Evaluation of alternative bacterial indicators for use in determining compliance with water quality criteria.

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Historical monitoring conducted for the evaluation of microbiological contamination and associated public health risks associated with contact recreation at bathing beaches along the Texas Gulf coast has been limited. Most monitoring conducted by environmental and public health agencies was conducted sporadically for purposes of determining trends and sources of microbiological contamination, designation and protection of contact recreation areas, and/or for classification of shellfish areas. This monitoring depended on the use of "indicator" organisms, that are used to detect the presence of contaminated water which may harbor pathogenic species. At present the primary indicator used to assess microbiological contamination is fecal coliform bacteria. Due to high variability and lack of specificity, the EPA and many states have however, proposed to utilize alternative indicators for marine waters including the Enterococci test. Our study examined the effect of utilizing alternative indicators, testing methodology, and propose criteria on the incidence of non-compliance with existing and proposed water quality standards. We conducted our study during the summer of 1998 within the Galveston Bay watershed at various beaches located throughout the estuary. Based on the results of our study, the use of Enterococci as an indicator may result in a higher incidence of non-compliance than traditional fecal coliform bacteria. Enterococci levels were also more variable than other indicators tested. For all indicators evaluated reliance on single point maximum values resulted in higher incidents of non-compliance than recommended geometric mean criteria. Due to the inherent variability of these indicators we recommend that only geometric mean based standards be used to determine compliance with designated uses. Further evaluation of bacteriological methods utilized during our study is warranted, due to potential elevated false positive rates that may have been induced by increased nutrient levels in un-diluted samples. Based on the findings of our study we recommend additional parallel monitoring to evaluate the performance of the testing methods.

Introduction

Each year millions of people visit beaches along the Gulf coast of Texas to swim, boat and fish. Baseline information on ambient levels of potential waterborne pathogens to evaluate potential risks of from various forms of contact recreation is needed. At present the most commonly used monitoring tool used by Texas state agencies to evaluate risks from waterborne pathogens is the fecal coliform test (TNRCC, 1997a). The designated uses of all Texas Gulf of Mexico beaches and many bay waters has been defined as contact recreation (TNRCC, 1997b). However, little intensive monitoring to determine compliance with bacteriological standards at Gulf beaches has occurred. Most microbiological monitoring in estuaries and Gulf beaches in Texas has been conducted by environmental and public health agencies. Historically this monitoring was conducted sporadically to determine trends in microbiological contamination, or to determine whether selected waterbodies met shellfish harvest designations. This monitoring depends on the use of "indicator" organisms (e.g. fecal coliform bacteria) that are used to detect the presence of contaminated water which may harbor pathogenic species. These indicators are formally incorporated into State of Texas water quality standards as promulgated by the Texas Natural Resource Conservation Commission (TNRCC)(TNRCC, 1997b).

There are at least two laboratory methods used by Texas state agencies to detect fecal coliform bacteria which includes the multiple tube fermentation method and the membrane filtration method (American Public Health Association, 1992). Both methods are approved by the EPA and yield comparable results. Separate water quality standards and testing methodology have been developed for protection of human health from shellfish consumption but are not being evaluated by this study.

In Texas, criteria that apply to waterbodies with a designated use of "contact recreation" are defined as a level of 200 fecal coliform colonies per 100 ml as a geometric mean based on a representative sampling of not less than five samples collected over not more than 30 days. Alternatively, fecal coliform levels shall not equal or exceed 400 colonies per 100 ml in more than 10% of all samples, but based on at least five samples, taken during any 30-day period. If ten or fewer samples are analyzed, no more than one sample can exceed 400 colonies per 100 ml. The fecal coliform indicator has been challenged as not being very predictive of the true risk from waterborne pathogens associated with human wastes (EPA, 1986). It has estimated that many false positives are a result of non-human sources of fecal coliform bacteria and/or thermophilic bacteria that grow on media used for the culture of fecal coliforms. Little intensive compliance monitoring (5 sample - 30 day) that would specifically evaluate whether a waterbody meets fecal coliform standards for contact recreation has been historically conducted by the state of Texas. This was due primarily to logistical and resource limitations.

The EPA has recommended alternative bacteriological water quality indicators. For marine and estuarine waters EPA has recommended the use of the Enterococci indicator test (EPA, 1986). Enterococci includes a subgroup of fecal streptococci bacteria (mainly *Streptococcus faecalis* and *Streptococcus faecium*) that is present in the intestinal tracts and feces of warm-blooded animals. The recommended Enterococci water quality criteria employs a geometric mean of 33 cfu/100 ml based on not less than 5 samples over a 30 day period. A maximum value of 104 cfu/100 ml (upper 75% confidence interval) is not to be exceeded for any one sample in marine waters.

The recommended indicator for freshwater is *Escherichia coli* (EPA, 1986). *E. coli* is a subgroup of fecal coliform bacteria present in the intestinal tracts and feces of warm blooded animals. The recommended water quality criteria is a geometric mean of 126 cfu/100 ml based on not less than 5 samples over a 30 day period. A maximum value of 235 cfu/100 ml (upper 75% confidence interval) of *E. coli* is not to be exceeded for any one sample in freshwater.

Based on previous epidemiological studies cited by the EPA, these two methods provide a better evaluation

of the risk, that is a stronger correlation with waterborne pathogens, from contact recreation (e.g. swimming). However, some researchers have indicated that the methodology used to develop the proposed marine criteria based on *Enterococci* was flawed due to combining of several distinct data sets and possible confounding variables including salinity (Fleisher, 1991).

The TNRCC has been in the process of evaluating the use of these alternative indicators and is near completion of a comprehensive review of the accuracy of various methods and their relation to health risks (Dean, 1998). The focus of that study was the evaluation of alternative bacteriological indicators and methods for assessing the risk to swimmers from contact with ambient waters. The TNRCC study is driven by the fact that the current fecal coliform based monitoring techniques are very non-specific, variable, and can detect non-human sources of bacteria (high false positive). Recent studies of Gulf beaches along the upper Texas coast showed that most beaches were supporting this designated use (Marks and Guillen, 1999). This was based however on single point sampling during each season and comparison of this value to the 400 cfu/100 ml screening value. The Texas General Land Office (TGLO) has recently initiated a bacteriological monitoring program along selected Texas beaches to further address this problem.

The primary objective of this study was to evaluate spatial trends in the various bacteriological indicators along the upper Texas coast, specifically the Galveston barrier island complex. A secondary objective was to compare the frequency of regulatory non-compliance with existing and proposed water quality standard indicators and standards. The final objective was to evaluate potential relationships of these indicators with other water quality indicators and general land use.

Study Area

Ten sites were selected within the Galveston Bay system and adjacent barrier island beaches (Fig. 1 and Table 1). Stations were established at San Jacinto River at Banana Bend (SJBB), San Jacinto River at I-10 (SJ10), near the mouth of Little Cedar Creek located in upper Galveston Bay (LCC), near the end of the Texas City Dike adjacent to upper Galveston Bay (TCD), Offatts Bayou on Galveston Island (OB), an enclosed pond near East Beach on Galveston Island (EBM), Stewart Beach on the ocean side of Galveston Island, an ocean beach near western end of Galveston Island, Jamaica Beach (JBG) and at a bay side marsh area near Jamaica Beach (JBM). These areas represent various locations that based on historical knowledge of the authors you would expect to find limited to heavy usage by fisherman, swimmers and small craft operators. In addition, these sites were selected to cover a range of salinities and loading by potential sources of fecal coliform bacteria.

Methods

Sampling for indicator bacteria and related variables was conducted according to TNRCC guidelines for evaluation of fecal coliform water quality criteria (TNRCC, 1997a). All sampling conducted during this survey was conducted within a 30 day period spanning July and/or August 1998. This represents a season of peak usage of these areas by fisherman, boaters swimmers, and tourists. Five individual samples were collected during the 30 day period at an interval of approximately 5-7 days apart.

In-situ water quality monitoring consisted of water temperature, dissolved oxygen, pH, conductivity, salinity and transparency measurements at each site. These measurements were made in-situ using a Hydrolab Surveyor II multi-parameter meter. All measurements were made at the surface in approximately 1-3 foot depth. Bacteriological samples were collected at the same location using a sterile plastic (Whirl-Pak©) 4oz bag. Bacteriological samples were collected at the surface in approximately 1 foot water depth. When all sampling was completed the bacteriological samples were transferred to ice chests with ice and taken to the lab for colony culture and enumeration. During some sampling events, additional water quality samples were

collected for nutrients and total suspended solid (TSS). These samples were analyzed by the TNRCC laboratory. Rainfall data was obtained from NOAA National Weather Service precipitation stations located near sampling sites. Twenty-four cumulative rainfall amounts were used for comparison to other variables.

Analysis of the fecal coliform bacteria was conducted according to EPA and TNRCC accepted methods (TNRCC, 1997a). In addition, analyses for E. Coli (freshwater) and Enterococci (marine waters) indicator bacterial assemblages was conducted using the IDEXX™ "defined substrate" method (Budnick et al., 1996; IDEXX, 1998b). The IDEXX™ developed E.coli method, called Colilert® , which has been approved by the EPA for drinking water and source water testing for presence/absence, uses a patented defined substrate technology (Dougherty, 1996). The nutrient indicator MUG (4-methyl-umbelliferyl- ß-d-glucoronide) is metabolized by the E.coli enzyme ß-glucoronidase As E. coli uses ß-glucuronidase to metabolize MUG a flourescent (under U-V light) by-product, 4-methyl-umbelliferone is produced (IDEXX, 1998a; Covert et al., 1992). The estimation of numbers of colonies is obtained using a most probable number (MPN) approach in which separate incubation wells are inoculated and then read. Previous studies have indicated that this method yields results that are statistically indistinguishable from the recommended approved EPA EC-medium plus MUG test (American Public Health Association, 1992) (Covert et al., 1992).

The Enterococci method developed by IDEXX™, uses the Enterolert® defined substrate system. This is a rapid 24-hr test that detects Enterococci in water. Enterolert uses 4-methylumbelliferyl-ß-D-glucoside as the defined substrate nutrient indicator (Chen et al. 1998; Verma 1998). This compound, when hydrolyzed by enterococcus ß-glucosidase, releases 4-methylumbelliferone which exhibits fluorescence under a UV_{365nm} lamp. The estimation of numbers of colonies is obtained using a most probable number (MPN) approach in which separate incubation wells are inoculated and then read. This technique is similar to the EPA approved Method 1600: membrane filter test method for Enterococci (EPA 1997). The same nutrient media, mEI using indoxyl ß-D glucoside, is used for culturing as in the IDEXX method. Results of side by side performance tests against the approved EPA method have yielded similar results that are highly correlated (Chen et al. 1998). The specificity and sensitivity of the IDEXX method was also good. The manufacturer of this product and technique recommends the dilution of marine and estuarine samples with deionized water ranging from a 1:20 to 1:1 ratio respectively. This information was not communicated to the researchers and so, the targeted 1:1 ratio (for estuaries) was not obtained. According to the manufacture, if phosphorus levels are "elevated", this could result in higher than usual values (false positives). We therefore evaluated the influence of nutrients (within the range observed) by linear correlation analysis.

Summary sample statistics were generated for each of the bacteriological indicators. When data were reported below the detection limit (e.g. <10 colonies/100 ml), one-half the detection limit (e.g. 5 colonies/100 ml) was generally substituted for purposes of data analysis. Pearson correlation coefficient were calculated between physico-chemical variables and indicator bacteria levels to determine if there was any relationship between these parameters.

Values collected during the intensive survey were compared to State of Texas fecal coliform criteria and EPA recommended criteria for E. coli and Enterococci (TNRCC, 1998) . E. coli and Enterococci values were compared to the recommended alternative standards developed by the EPA (EPA, 1986). The recommended freshwater and marine standard for E. coli and Enterococci is a geometric mean of 126 CFU/100 ml and 33 cfu/100 ml respectively (EPA, 1986). This is based on a sample size of at least 5 samples within a 30 day period. Both of these standards also utilize various confidence intervals for establishing a one sample maximum standard based on anticipated waterbody use. For example, the maximum single measurement sample allowed for a designated bathing beach is the one-sided 75% confidence interval (C.I.) for each indicator. Default log standard deviations are provided to calculate this if site specific data are lacking (EPA, 1986). Using these default values the 75% confidence interval value for E. coli and Enterococci is 235 and

104 cfu/100 ml respectively. Higher C.I. values are provided for less frequently used waterbodies (e.g. 95% upper C.I. for infrequently used areas).

Results

Water temperature varied little between stations and was uniformly high (>28C). The pH of each station were within normal ranges (6.8-8.5 s.u.) encountered in estuarine systems. The majority of stations exhibited elevated salinities greater than 25 ppt (Figure 2). However, Little Cedar Creek, San Jacinto River at I-10, and San Jacinto River at Banana Bend each exhibited salinities lower than 10 ppt. The station at Seabrook exhibited intermediate salinities at approximately 15 psu. The lowest salinity was exhibited at Banana Bend with values <= 5 psu. Dissolved oxygen levels were quite variable and ranged between 20 and 3 mg/l. The majority of values fell between 5 and 10 mg/l. The Offatts Bayou site had the most variable dissolved oxygen readings.

Total suspended solids (TSS) was not always collected at each site. Based on the data collected TSS varied considerably between stations. Highest TSS (400 mg/l) was observed at the Stewart Beach station and reflects the high turbidity generated as a result of wave action. Lowest values (<27 mg/l) were observed at the Seabrook and San Jacinto River stations.

Ammonia nitrogen levels were fairly low throughout the study (<0.03). Levels were however, elevated at the Little Cedar Creek station, approaching 0.17 mg/l. Intermediate levels (0.09-0.12 mg/l) were observed at the San Jacinto River stations. Combined nitrate and nitrite (TIN) levels were all generally below 1.0 mg/l at each station. However, elevated levels (>2 mg/l) were observed at the Little Cedar Creek and San Jacinto River stations.

Total phosphorus levels were generally low (< 0.5 mg/l) throughout the study period. Dissolved orthophosphate levels were also generally below 0.5 mg/l. Highest (>0.98 mg/l) total phosphorus and orthophosphate levels were generally observed at the Little Cedar Creek and San Jacinto River at I-10 stations.

Rainfall amounts were minimal during the survey period. The majority of sites did not register any rainfall during the survey. The highest 24 amount of rainfall reported was at a weather station near the Texas City dike. This amounted to 0.86 inches.

Fecal coliform levels varied considerably among stations (Fig. 3). Highest levels were generally found at the San Jacinto River stations; and at the Little Cedar Creek, Offatts Bayou, and Seabrook Shoreline stations. Fecal coliform 5 sample, 30 day criteria were exceeded at both the Little Cedar Creek, San Jacinto River at I-10 and Offatts Bayou stations (Table 2). In addition, violations of the single sample criteria were also observed at the same stations, for a total of 5 incidents.

E. coli levels fluctuated between stations (Fig. 4). Lowest levels were generally observed at the Texas City Dike and Seabrook shoreline stations. Proposed E. coli geometric mean criteria were exceeded at 6 stations (Table 2). Proposed E. coli single sample criteria were exceeded at 8 stations, for a total of 21 incidents. The Texas City dike and San Jancinto River stations although not violating the geometric mean criteria, did violate the single point criteria.

Lowest Enterococci levels were generally observed at the San Jacinto River Banana Bend and Seabrook Shoreline stations (Fig. 5). All other stations exhibited similar geometric means and considerable variability in individual measurements. Proposed Enterococci geometric mean criteria were violated at 8 stations. In addition, the single value criteria was violated at the same 8 stations, including a total of 22 incidents (Table

2). The Seabrook and San Jacinto River at I-10 stations were the only stations were criteria were not exceeded.

Overall, the only two stations not exceeding any of the existing or proposed criteria were the Seabrook shoreline and San Jacinto River at I-10 locations (Table 2).

Fecal coliform levels exhibited significant negative correlations with pH and positive correlations with dissolved oxygen (Table 3). This may be due to the higher fecal coliform levels observed at stations with lower pH and higher dissolved oxygen. These stations include the upper San Jacinto River stations. E. coli levels exhibited significant positive correlations with salinity (Table 3). Higher E. coli levels were generally observed at stations exhibiting higher salinities. (Figs. 2 and 6). Enterococci levels exhibited negative correlations with water temperature, and positive correlations with salinity and pH. Higher Enterococci levels were generally found at stations exhibiting lower temperatures, and higher salinities and pH (Figs. 2 and 7). We did not observe any significant correlation between any bacterial indicator and nutrients or TSS (Table 3). This is important since we were concerned with the possible influence of added nutrients on the survival of bacteria. Enterococci and E. coli levels exhibited positive correlations (Table 3). All significant correlations reported were low ® <0.50) values.

Although all methods yielded negative results when exposed to blank samples, the results from exposure to both positive and negative controls are equivocal (Table 4). This is partly due to the high detection limits used by the fecal coliform test which included the lower range of values that were possible for the E. coli, Klebsiella and Pseudomonas cultures. In addition, low level growth (< 5 cfu/100 ml) was observed using the E. coli and Enterococci test when incubating both negative control cultures (Pseudomonas and Klebsiella). The E. coli test did record a total of 6.2 cfu/100 ml when exposed to the E. coli culture. If all the cultures contained similarly low amounts of bacteria, this would explain the below detection limit numbers seen with the fecal coliform test. In all cases, none of the indicator tests showed values that would exceed any criteria level.

Conclusions

This study illustrate some of the primary issues when dealing with bacteriological indicators. Sampling variability is often very high. As a result, relying on a single sample criteria can result in many sites being classified as not attaining compliance with standards based on short term fluctuations in bacteriological populations and water masses. This also results in many sites violating standards more frequently in comparison to using the geometric mean value. Variability between replicate samples was identified as one of the most greatest sources total variation in a study in Southern California waters (McGee et al., 1999).

The fecal coliform criteria has a long history of use within the regulatory arena, and has been widely used by the state of Texas for the determination of compliance of water quality standards and attainment of "fishable/swimmable" designated use. However, as illustrated in our study, this less specific standard exhibited very poor correlation with any of the EPA proposed indicators. Surprisingly however, the fecal coliform indicator yielded fewer violations than the preferred alternative indicators. The higher growth and violation of proposed criteria could be partially explained by increased nutrients and salinity as a result of using undiluted samples. However, over the range of nutrients observed in our study we failed to observe any relation with any of the indicators and criteria evaluated. There was a higher growth rate observed in samples with higher salinities. However, this correlation was relatively low ® = 0.31-0.33).

The IDEXX bacteriological indicator test methods were relatively easy to use, utilized proven technology and has documented performance data that supports it's specificity and sensitivity. We however, recommend that future testing and use of the IDEXX system or any other indicator employ blanks, positive and negative

biological controls, and utilize a series of dilutions to test the effect of salinity and nutrients on viability. Due to the inherent variability of these indicators we recommend that only geometric mean based standards be used to determine compliance with designated uses. This would mean continued sampling at a higher intensity (5 sample, 30 day) than traditional routine (1 sample) monitoring schemes. Further testing of these alternative protocol and criteria during winter months is also warranted to evaluate the effect of temperature on viability and standards violation.

Based on the findings of our study we recommend additional parallel monitoring to evaluate the performance of the testing methods.

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Table 1. Description of stations sampled during this study.

Station Description	TNRCC Segment Number and Description	Potential Sources of Coliforms near site (maximum average flow reported 12 months)	No. and permitted volume (MGD) of WWTP outfalls in segment
San Jacinto River at Banana Bend	1001, San Jacinto R. 17 miles	Onsite septic tanks, suburban runoff, vessel traffic	13 (11.01)
San Jacinto R. at I-10 City of Baytown	1001, San Jacinto R. 17 miles	Several City of Baytown WWTP (3.21 MGD), suburban runoff, vessel traffic	13 (11.01)
Mouth of Little Cedar Creek. City of LaPorte, TX	2421, Upper Galveston Bay, 108.2 sq. miles	City of LaPorte WWTP (5.5 MGD), suburban runoff	6 (12.59)
Seabrook Shoreline @ Toddville Rd. City of Seabrook	2421, Upper Galveston Bay, 108.2 sq. miles	suburban runoff	6 (12.59)
Offatts Bayou @ XXst. Street. City of Galveston	2424, West Bay, 69.3 sq. miles	suburban runoff, City of Galveston WWTP., (2.9 MGD)	13 (16.95)
Stewart Beach at Seawall Blvd. City of Galveston	2501, Gulf of Mexico, 3,879 sq. miles	beach runoff, wildlife, no point sources in vicinity	3 (0.04 MGD)
East Bay Tidepool @ Jetty Rd.	2439, Lower Galveston Bay , 139.6 sq. miles	wildlife, no point sources	6 (10.32)
Jamaica Beach subdivision, City of Jamaica Beach	2424, West Bay, 69.3 sq. miles	Jamaica Beach WWTP (0.11 MGD), suburban runoff, wildlife	6 (12.59)
Jamaica Beach Marsh	2424, West Bay, 69.3 sq. miles	wildlife	6 (12.59)

Table 2. Number of violations using single sample and geometric mean existing and proposed criteria.

	HDL FC GM	DL FC GM	FC Single	HDL EC GM	DL EC GM	EC Single	Entero. GM	Entero. S
TX City	0	0	0	0	0	1	1	
Seabrook	0	0	0	0	0	0	0	
Little Cedar Crk.	1	1	2	1	1	3	1	
SJR @ I-10	1	1	2	0	0	1	1	
SJR @ BB	0	0	0	0	0	0	0	
Offatts B.	1	1	1	1	1	4	1	
J.B. Marsh	0	0	0	1	1	3	1	
J. Beach	0	0	0	1	1	3	1	
Stewart B.	0	0	0	1	1	3	1	
E.B. Marsh	0	0	0	1	0	1	1	
Total	3	3	5	5	5	19	8	

DL = violations based on using the detection limit when values less than detection limit observed.

HDL = violations based on using half of the detection limit when values less than detection limit observed.

Single criteria: FC = 400; EC = 235; Entero. = 104 cfu/100 ml

Geometric mean criteria: FC = 200; EC = 126, Entero. = 33 cfu/100 ml

FC=fecal coliform, EC = E.coli, Entero.= Enterococci

GM = based on geometric mean, Single = based on single observations

Table 3. Significant ($p < 0.001$) correlation coefficients observed during the study.

	Fecal coliform	E. coli	Enterococci
E. coli		1.0	0.461
Enterococci		0.461	
Temperature			-0.36
Salinity		0.31	0.33
Dissolved oxygen	0.42		
pH	-0.28		0.281

Table 4. Results of QA/QC testing of various bacteriological indicators.

	Fecal coliform	E. coli	Enterococci
Blank	<10	0	0
E. coli (1-50 cells) positive control: Fecal coliform + E. coli; negative control: Enterococci.	<10	6.2	5.3
Klebsiella sp (1-50 cells) positive control: Fecal coliform; negative control: E. coli + Enterococci.	<10	5.3	5.3
Pseudomonas sp. (1-50 cells) negative control: all indicators.	<10	1	6.4

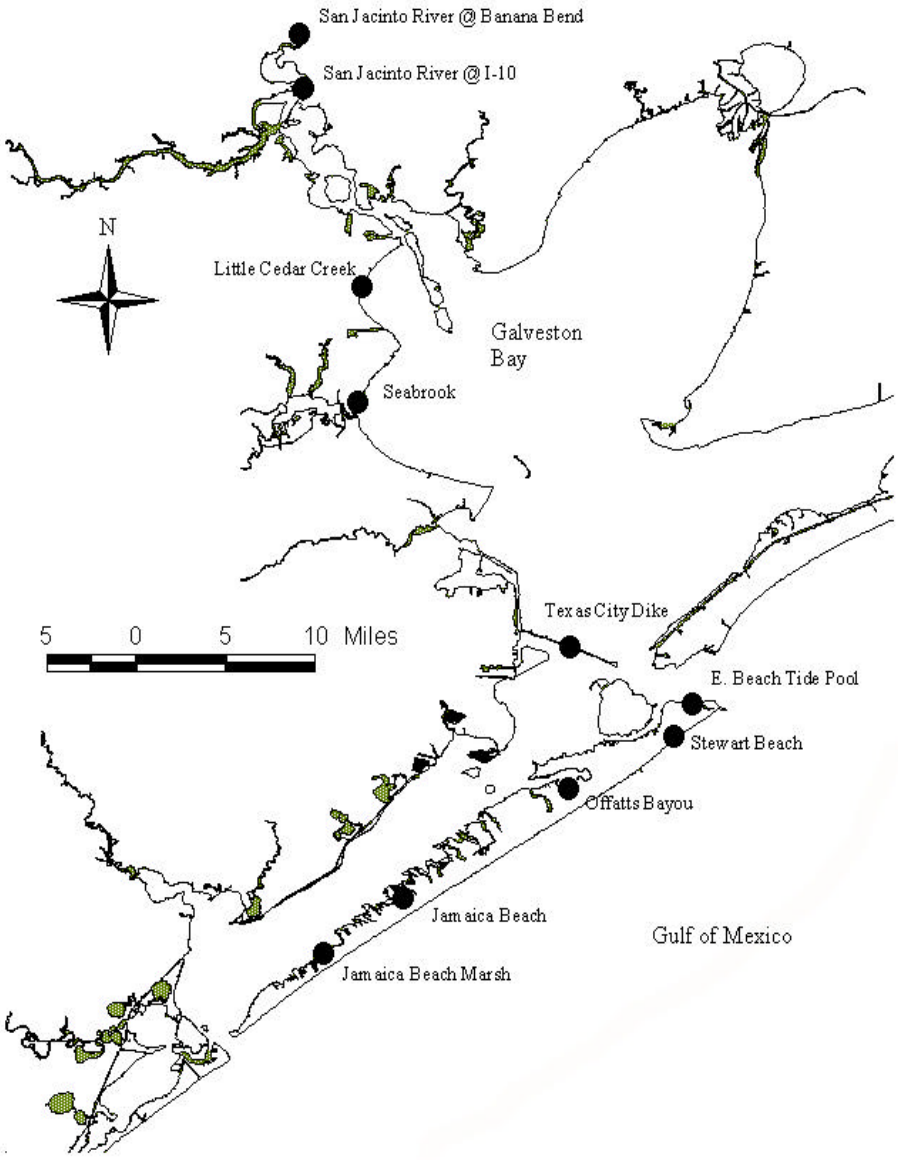
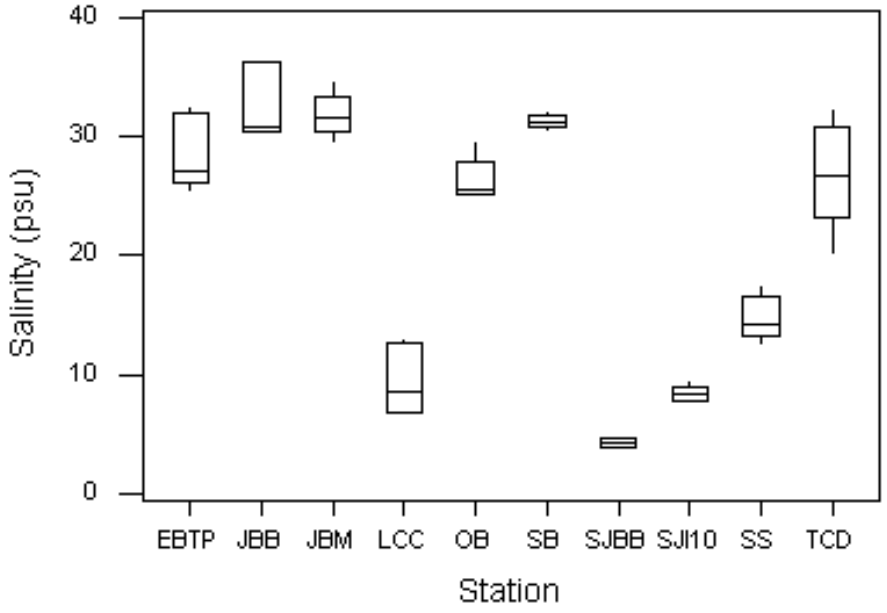


Figure 1. Location of sampling stations.


Salinity (psu)
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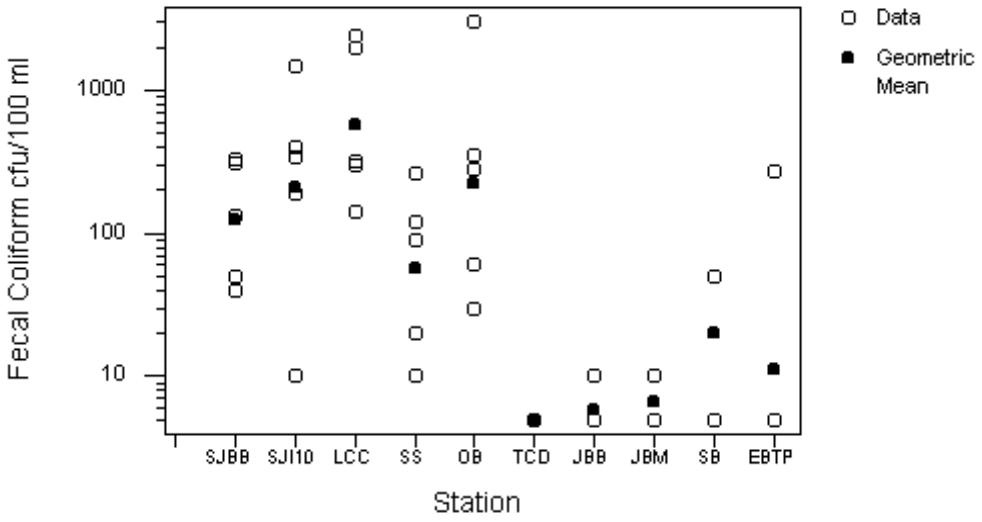
Fecal Coliform cfu/100 ml
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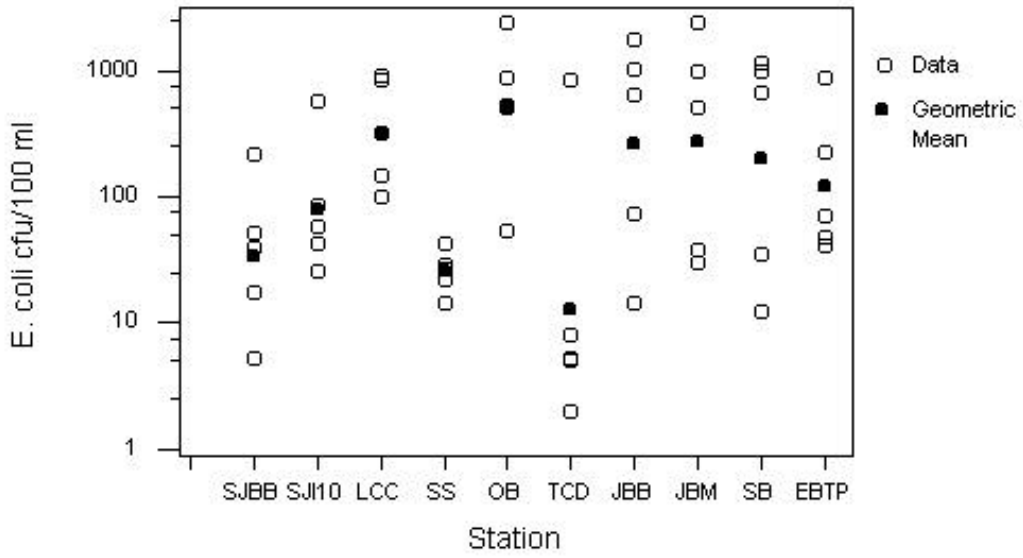
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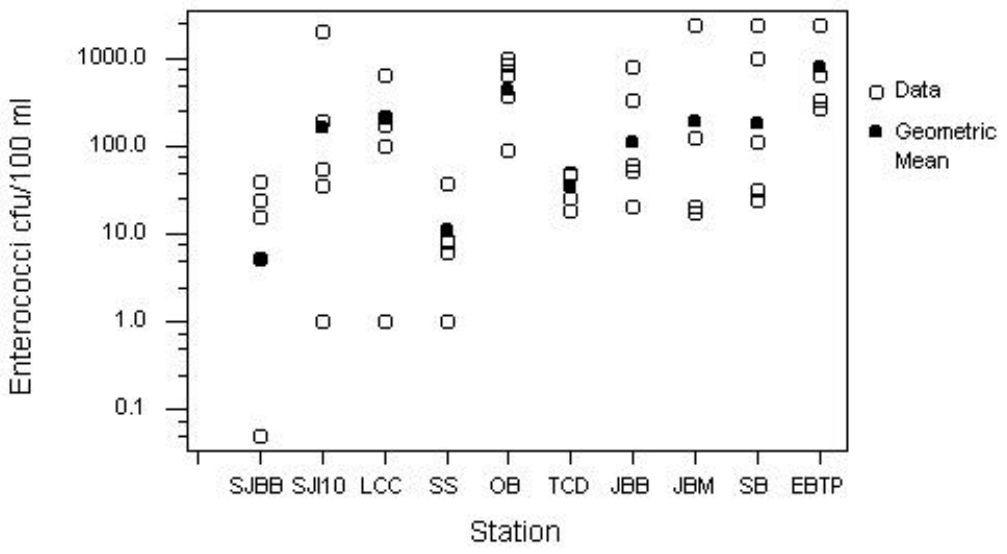
Enterococci cfu/100 ml
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