeiffer et al., 2003; Tamplin et al., 1982) significant relationships were shown between water temperature, salinity, and levels of *V. vulnificus* in this study. In these other studies, *V. vulnificus* was most commonly isolated when water temperatures exceeded 15° C and salinities ranged from 5 to 30 ppt. Water temperatures less than 10° C had a negative effect on isolation of *V. vulnificus.* In the current study, water temperatures ranged from 9.71 to 31.35° C, and salinities ranged from 0.62 to 52.67 ppt). *V. vulnificus* was isolated at some level each sampling event, regardless of water temperature and salinity levels. However, overall levels of *V. vulnificus* increased with water temperature and decreased with salinity. That *V. vulnificus* was isolated when water temperature was 9.71° C (February 2007 at Copano Bay near Bayside) is of particular interest since *V. vulnificus* is believed to enter the viable but non-culturable (VBNC) state during colder months (i.e., in cooler waters below 10° C) (Oliver and Bockian, 1995; Oliver et al., 1995; Oliver, 1999; Weichart, 1999; Whitesides and Oliver, 1997). Oliver and Bockian (1995) were able to induce the VBNC state by a reduction of ambient temperature to 5° C, with cells becoming nonculturable within 7 days. Since water temperatures in south Texas waters normally do not stay below 10° C for a long period of time, *V. vulnificus* isolates in the Coastal Bend region may not all enter a VBNC state. Although water temperature was 9.71° C at Copano Bay near Bayside in February 2007, levels of *V. vulnificus* were 3.90×10^3 CFU/100mL.

Numbers of *V. vulnificus* were positively correlated with water temperature and negatively correlated with dissolved oxygen, similar to findings by Pfeffer et al. (2003) in a study of *V. vulnificus* in estuarine waters of eastern North Carolina. Water temperature ranged from 15 - 27°C in their study and was the factor most highly correlated with isolation of *V. vulnificus.* Pfeffer et al. also found a negative correlation between isolation of *V. vulnificus* and dissolved oxygen levels as in the present study.

While most environmental studies have used correlation analyses alone to establish significant relationships between environmental and bacteriological factors, multiple regression analyses were completed in the present study to evaluate which variables are responsible for the variability in the isolation of *V. vulnificus* in south Texas coastal waters. Water temperature and salinity accounted for most (48%) of the variability in numbers of *V. vulnificus.* Water temperature was the environmental factor most highly correlated with presence of *V. vulnificus*. Salinity had a significant positive correlation with the residuals from the model including all other variables. The other 52% of the variability in the isolation of *V. vulnificus w*as caused by unknown factors. Dissolved oxygen, which was not included in the multiple regression model, was negatively correlated with *V. vulnificus.* Thus, it appears that no single parameter is primarily responsible for the isolation of *V. vulnificus* from south Texas coastal waters.

Other studies have used only water temperature to explain the variability in *V. vulnificus* levels (Motes et al., 1998; Pfeiffer et al., 2003; Randa et al., 2004). Motes et al. were able to use water temperature to explain 60% of the change in frequency of *V. vulnificus* isolation from oysters while Randa et al. were able to explain 60% of the variability in concentration of this organism in water with water temperature (range from 6 - 28°C). In the study by Pfeffer et al., water temperature was able to explain 47% of the variability in *V. vulnificus* isolation (ranging from 15 - 27°C).

Of the other environmental parameters measured in this study a negative correlation was found between dissolved oxygen levels and levels of *V. vulnificus,* similar to the negative correlation found by Pfeffer et al. (2003). There was no significant correlation between pH and numbers of *V. vulnificus* in the present study. This contrasts with earlier studies conducted by Oliver et al. 1982, 1983 and Tamplin et al. 1982. However, the pH range in the present study was less than 1 s.u. (7.51 – 8.45), and within the optimum pH range for this organism (Thompson et al., 2006) whereas in the studies by Oliver et al., pH ranged from 6.8 to 8.0 s.u.

Enterococci levels ranged from <1 to 1.06 X 10^3 CFU/100mL at the six sites. The highest numbers of *Enterococcus* sp. were found at Cole Park in September 2006 following a light rainfall the previous week. Two storm drains are located at Cole Park which carry runoff into Corpus Christi Bay. Levels of enterococci were significantly correlated with numbers of *V. vulnificus*. This suggests that enterococci could be used as an indicator for *V. vulnificus* levels. However, a longer term study, designed specifically to examine this relationship, would be needed before any recommendation could be made on use of *Enterococcus* sp. as an indicator of elevated levels of V. vulnificus.

RECOMMENDATIONS

- In order to identify other parameters influencing levels of *V. vulnificus* in south Texas coastal waters a longer term study at different sampling sites should be conducted, to include additional parameters such as nutrient levels. Relationships between environmental factors and incidence of *V. vulnificus* could be used to develop a predictive model for elevated levels of the organism which could be used to advise the public of increased risk under certain conditions.

- The finding that levels of enterococci were correlated with numbers of *V. vulnificus* demonstrates a need for additional studies designed to further examine this relationship and its possible use in indicating elevated V. vulnificus levels in recreational waters.

- This study assessed V. *vulnificus* levels only in the water column. To more fully understand the ecology of the organism, populations in sediment and oysters should also be enumerated to determine distribution and levels of *V. vulnificus* in these habitats.

- This study did not examine diversity of biotypes or strains of environmental *V. vulnificus*. While all strains are thought to be virulent there may be significant differences between many of the environmental strains and clinical strains shown to cause wound infections. A subsequent study assessing diversity and characteristics of environmental strains compared to clinical strains could provide a greater understanding of the level of risk to the public from exposure to *V. vulnificus* in coastal waters.

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APPENDIX

Other physical water quality parameters from Bird Island, Nueces Bay, and Cole Park for August 2006 through July 2007.

Date	Time	Station ID	Total Depth (m)	Depth Sample Collected (m)	Air Temp. (°C)	Wind Int.	Wind Dir.
08/15/06	838	Bird Island	0.40	0.133	28.2	Moderate	SE
09/06/06	810	Bird Island	0.40	0.133	23.9	Moderate	N
10/04/06	750	Bird Island	0.46	0.153	26.7	Slight	E
11/08/06	743	Bird Island	0.45	0.150	23.2	Slight	SE
12/06/06	753	Bird Island	0.66	0.220	20.3	Calm	SE
01/03/07	835	Bird Island	0.51	0.170	16.4	Moderate	NE
02/07/07	755	Bird Island	0.49	0.163	16.2	Slight	SE
03/07/07	740	Bird Island	0.57	0.190	18.9	Slight	SE
04/04/07	802	Bird Island	0.48	0.160	23.7	Slight	NE
05/09/07	845	Bird Island	0.51	0.170	24.6	Moderate	SE
06/06/07	910	Bird Island	0.48	0.160	28.3	Slight	SE
07/05/07	904	Bird Island	0.51	0.170	25.7	Slight	SE
08/15/06	947	Nueces Bay	0.40	0.133	28.2	Moderate	SE
09/06/06	926	Nueces Bay	0.40	0.133	23.9	Moderate	N
10/04/06	906	Nueces Bay	0.46	0.153	26.7	Slight	E
11/08/06	854	Nueces Bay	0.45	0.150	23.2	Slight	SE
12/06/06	908	Nueces Bay	0.66	0.220	20.3	Calm	SE
01/03/07	955	Nueces Bay	0.51	0.170	16.4	Moderate	NE
02/07/07	904	Nueces Bay	0.42	0.140	19.3	Slight	SE
03/07/07	846	Nueces Bay	0.38	0.127	21.3	Slight	SE
04/04/07	919	Nueces Bay	0.52	0.173	22.5	Moderate	NE
05/09/07	954	Nueces Bay	0.54	0.180	25.5	Slight	SE
06/06/07	1015	Nueces Bay	0.48	0.160	29.4	Slight	SE
07/05/07	1017	Nueces Bay	0.54	0.180	26.1	Slight	SE
08/15/06	1022	Cole Park	0.50	0.167	30.3	Moderate	SE
09/06/06	1002	Cole Park	0.54	0.180	27.4	Slight	N
10/04/06	943	Cole Park	0.52	0.173	28.4	Moderate	E
11/08/06	926	Cole Park	0.72	0.240	25.8	Slight	SE
12/06/06	940	Cole Park	0.66	0.220	20.4	Slight	SE
01/03/07	1027	Cole Park	0.57	0.190	15.4	Strong	NE
02/07/07	937	Cole Park	0.54	0.180	21.4	Slight	SE
03/07/07	915	Cole Park	0.60	0.200	21.2	Slight	SE
04/04/07	956	Cole Park	0.46	0.152	23.8	Strong	NE
05/09/07	1027	Cole Park	0.60	0.200	26.0	Slight	SE
06/06/07	1042	Cole Park	0.51	0.170	29.7	Moderate	SE
07/05/07	1050	Cole Park	0.66	0.220	27.9	Slight	SE

Other physical water quality parameters from Bird Island, Nueces Bay, and Cole Park for August 2006 through July 2007.

Date	Time	Station ID	Pres. Weather	Water Color	Water Odor	Water Surface	Tidal Stage
08/15/06	838	Bird Island	Cloudy	Green	None	Ripples	Rising
09/06/06	810	Bird Island	Cloudy	Green-Brown	None	Ripples	Rising
10/04/06	750	Bird Island	Clear	Green	None	Ripples	Rising
11/08/06	743	Bird Island	Clear	Green-Brown	None	Ripples	Falling
12/06/06	753	Bird Island	Overcast	Green	None	Calm	Rising
01/03/07	835	Bird Island	Cloudy	Green	None	Ripples	Rising
02/07/07	755	Bird Island	Cloudy-Fog	Green	None	Ripples	Falling
03/07/07	740	Bird Island	Clear	Green	None	Ripples	Rising
04/04/07	802	Bird Island	Cloudy	Green	None	Ripples	Rising
05/09/07	845	Bird Island	Cloudy	Green	None	Ripples	Falling
06/06/07	910	Bird Island	Clear	Green	None	Ripples	Falling
07/05/07	904	Bird Island	Cloudy	Yellow-Brown	None	Ripples	Falling
08/15/06	947	Nueces Bay	Cloudy	Green	None	Ripples	Rising
09/06/06	926	Nueces Bay	Cloudy	Green-Brown	None	Ripples	Rising
10/04/06	906	Nueces Bay	Clear	Green	None	Ripples	Rising
11/08/06	854	Nueces Bay	Clear	Green-Brown	None	Ripples	Falling
12/06/06	908	Nueces Bay	Overcast	Green	None	Calm	Rising
01/03/07	955	Nueces Bay	Cloudy	Green	None	Ripples	Rising
02/07/07	904	Nueces Bay	Overcast	Grey	None	Calm	Falling
03/07/07	846	Nueces Bay	Clear	Brown	None	Ripples	Rising
04/04/07	919	Nueces Bay	Overcast	Brown	None	Ripples	Rising
05/09/07	954	Nueces Bay	Cloudy	Brown	None	Ripples	Rising
06/06/07	1015	Nueces Bay	Clear	Brown	None	Ripples	Rising
07/05/07	1017	Nueces Bay	Cloudy	Brown	None	Ripples	Rising
08/15/06	1022	Cole Park	Cloudy	Green	Musky	Ripples	Rising
09/06/06	1002	Cole Park	Clear	Green	None	Ripples	Rising
10/04/06	943	Cole Park	Clear	Green	None	Waves	Rising
11/08/06	926	Cole Park	Clear	Green	Rotten Eggs	Ripples	Falling
12/06/06	940	Cole Park	Overcast	Green	None	Calm	Rising
01/03/07	1027	Cole Park	Overcast	Green	None	Waves	Rising
02/07/07	937	Cole Park	Cloudy	Green	None	Ripples	Falling
03/07/07	915	Cole Park	Cloudy	Green	None	Ripples	Falling
04/04/07	956	Cole Park	Overcast	Brown	Musky	Waves	Falling
05/09/07	1027	Cole Park	Cloudy	Green	Musky	Ripples	Rising
06/06/07	1042	Cole Park	Cloudy	Green	None	Ripples	Falling
07/05/07	1050	Cole Park	Cloudy	Green-Brown	None	Ripples	Falling

Other physical water quality parameters from Redfish Bay, Copano Bay, and Bayside for August 2006 through July 2007.

Date	Time	Station ID	Total Depth (m)	Depth Sample Collected (m)	Air Temp. (°C)	Wind Int.	Wind Dir.
08/23/06	723	Redfish Bay	0.36	0.120	27.0	Slight	SE
09/13/06	711	Redfish Bay	1.10	0.367	25.3	Slight	NW
10/11/06	710	Redfish Bay	1.20	0.400	22.6	Slight	S
11/15/06	709	Redfish Bay	1.04	0.347	20.6	Strong	NW
12/13/06	804	Redfish Bay	1.00	0.333	17.9	Slight	N
01/11/07	715	Redfish Bay	1.05	0.350	18.9	Moderate	SE
02/14/07	731	Redfish Bay	0.80	0.267	6.8	Moderate	NE
03/21/07	719	Redfish Bay	1.20	0.400	22.7	Moderate	SE
04/11/07	723	Redfish Bay	1.25	0.417	21.5	Slight	NW
05/16/07	831	Redfish Bay	1.25	0.417	24.7	Slight	NE
06/13/07	834	Redfish Bay	1.40	0.467	27.7	Slight	SW
07/11/07	827	Redfish Bay	1.35	0.450	27.9	Moderate	SE
08/23/06	833	Copano Bay	1.15	0.383	27.8	Moderate	SE
09/13/06	806	Copano Bay	1.15	0.383	25.6	Slight	NW
10/11/06	807	Copano Bay	1.10	0.367	26.3	Slight	S
11/15/06	806	Copano Bay	1.05	0.350	18.7	Strong	NW
12/13/06	859	Copano Bay	0.85	0.283	16.5	Slight	N
01/11/07	807	Copano Bay	0.80	0.267	18.2	Moderate	SE
02/14/07	835	Copano Bay	0.80	0.267	6.0	Moderate	NE
03/21/07	815	Copano Bay	1.05	0.350	22.1	Moderate	SE
04/11/07	817	Copano Bay	1.10	0.367	19.9	Slight	NW
05/16/07	921	Copano Bay	1.10	0.367	25.8	Slight	NE
06/13/07	933	Copano Bay	1.00	0.333	28.4	Slight	SW
07/11/07	923	Copano Bay	1.20	0.400	29.7	Moderate	SE
08/23/06	940	Bayside	0.60	0.200	28.5	Slight	SE
09/13/06	924	Bayside	0.42	0.173	26.6	Slight	NW
10/11/06	935	Bayside	0.55	0.183	22.9	Slight	S
11/15/06	925	Bayside	0.48	0.160	21.8	Strong	NW
12/13/06	1021	Bayside	0.46	0.153	18.2	Slight	N
01/11/07	925	Bayside	0.46	0.153	20.0	Moderate	SE
02/14/07	947	Bayside	0.44	0.147	6.8	Moderate	NE
03/21/07	927	Bayside	0.57	0.190	22.0	Moderate	SE
04/11/07	927	Bayside	0.58	0.193	21.9	Slight	NW
05/16/07	1034	Bayside	0.48	0.160	28.5	Slight	NE
06/13/07	1043	Bayside	0.48	0.160	32.1	Calm	SW
07/11/07	1031	Bayside	0.57	0.190	30.0	Moderate	SE

Other physical water quality parameters from Redfish Bay, Copano Bay, and Bayside for August 2006 through July 2007.

Date	Time	Station ID	Pres. Weather	Water Color	Water Odor	Water Surface	Tidal Stage
08/23/06	723	Redfish Bay	Cloudy	Green-Brown	None	Ripples	Rising
09/13/06	711	Redfish Bay	Cloudy	Brown	Musky	Ripples	Falling
10/11/06	710	Redfish Bay	Cloudy	Green	Musky	Ripples	Falling
11/15/06	709	Redfish Bay	Clear	Brown	None	Ripples	Rising
12/13/06	804	Redfish Bay	Clear	Green	Musky	Ripples	Falling
01/11/07	715	Redfish Bay	Cloudy	Green	None	Ripples	Rising
02/14/07	731	Redfish Bay	Cloudy	Brown	None	Ripples	Rising
03/21/07	719	Redfish Bay	Cloudy	Green-Brown	None	Ripples	Falling
04/11/07	723	Redfish Bay	Overcast; Foggy	Green	None	Ripples	Rising
05/16/07	831	Redfish Bay	Clear	Green	None	Ripples	Falling
06/13/07	834	Redfish Bay	Clear	Green	None	Ripples	Falling
07/11/07	827	Redfish Bay	Cloudy	Green	None	Ripples	Rising
08/23/06	833	Copano Bay	Clear	Green	None	Ripples	Rising
09/13/06	806	Copano Bay	Cloudy	Green	Musky	Ripples	Falling
10/11/06	807	Copano Bay	Clear	Green-Brown	None	Ripples	Falling
11/15/06	806	Copano Bay	Clear	Brown	None	Waves	Rising
12/13/06	859	Copano Bay	Clear	Green	None	Ripples	Falling
01/11/07	807	Copano Bay	Clear	Green	None	Ripples	Rising
02/14/07	835	Copano Bay	Cloudy	Green-Brown	None	Ripples	Rising
03/21/07	815	Copano Bay	Cloudy	Brown	None	Ripples	Rising
04/11/07	817	Copano Bay	Cloudy	Green	None	Ripples	Rising
05/16/07	921	Copano Bay	Clear	Green	None	Ripples	Rising
06/13/07	933	Copano Bay	Clear	Green	None	Ripples	Rising
07/11/07	923	Copano Bay	Cloudy	Brown	None	Ripples	Rising
08/23/06	940	Bayside	Cloudy	Green	None	Ripples	Rising
09/13/06	924	Bayside	Cloudy	Green	None	Ripples	Falling
10/11/06	935	Bayside	Clear	Green-Brown	Musky	Ripples	Falling
11/15/06	925	Bayside	Clear	Green-Brown	None	Waves	Rising
12/13/06	1021	Bayside	Clear	Green	None	Ripples	Falling
01/11/07	925	Bayside	Clear	Green	None	Ripples	Rising
02/14/07	947	Bayside	Cloudy	Brown	None	Ripples	Rising
03/21/07	927	Bayside	Cloudy	Brown	None	Ripples	Falling
04/11/07	927	Bayside	Clear	Green-Brown	None	Ripples	Rising
05/16/07	1034	Bayside	Cloudy	Green-Brown	None	Ripples	Falling
06/13/07	1043	Bayside	Clear	Green	None	Ripples	Falling
07/11/07	1031	Bayside	Cloudy	Brown	None	Ripples	Rising