Healthy Beaches Tampa Bay

Microbiological Monitoring of Water Quality Conditions and Public Health Impacts

Final Project Report

1999-2000

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Executive Summary – Healthy Beaches Tampa Bay

I. Introduction

Clean beaches and the recreational activities associated with them form the backbone of the tourist industry in the Tampa Bay region. Risks to swimmers using polluted beaches has been a major issue associated with the setting of ambient water quality standards and discharge limits to recreational sites. Prevention of disease associated with recreational waters depends on the use of appropriate fecal indicators. Suitable indicators should mirror the source and fate of common human fecal pathogens, in other words, they should come from the same general source as pathogens and die off at a similar rate when exposed to environmental variables such as salinity, temperature and sunlight. However, the finding that the most widely used fecal contamination indicator, fecal coliforms, and more specifically *E. coli*, grow naturally on vegetation in warm climates clearly brings into question whether these or other indicators developed for temperate climates are applicable in Florida and other southeastern areas. (Fujioka et al, 1999) In addition, total and fecal coliform bacterial indicators have not been able to consistently indicate the persistence of pathogens, especially viruses, in surface waters. F-specific RNA coliphage, enterococci and *Clostridium perfringens* have been suggested as alternative indicators of fecal contamination and public health risks.

In order to ascertain the validity of these proposed indicators of fecal pollution, this study examined traditional and alternative pollution indicators, as well as the presence of pathogenic viruses, and their association with environmental variables (salinity, rainfall, stream flow) in fresh and marine water systems of the Tampa Bay area. From this and other available information, recommendations could be make as to the applicability of these indicators. The final goal of this project was to form the baseline for other studies and help to develop a long-term strategy for addressing or enhancing Florida water quality.

II. Goals of Healthy Beaches Tampa Bay and Sampling Strategy

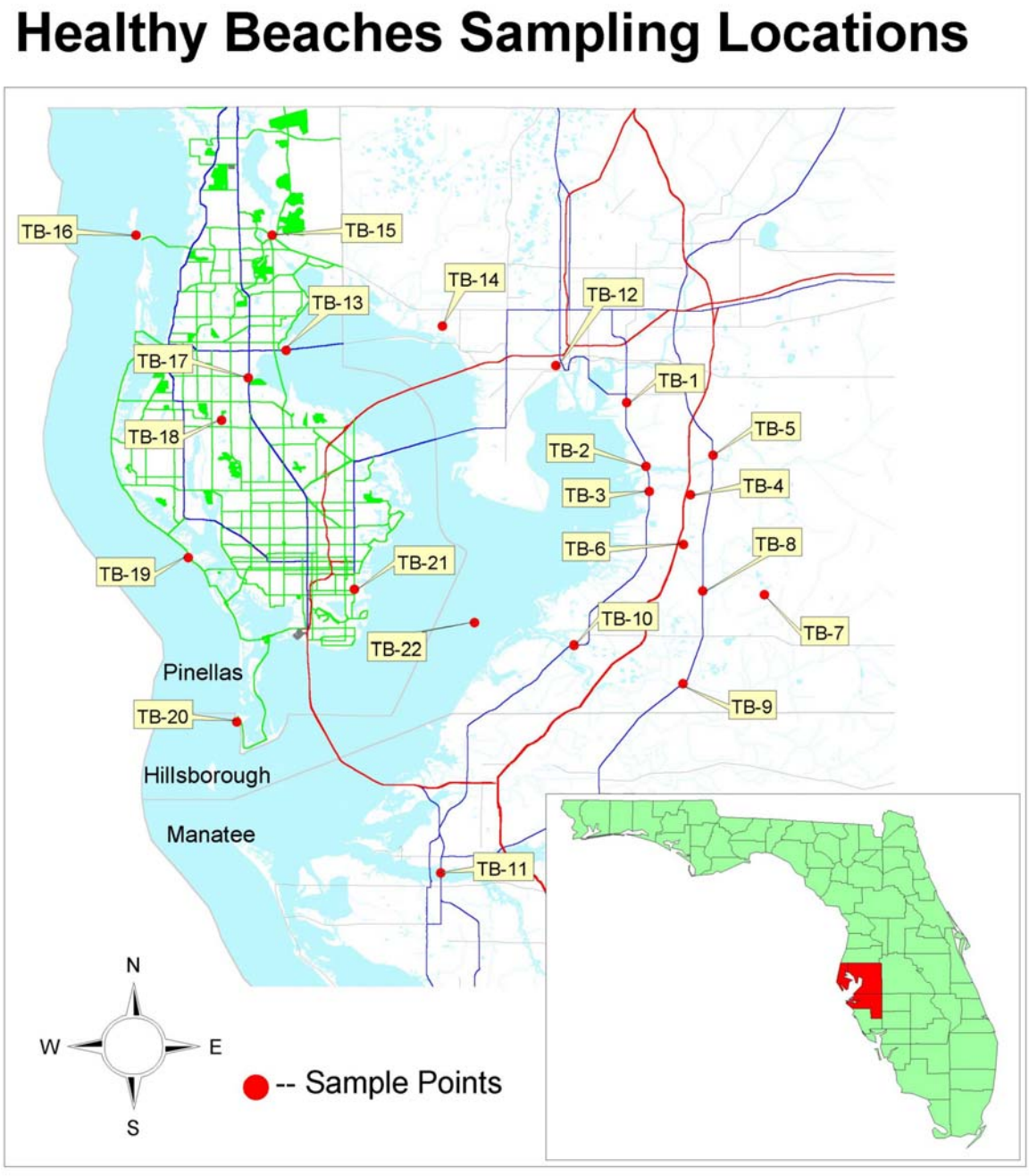
The goals of this study were:

1) To determine appropriate indicators for microbiological water quality for recreational sites in area beaches and for Tampa Bay.
2) To determine the occurrence of pathogens along with indicators in Tampa Bay watersheds and area beaches, their associated sources (animal vs human), public health risks and potential for management.

Twenty-two sites were chosen in Tampa Bay for this study with the assistance of an advisory council. Figure A shows their location along Tampa Bay. Four beach sites were chosen to represent several different beach types, including urban (TB13 Courtney Campbell Causeway beach), heavy boat use (TB19 John's Pass), recreational site in rural area (TB20 North Beach, Ft. Desoto) and pristine unpopulated beach (TB16 Honeymoon

Island). The Alafia watershed was represented by sites TB2 and TB5, the Little Manatee by sites TB9 and TB10, the Manatee watershed by site TB11 and the Hillsborough watershed by site TB12. The Bullfrog Creek sub-basin was chosen for in-depth monitoring due to the history of heavy pollution in the system, and included sites TB3, TB4, TB6, TB7 and TB8 (See Figure B). The Delaney Creek sub-basin was represented by site TB1. The remaining sites were located in Pinellas county, which cannot be divided into distinct watersheds, but is rather several non-continuous creek and wetland systems. These sites included TB14 Sweetwater Creek, TB15 Lake Tarpon Canal, TB17 Allen's Creek, TB18 Joe's Creek/Cross Bayou and TB21 Salt Creek.

Figure A
Tampa Bay Sampling Sites



Graphic produced by Pinellas County Health Department - Environmental Engineering Division

Figure B
Bullfrog Creek Sampling Sites in Detail

Sampling extended from June 1999 to August 2000, and each site was sampled for traditional and alternative fecal indicators, which included Fecal Coliforms, *E.coli*, Enterococci, *Clostridium perfringens* and Coliphage. Physical parameters were measured at the time of sampling as well, and included temperature, pH, turbidity and salinity. Out of the 22 total sites, 10 were chosen for in-depth testing (including antibiotic resistance analysis, ribotyping of *E. coli* isolates and *Bacteroides fragilis* phage assay for differentiating animal and human contamination, and human pathogenic enteroviruses). These sites were monitored 6 times throughout the study. The sites chosen for in-depth study in Hillsborough County included all sites along Bullfrog Creek: TB3, TB4, TB6, TB7, TB8. In Pinellas County, the sites included TB13 Courtney Campbell Causeway, TB14 Sweetwater Creek, TB17 Allen's Creek, TB19 John's Pass Beach and TB20 North Beach, Ft. DeSoto. Twenty parasite (*Cryptosporidium* and *Giardia*) samples were collected and analyzed for the 10 in-depth sites as well, one set every 6 months during the study.

The following table (Table A) gives the fecal indicator guidelines and levels used for the comparison of the data in this study. For the individual sampling results, the single sample guideline was used for Fecal Coliforms and Enterococci. No single sample guidelines are given for *E.coli, Clostridium perfringens* and Coliphage. In these cases, the geometric mean guideline was used. For the site to site comparisons, the geometric mean of all the results obtained throughout the study were used and compared to the geometric mean guidelines given.

Table A Indicator Guidelines used in this study

Fecal Coliforms	EPA and the state of Florida recommended guidelines for a single sample of 800 cfu/100 mL, for a geometric mean, 200 cfu/100 mL
E.coli	EPA recommended guideline for a geometric mean sample 126 cfu/100 mL
Enterococci	EPA recommended guidelines for a single sample of 104 cfu/100 mL, for a geometric mean , 33-35 cfu/100 mL for marine and fresh water respectively.
C. perfringens	Guidelines used by state of Hawaii based on research by Dr. Roger Fujioka et al at the University of Hawaii of 50 cfu/100 mL for fresh and brackish water and 5 cfu/100 mL for marine waters.
Coliphage	Level used - 100 pfu/100 mL based on previous research by Dr. Joan Rose, USF

III. Material and Methods

Samples were collected using sterile 1 L plastic bottles and placed on ice for transportation to the lab. Samples were processed within 8 hours of collection. For each bacterial indicator, volumes of the water sample were analyzed using membrane filtration. The filters were then placed on the appropriate media for each individual bacterial indicator assay. Coliphage were enumerated using the standard overlay technique according to the Standard Methods for Examination of Water and Wastewater, APHA, 1989. Culturable Enteroviruses were detected by cell culture methods, (Standard Methods for Examination of Water and Wastewater, 1989), and Protozoan analysis was carried out using filtration and immunofluorescence microscopy techniques (Proposed ICR Protozoan Method for Detecting *Giardia* cysts and *Cryptosporidium* oocysts in Water by Fluorescent Antibody Technique, Standard Methods for the Examination of Water and Wastewater, 18th ed. Supplement).

For Antibiotic Resistance Analysis (ARA), Fecal coliform isolates were picked from filters incubated with mFC medium (see Fecal Coliforms). The antibiotic resistance pattern of each isolate was compared isolates from known sources (cattle, wild animals, human, etc.) using discriminant analysis. The molecular ribotyping of *E.coli* isolates was accomplished by the method of Parveen *et al* (1997).

IV. Results and Discussion

A) Indicators

As the results were analyzed it became clear that there were three distinct groupings, the rural sites (characterized by more septic tanks and agriculture), the urban sites (characterized by high density land use and storm water control) and the beach sites. The rural sites included Delaney Creek (TB1), the Alafia River (TB2 and TB5), the Bullfrog Creek system (TB3, TB4, T6, TB7 and TB8), the Little Manatee River (TB9 and TB10) and the Manatee River (TB11). The urban sites included the Hillsborough River (TB12), Sweetwater Creek (TB14), Tarpon Lake Canal (TB15), Allen's Creek (TB17), Joe's Creek/Cross Bayou (TB18) , and Salt Creek (TB21). The four beach sites were the Courtney Campbell Causeway Beach (TB13), Honeymoon Island (TB16), John's Pass (TB19) and North Beach at Ft. Desoto (TB20).

In the rural site grouping, site TB4 Bullfrog Creek consistently had high levels of indicators except for *C. perfringens.* Sites TB6 and TB7 along Bullfrog Creek generally had high levels of Fecal Coliforms, *E.coli*, Enterococci and Coliphage as well. Site TB5 Alafia River showed moderate levels of indicators, and sites TB2, TB8, TB9, TB10 and TB11 showed less contamination. Site TB1 Delaney Creek had high levels of *E.coli,* Enterococci and Coliphage, but low levels of Fecal Coliforms and *C. perfringens.* Site TB3 Bullfrog Creek had the highest levels detected for *C.perfringens*.

For the urban site grouping, site TB14 Sweetwater Creek had the highest levels of indicators except for *C. perfringens*. Site TB17 Allen's Creek showed moderate levels of indicators, and sites TB15, TB12, TB18 and TB21 showed slightly less contamination.

Sites TB17 Allen's Creek and TB18 Joe's Creek had the highest levels detected for *C. perfringens*.

For the beach sites, TB13 Courtney Campbell Causeway beach had the highest levels of indicators followed by TB20 Ft. Desoto and TB16 Honeymoon Island. *Clostridium perfringens* was only found consistently at TB13 Courtney Campbell Causeway Beach, the most urban-located beach in the study. *Clostridium perfringens* only occurred once at TB20 North Beach, twice at TB19 John's Pass and was never detected at TB16 Honeymoon Island. Coliphage showed a similar pattern in regard to the beach sites. The control site, TB22, had indicator levels below all guidelines for the entire length of the study.

For sites exceeding the suggested geometric guidelines, the two consistently high sites were TB4 Bullfrog Creek and TB14 Sweetwater Creek. The remaining sites along Bullfrog Creek (TB3, TB6, TB7 and TB8) were next among the highest sites when comparing indicator levels. Sites TB16 Honeymoon Island, TB19 John's Pass and TB20 Ft. Desoto were among the lowest sites when comparing geometric means of indicator levels.

Among most of the sites, a peak in indicator values occurred in September and October of 1999, and again in March of 2000. Overall, however, most rural sites show a stronger seasonal increase in indicator levels during the winter and early spring months while most urban sites were fairly consistent throughout the year. When looking at the seasonal graphs for each site, those located in rural areas show *C. perfringens* and coliphage occurring primarily in the winter and early spring months, whereas highly developed urban areas show these indicators occurring throughout the year. The exception to this is the Bullfrog Creek system, which shows indicator levels similar to that of urban sites. In addition, Fecal coliforms and *E.coli* levels were shown to peak without a corresponding peak in the other indicators.

When using statistical correlation, the strongest relationship between indicators was found with Fecal Coliforms and *E. coli*, which is expected due to the fact that *E.coli* makes up the largest percentage of the Fecal Coliform group. The second strongest link was between Coliphage and Enterococci, followed by Enterococci and Fecal Coliforms and *E.coli*, with *Clostridium perfringens* showing the weakest correlation when compared with the other indicators. The *Bacteroides fragilis* phage correlated best with Enterococci and Coliphage.

B) Pathogens

The 10 in-depth sites were monitored for the presence of Enteroviruses (a group of human viruses found in feces which include Poliovirus, Coxsackieviruses and Echoviruses). The highest number of virus isolations occurred in September and October 1999 (with 3 and 4 sites positive out of 5, respectively), which corresponds to the indicator peak found in the rural sites during October 1999, and the September 1999 peak found in the urban and beach sites. The virus levels ranged from 1.1 to 27.1 MPN-PFU/100 L. Bullfrog Creek overall showed consistent Enterovirus results, with TB3 and

TB4 showing the highest percentage of positive results. The two urban sites and the three beach sites had 1-2 positive results during the length of the study.

For the Protozoan parasites, 20 samples were collected from the in-depth sites (10 sites sampled 2 times during the study). No *Giardia* were detected during the study. Sites TB3, TB4, TB7 and TB8 along Bullfrog Creek all showed the presence of *Cryptosporidium* with results of 3.48 oocysts per 100 L of water for TB7, 7.03 oocysts/100 L for TB8, 124.4 oocysts/100 L for TB4 and 470 oocysts/100 L for TB3. Each site tested positive for *Cryptosporidium* only once during the study. (See Table B)

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Table B Percentage of Enterovirus and Parasite Positives by Site

		Viruses	**Parasites**
Site Type	**Site**	+ virus out of total samples collected	+ *Crypto* out of total samples collected
Rural	TB3 Bullfrog	4 of 5	1 of 2
	TB4 Bullfrog	4 of 6	1 of 2
	TB6 Bullfrog	3 of 6	0 of 2
	TB7 Bullfrog	2 of 6	1 of 2
	TB8 Bullfrog	2 of 6	1 of 2
Urban	TB14 Sweetwater	2 of 6	0 of 2
	TB17 Allen's	2 of 6	0 of 2
Beach	TB13 Courtney C.	2 of 6	0 of 2
	TB19 John's Pass	1 of 6	0 of 2
	TB20 Ft. DeSoto	1 of 6	0 of 2

C) Predicting pathogen presence (Enterovirus) with Indicators

The indicators were compared to the presence of Enteroviruses using statistical correlations. The strongest correlation existed between Enterovirus and Enterococci, with an r value of 0.553 ($p=<0.001$), followed by Coliphage (r value=0.457, $p=<0.001$), Fecal Coliforms (r value=0.442, p=0.001) and *E.coli* (r value=0.370, p=0.010). *Clostridium perfringens* showed no correlation to the presence of Enteroviruses. These correlations are low, with the highest r value only at 0.553, but this is not uncommon for environmental samples.

The presence or absence of enteroviruses was compared against the suggested guidelines for the indicators included in this study (See Table 1) for all samples with both enterovirus and indicator data. When the indicators were below the suggested guidelines, suggesting that the water was safe, the percentage of positive Enterovirus results were 16% for Fecal Coliforms, 19% for Enterococci, 22% for Coliphage and 30% for *Clostridium perfringens*. The percentages improved when multiple indicators were used. Combining Fecal Coliforms and Enterococci or Fecal Coliforms and Coliphage reduce that percentage to 6% and 9%, respectively.

D) Fecal Coliform Source Tracking

The most striking finding of this study was the extent to which wild animals dominate as a source of fecal coliforms and *E.coli*, in 73.6% of all samples, the majority of isolates were identified as nonhuman. All of the source-specific methods used in the study indicate that human pollution is significantly impacting the Bullfrog Creek Watershed. The consistent impact from human sources is less clear at the Pinellas county sites, although there were days when "spikes" of human isolates dominated the sites. The percentage of isolates identified as human by antibiotic resistance analysis was significantly correlated with enterovirus counts, but the percentage of isolates identified as human by ribotyping was not significantly correlated with enterovirus counts. This discrepancy points to the need for including the fingerprints of more isolates from known, local sources in the respective databases.

E) Climate and Indicators

The Fall peak in fecal indicator levels corresponded to the end of the rainy season, however, the Spring peak could not be linked to rainfall or stream flow parameters. A lag time beyond 30 days existed when rainfall was compared to the indicators, but localized peaks associated with rainfall events may still occur within individual watersheds.

Total rainfall rather than average rainfall was better than stream flow for predicting indicator level peaks overall. For Enterococci, the 7 day total rainfall value was useful, but for coliphage, the 3 day total was better perhaps because of the decreased survival of this indicator in warm tropical waters. Average rainfall for beach sites was useful only when looking widely at the Bay, not for the individual sites. Enterococci compared to the 10 day total rainfall value was the only useful indicator correlation at the beach sites.

Negative correlations to rainfall and stream flow suggest that in some watersheds dilution due to increased rainfall and stream flow will actually decrease the number of phage and *Clostridium*. Both coliphage and *Clostridium* were found in low numbers compared to the other indicators. Sources are more likely to be related to feces compared to coliforms and Enterococci, which might have a soil or vegetative source. And while *Clostridium* could accumulate in sediments and does survive for extended periods of time, the low concentrations make it susceptible to non-detects when fresh water increases.

Binary Logistic Regressions were used to determine the relationship between rainfall, stream flow and the presence of Enterovirus. A slightly significant logistic regression result occurred within the beach site grouping between the 7 day average rainfall values and the presence of Enterovirus, resulting in a 64.3% concordant percentage, 30.4% discordant percentage and a 5.4% tie. Salinity and Enterovirus in this same beach grouping resulted in a concordant percentage of 69.6%, 26.8% discordant percentage and a 3.6% tie. No other significant relationship was found between the climate factors used in the study, and the presence or absence of Enterovirus. The virus data set for this study is small, however, and a more intensive virus sampling regime may be needed for a more

accurate statistical analysis of climate factors and their contribution to virus water quality on the beaches.

V. Recommendations

What indicators are appropriate for Tampa Bay?

- The use of two indicators, both the fecal coliform bacteria and enterococci on a routine basis is warranted based on the results of this study. *E.coli* appears to be of little added value in either marine or fresh waters.
- Source tracking using multiple antibiotic resistance for fecal coliform bacteria should be included and a large catalog and repository for Tampa Bay should be built and supported.
- Coliphage should be added as a third indicator in areas with fresh water inputs during the study of storm events on water quality.
- *Clostridium perfringens* and *Bacteroides* phage, while indicative of fecal pollution, only have limited added value as alternative indicators.
- *Clostridium* may be useful during one-time sanitary surveys.
- *Bacteriodes* will be useful in studying wastewater facilities (disinfected wastewater) and septic tank inputs into common warm marine waters.
- Biological Source Tracking is a very useful tool, and a large database for Tampa Bay should be built and supported.

The continued use of fecal coliform bacteria is supported but only with the addition of enterococci, as well as characterization of the types of fecal coliform bacteria found using the source tracking techniques. Coliphage as a third indicator should be added during specialized surveys. This approach will be useful in demonstrating risk, seasonal variability, sources and the data can be used to make both short-term and long-term management decisions on the watershed.

What levels are appropriate for Tampa Bay?

- The 104 CFU single sample level and geometric mean of 35 CFU associated with Enterococci is partially supported by this study for the fresh water tributaries. However the 200 and 800 CFU for the fecal coliform bacteria are not and may be too stringent. A set of values for the fecal coliform bacteria can not be supported at this time.
- A greater database is needed at the contrasting beaches to make recommendations for beach water quality monitoring and levels.

Is pathogen monitoring warranted?

- Viruses have been the group of pathogens which have shown the most value in marine waters as a benchmark to compare to the indicators representing human health risks.

- Risk assessment models suggest that the likelihood of becoming ill is 1/1000 to 1/10,000 if ingesting water at the levels recorded on the beaches from a single swimming event. In order to further define this risk, virus testing is warranted, as a part of any particular beach study.
- Enteroviruses were found in Tampa Bay sites in 39% (23 out of 59 samples) of the samples tested, but at 100% of the sites tested. In other words, at least one positive result occurred at every site tested at some time during the study.

What other information is needed to move into Phase II Healthy Beaches?

- A more detailed study directly on the beaches is needed.
- Specifically working with a transport model, a temporal and spatial study is needed, this can be accomplished using indicators. The current data set could be used to support an initial study, however more data are needed on the beaches.

Are the data and recommendations for Tampa Bay useful for a State-wide program?

- Yes, state, local and private agencies involved in water quality studies (wastewater, stromwater, septage etc), should move immediately to monitoring for both enterococci and fecal coliform bacteria as well as contributing to a state-wide database on the characterization of "source-tracking" isolates. Virus testing should be built into specialized studies.

Perspective and Future Directions: Healthy Beaches Phase II and beyond

Because most pathogens are host-specific, the goal of this study has been to access the risk of human disease by measuring pollutants of human origin. However, a great deal of additional work remains in order to protect public health and enhance the environment, including:

- Modeling of conditions that determine pollution events to provide ways to predict, avoid and mitigate. Healthy Beaches Phase II has been proposed to address modeling and risk assessment. (Proposal is included in Appendix XII)
- Development of technology and methods such as biosensors to enable the rapid measurement of indicators or actual pathogens.
- Better understanding and response to waterborne diseases not necessarily of human origin, such as those that cause wound infections, animal parasites such as *Giardia* and *Cryptosporidium*, organisms from animal waste (e.g. *E.coli* 0157:H7), and natural organisms such as *Vibrio vulnificus* and harmful algal blooms.
- Development of a comprehensive database of Enterococci and Fecal Coliforms for use in biological source tracking, and development of methods to quickly perform the analysis locally.
- Increase efforts to eliminate or reduce identified causes of pollution, such as septic tanks, leaking sewer collections systems, failing lift stations, provision of sanitary facilities at beaches, and selected sources of animal pollution.

- Develop statutes, guidelines, methods and education programs so that the public will be aware of risks and take action accordingly as it is not possible to obtain a natural environment that is entirely risk free.
- Undertake a risk assessment investigation specific to warm climates areas, including epidemiological methods, to quantify the relationship between exposure to various concentrations of pathogens and the associated risk of acquiring disease.

I. Introduction

Tampa Bay is located on the west central coast of Florida, opening to the Gulf of Mexico. This is a shallow subtropical estuary, one of the largest in the southeastern U.S. It is valued for its ecosystem, fisheries, recreational uses and as a port. The drainage basin is approximately 2300 square miles and includes 9 major and 76 minor sub-basins. The major tributaries in the Bay are the Hillsborough, Alafia, Little Manatee and Manatee Rivers, while minor systems include Alligator Creek, Joe's Creek (Pinellas County), Rocky Creek, Double Branch Creek, Sweetwater Creek (northwest Hillsborough County), Tampa Bypass Canal, Delaney Creek, Bullfrog Creek (central and south Hillsborough County), and Frog Creek (Manatee County). Freshwater inputs are highly significant to the Bay and are associated with rainfall, with about 60% of the annual precipitation occurring from June to September. Along with this freshwater input is the input of contaminants originating from point and non-point sources.

The estimated population in Florida by the year 2010 is 16 million people. Growth in the counties around Tampa Bay has been significant (about 1.8 million live around the Bay) and it is estimated that Pinellas, Hillsborough and Manatee will gain as many as 500,000 people in the next 10 to 15 years. Increased wastewater originating from treatment plants and septic tanks as well as increased biosolids will need to be managed. Increased urbanization has and will continue to alter the watershed as well as the freshwater flows to the Bay. Non-point source loading rates for nitrogen, phosphorous, metals, BOD and suspended solids have been estimated with current, and changes to, land use, however, more recent microbial contaminants have been identified as high priority risk to waters in coastal communities. In particular, public health issues have been highlighted by the *Clean Water Initiative* as a result of poor environmental conditions in coastal waters due to increased population growth and urbanization.

Clean beaches and the recreational activities associated with them form the backbone of the tourist industry in the Tampa Bay region. Water quality at beaches ranges from excellent (i.e. most of the Gulf beaches, seldom closed due to water quality) to moderate (beaches on Tampa Bay and inland waterways that are periodically closed) to poor (lakes and other freshwater environments which have been permanently closed). The moderate quality beaches of particular interest (having received media attention associated with closures) include Fred Howard Park in Tarpon Springs, beaches along the Courtney Campbell and Gandy Causeways, and North Shore Beach in St. Petersburg. Bodies of water permanently closed to swimming include Brooker Creek Park at Lake Tarpon, the Boy Scout Camp at Lake Chautaqua and Wall Springs. The latter is of particular interest because it represents a growing problem with springs in Florida, that is, deterioration of groundwater quality.

Risks to swimmers using polluted beaches has been a major issue associated with the setting of ambient water quality standards and discharge limits to recreational sites. Public health concerns in recreational waters in the tropics and subtropics differ from those of cooler waters. Prevention of disease depends on the use of appropriate fecal

indicators. However, the finding that the most widely used fecal contamination indicator, fecal coliforms and more specifically *E. coli*, grow naturally on vegetation in warm climates clearly brings into question whether these or other indicators developed for temperate climates are applicable in Florida and other southeastern areas. (Fujioka et al, 1999)

In recent years, total and fecal coliform bacterial indicators have not been able to consistently indicate the persistence of pathogens, especially viruses in surface waters. F-specific RNA coliphage, enterococci and *Clostridium perfringens* have been suggested as better indicators of fecal contamination and public health risks. Table 1 outlines some advantages and disadvantage of these traditional and alternative indicators.

Table 1 Indicators of fecal contamination

Indicator	**Advantage**	**Disadvantage**	**Potential Use for Tropical Waters**
Fecal Coliforms	Historical database. Good relationship to rainfall.	Found to be ubiquitous in water and other environments, no relationship to pathogens. Variability in levels is great, regardless of pollution source.	Useful for source tracking using antibiotic resistant markers. Resolution to the source genus level (dog, human, cow, bird and soil).
E.coli	Is a potential pathogen, indicates potentially greater risk, and due to liability cannot be ignored	Most of the *E.coli* is harmless, can have a variety of sources including soil.	Applicable to biosensors for pathogen detection. (eg. 0157:H7) Ribotyping can be used to differentiate animal and human sources; correlates well with detection of human sources of pollutants in fresh/ low salinity waters.
Enterococci	Found in large numbers relative to some alternative indicators, good correlation with enteric viruses	Has both environmental and fecal sources. Its common occurrence in tropical soils is not well known.	Generally a good fecal indicator for both fresh and marine water.

Clostridium perfringens	In low dilution areas, with waterways which are estuarine, less influence by salinity	Low levels. Little relationship to pathogens.	Limited application, good in transects in watersheds from fresh to saline.
Coliphage	Appear to be a better indicator in fresh water systems, only in one watershed could the phage be related to enteric viruses.	Survives very poorly in warm marine waters.	Indication of fresh water inputs; can be typed for animal/human distinction.
Bacteroides fragilis phage	Survives well in warm marine waters. Can identify human impact within 48 hours.	Found in low numbers in wastes. Appears susceptible to chlorination process in wastewater plants.	In areas of low dilution, can indicate human septic wastes, as not found in treated-disinfected sewage;applicable to warm marine waters.

a) Water Quality Studies in Florida

Our laboratories have been involved in the study of microbial quality of Florida waters since 1992. (Paul et al., 1995) Studies have been conducted in the Florida Keys and more recently along the Pithlachascotee River, in Homosassa Springs, Satasota County along the Phillippi Creek and in Charlotte Harbor for microbial contaminants associated with public health risks (Rose et al.,1995, Lipp et al.,2000:2000a, Griffin et al.,1999-2000). Both traditional and alternative indicators in addition to pathogen monitoring for viruses and parasites have been used in these studies.

Fecal contamination associated with non-point sources (septic tanks ands storm water) all along the Phillippi Creek was evident based on the use of four indicators of pollution (fecal coliform bacteria, coliphage, Enterococci and *Clostridium perfringens*). The water quality could not meet Florida State standards or Federal guidelines for safe swimming. The Enteric protozoa, *Cryptosporidium* and *Giardia,* were detected, but more significantly, human viruses were detected in 91% of the sites sampled.

In comparing indicator organisms against pathogens, 64.7% of the samples with pathogens had >100 cfu/100 mL for fecal coliforms and levels of bacteria were highest during the rainy season and at areas with the greatest density of septic tanks. In contrast, *Clostridium* and coliphage were found at lower numbers. In 41% of the samples

containing pathogens, these indicators were at non-detectable levels. Enterococci proved to be the most accurate indicator of pathogen presence, as 76% of the samples containing pathogens contained >35 cfu/100 mL. All four indicators, however, could be used in cluster analysis to pinpoint high pollution waterways. (Lipp et al.,2000)

Studies along the Pithlachascotee River showed significantly less contamination compared to Phillippi Creek using similar bacterial and viral indicators (values were on average 2 to 10 times lower). No protozoa were detected. Peaks of contaminants were associated with rainfall events, and in this study, subsurface contamination was indicated in the upper reaches and most urbanized sites of the river. Management of septic tanks and storm waters contributed to an improved water quality compared to areas like Phillippi Creek.

Water quality in Charlotte Harbor was studied for one year primarily at sites with salinities greater than 15 ppt. (Lipp et al.,2000a) All four fecal indicators (see above) were correlated with stream flows from the Myakka and Peace Rivers. Numbers of Enterococci were highly correlated with the freshwater flows, and Enterococci proved to be a good indicator of virus contamination, with 87% of virus-positive samples containing *Enterococcus* levels of >35cfu/100 mL El Niño related rain events in November, December, January and February were associated with human virus detection and increased virus indicators (coliphage) at near shore and off shore sites. In this case, the coliphage accurately predicted the presence of human viruses at levels greater than 100 pfu/100 mL. In addition, the indicators were shown to accumulate in the sediments. Levels were 10 to 100x higher than in the water column with the exception of the coliphage (which may be due to the rapid die-off).

Human enteric viruses were detected by RT-PCR in 95% of the canals in the Florida Keys influenced by septic systems (Griffin et al.,1999). *Clostridium* was detected in 63% of the sites, however, concentrations were very low. Only once was greater than 26 cfu/100 mL detected. Coliphage were detected in only 10% of the sites, while approximately 79% of the sites were positive for fecal coliforms, *E.coli*, and Enterococci. Forty seven percent of the sites had Enterococci levels greater than 35 cfu/100 mL, and 21% of the sites had fecal coliforms >200 cfu/100 mL. *E.coli* constituted 13-99% of the fecal coliform population at each site. Increasingly, in later studies (unpublished data), no culturable viruses were detected until the winter months. This finding can be attributed to considerably cooler water temperatures, since coliphage were found to die off rapidly in warm saline waters such as those found in the Florida Keys.

The use of F+ specific coliphage was applied in Homosassa Springs, Florida to identify the impact of an animal park on the water quality (Griffin et al., 2000). Rainfall significantly influenced the levels of all four indicators. Fecal coliforms were found throughout the water way at around 100 cfu/100 mL, but were higher (~3000 cfu/100 mL) at areas directly influenced by discharges from the animal holding pens. *Clostridium* and enterococci were also elevated in these areas. Coliphage were

consistently recovered from this cool, freshwater system. A coliphage enrichment procedure followed by genotyping indicated that animals were the likely source of the contamination.

To date, extensive investigations into the range of microbial contaminants, the sources and public health risks have not been undertaken in Tampa Bay, however, studies on fecal coliform bacteria showed that Bullfrog Creek and Delaney Creek were the most heavily contaminated, followed by the Lake Thonotosassa tributaries. These levels were in exceedance of the Florida standards for recreational waters and in violation of Florida State Standards for Safe Swimming (200CFU/100ml). (EPC-Hillsborough County Report 1995-1997)

b) Goals of Healthy Beaches Tampa Bay

The goals of this study were:

3) To determine appropriate indicators for microbiological water quality for recreational sites in area beaches and for Tampa Bay.
4) To determine the occurrence of pathogens along with indicators in Tampa Bay watersheds and area beaches, their associated sources (animal vs human), public health risks and potential for management.

This study examined alternative pollution indicators and their association with environmental variables (salinity, rainfall, stream flow) in key basins. The study examined the water quality of near shore to off shore sites in the Bay, tributaries, beach sites, estuarine sites and freshwater sites along with uses and sources of contamination. These data are to be used to better communicate the potential public health risks for recreation in Tampa Bay and for improved monitoring, remediation and management programs. The final goal of this project was to form the baseline for other studies and help to develop a long-term strategy for addressing or enhancing Florida water quality.

The specific tasks included:

- An historical literature review and summary of existing studies on fecal indicators in Tampa Bay.
- A survey of Tampa Bay and area beaches for fecal indicators including coliforms, *E. coli*, coliphage, *Bacteroides fragilis* phage, *Clostridium* and Enterococci.
- A study of antibiotic resistance markers, delineating areas of human versus animal wastes.
- A study of the occurrence of human pathogens (enteric viruses, *Cryptosporidium* and *Giardia*) to assess the potential public health risks.
- Development of data bases for incorporation into water quality hydrodynamic modeling for evaluating ecosystems and public health.
- Discussion of strategies for the future to meet the long term goals for Healthy Beaches Tampa Bay and the State of Florida
- Meetings with an Advisory committee that assisted with review, communication and strategy development.

II. Approaches

a) Advisory Council

An advisory committee was set up headed by Ms. Holly Greening of the Tampa Bay Estuary Program to provide assistance in site selection, aid in community awareness, and to review the results of the study.

b) Literature Review

An retrospective analysis of the historical fecal coliform data from Tampa Bay was performed by Dr. Erin Lipp at USF. The report is included in Appendix I.

c) Sampling sites and watershed descriptions

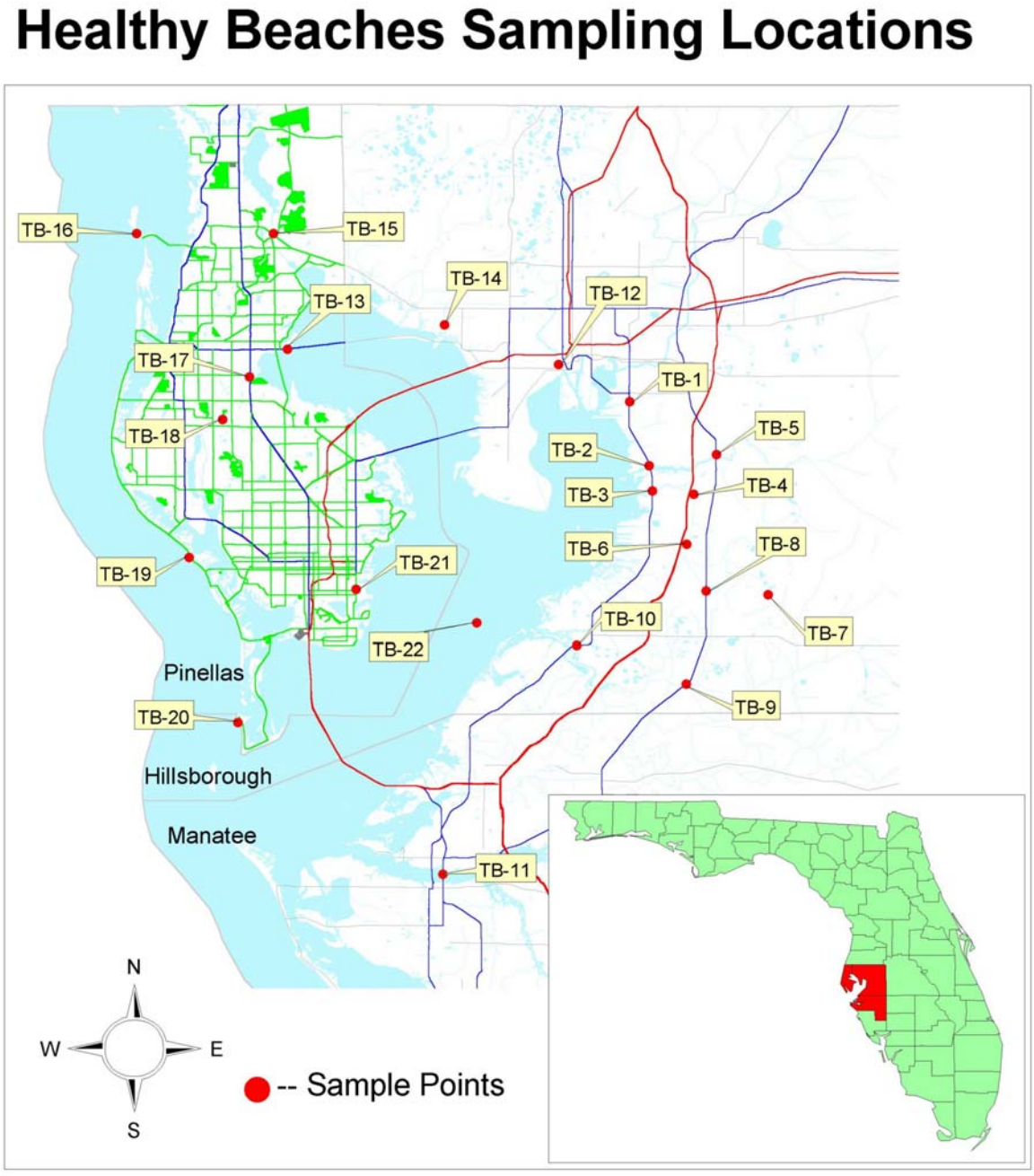
Twenty-two sites were chosen in Tampa Bay for this study with the assistance of the advisory council. The final choices were based on watershed representation, areas of concern in regard to pollution, accessibility and previously sampled sites. Table 2 gives an overview of the sites selected, and Figure 1 shows their location along Tampa Bay. Eleven sites of primarily rural or suburban land use were chosen in Hillsborough and Manatee counties. Six sites were located in highly urban areas (mainly in Pinellas county with the exception of Sweetwater Creek -TB14 and the Hillsborough River -TB12), and 4 beach sites were chosen to represent several different beach types, including urban (TB13 Courtney Campbell Causeway beach), heavy boat use (TB19 John's Pass), recreational site in rural area (TB20 North Beach, Ft. DeSoto) and pristine unpopulated beach (TB16 Honeymoon Island). A detailed listing of the sampling sites and directions for each are found in Appendix II, and the GIS locations of each of these sites are in Appendix III.

Tampa Bay can be divided into five areas, which include four distinct watersheds in Hillsborough county and the peninsula of Pinellas county. Several small sub-basins are located around the Bay as well. The Alafia watershed was represented by sites TB2 and TB5, the Little Manatee by sites TB9 and TB10, the Manatee watershed by site TB11 and the Hillsborough watershed by site TB12. The Bullfrog Creek sub-basin was chosen for in-depth monitoring due to the history of heavy pollution in the system. Sites TB3, TB4, TB6, TB7 and TB8 were located along this system (See Figure 1a). The Delaney Creek sub-basin was represented by site TB1. The remaining sites were located in Pinellas county, which cannot be divided into distinct watersheds, but is rather several non-continuous creek and wetland systems. A detailed description of the major watersheds can be found in Appendix IV.

Table 2 Sampling Site Overview

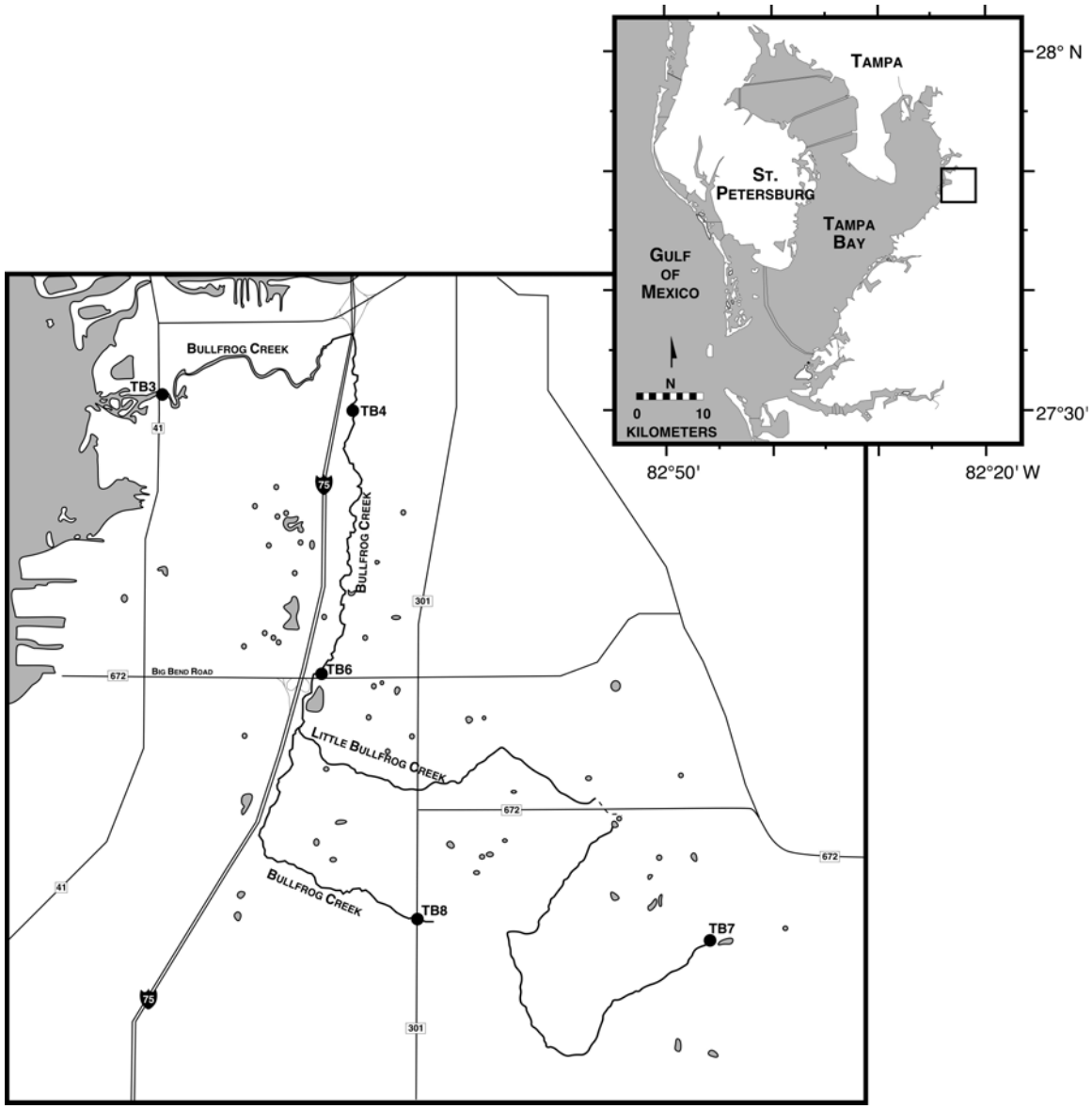
Watershed Description	Site ID	Watershed	Site description
Rural	TB1	Delaney	Sub-basin system in central Hills. Co.
	TB2	Alafia	Mouth of Alafia River
	TB3	Bullfrog	Sub-basin system in central Hills. Co.
	TB4	Bullfrog	Further inland site along Creek
	TB5	Alafia	Inland on Alafia River
	TB6	Bullfrog	Junction of Little and Big Bullfrog
	TB7	Bullfrog	Little Bullfrog headwater
	TB8	Bullfrog	Big Bullfrog headwater
	TB9	L. Manatee	Inland site along Little Manatee River
	TB10	L. Manatee	Mouth of Little Manatee River
	TB11	Manatee	Mouth of Manatee River
Urban	TB12	Hillsb.	Downtown Tampa, Univ of Tampa
	TB14	Sweetwater	Sub-basin, Highly developed area
	TB15	Pinellas	Lake Tarpon Bypass Canal
	TB17	Pinellas	Allen's Creek ,US 19 in Clearwater
	TB18	Pinellas	Joe's Creek and Cross Bayou, St. Pete
	TB21	Pinellas	Salt Creek south of downtown St.Pete
Beach	TB13	Pinellas	Clearwater /Courtney C. Causeway
	TB16	Pinellas	Honeymoon Island, upper beach
	TB19	Pinellas	John's Pass, Inter coastal, boat traffic
	TB20	Pinellas	North Beach, Ft. DeSoto, inside beach
Control	TB22	Bay	Midwater, center of Tampa Bay

Figure 1
Tampa Bay Sampling Sites



Graphic produced by Pinellas County Health Department - Environmental Engineering Division

Figure 1a
Bullfrog Creek Sampling Sites in Detail



d) Sampling Schedule

Each site was sampled monthly for a period of approximately one year for traditional and alternative fecal indicators, which included Fecal Coliforms, *E.coli*, Enterococci, *Clostridium perfringens* and Coliphage. Physical parameters were measured at the time of sampling as well, and included temperature, pH, turbidity and salinity. A list of the physical measurements, as well as the date and time of each sample, is found in Appendix V.

Out of the 22 total sites, 10 were chosen for in-depth testing (including antibiotic resistance analysis, ribotyping of *E. coli* isolates, enterovirus detection and *Bacteroides fragilis* phage assay). These sites were monitored 6 times throughout the study. The sites chosen for in-depth study in Hillsborough County include all sites along Bullfrog Creek: TB3, TB4, TB6, TB7, TB8. In Pinellas County, the sites include TB13 Courtney Campbell Causeway, TB14 Sweetwater Creek, TB17 Allen's Creek, TB19 John's Pass Beach and TB20 North Beach, Ft. DeSoto. Table 3 gives an overview of the sampling strategy for the study.

Table 3 Overview of Sampling Strategy

Sampling sites	**Number of samplings in study and tests performed**		
	Indicators (9-12)	**In-depth tests (6)**	**Parasites (2)**
TB1 Delaney Creek	X		
TB2 Alafia River	X		
TB5Alafia River	X		
TB9 Little Manatee River	X		
TB10 Little Manatee River	X		
TB11 Manatee River	X		
TB12 Hillsborough River	X		
TB15 Lake Tarpon Canal	X		
TB18 Joe's Creek	X		
TB21 Salt Creek	X		
TB22 Control Site	X		
TB3 Bullfrog Creek	X	X	X
TB4	X	X	X
TB6	X	X	X
TB7	X	X	X
TB8	X	X	X
TB13 Courtney Campbell	X	X	X
TB14 Sweetwater Creek	X	X	X
TB16 Honeymoon Island	X	X	X
TB17 Allen's Creek	X	X	X
TB19 John's Pass	X	X	X
TB20 Ft. DeSoto	X	X	X

e) Materials and Methods

Sample Collection

Grab samples were collected in sterile 1 L plastic bottles and placed on ice for transportation to the lab. Samples were processed within 8 hours of collection.

Fecal Indicators

For each bacterial indicator assayed, volumes of the water sample were filtered through a 0.45μm pore size membrane filter (Osmonics) using a 47mm Gelman filter funnel fitted to a vacuum manifold. Sample volumes were determined by the fecal contamination level at each site. The filters were then placed on the appropriate media as described below for each individual bacterial indicator assay.

Fecal coliforms were enumerated according to the Standard Methods for Examination of Water and Wastewater, APHA, 1989 (71). Water samples were filtered as described above and placed on mFC agar plates (Difco). Plates were then incubated for 18 to 24 hours at 44.5^{o}C in a water bath. The dark blue colonies were counted as fecal coliforms.

Enterococci were enumerated using Method 1600, USEPA. (72) Water samples were filtered as described above. The filters were placed on mEI agar plates (Difco) and incubated for 18 to 24 hours at 41 ^{o}C. Those colonies exhibiting a blue halo were counted as enterococci.

E. coli were enumerated by taking those plates positive for fecal coliforms, transferring the membrane filter to EC with MUG media (Difco), and incubating for an additional 24 hours at 37^{0}C. Colonies that fluoresced under UV light were counted as *E. coli*. and isolated for ribotyping and antibiotic resistance assessment for source identification.

C. perfringens were enumerated by filtering water samples as described above. The filter were then placed on mCP agar plates (acumedia- Baltimore, Maryland) and incubated anaerobically in GasPak jars (BBL GasPak, Becton Dickinson) for 18 to 24 hours at 45^{0}C. Yellow colonies that turned pink or red when exposed to ammonium hydroxide fumes were counted as *C. perfringens.*

Coliphage were enumerated according to the Standard Methods for Examination of Water and Wastewater, APHA, 1989. A 1ml aliquot of the water sample was added to a 1 ml aliquot of a log phase *E. coli* host bacterial culture in a tube of melted soft TSA agar and overlayed onto a TSA plate. The agar was allowed to solidify, and the plate was incubated for 18 to 24 hours at 37^{0}C. Each sample was assayed using 10 replicate plates. Phage concentration of the samples were calculated by using the number of plaques that appeared on the bacterial lawn of each plate.

Culturable Enteroviruses

Concentrated water samples from absorption/elution using the filterite filter were prefiltered through a 0.2 μm filter (25mm, Corning) then stored at -70^{0}C (Standard Methods, 1989; Jiang et al., 1992). The samples were then quickly melted in a 37^{0}C water bath before inoculation onto cells and kept on ice during the processing. One milliliter of sample was inoculated onto each of a total of twenty 25mm^{2} bottles with a Buffalo green monkey (BGM) kidney cell monolayer without cell culture media. After the bottles with the sample were incubated with the cell side down at 37^{0}C for two hours,

maintenance medium (E-MEN with 5% fetal calf serum) was added to each bottle. The bottles were incubated at 37^0C for two weeks and evaluated daily for cell destruction caused by viruses known as cytopathic effects (CPE). Both positive and negative samples were frozen at -70^0C and thawed at 37^0C before being transferred (1ml of each) to a 13x100mm tube with a new BGM monolayer. The tubes were incubated at 37^0C for two more weeks and examined for CPE each day (Standard Methods for Examination of Water and Wastewater, 1989).

Protozoan Analysis

Samples were processed and assayed using filtration and immunofluorescence microscopy techniques (Federal Register/Vol. 59, No. 28/February 10, 1994 Appendix C to Subpart M – Proposed ICR Protozoan Method for Detecting *Giardia* cysts and *Cryptosporidium* oocysts in Water by Fluorescent Antibody Technique. D-19 Proposal P 229, Proposed Test Method for *Giardia* cysts and *Cryptosporidium* oocysts in Low Turbidity Water by a Flourescent Antibody Procedure. 1992. Annual Book of ASTM Standards, ASTM, Philadelphia, PA. Section 9711, Pathogenic Protozoa, Proposed Method for *Giardia* and *Cryptosporidium* spp. 1993. Standard Methods for the Examination of Water and Wastewater, 18th ed. Supplement, APHA, AWWA, WEF, Washington , DC.)

Between 160 and 400 L (35 to 630 gallons) were collected from the 10 sites. Samples were collected by filtration through a 1.0 μm 10 inch yarn wound filter cartridge. Volumes were monitored by attached flow meters. After collection, the filters were placed on ice for transport to the USF lab, then cut apart and washed to collect the material from the filter and recover protozoan cysts and oocysts. Final washed volume was then centrifuged to a concentrated pellet representing the initial volume of water collected.

The final concentrates were examined using immunofluorescence microscopy with monoclonal antibodies (Mab) specific to the oocyst and cyst wall which are labeled with a specific stain. Equivalent volumes from the concentrates examined under the microscope were calculated and the concentrations of cysts and oocysts per 100 L were determined.

Antibiotic Resistance Analysis

Antibiotic Resistance Analysis (ARA) – *E.coli* isolates were picked from filters incubated with mFC medium (see Fecal Coliforms). Isolated colonies were transferred individually to microtitre dish wells containing EC broth. Microtitre dishes were incubated for 24 hours at 44.5^0 C. The antibiotic resistance pattern (ARP) of each isolate was defined by the growth on at least eight antibiotics at four concentrations each, which was accomplished by replica plating isolates from the microtitre dishes to TSA plates which were each amended with one antibiotic. The antibiotic used were amoxicillin, cephalothin, erythromycin, ofloxacin, tetracycline, gentamicin, chlortetracyline and moxalactam. The ARP of each isolate was compared to ARP's of isolates from known sources (cattle, wild animals, human, etc.) by discriminant analysis. Discriminant analysis assigned each isolate to a source category based on the similarity of the isolate's ARP to those from known sources in the database.

Ribotyping

Ribotyping of *E.coli* isolates was accomplished by the method of Parveen *et al* (1997). Chromosomal DNA was extracted from *E.coli* isolates and digested with *Hind*/III. Fragments were separated by agarose electrophoresis. The cDNA from the *E.coli* 16S rDNA was labeled with digoxigenin-dUTP and used as probes. *E.coli* ribotype profiles were then compared to those of Dr. George Lukasik's known source library by discriminant analysis. This library was developed in the laboratory of Dr. Mark Tamplin, formerly of University of Florida, now with the USDA.

III. Results

A) Sampling Summary

Sampling began in June 1999 and ended in August 2000. The total sampling sites (22) were divided into two groups, and a group was sampled every other week. The control site was sampled according to the Environmental Protection Commission of Hillsborough County's monthly sampling schedule, and brought to the University of South Florida, St. Petersburg campus on the day of collection. Sampling was extended beyond the initial 12 month period to compensate for missed specimens due to weather or scheduling conflicts. A total of 60 Enterovirus samples were collected and analyzed for the 10 in-depth study sites over the 15 month sampling period, and 20 total parasite samples were collected and analyzed for the 10 in-depth sites as well, one set every 6 months during the study.

The following table (Table 4) gives the fecal indicator guidelines and levels used for the comparison of the data in this study. For the individual sampling results, the single sample guideline was used for Fecal Coliforms and Enterococci. No single sample guidelines are given for *E.coli, Clostridium perfringens* and Coliphage. In these cases, the geometric mean guideline was used. For the site to site comparisons, the geometric mean of all the results obtained throughout the study were used and compared to the geometric mean guidelines given.

Table 4 Indicator Guidelines used in this study

Fecal Coliforms	EPA and the state of Florida recommended guidelines for a single sample of 800 cfu/100 mL, for a geometric mean, 200 cfu/100 mL
E.coli	EPA recommended guideline for a geometric mean sample 126 cfu/100 mL
Enterococci	EPA recommended guidelines for a single sample of 104 cfu/100 mL, for a geometric mean , 33-35 cfu/100 mL for marine and fresh water respectively.
C. perfringens	Guidelines used by state of Hawaii based on research by Dr. Roger Fujioka et al at the University of Hawaii of 50 cfu/100 mL for fresh and brackish water and 5 cfu/100 mL for marine waters.
Coliphage	Level used - 100 pfu/100 mL based on previous research by Dr. Joan Rose, USF

B) Results of Traditional and Alternative Indicators

The sampling sites were divided up into the categories of rural (which included suburban areas), urban and beach sites. The arithmetic and geometric averages for each individual indicator at each sampling site were calculated. Less than values, or those values that fell below the limit of detection for that indicator, were changed to zeros to calculate the averages. Tables with each individual sampling and the results of the indicator data can be found in Appendix VI. Below are summary tables (Tables 4 through 8) for each indicator (Fecal Coliforms, *E.coli,* Enterococci, *Clostridium perfringens* and Coliphage). The sites are divided into rural, urban and beach sites, with the control site at the bottom of the table. The site designations are given, and the total number of samples is listed under the "n" column. The range is given to show the lowest and highest indicator result seen in the course of the study. The percent Positive column is based on the number of specimens in which the indicator was detected at any level above the detection limit. The last two columns are the arithmetic and geometric mean of the indicator for the entire period of the study.

For Table 5, Fecal coliforms, the results ranged from <1 cfu/100 mL at the control site to 174,900 cfu/100 mL at TB4 Bullfrog Creek. In the rural sites, the percentage of positive results was generally 100%, with slightly lower percentages for the urban sites, and lower still for the beach sites. The only exception to this was TB13 Courtney Campbell Causeway beach, with fecal coliforms present 100% of the time during the sampling period. The arithmetic mean ranged from 0.4 cfu/100 mL at the control site to 22,687 cfu/100 mL at TB4 Bullfrog Creek, and the geometric mean ranged from 0.2 cfu/100 mL to 5032 cfu/100 mL at the control site and TB4 Bullfrog Creek, respectively In Table 6 for *E.coli,* results are very similar to the fecal coliforms trends as described above.

Enterococci in Table 7 ranged from <2 and <4 cfu/100 mL at several sites (including 3 out of the 4 beach sites) to 135,650 cfu/100 mL at TB4 Bullfrog Creek. In both the rural and urban sites, Enterococci was found generally 100% of the time, but only 38 to 92% of the time at the beach sites. The arithmetic mean for enterococci ranged from 0.3 cfu/100 mL at the control site to 14,520 cfu/100 mL at TB4 Bullfrog Creek, and the geometric mean ranged from 0.2 cfu/100 mL at the control site to 3009 cfu/100 mL at TB4 Bullfrog Creek.

For Table 8, *Clostridium perfringens*, the results ranged from below the detection limit for all sites of the study to 160 cfu/100 mL at TB4 Bullfrog Creek. *Clostridium* was never found 100% of the time at any site, and the higher percentages occurred in the rural sites as well as the urban sites. The percentage for the beach sites were very low with the exception of TB13 Courtney Campbell Causeway beach, in which *C. perfringens* was detected 58% of the time. The arithmetic mean ranged from below the detection limit for TB16 Honeymoon Island and the control site to 32.7 cfu/100 ml at TB4 Bullfrog Creek, and the geometric mean ranged from below the detection limit for TB16 Honeymoon Island and the control site to 11.3 cfu/100 mL at TB3 Bullfrog Creek. For the 4 beach sites, *C.perfringens* was only found consistently at TB13 Courtney Campbell Causeway Beach, the most urban-located beach in the study. *C.perfringens* only occurred once at

TB20 North Beach, twice at TB19 John's Pass, and was never detected at TB16 Honeymoon Island.

For the final indicator, Coliphage, Table 9 shows the results ranged from below the detection limit for most of the sampling sites to 28,180 pfu/100 mL for TB4 Bullfrog Creek. The percentage of positive samples ranged from 54 to 100% for both the urban and rural sites, but showed very low percentages for the beach sites. Coliphage showed a similar pattern to *Clostridium* in regard to the beach sites. The arithmetic mean ranged from below the detection limit for TB16 Honeymoon Island and the control site to 2937 pfu/100 mL at TB4 Bullfrog Creek, and the geometric mean ranged from below the detection limit for TB16 Honeymoon Island and the control site to 911 pfu/100 mL at TB4 Bullfrog Creek.

Table 5 Fecal Coliform Averages for all Sites

	Site	n	Range (cfu/100mL)	% Pos	Arithmetic Avg (cfu/100mL)	Geo. Avg (cfu/100mL)
Rural	TB1	12	55 - 24,450	100	3045	472
	TB2	12	<10 - 7415	83	804	58
	TB3	12	100 - 16,350	100	2998	913
	TB4	13	550 - 174,900	100	22,687	5032
	TB5	12	35 - 5300	100	1223	664
	TB6	13	300 - 110,200	100	9997	1688
	TB7	13	12 - 13,850	100	4057	977
	TB8	13	90 - 6050	100	755	296
	TB9	12	100 – 10,250	100	1510	455
	TB10	12	10 – 4140	100	525	106
	TB11	11	<10 – 3890	91	402	34
Urban	TB12	10	<10 – 3400	90	1194	265
	TB14	13	80 – 33,150	100	9421	3655
	TB15	10	35 – 40,000	100	4187	298
	TB17	13	<10 – 23,700	92	3377	665
	TB18	10	<10 – 3115	90	501	114
	TB21	11	<10 – 6100	82	1656	185
Beach	TB13	13	15 – 26,900	100	3057	300
	TB16	10	<4 – 4745	80	523	17
	TB19	12	<10 – 13,240	83	1438	53
	TB20	13	<4 – 10,900	54	1574	25
Control	TB22	11	<1 - 4	18	0.4	0.2

Table 6 *E.coli* Averages for all Sites

	Site	n	Range (cfu/100mL)	% Pos	Arithmetic Avg (cfu/100mL)	Geo. Avg (cfu/100mL)
Rural	TB1	10	50 – 24,450	100	3589	561
	TB2	10	0.5 – 7415	100	831	63
	TB3	11	75 – 16,350	100	2846	592
	TB4	11	50 – 174,900	100	23,948	2302
	TB5	10	85 – 5300	100	1250	653
	TB6	11	350 – 110,200	100	10,893	1231
	TB7	10	175 – 17,200	100	3617	844
	TB8	10	70 – 900	100	233	214
	TB9	10	45 – 10,250	100	1622	336
	TB10	10	10 – 4140	100	534	87
	TB11	10	<10 – 3890	90	407	19
Urban	TB12	8	15 – 3200	100	768	264
	TB14	11	80 – 15,100	100	3789	1378
	TB15	8	45 – 235	100	111	57
	TB17	12	<10 –23,700	92	2476	314
	TB18	9	<10 – 3115	89	466	96
	TB21	10	<10 – 5340	90	1362	222
Beach	TB13	11	10 – 26,900	100	3460	231
	TB16	9	<4 – 4745	78	559	16
	TB19	12	<10 – 13,240	83	1400	42
	TB20	12	<4 – 10,900	50	1643	26
Control	TB22	7	<1 - <10	0	0	0

Table 7 Enterococci Averages for all Sites

	Site	n	Range (cfu/100mL)	% Pos	Arithmetic Avg (cfu/100mL)	Geo. Avg (cfu/100mL)
Rural	TB1	12	40 –12,300	100	1542	419
	TB2	12	<4 – 496	83	70	15
	TB3	13	10 – 17,200	100	1609	189
	TB4	13	134 – 135,650	100	14,520	3009
	TB5	12	2 – 6350	100	1144	269
	TB6	13	68 – 43,000	100	7738	1065
	TB7	13	44 – 31,650	100	3852	745
	TB8	13	42 – 17,850	100	1593	231
	TB9	12	20 – 17,200	100	1962	216
	TB10	12	10 – 2905	100	305	55
	TB11	11	<4 – 102	82	18	7
Urban	TB12	10	2 – 585	100	157	65
	TB14	13	5 – 35,000	100	5779	940
	TB15	10	8 – 236	100	70	37
	TB17	13	14 – 720	100	205	109
	TB18	10	8 – 124	100	52	33
	TB21	11	<4 – 1270	82	146	19
Beach	TB13	13	<10 – 600	92	123	47
	TB16	10	<2 – 557	60	58	3
	TB19	13	<4 – 28	77	5	3
	TB20	13	<4 – 77	38	7	0.9
Control	TB22	10	<4 - 1	30	0.3	0.2

Table 8 ***Clostridium perfringens*** **Averages for all Sites**

	Site	n	Range (cfu/100mL)	% Pos	Arithmetic Avg (cfu/100mL)	Geo. Avg (cfu/100mL)
Rural	TB1	11	<4 – 32	64	7.8	3.7
	TB2	11	<4 – 14	64	4.1	2.3
	TB3	12	<4 – 50	83	20.8	11.3
	TB4	13	<4 – 160	62	32.7	7.8
	TB5	11	<4 – 46	64	9.5	3.4
	TB6	13	<4 – 46	54	12.5	4.3
	TB7	13	<4 – 32	77	7.9	4.0
	TB8	13	<4 – 16	62	4.8	2.5
	TB9	11	<4 – 16	45	3.5	1.5
	TB10	11	<4 – 14	45	2.9	1.2
	TB11	11	<4 – 6	45	1.2	0.7
Urban	TB12	9	<4 – 22	56	4	1.7
	TB14	12	<4 – 148	75	25	7.4
	TB15	8	<4 – 34	75	8.4	4.1
	TB17	12	<2 – 26	67	7.8	3.7
	TB18	8	<2 – 30	63	11.1	4.7
	TB21	9	<2 – 18	22	2.4	0.7
Beach	TB13	12	<4 – 52	58	11	3.5
	TB16	8	<2 - <4	0	0	0
	TB19	12	<2 - <4	17	0.5	0.3
	TB20	12	<2 – 10	8	0.8	0.2
Control	TB22	8	<2 - <4	0	0	0

Table 9 Coliphage Averages for all Sites

	Site	n	Range (pfu/100mL)	% Pos	Arithmetic Avg (pfu/100mL)	Geo. Avg (pfu/100mL)
Rural	TB1	11	50 – 7560	100	1226	456
	TB2	11	<10 – 140	73	38	12
	TB3	13	20 – 1850	100	455	202
	TB4	13	110 – 28,180	100	2937	911
	TB5	11	<10 – 980	91	206	68
	TB6	13	90 – 22,920	100	2341	497
	TB7	13	<10 – 3680	92	426	110
	TB8	13	<10 – 1680	85	170	32
	TB9	11	30 – 1470	100	430	232
	TB10	11	<10 – 1080	64	115	9
	TB11	11	<10 – 20	54	6	3
Urban	TB12	10	<10 – 260	80	60	24
	TB14	12	70 – 2650	100	653	380
	TB15	9	<10 – 30	67	12	6
	TB17	13	<10 – 120	85	35	17
	TB18	9	<10 – 220	89	77	36
	TB21	10	<10 – 230	80	33	10
Beach	TB13	13	<10 – 20	31	4	1
	TB16	9	<10	0	0	0
	TB19	13	<10 – 20	23	3	0.8
	TB20	13	<10 – 20	8	2	0.3
Control	TB22	9	<10	0	0	0

Table 10 gives a summary of the individual sampling events that exceeded the suggested indicator guidelines for Fecal Coliforms, *E. coli,* Enterococci, *Clostridium perfringens* and Coliphage. For each indicator, a column is given to show the percentage of the samples that exceeded the suggested guidelines for single samples found in Table 4. For those indicators that did not have a single sample guideline (*E.coli, C.perfringens* and coliphage), the geometric mean guideline was used. Sites TB4 Bullfrog Creek and TB14 Sweetwater Creek consistently had high levels of indicators except for *C. perfringens.* Sites TB6 and TB7 along Bullfrog Creek generally had high levels of Fecal Coliforms, *E.coli*, Enterococci and Coliphage as well. The other sites showed less contamination, with TB17 Allen's Creek and TB5 Alafia River showing moderate levels of indicators. Site TB1 Delaney Creek had high levels of *E.coli* , Enterococci and Coliphage, but low levels of Fecal Coliforms and *C. perfringens.* Sites TB3 Bullfrog Creek, TB17 Allen's Creek and TB18 Joe's Creek had the highest levels detected for *C.perfringens*. For the beach sites, TB13 Courtney Campbell Causeway beach had the highest levels of indicators followed by TB20 Ft. DeSoto. The control site, TB22, had indicator levels below all guidelines for the entire length of the study.

Table 10 Summary of samples exceeding the suggested single sample Indicator guidelines

	Site	**Fecal Coliforms**	***E.coli***	**Enterococci**	***C.perfingens***	**Coliphage**
Rural	TB1	17%	90%	75%	0%	91%
	TB2	8%	40%	17%	27%	9%
	TB3	33%	82%	46%	67%	62%
	TB4	77%	91%	100%	23%	100%
	TB5	42%	90%	67%	0%	36%
	TB6	69%	100%	85%	0%	92%
	TB7	38%	100%	92%	0%	62%
	TB8	15%	60%	62%	0%	23%
	TB9	25%	60%	58%	0%	73%
	TB10	17%	30%	25%	18%	9%
	TB11	9%	10%	0%	9%	0%
Urban	TB12	40%	75%	40%	11%	10%
	TB14	85%	91%	85%	17%	75%
	TB15	10%	25%	20%	0%	0%
	TB17	54%	83%	62%	42%	8%
	TB18	20%	33%	10%	50%	22%
	TB21	27%	70%	18%	11%	10%
Beach	TB13	31%	55%	38%	25%	0%
	TB16	10%	22%	10%	0%	0%
	TB19	17%	25%	0%	0%	0%
	TB20	31%	33%	0%	8%	0%
Control	TB22	0%	0%	0%	0%	0%

C) Comparison of Sites

Geometric Mean Graphs

The graphs in Figures 2 through 6 show the geometric mean of each indicator. For each indicator graph, the X, or bottom, axis shows all the sampling sites for the study, and the Y, or left, axis shows the colony forming units (CFU) for the bacterial indicators and the plaque forming units (PFU) for the virus indicator coliphage per 100 mL of water. These data have not been log transformed, it is given in the actual numbers of organisms per 100 mL of water.

For Figure 2 (Fecal Coliforms), sites TB1, TB3, TB4, TB5, TB6, TB7, TB8 and TB9, TB12, TB13m TB14 and TB15 and TB17 all exceed the EPA's suggested guideline of 200 cfu/100 mL for a geometric mean result. Sites TB4 Bullfrog Creek and TB14 Sweetwater Creek are the two most contaminated in regard to fecal coliforms.

In Figure 3 (*E.coli*), sites TB1, TB3, TB4, TB5, TB6, TB7, TB8 andTB9, TB12,TB13 and TB14, TB17 and TB21 all exceed EPA's suggested guideline of 126 cfu/100 mL for a geometric mean result. Sites TB4 and TB6 along Bullfrog and TB 14 Sweetwater Creek have the highest averages for this indicator**.**

For Figure 4 (Enterococci), sites TB1, TB3, TB4, TB5, TB6, TB7, TB8, TB9 and TB10, TB12, TB13, TB14 and TB15, and TB17 all exceed the suggested guideline of 104 cfu/100 mL for a geometric mean result. Sites TB4, TB6 and TB7 along Bullfrog Creek and TB14 Sweetwater Creek are the highest sites for this indicator.

In Figure 5 (***Clostridium perfringens*****),** none of the sites exceed the recommended guideline (Fujioka et al 1985) for recreational water for a geometric result.

Finally, in Figure 6 (Coliphage), sites TB1, TB3 , TB4, TB6, TB7, TB9 and TB14 all exceed 100 pfu/100 mL. Site TB4 Bullfrog Creek shows the highest mean level of coliphage.

As previously mentioned, Fecal Coliforms, *E.coli* and Enterococci could be detected in almost all samples. Thirteen of the 22 sites were above the geometric suggested guideline for both *E.coli* and Fecal Coliforms, and 14 of the 22 sites were above the geometric suggested guideline for Enterococci. However, none of the beach sites with the exception of TB13 Courtney Campbell Causeway beach were above the fecal coliform and *E.coli* suggested geometric mean guidelines.

The two consistently high sites for all of the indicators were the rural siteTB4 Bullfrog Creek and the urban site TB14 Sweetwater Creek. The remaining sites along Bullfrog Creek (TB3, TB6, TB7 and TB8) are the next highest sites in regards to geometric indicator means. The low sites were TB2 Alafia River, TB11 Manatee River, TB18 Joe's Creek and TB22 Control site. The beach sites TB16 Honeymoon Island, TB19 John's Pass and TB20 North Beach, Ft. DeSoto were also among the lowest geometric means for the indicators used, but the urban beach site, TB13 Courtney Campbell, did show a geometric mean above the recommended guideline for all indicators except *C. perfringens* and Coliphage. The lower levels of coliphage in this case may be due to the short survival time of the phage in warm saline waters.

Figure 2 Fecal Coliforms, Geometric Means by Site

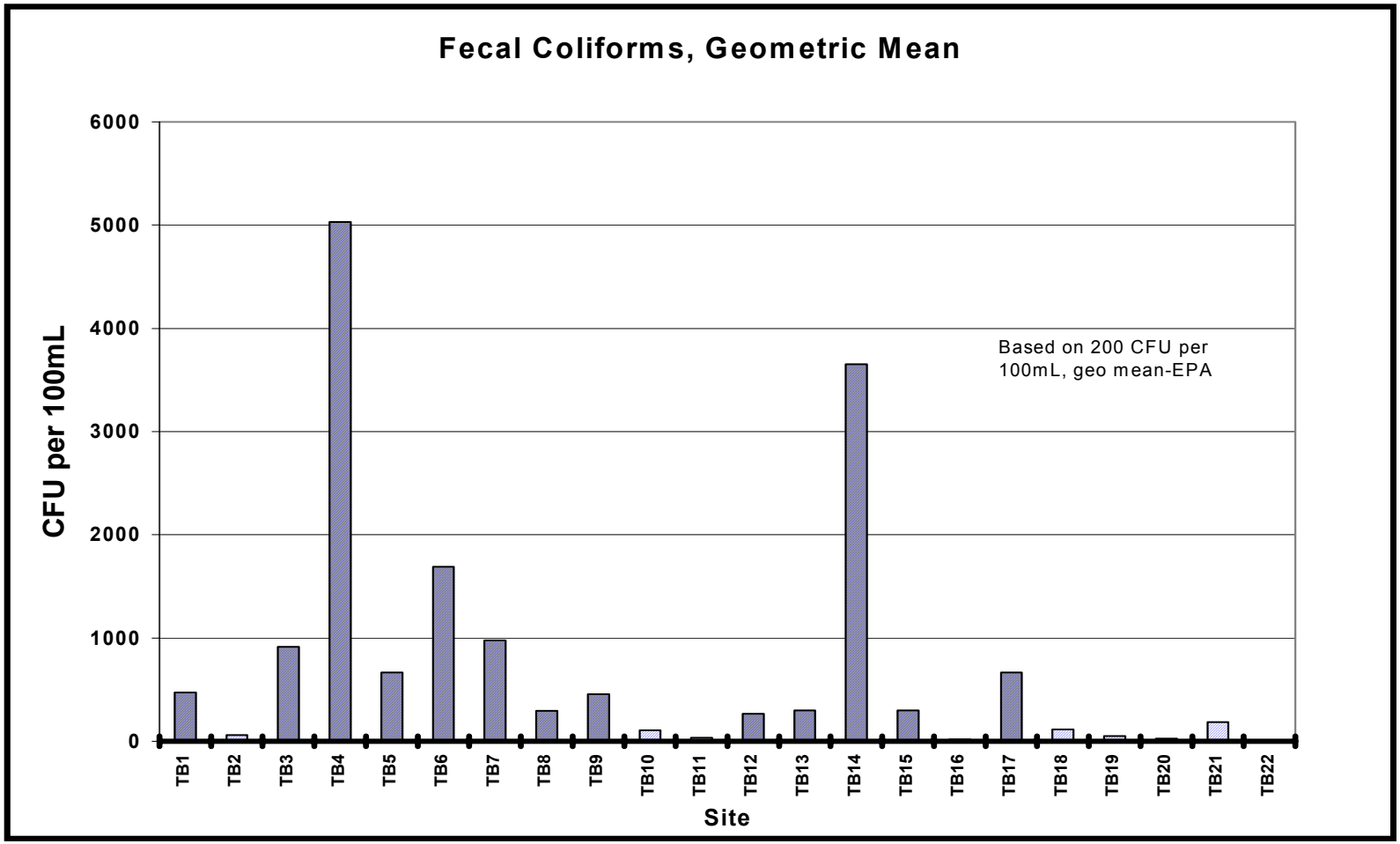


Figure 3 *E.coli* Geometric Means by Site

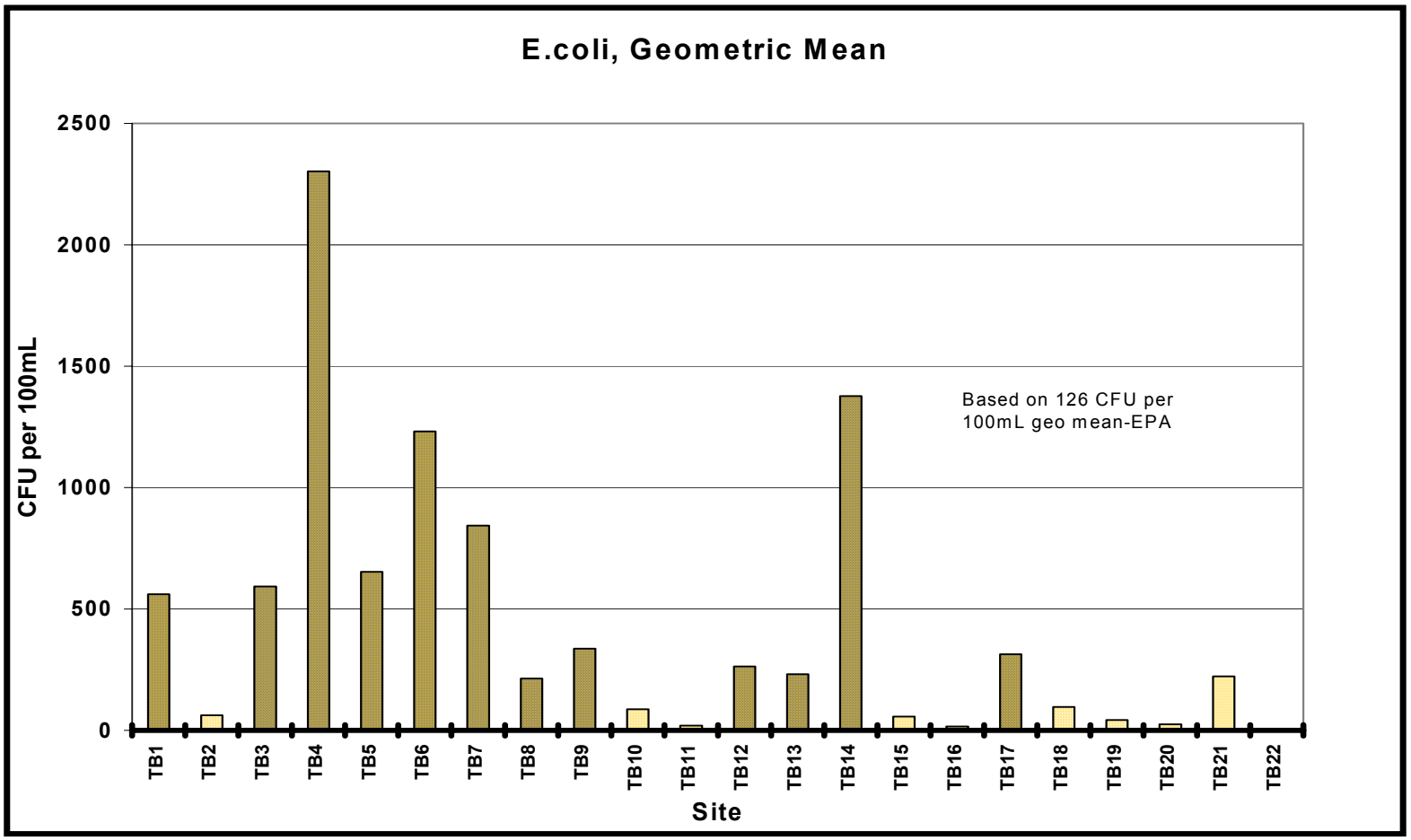


Figure 4 Enterococci Geometric Means by Site

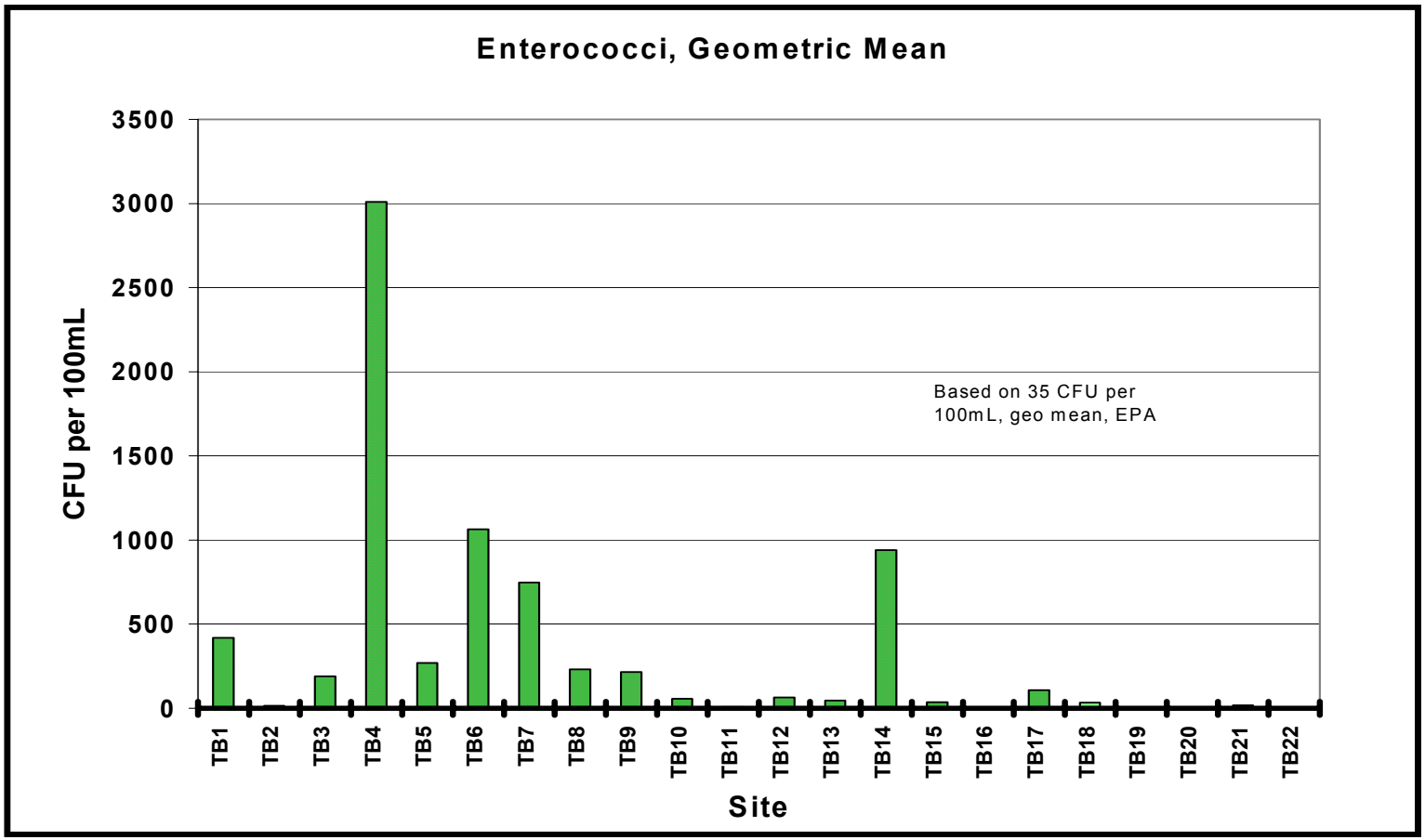


Figure 5 ***Clostridium perfringens*** **Geometric Means by Site**

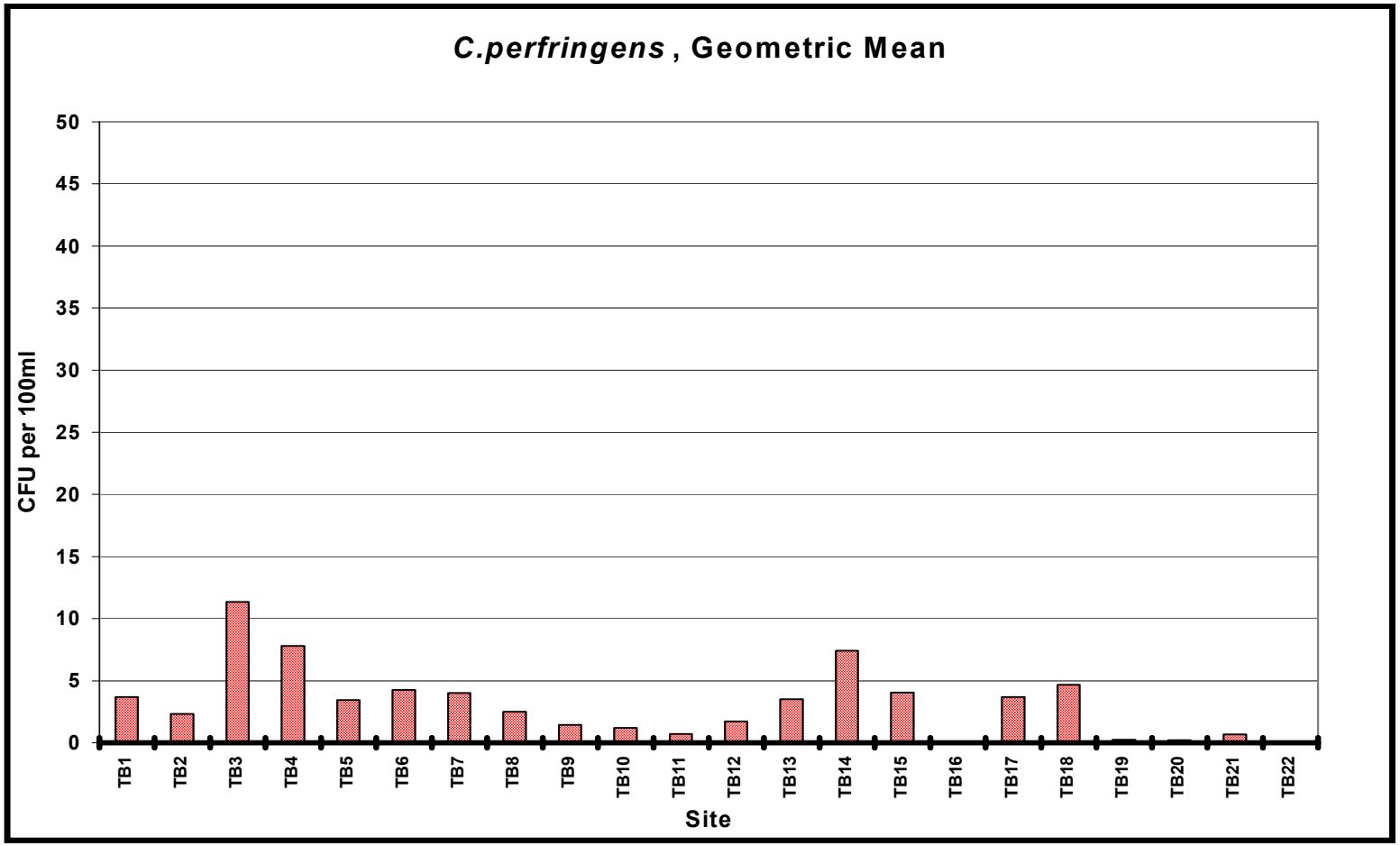
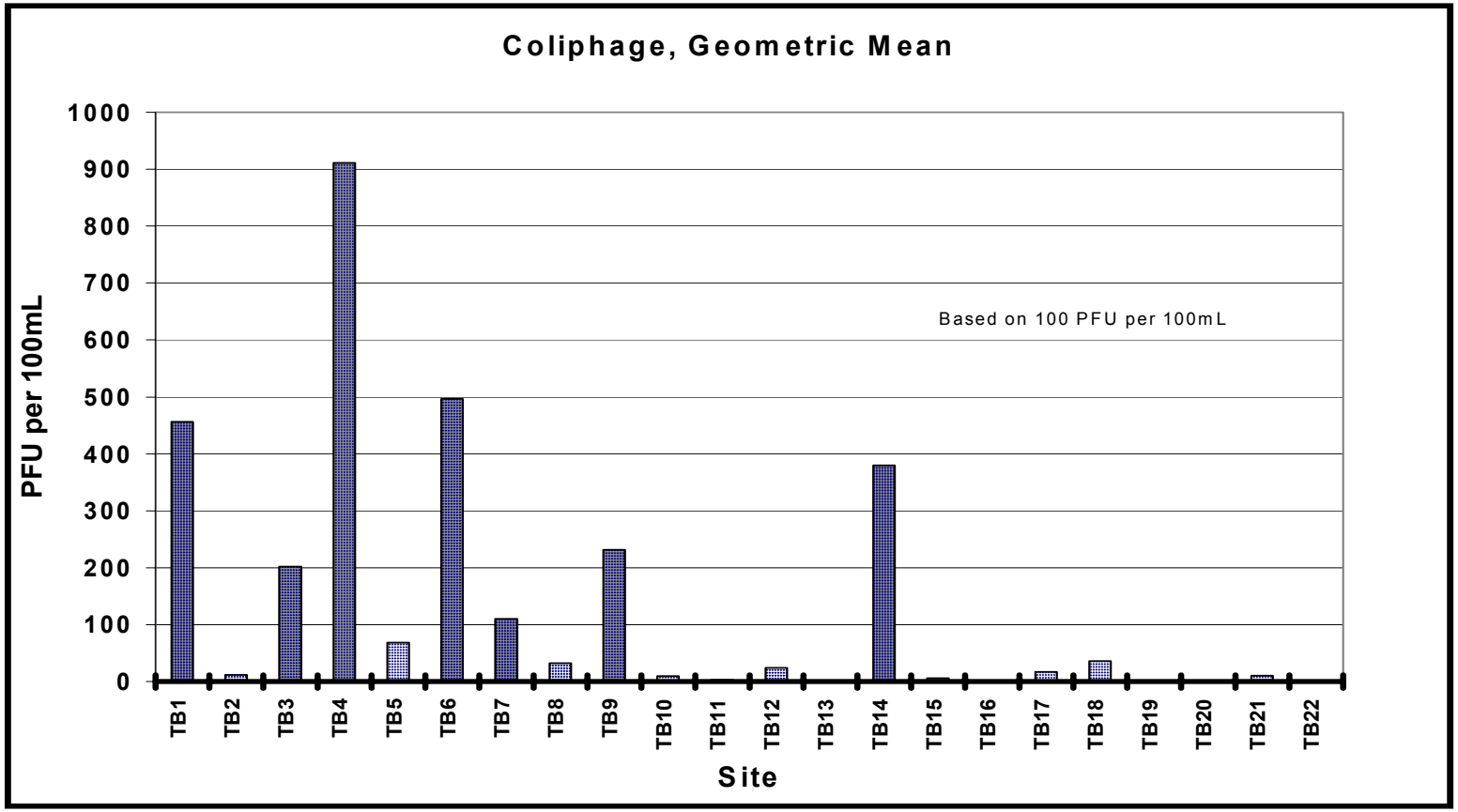


Figure 6 Coliphage Geometric Means by Site



Seasonal Graphs of Indicator Data

The indicator values for each month of the study for each individual site are represented on a bar graph to illustrate the seasonal changes for each sampling site. For each graph, the X, or bottom, axis shows the months of the study, and the Y, or left, axis shows the colony forming units (CFU) for bacteria and the plaque forming units (PFU) for viruses per 100 mL of water. The CFU and PFU/100 mL are log transformed in order to compare the indicators on one graph. For example, the suggested guideline for fecal coliforms is 800 cfu/100 mL for a single sampling, which is equivalent to 2.9 on the log transformed scale. For Enterococci, the suggested level of 104 cfu/100 mL is equivalent to 2.0 on the log transformed scale. Fecal coliforms are represented by the first bar in the grouping, *E.coli* by the second bar, Enterococci by the third bar, *C. perfringens* by the fourth bar and Coliphage by the fifth and last bar in each grouping on the graph.

Rural Sampling Sites

Sampling began in June of 1999. For the rural sites TB1 through TB11 (Figures 7 to 17), peaks in indicator levels were generally seen in October 1999 and March 2000, and a reduction in indicator numbers occurred during the summer months. While high levels of indicators were found through out the year in Bullfrog Creek (TB3, TB4, TB6-TB8), similar peaks were found in Oct 1999 and March 2000. Some of the peaks only involved Fecal Coliforms and *E.coli*, while others involved the entire group of indicators.

Urban Sampling Sites

The urban sites consisted of TB12 Hillsborough River, TB14 Sweetwater Creek, TB15 Lake Tarpon, TB17 Allen's Creek, TB18 Joe's Creek and TB21 Salt Creek. (Figures 18 to 23) The peaks in these sites are more sporadic than previously seen in the rural sites. For TB12, peaks occurred in December 1999 and March 2000, while TB14 and TB18 only showed a peak in Dec 1999 and TB15 only in March 2000. Other isolated peaks occurred in some of the sites. All but one of the indicator peaks in the urban site grouping involved only Fecal Coliforms and *E.coli*. For the urban sites, indicator levels were fairly consistent through out the year, with a slight seasonal drop in the summer months occurring only at TB18 Joe's Creek.

Beach Sampling Sites

The beach sites consisted of an urban beach (TB13 Courtney Campbell Causeway beach), a high boat traffic beach area (TB19 John's Pass), a rural beach area with heavy recreational use (TB20 Ft. DeSoto) and a pristine beach with a high bird population and no swimming (TB16 Honeymoon Island). The beach at TB13 was the only beach site where all 5 indicators used in the study appeared. Peaks of Fecal coliforms and *E.coli* occurred in September 1999 and March 2000 at this site, with *Clostridium perfringens* and coliphage appearing sporadically through out the study. For TB19, peaks in the levels of fecal coliforms and *E.coli* occurred in September 1999, December 1999 and February 2000, with *Clostridium perfringens* and coliphage occurring only in the winter months. At TB20, high peaks of Fecal coliforms and *E.coli* were found in September and

December 1999, and February and March 2000. In this case, the indicators are rather sporadic and do not occur with any consistency. The last beach site, TB16, had only Fecal Coliforms, *E.coli,* and Enterococci occurring, with peaks in August, October and December 1999.

Control Sampling Site

Fecal Coliforms were only detected during July 1999, and Enterococci in August 1999, January and March 2000. These indicators occurred only in very low numbers. This site was not sampled December 1999 and June-August 2000.

Figure 7 Indicator Levels in TB1 Delaney Creek

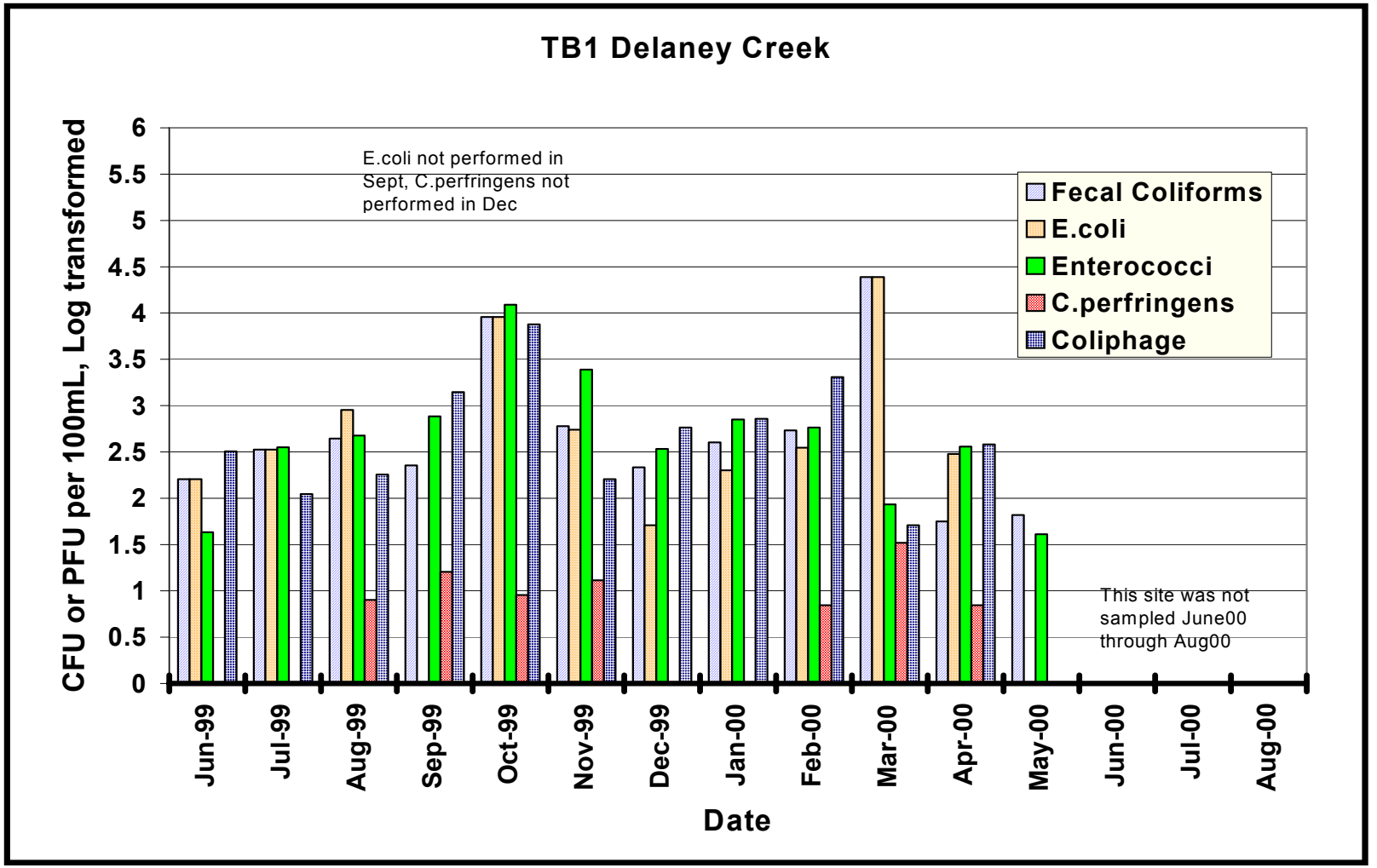


Figure 8 Indicator Levels in TB2 Alafia River

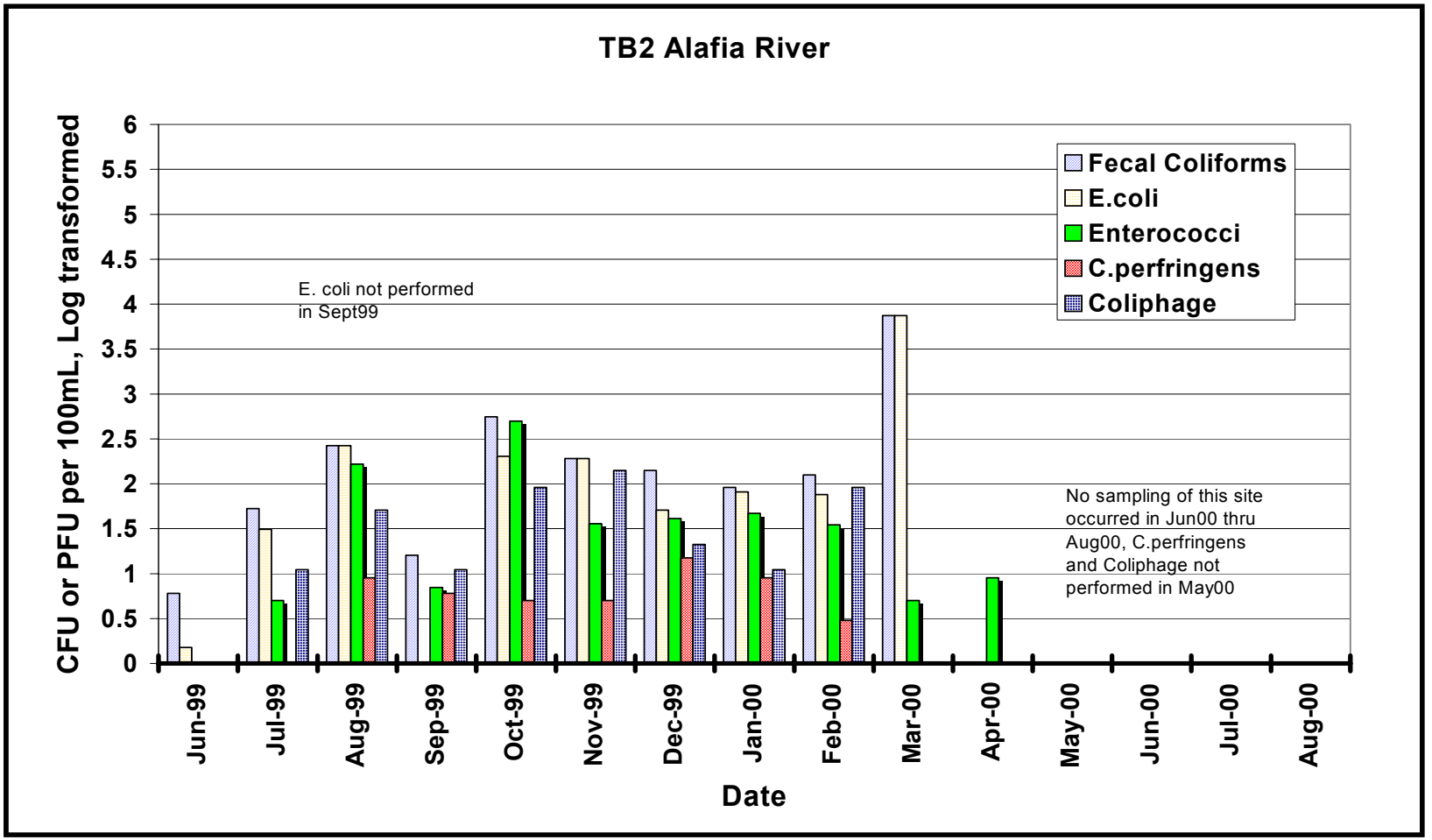


Figure 9 Indicator Levels in TB3 Bullfrog Creek

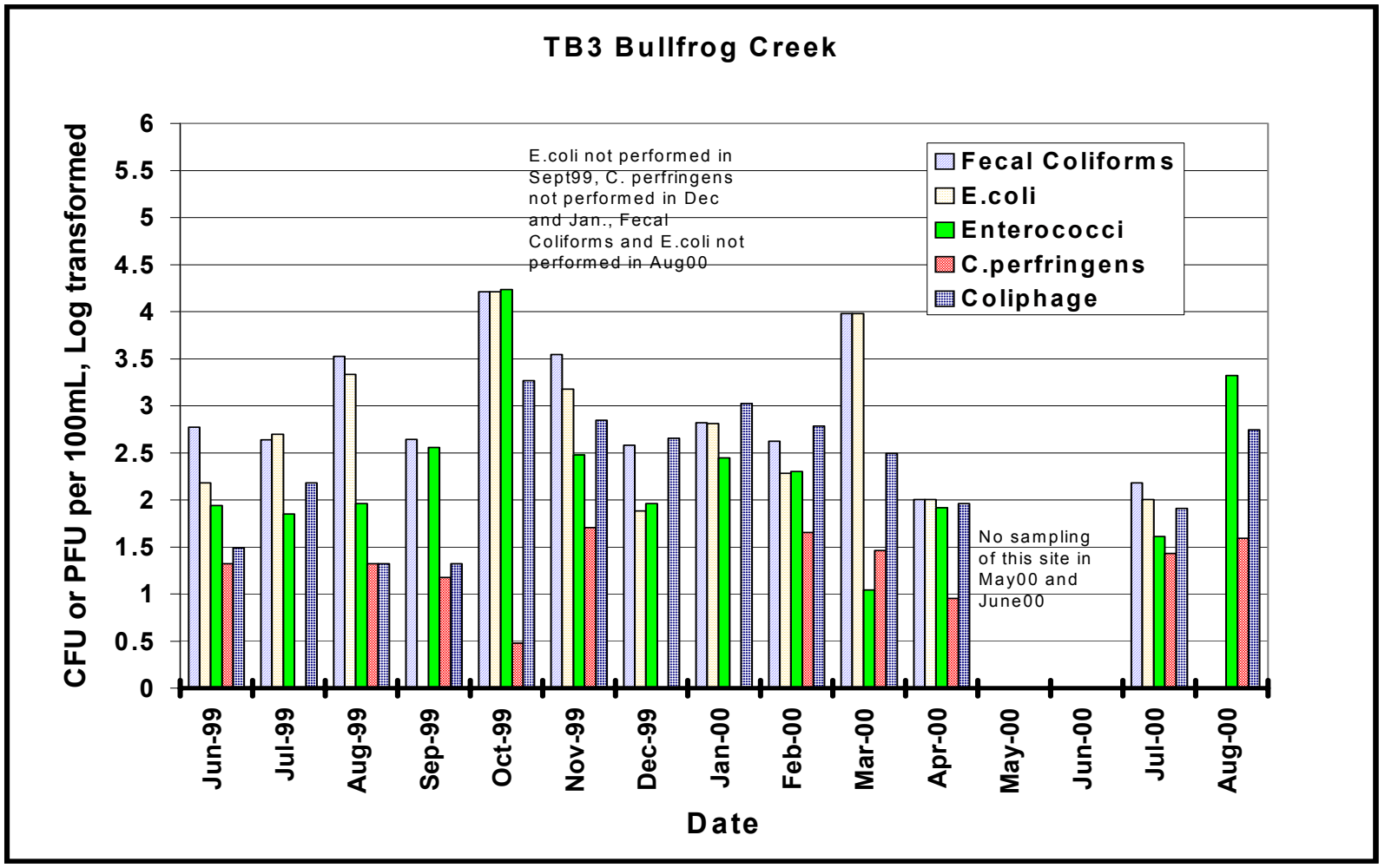


Figure 10 Indicator Levels in TB4 Bullfrog Creek

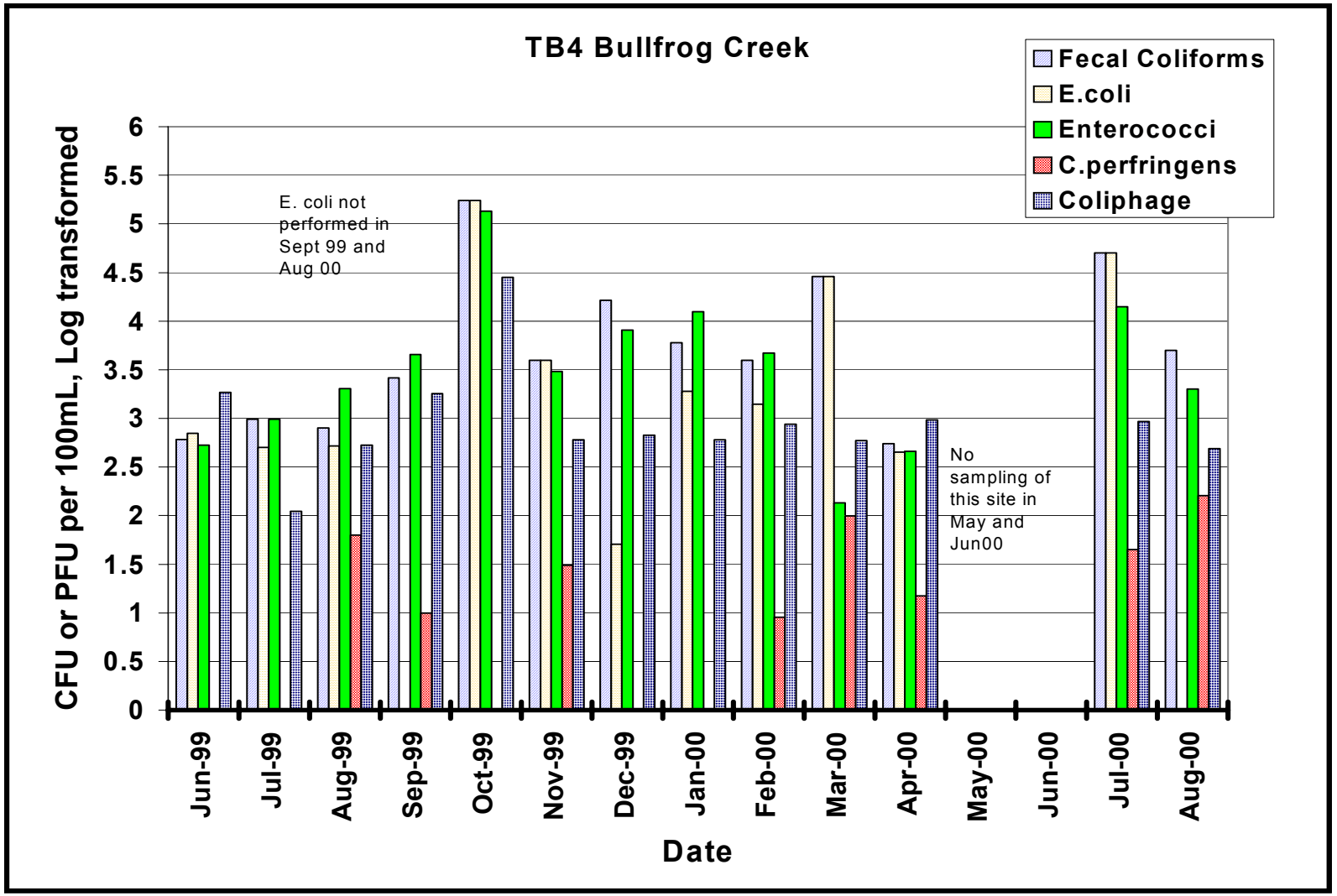


Figure 11 Indicator Levels in TB5 Alafia River

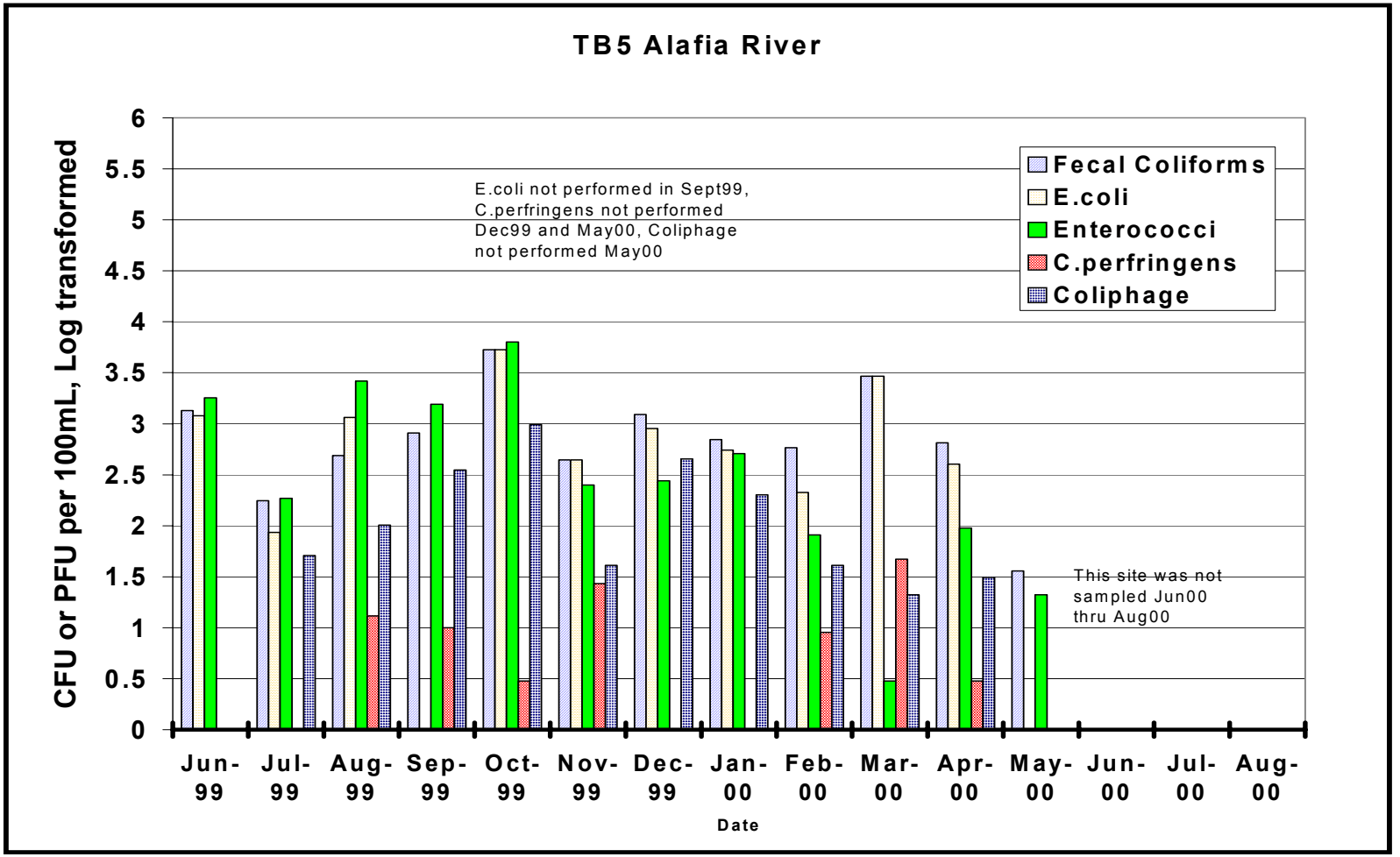


Figure 12 Indicator Levels in TB6 Bullfrog Creek

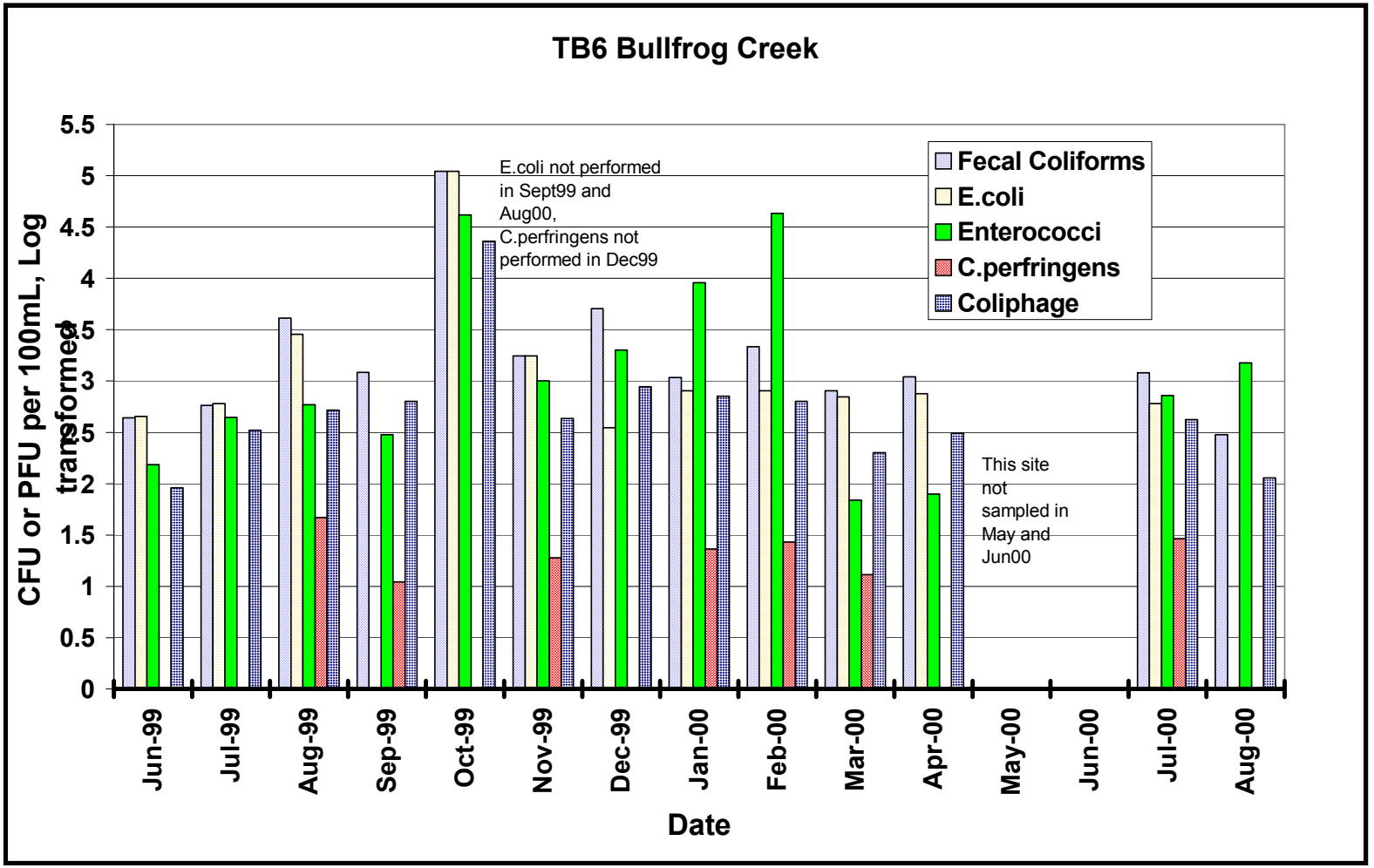


Figure 13 Indicator Levels in TB7 Bullfrog Creek

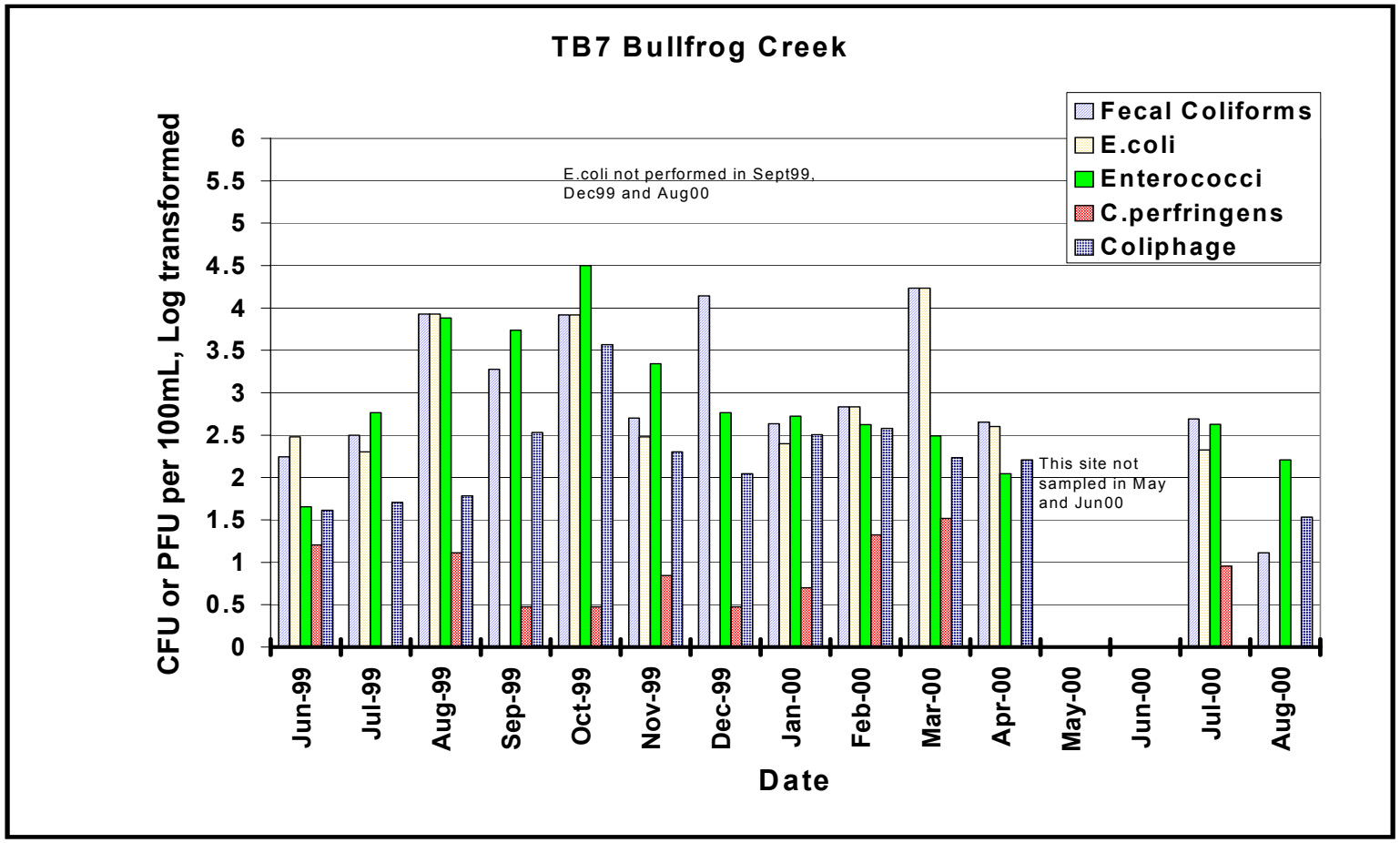


Figure 14 Indicator Levels in TB8 Bullfrog Creek

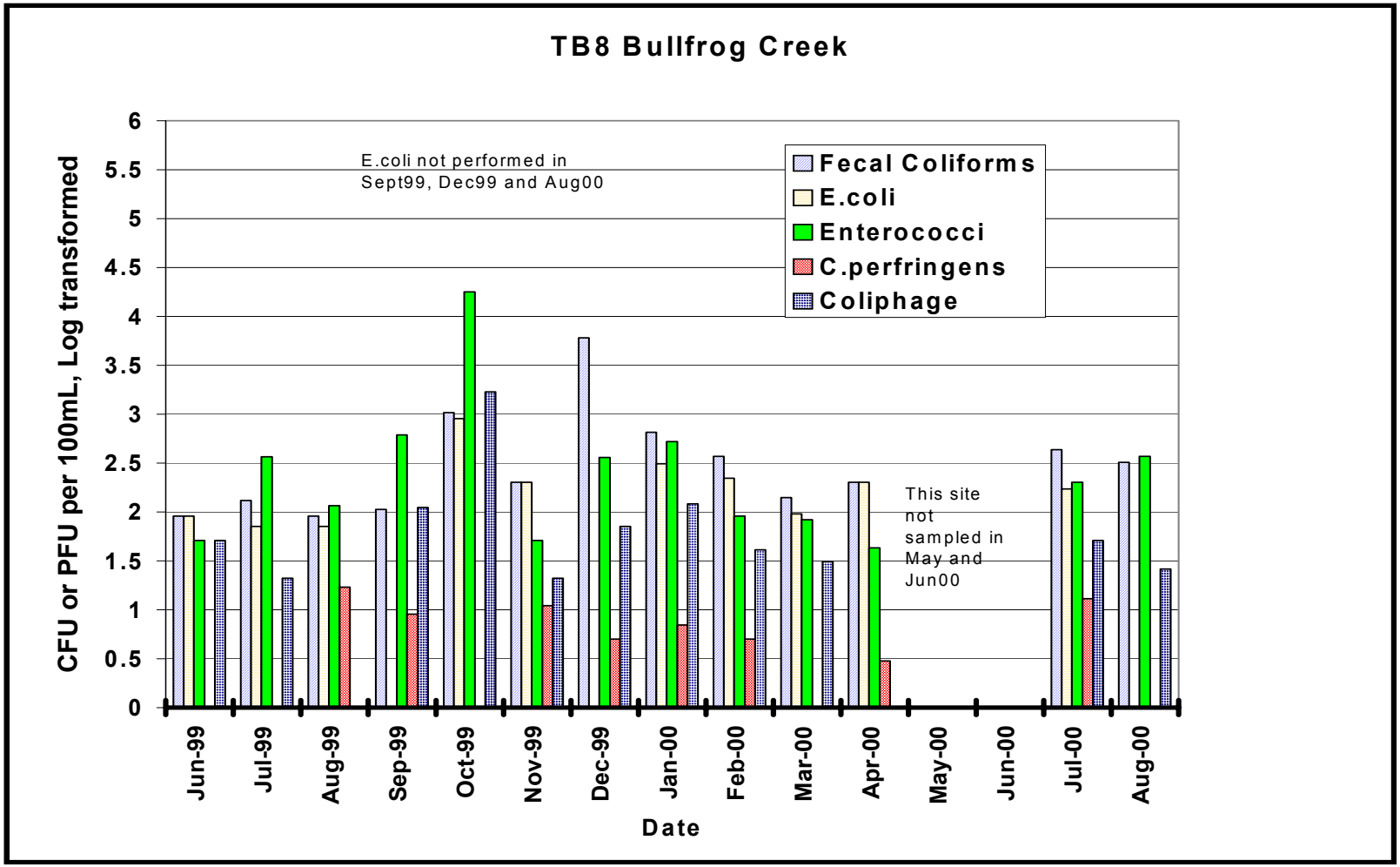


Figure 15 Indicator Levels in TB9 Little Manatee River

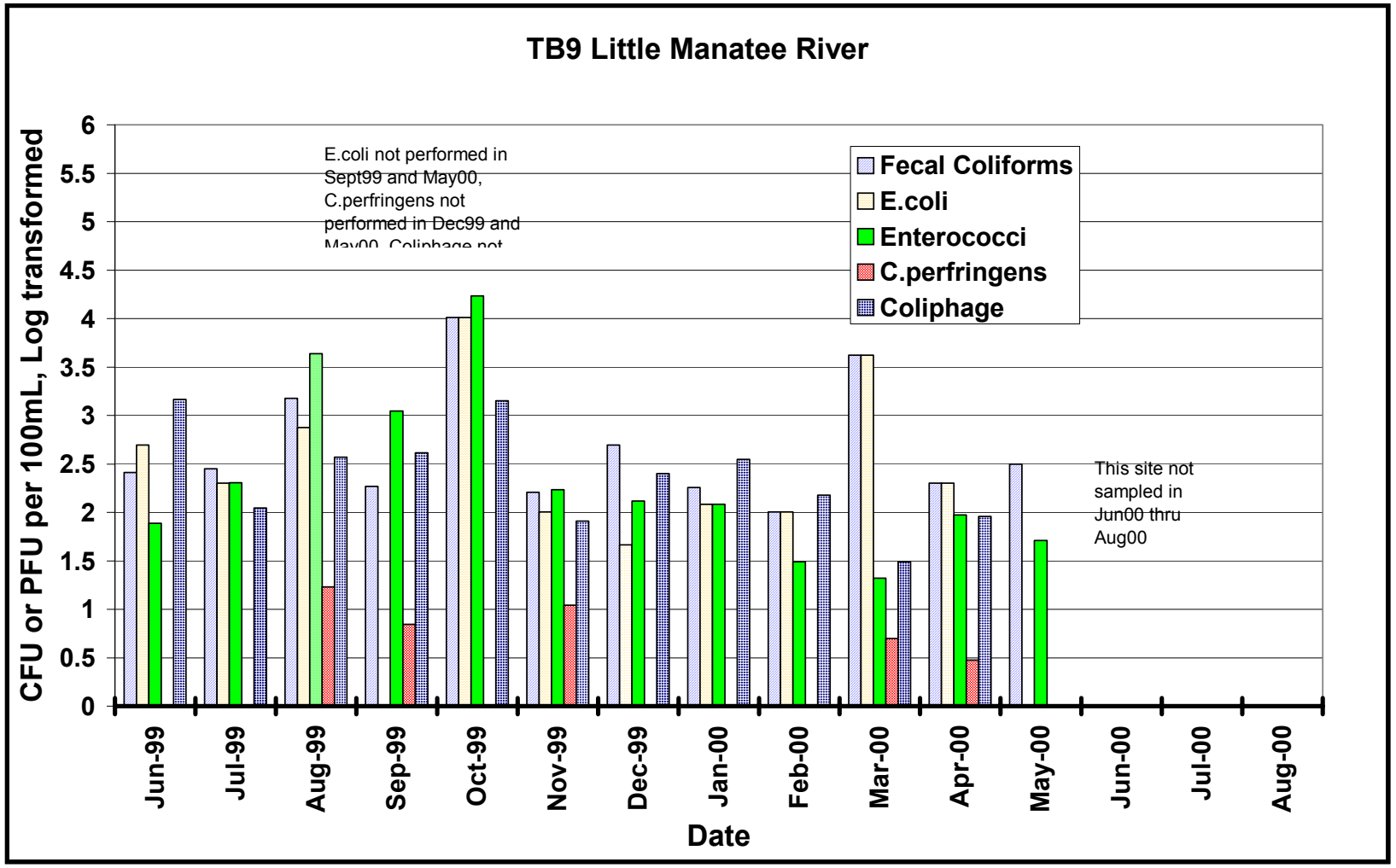


Figure 16 Indicator Levels in TB10 Little Manatee River

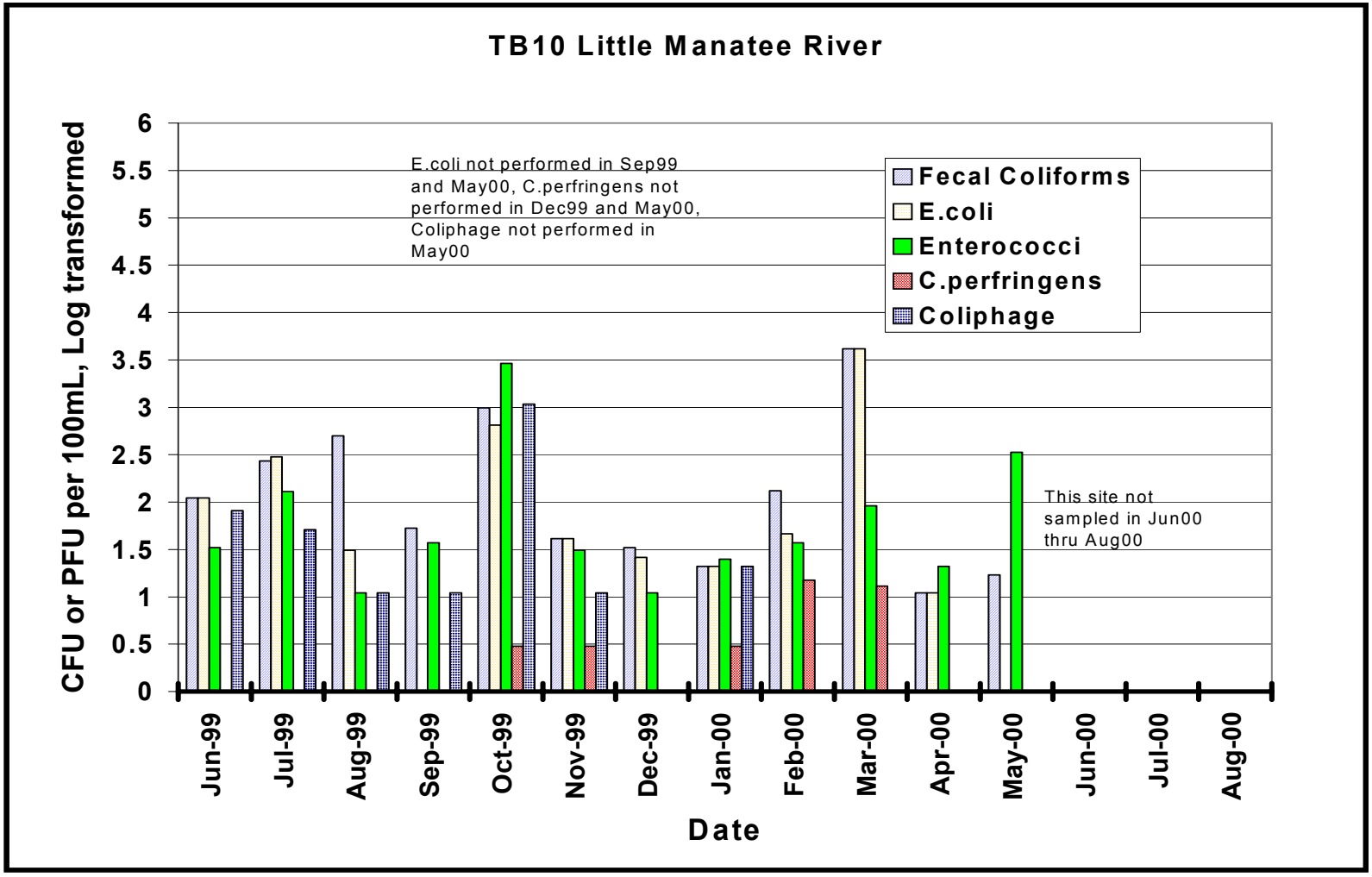


Figure 17 Indicator Levels in TB11 Manatee River

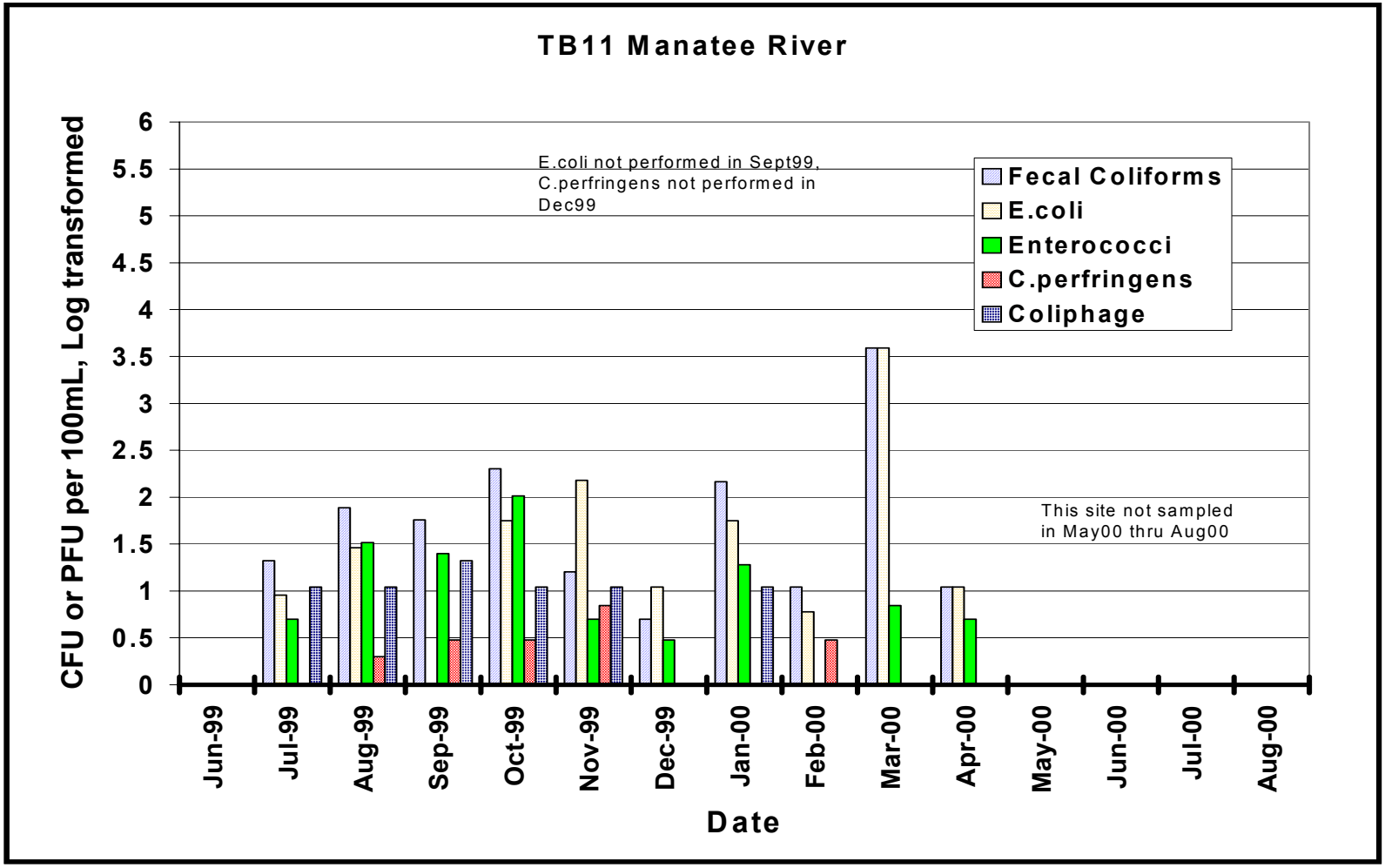


Figure 18 Indicator Levels in TB12 Hillsborough River

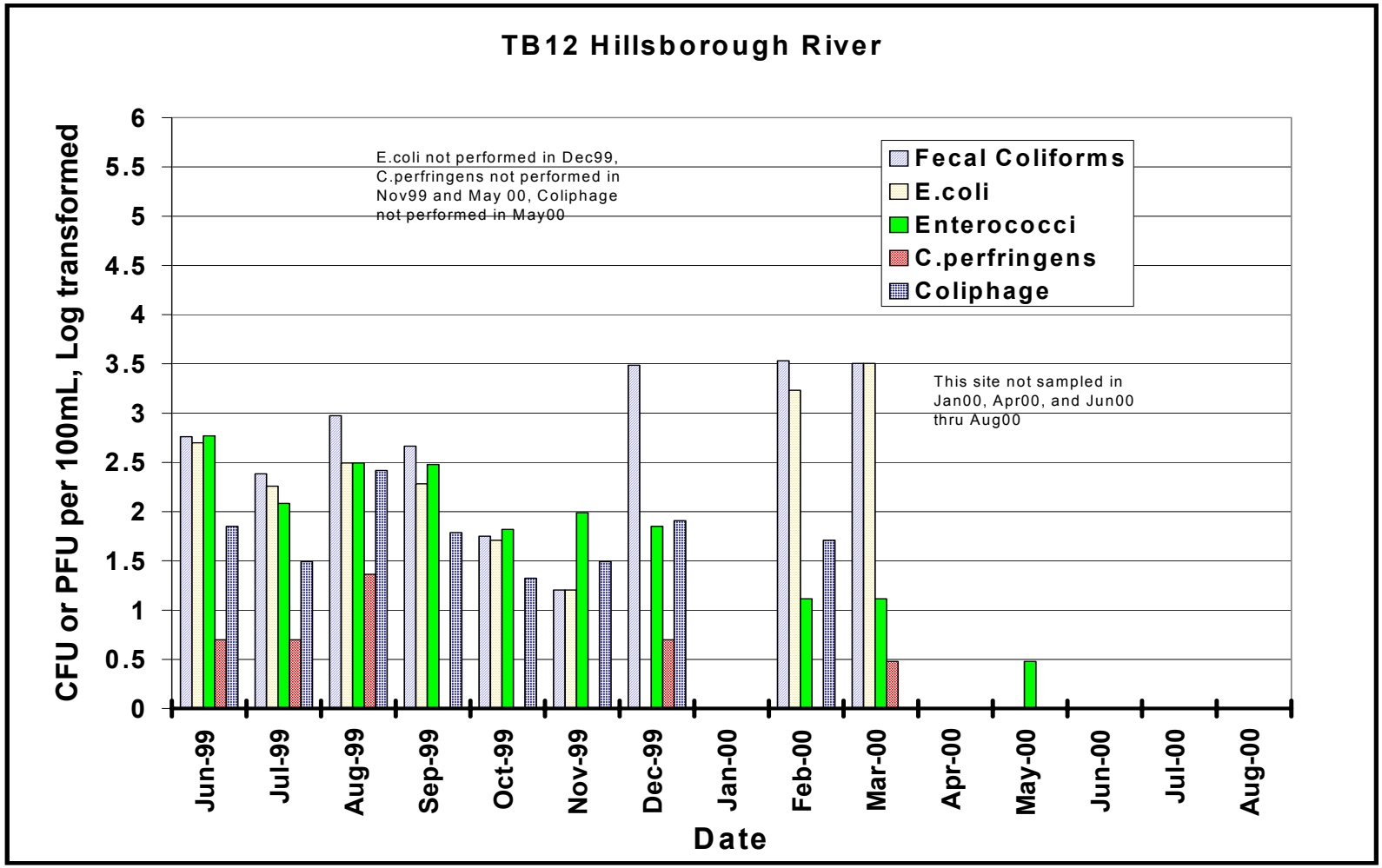


Figure 19 Indicator Levels in TB14 Sweetwater Creek

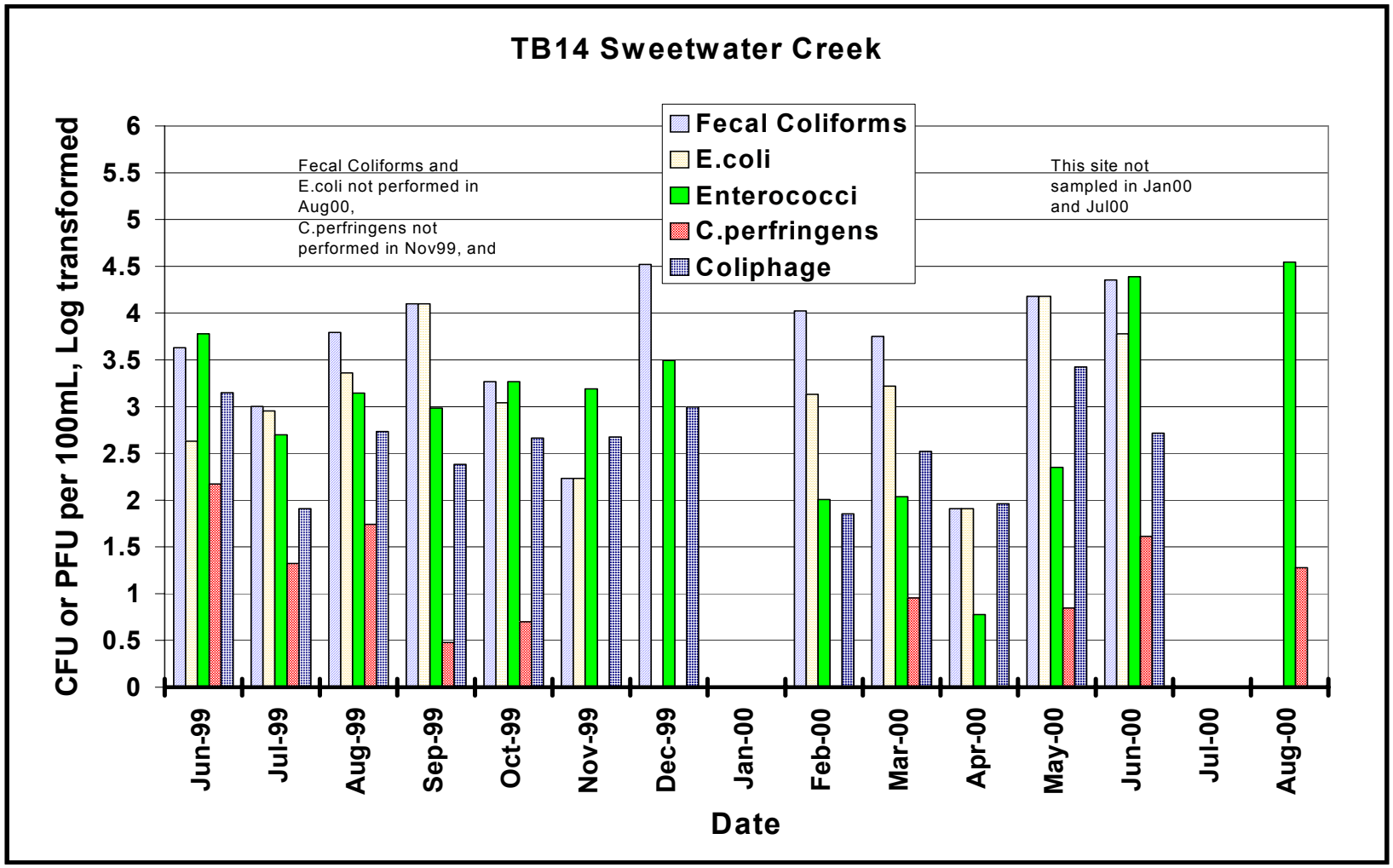


Figure 20 Indicator Levels in TB15 Lake Tarpon Canal

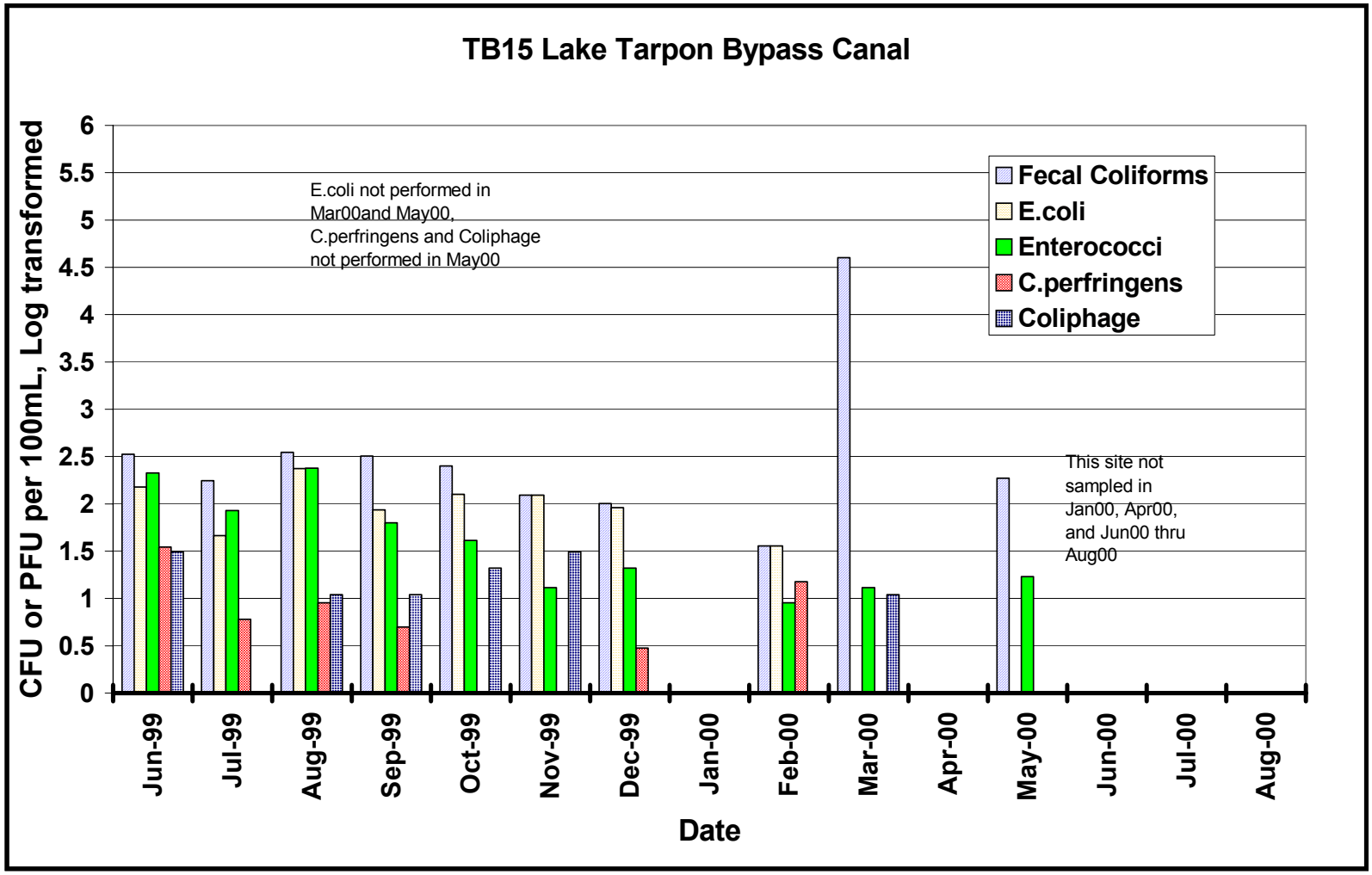


Figure 21 Indicator Levels in TB17 Allen's Creek

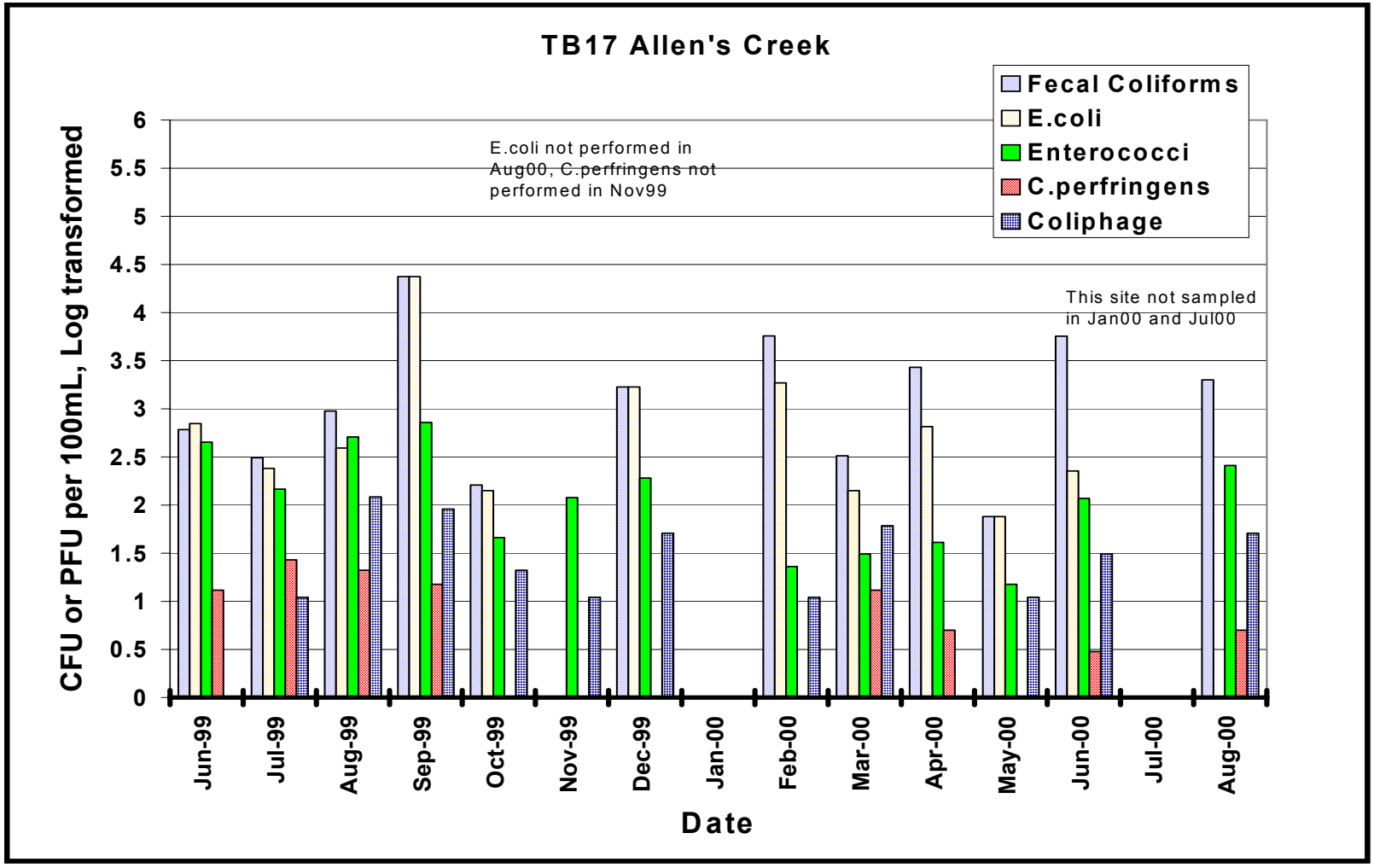


Figure 22 Indicator Levels in TB18 Joe's Creek

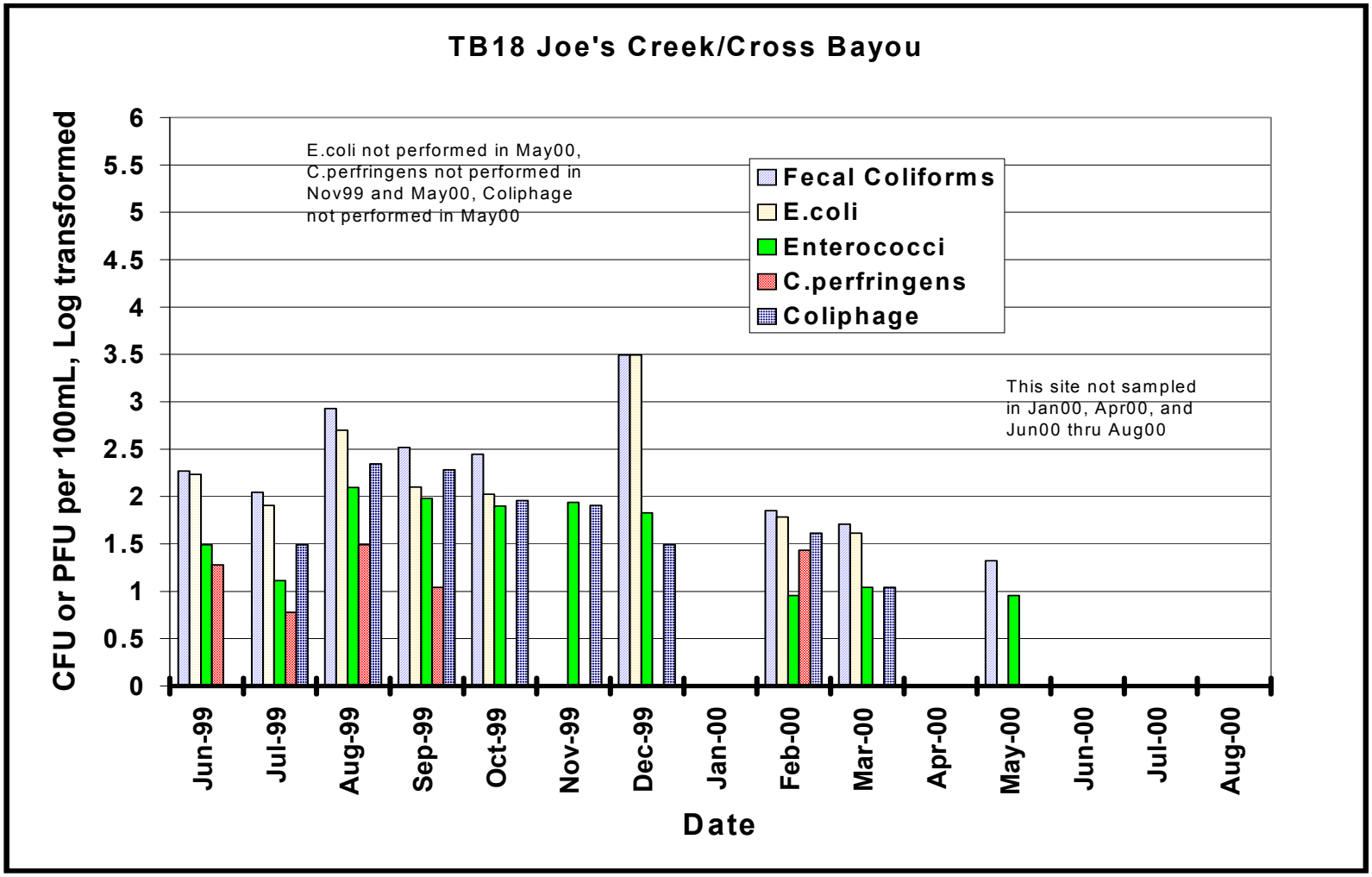


Figure 23 Indicator Levels in TB21 Salt Creek

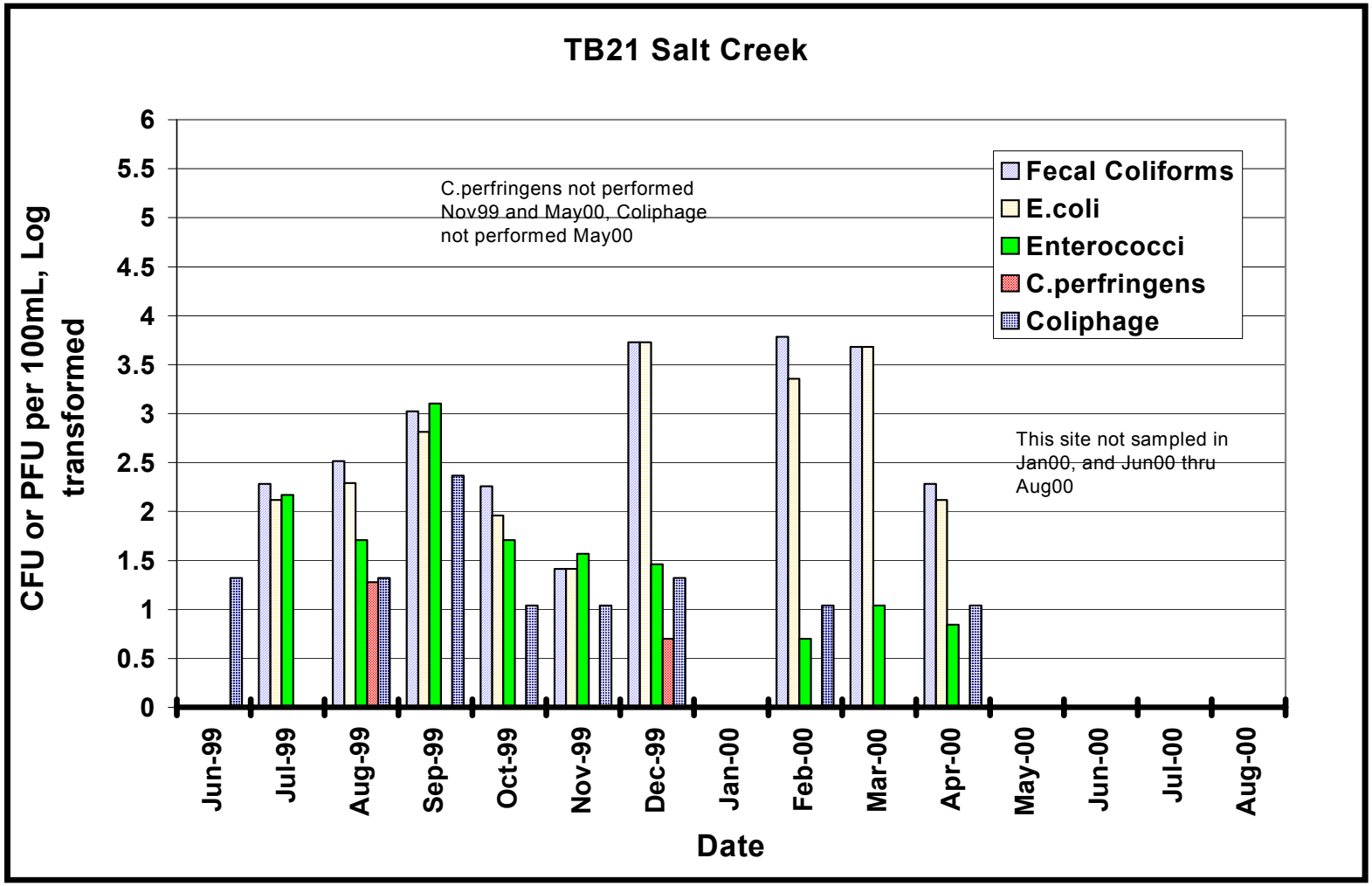


Figure 24 Indicator Levels in TB13 Courtney Campbell Causeway beach

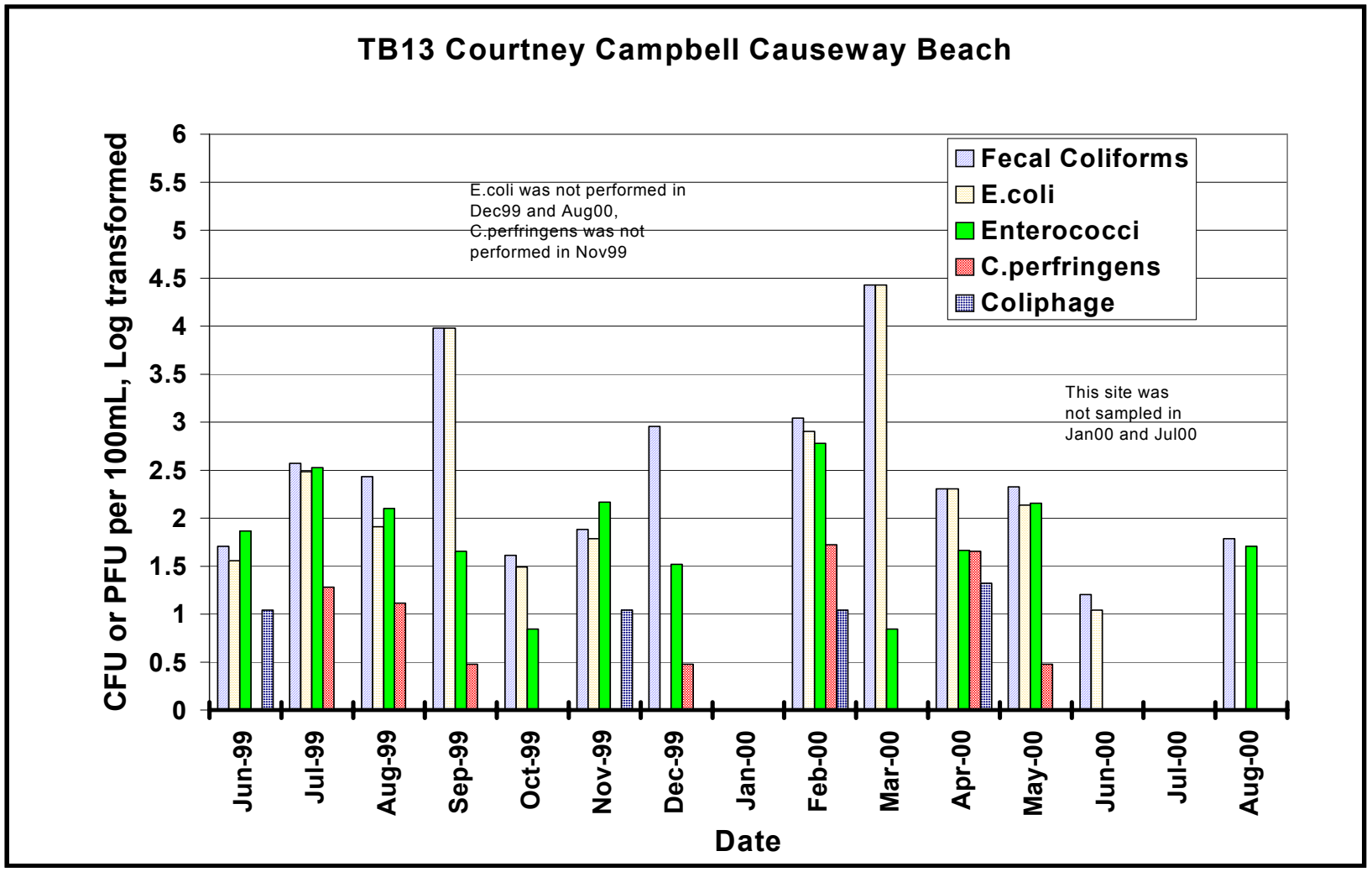


Figure 25 Indicator Levels in TB19 John's Pass

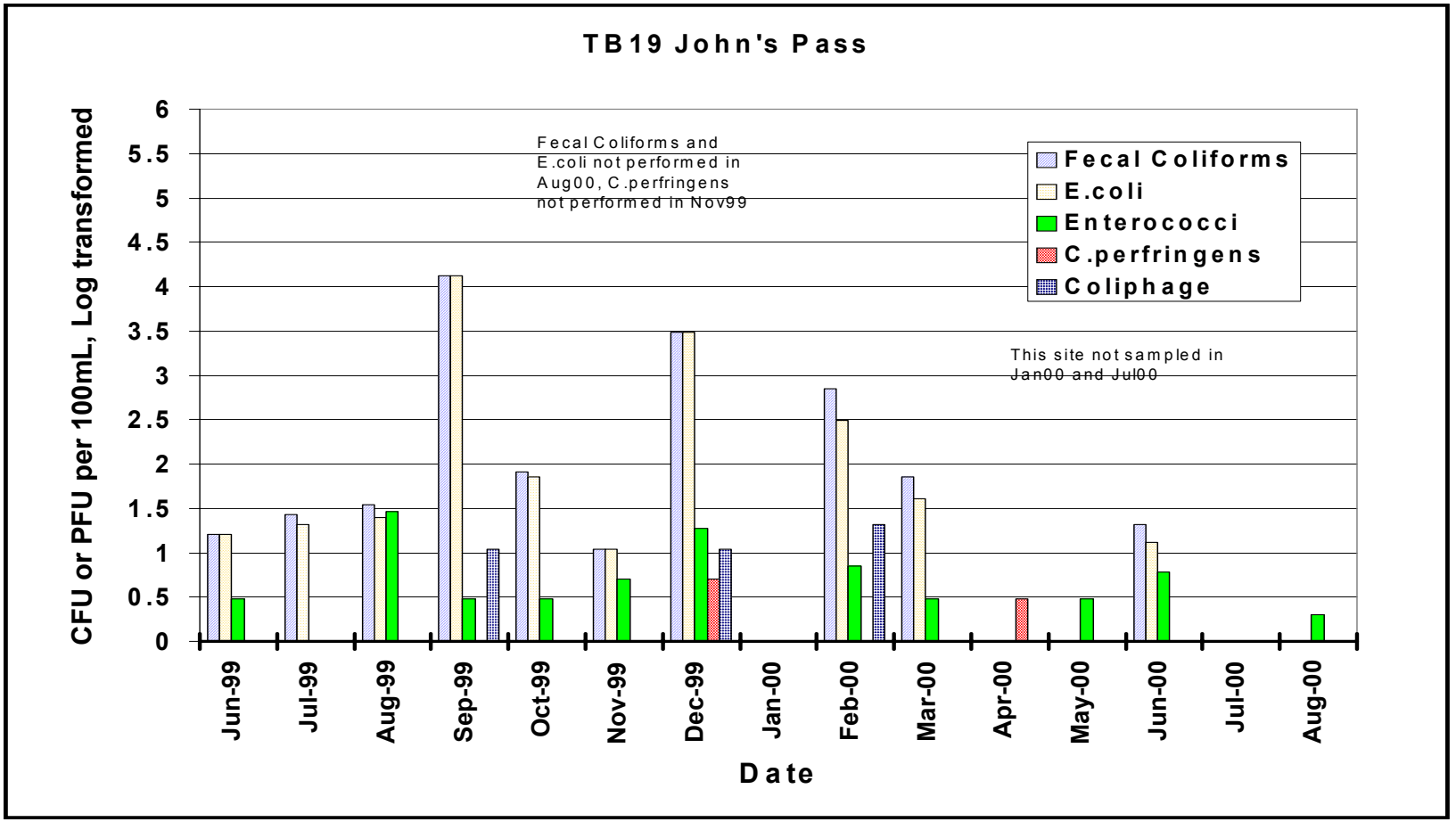


Figure 26 Indicator Levels in TB20 North Beach

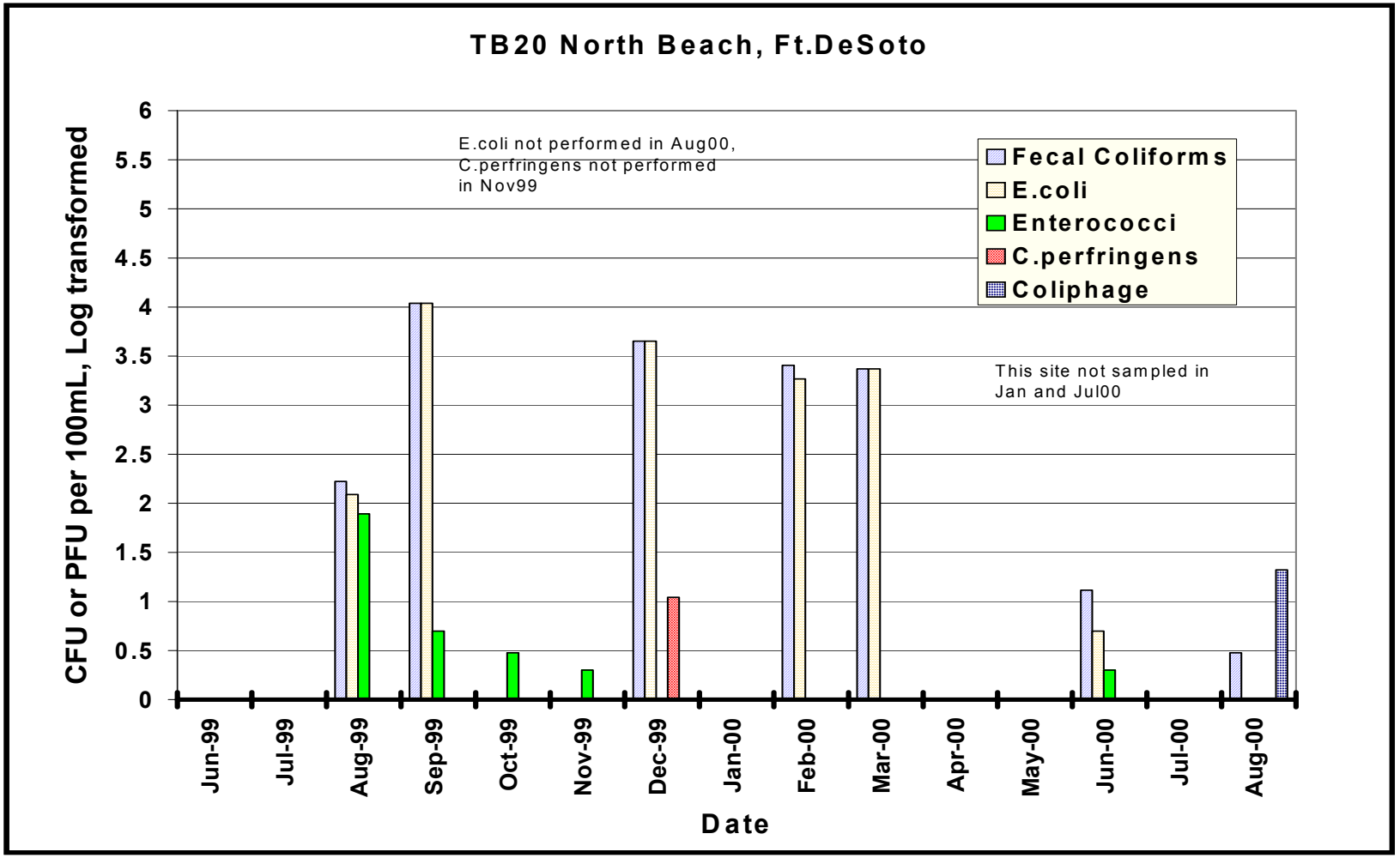


Figure 27 Indicator Levels in TB16 Honeymoon Island

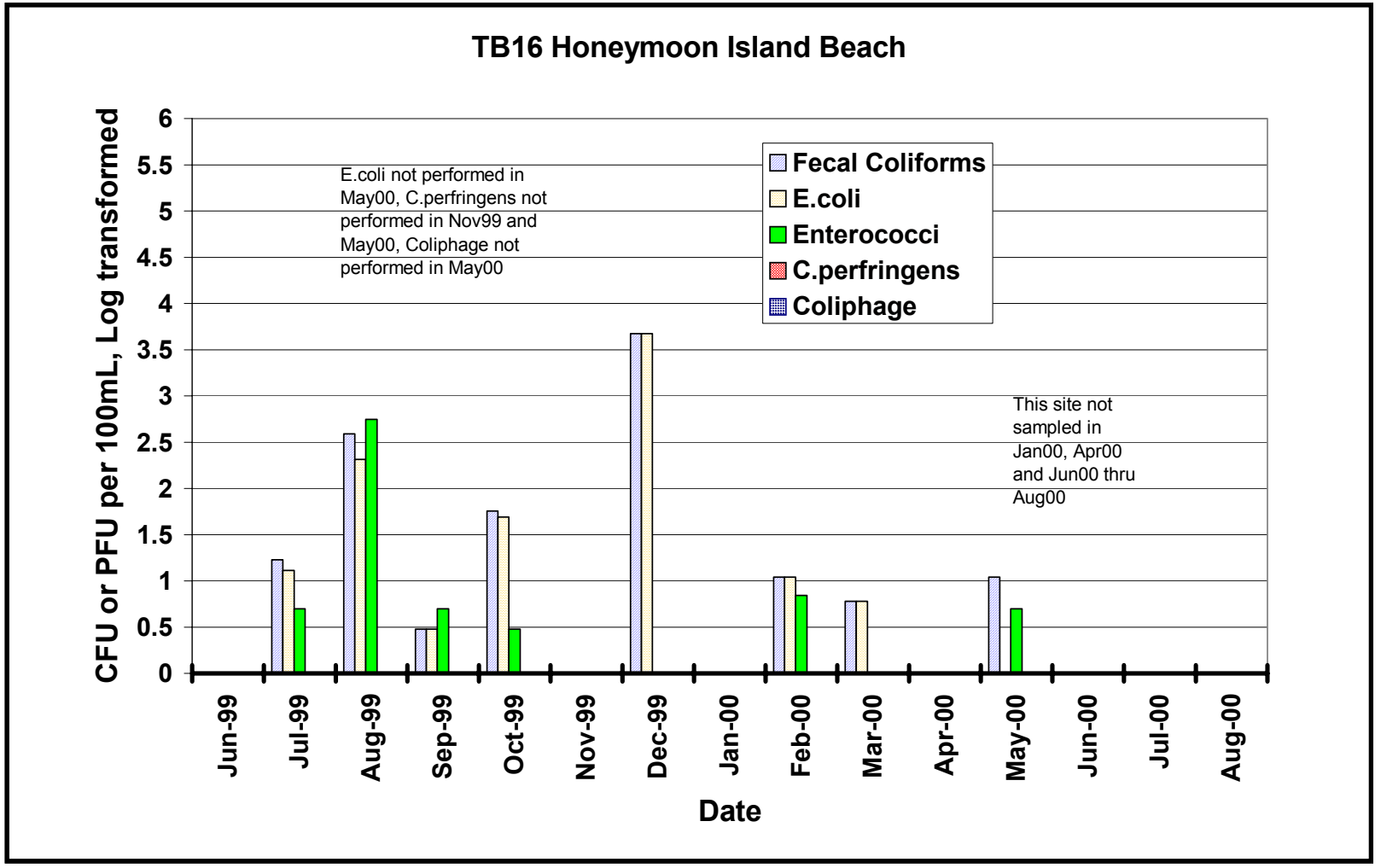
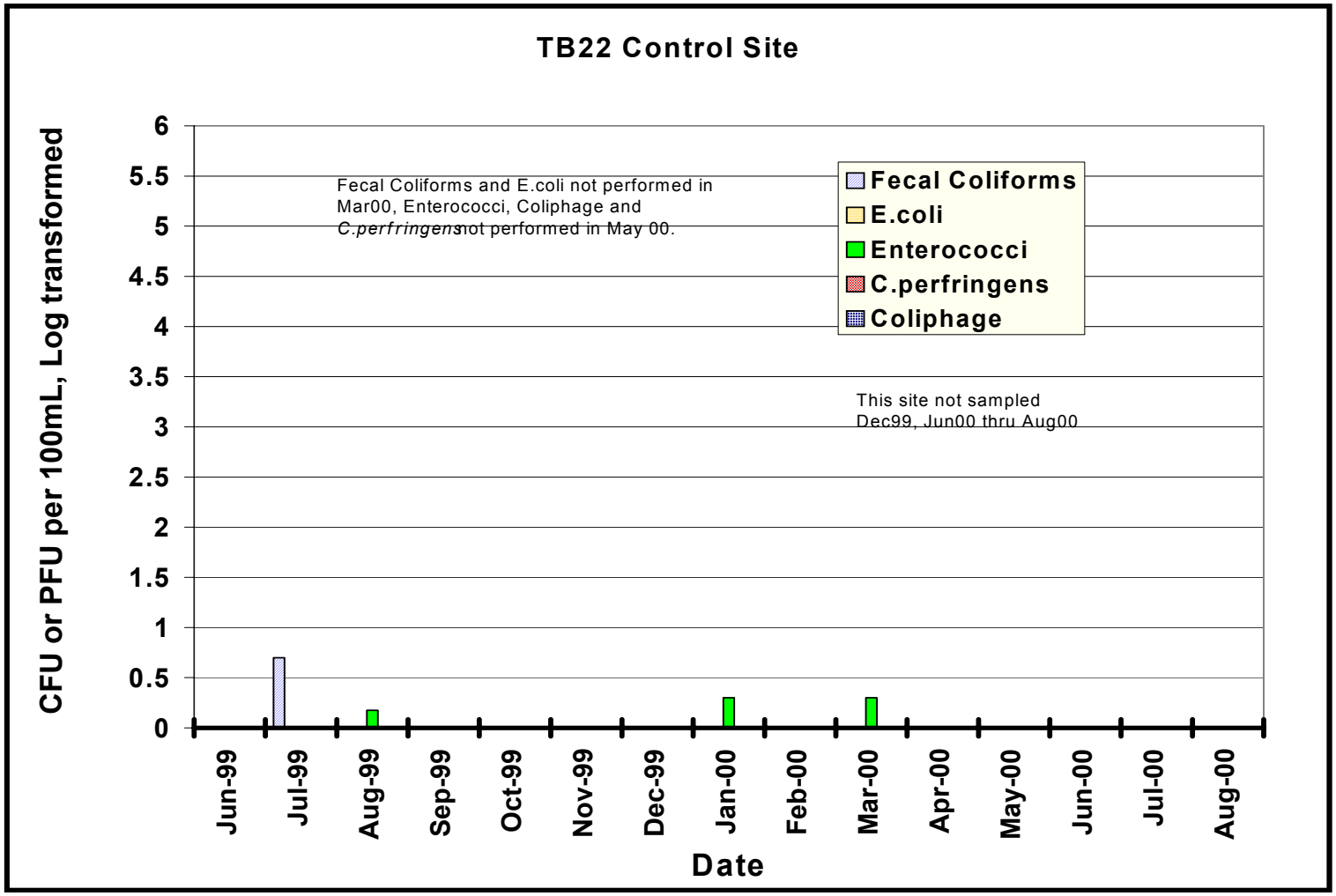


Figure 28 Indicator Levels in TB22 Control Site



Discussion of Indicator Data

The highest individual sampling result for Fecal Coliforms occurred at TB4 Bullfrog Creek, with 174,900 cfu/100 mL. The highest arithmetic and geometric mean also occurred at site TB4, with 22,687 cfu/100 mL and 5032 cfu/100 mL, respectively. *E.coli* resulted in very similar numbers, again with site TB4 Bullfrog Creek. For Enterococci, the highest individual sampling result occurred at TB4, with 135,650 cfu/100 mL, and the high arithmetic mean of 14,520 cfu/100 mL and the geometric mean of 3009 cfu/100 mL occurring at TB4 as well. *Clostridium perfringens* had the highest individual sampling result of 160 cfu/100 mL at TB4, and the highest arithmetic mean of 32.7 cfu/100 mL occurring at TB4. The highest geometric mean, however, occurred at TB3 Bullfrog Creek, with a result of 11.3 cfu/100 mL. The highest individual sampling result for Coliphage occurred at TB4 with a result of 28,180 pfu/100 mL, and the highest arithmetic and geometric mean occurred at this site as well, with 2937 pfu/100 mL and 911 pfu/100 mL, respectively.

For sites exceeding the suggested geometric guidelines, the two consistently high sites were TB4 Bullfrog Creek and TB14 Sweetwater Creek. The remaining sites along Bullfrog Creek (TB3, TB6, TB7 and TB8) were next among the highest sites when comparing indicator levels. Sites TB16 Honeymoon Island, TB19 John's Pass and TB20 Ft. DeSoto were among the lowest sites when comparing geometric means of indicator levels.

The months of Mar 00 and Dec 99 were the worst in terms of the most sites exceeding the Fecal Coliform guideline, with 15 and 13 sites respectively out of 22. Sep and Oct 99 follow closely behind with 10 sites out of 22 for both months. The months of Aug 99 and Jul 99 were the worst in terms of the most sites exceeding the Enterococci guideline, with 15 and 13 sites respectively out of 22. Sep, Oct and Nov 99 follow closely behind with 12, 11 and 10 sites out of 22 respectively.

The months of Sep 99 and Oct 99 were the worst in terms of the most sites exceeding the Coliphage guideline, with 10 sites out of 22 for both months. Aug 99, Dec 99 and Jan 00 follow closely behind with 8 sites out of 22 for all three months.

When looking at the seasonal graphs for each site, those located in rural areas show *C. perfringens* and coliphage occurring primarily in the winter and early spring months, whereas highly developed urban areas show these indicators occurring throughout the year. The exception to this is the Bullfrog Creek system, which shows indicator levels similar to that of urban sites. Most rural sites show a seasonal increase in indicator levels during the winter and early spring months, however, most urban sites are fairly consistent throughout the year. Fecal coliforms and *E.coli* levels were shown to peak without a corresponding peak in the other indicators.

E) Bacterial Source Tracking

Background

The goal of bacteriological water quality testing is to predict the risk of disease based on measured levels of bacteria and/or bacterial products. This goal has been elusive, in part because of the limitations of the methods we use. It is difficult, time-consuming and expensive to directly quantify disease-causing bacteria and viruses, and virtually impossible to test for all possible pathogens in a water sample. Thus, we quantify indicator bacteria, whose presence more-or-less reflects the probability that there are pathogens in the water. The problem with the fecal coliform indicator, in a nutshell, is that it is a poor predictor some human pathogens, particularly enteric viruses and protozoa, and may well be present in waters where there are few or no viral, bacterial or protozoan pathogens.

One of the major reasons that fecal coliforms are inadequate indicators is that they are present in the gastrointestinal tract of all warm-blooded animals. Some animal feces, i.e. those of humans, cattle, and swine, have a higher probability of containing human pathogens than the feces of most other species, therefore these animals are included in the "high risk" group. Very low levels of fecal indicator bacteria from a high risk animal group would indicate a greater potential health hazard than higher levels of indicator bacteria from a low risk animal group. Currently, there is no routine testing method that can be used to determine the origin of fecal indicator bacteria, however, such a method would allow much more accurate risk assessment than we can achieve with standard testing methods. It would also allow regulatory agencies to more effectively identify and eliminate the source of bacterial contamination to natural waters.

The enterococci (*Enterococcus* species) are another major group of indicator bacteria that have been adopted by the EPA and some other regulatory agencies as a bacteriological water quality indicator, particularly for marine and estuarine waters. Some studies have indicated that enterococcus levels are more closely correlated with cases of gastroenteritis in recreational waters than other indicator organisms (Cabelli *et al*., 1979; Cabelli,1983). The enterococci also survive longer under some environmental conditions, i.e. in saline waters, than fecal coliforms. However, the enterococci share the major disadvantage of the fecal coliform group; they are shed in the feces of all warm-blooded animals and therefore provide no indication of the source of fecal contamination.

Bacterial Source Tracking. Bacterial Source Tracking (BST) is a term that refers to a group of methods that are used to type, or fingerprint, indicator bacteria such as fecal coliforms in order to determine their source, i.e. from human, dog, wild animal, etc. BST techniques that are currently in use measure characteristics such as antibiotic resistance to generate the fingerprint streams (Hagedorn *et al.,* 1999; Harwood *et al*., 2000; Wagner and Harwood, 1999; Wiggins, 1996; Wiggins *et al.,* 1999), or they may analyze differences at the level of the DNA fingerprint, as in ribotyping (Parveen *et al.,*1999. All

BST techniques rely on the establishment of a large database of "fingerprints" of indicator bacteria from known sources, i.e. humans, cattle, wild animals, etc. Fingerprints of bacteria isolated from water samples can then be statistically compared to the fingerprints in the database, allowing the investigator to determine the source of fecal contamination to the water.

Bacterial resistance to antibiotics can be used to differentiate between indicator bacteria that are shed in the feces of various animals. Humans are exposed to a different set of antibiotics than are cattle, and poultry to a different set, and so on, while wild animals have relatively little exposure to antibiotics. Bacteria rapidly develop resistance to specific antibiotics when the animals in which they live are frequently treated with those antibiotics. It has been shown that antibiotic resistance patterns are significantly different in fecal indicator bacteria from different host sources, and that these differences are consistent enough that they can be used to predict the source of bacteria isolated from rivers and streams (Hagedorn *et al.,* 1999; Harwood *et al*., 2000; Wagner and Harwood, 1999; Wiggins, 1996; Wiggins *et al.,* 1999).

The method, termed **antibiotic resistance analysis** (ARA) is carried out by first developing a database of antibiotic resistance patterns (ARPs) of indicator bacteria from known animal sources. Other investigators currently using this technique (Hagedorn and Wiggins) use fecal streptococci or enterococci as the indicator organism. Our laboratory uses both enterococci and fecal coliforms. In order to construct a database, fecal coliforms (for example) are isolated by membrane filtration from feces obtained from known animal sources. These isolates are plated on a battery of antibiotic-containing media and are scored positive or negative for growth on each plate. Currently we are using eight different antibiotics at four concentrations each, so the ARP of each isolate consists of 32 data points. The procedure for determining the ARPs of isolates requires four to five days.

ARPs of bacteria from known sources are entered in a spread sheet. Discriminant analysis, a form of multiple analysis of variance, is used to analyze the data. Discriminant analysis uses the ARPs from known sources to generate the predictive equations (the "classification rule") that will be used to classify unknown isolates by source. The accuracy of the database is assessed by using ARPs of the isolates from known sources as test data. This procedure generates a source-by-source matrix that provides the rate of correct classification for each source. Below is an example. The top number is number of isolates, the bottom is percent classified in a given category.

Table 11 Fecal Coliform Database Correct Classification Rates

Fecal Coliforms From SOURCE	bird	cow	dog	huma	pig	Total
bird	258	10	1	1	2	272
	94.85	3.68	0.37	0.37	0.74	100.00
cow	6	219	17	25	37	304
	1.97	72.04	5.59	8.22	12.17	100.00
dog	3	9	273	0	3	288
	1.04	3.13	94.79	0.00	1.04	100.00
huma	21	15	23	246	12	317
	6.62	4.73	7.26	77.60	3.79	100.00
pig	4	55	2	20	252	333
	1.20	16.52	0.60	6.01	75.68	100.00
Total	292	308	316	292	306	1514
Percent	19.29	20.34	20.87	19.29	20.21	100.00

In this case 258 out of 272 (94.9%) isolates obtained from bird feces were classified as Source = bird by the database. Reading diagonally, 72% of cow isolates were classified correctly, 94.8% of dog isolates, 77.6% of human isolates and 75.7% of pig isolates were classified correctly. The average rate of correct classification (ARCC) indicates the overall accuracy of the database. For this database the ARCC is 83%. When the database is sufficiently representative of ARPs in the area, it can be used to predict the animal source of indicator bacteria that are contaminating the waters under investigation.

The ARA database used in this study contains fingerprints from 3309 fecal coliform isolates, of which 1154 are from humans and the remainder are from chickens, cattle, dogs, pigs and wild animals (mostly wild birds and raccoons). Generally, 48 isolates per site were analyzed, although there were exceptions when few fecal coliforms were isolated from the samples. *E. coli* isolates were differentiated from other fecal coliforms by the MUG test, and this data was also analyzed by ARA. Because the *E. coli* results are virtually indistinguishable from the fecal coliform results, the fecal coliform data is presented here.

Ribotyping was performed by the method of Parveen *et al.* (1999), using *E. coli* as the indicator organism. This database contains isolates from human and nonhuman (mostly wild) sources. 1 – 5 *E. coli* isolates per sample were analyzed

Enteroviruses are viruses that are thought to exclusively colonize humans, thus, they are specific indicators of human contamination. In this study, entervirus counts carried out on human cells lines were conducted to indicate the prevalence of human contamination at the Healthy Beaches sites.

Five sites in each area (the Bullfrog Creek watershed and Pinellas County) were chosen for the BST analysis. The Bullfrog Creek sites were chosen to represent a course from the headwaters to the mouth of the creek, while the Pinellas County sites were chosen to represent freshwater (TB 14 and TB17, Gulf of Mexico (TB 19 and TB 20) and Tampa Bay (TB13) environments. Each site was sampled six times over the course of the study.

Identification of Pollution from Human Sources. All sites displayed some level of human fecal pollution according to the three methods used (ribotyping, ARA and enterovirus counts). The three different methods did not always coincide on their prediction of the presence or absence of human contamination (see below), however the data collected over the course of the study unambiguously documents the presence of human fecal sources. A value called the Impact of Human Pollution (IHP) was devised in order to represent the combined results of the three methods of human source identification for each site. The percentage of human-positive sampling events for each site was first calculated for each site for each of the three methods. These values were added, then multiplied by 0.5 (for ease of graphical representation). For example, TB3 was human-positive by ribotyping in 33.3% of samples, by ARA in 80% of samples, and by enterovirus counts in 66.7% of samples. The IHP is calculated as (0.333 + 0.80 + 0.667)/2 = 0.90.

Overall, sites in Bullfrog Creek (Figure 29) were more frequently impacted by human sources than sites in Pinellas County (Figure 30). Sites TB3 and TB4 in the Bullfrog Creek watershed were the sites most consistently impacted by human pollution. Bullfrog Creek is bordered by fairly intensive residential and commercial land use at TB3, and all wastewater treatment is by onsite wastewater treatment and disposal systems (OSTDS). TB4 is the closest downstream site to a septage spreading site that is now closed, but which was operational during the course of the study. While TB3 is the site closest to the mouth of Bullfrog Creek, TB8 is the site located at the headwaters, and Figure 1 reveals a steady decrease in human impact from urbanized TB3 to the rural TB8. A possible source of sporadic human contamination at TB8 is a residence with an OSTDS.

The site most impacted in Pinellas County was Allan's Creek (TB 17), followed closely by Sweetwater Creek (TB14) and the Courtney Campbell Causeway (TB13). The Gulf of Mexico beach sites included John's Pass (TB19) and Fort DeSoto (TB20), which showed lower levels of human contamination than the freshwater and brackish water sites.

The Index of Human Pollution was significantly correlated with both *Enterococcus* (Figure 31) and fecal coliform (Figure 32) counts. For these analyses indicator organism counts are expressed as the geomean for each each site over the course of the study. Linear regression analysis yielded values of $P < 0.05$, $r^2 = 0.5023$ for IHP vs. *Enterococcus* counts, and $P < 0.05$, $r^2 = 0.4832$ for IHP vs. fecal coliforms counts. Of the individual source-specific techniques, only the percent enterovirus – positive sites was correlated with the average *Enterococcus* count for each site ($P < 0.05$, $r^2 = 0.4292$). Two of the three source-specific methods correlated with average fecal coliform numbers; enterovirus ($P < 0.05$, $r^2 = 0.4728$) and ribotyping ($P < 0.05$, $r^2 = 0.4265$) were significantly correlated, while ARA was not.

Agreement Between Source Tracking Methods. Agreement between the two bacterial source tracking methods (antibiotic resistance analysis and ribotyping) and enterovirus counts was assessed for each sampling event (Figure 33). Sites were scored positive for human impact when >20% of isolates were identified as human by ribotyping and by ARA, and when any enterovirus counts were detected. Sites were scored negative for human impact when <20% of isolates were identified as human by ribotyping and by ARA, and when no enterovirus counts were detected (<1/100 ml). Ribotyping and ARA results agreed for 31 of 53 samples (58.5%). Ribotyping and enterovirus results agreed for 29 of 52 (55.8%) samples. ARA and enterovirus results agreed most frequently, as positive results at the same sites were noted for 38 of 55 sampling events (69.1%). All three methods agreed for 21 of 51 samples (41.2%). There was no correlation between the percent of isolates identified as human by ribotyping and enterovirus counts. The Spearman rank correlation test (used for non-normally distributed data) showed a significant correlation between the percent of isolates identified as human by ARA and enterovirus counts ($p < 0.05$; $r = 0.324$). Fisher's exact test for difference in frequency showed no significant difference in the percent of sites where methods agreed when comparisons were made between ARA vs. ribotyping, ARA vs. enterovirus, and ribotyping vs. enterovirus.

Other Sources of Fecal Coliforms. Wild animals were the dominant source of fecal coliforms identified by antibiotic resistance analysis at each site (Figure 34). At TB 3, TB4 and TB6, human sources were almost equal those of wild animals. Recall that the Index of Human Pollution is also high at each of these sites. More isolates were assigned to the chicken and cattle categories in the Bullfrog Creek watershed (TB3 – TB8) than in the Pinellas County sites, which can be attributed to the agricultural activity that dominates the upper reaches of the watershed. In fact, chicken and cattle are insignificant sources (<10%) of isolates except at TB19, where 24.6% of all isolates were identified as from cattle. At only one sample event on 6/20/00 were cow isolates identified at TB19, when 48 of 48 fecal coliforms were typed as cattle isolates. One can speculate that this event may have been due to a hamburger wrapper from picnickers entering the water or some other unusual event. When a sporadic spike of contamination from a particular source occurs it is of less conern overall to watershed management than persistent input from a particular source. At the Pinellas County sites (13 – 20), wild animal sources were

particularly dominant (Figure 34). A prominent dog signal is present at TB20 (Fort DeSoto), which may be due to the practice of exercising dogs on the beach.

Discussion

This study has shown that an approach that combines several source identification techniques can be useful for watershed assessment and management. Perhaps one of the most striking findings of this study is the extent to which wild animals dominate as a source of fecal coliform and *E. coli* isolates. Over the course of the study, wild animal isolates dominated each site according to ARA. The ribotyping analysis agreed; in 73.6% of all samples (n=53) the majority of isolates were identified as nonhuman.

Nevertheless, all of the source-specific methods used in this study indicate that human pollution is significantly impacting the Bullfrog Creek watershed. This study suggests that OSTDS and associated activities may be a significant source of pollution to the Bullfrog Creek watershed, as there is no central sewer infrastructure in the watershed and there was at least one operational septage spreading site between TB4 and TB6.

The consistent impact from human sources is less clear at the Pinellas County sites, although there are days when "spikes" of human isolates dominate these sites. For example, on 6/20/00 TB 17 (Allan's Creek) 94% of fecal coliforms were identified as human isolates by ARA, 80% of isolates were human by ribotyping, and enterovirus were detected. However, such events are atypical of the Pinellas Creek sites, and were more likely to be observed at the Bullfrog Creek sites.

The advantage to using a "toolbox" approach, where several source tracking methods are combined, is clear from this study. Identification of enterovirus in water represents a rigorous test for human contamination, however this technique is very expensive, and a negative result does not preclude human contamination. Two major advantages to ARA as performed in this study include (1) the fact that 48 – 96 isolates per sample can be readily analyzed, thus providing the investigator some assurance that the population is adequately sampled, and (2) non-human sources of contamination can also be identified. Ribotyping can also be used to identify non-human sources if the database is constructed adequately for that purpose; both Dr. Lukasik and Dr. Harwood are working on ribotyping databases to identify multiple sources.

The percentage of isolates identified as human by ARA was significantly correlated with enterovirus counts, but the percentage of isolates identified as human by ribotyping was not significantly correlated with enterovirus counts. This discrepancy points to the need for including the fingerprints of more isolates from known, **local** sources in the respective databases. In the case of ARA, we have seen dramatic improvements in correct classification rates by adding fingerprints from local sources. The genetic and phenotypic variability of indicator bacteria such as *E. coli* is quite great, therefore any information that can be obtained on the fingerprints of actual contamination sources to a watershed is

extremely valuable. Encouragingly, ribotyping, ARA and enterovirus counts agreed on the presence/absence of human sources in 41.2% of samples. The probability of the three methods agreeing by chance alone is 0.125 (0.5 X 0.5 X 0.5), therefore the three methods agree on the presence of contamination far more frequently than would be predicted by a purely stochastic process.

Future Directions

Databases (ribotyping and ARA) are currently under construction that will be more accurate than those used in this study. Although the current databases are valuable tools for environmental analysis, correct classification rates for isolates from known sources can be improved. We are also very interested in analysis of *E. coli* from sediments, as it has been shown that long survival times can be attained by fecal coliforms deposited to sediments. During times when sediment bacteria are resuspended by waves, current action, or human/animal activities, these organisms may comprise a significant portion of the population in surface water. We are also investigating the growth and survival of fecal coliforms, *E. coli* and *Enterococcus* species in the subtropical waters, soils and sediments of Florida.

Figure 29

Bacterial Source Tracking: Comparison of ARA, RT and Enterovirus in Bullfrog Creek Sites

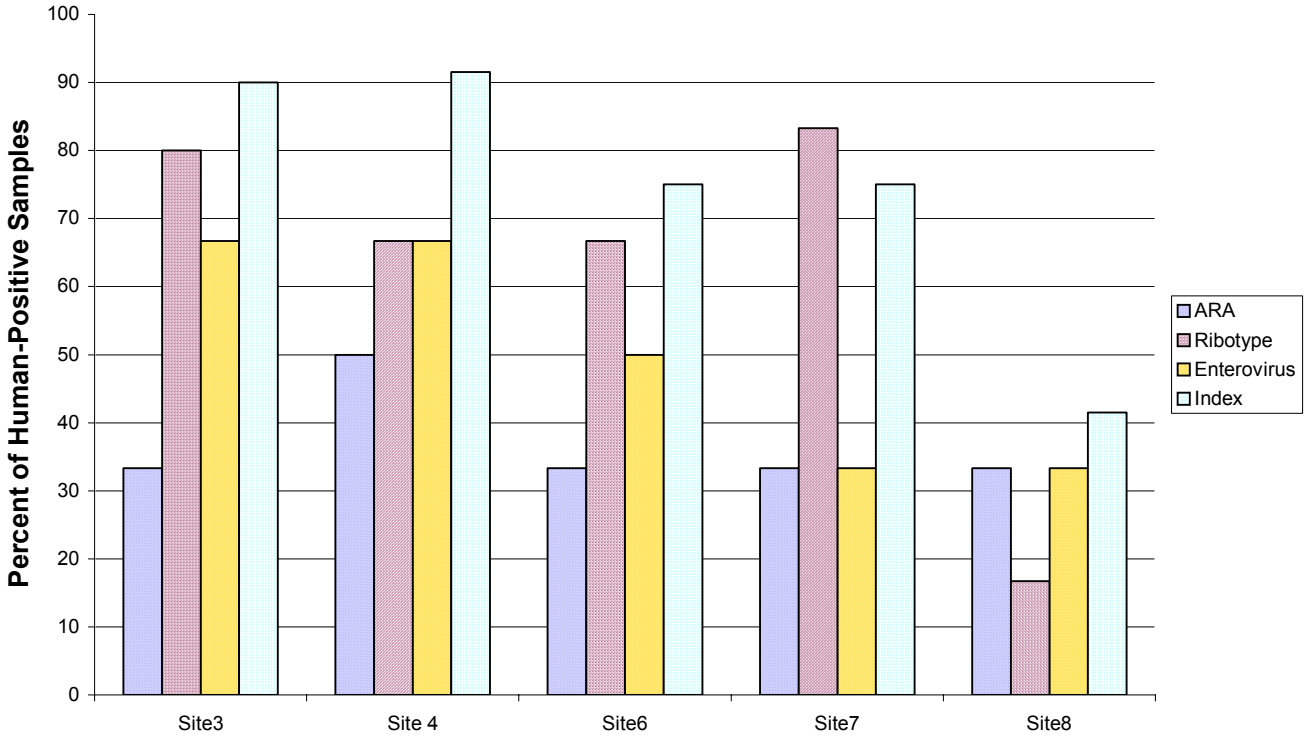


Figure 30

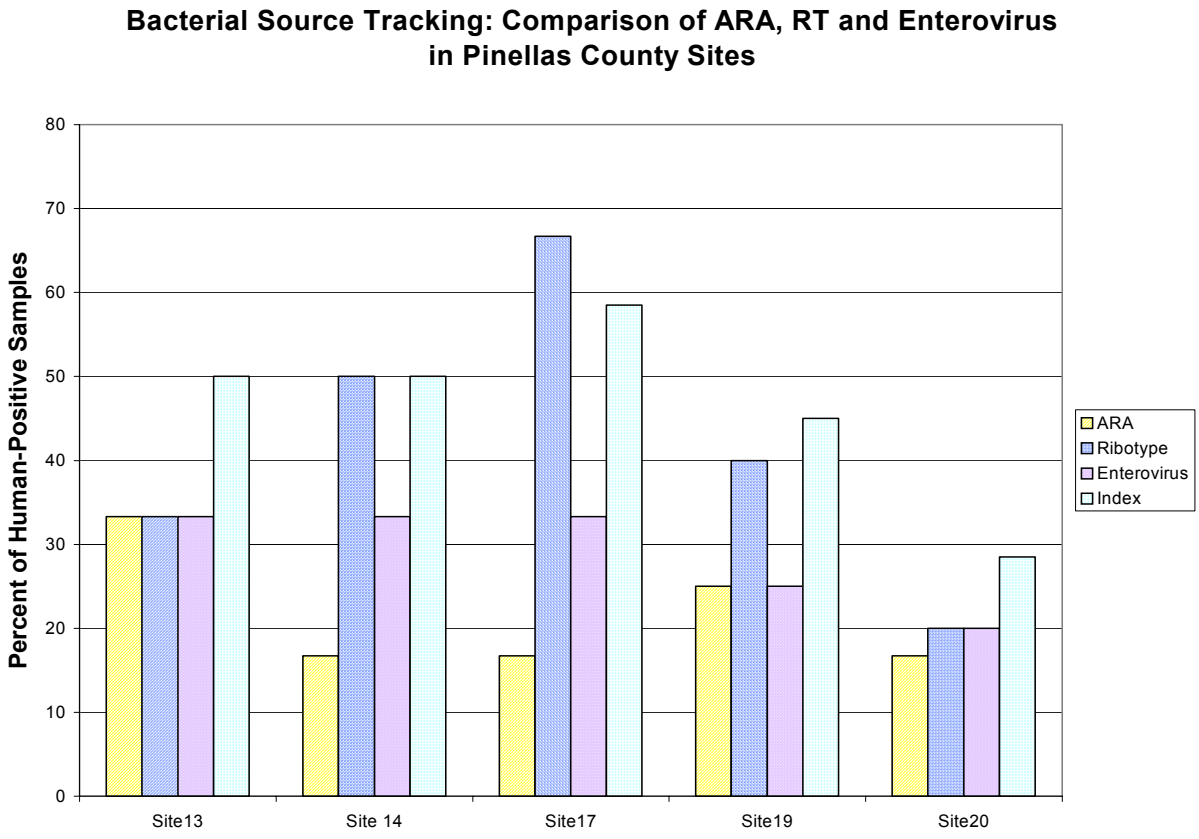

Bacterial Source Tracking: Comparison of ARA, RT and Enterovirus in Pinellas County Sites
Percent of Human-Positive Samples
80
70
60
50
40
30
20
10
0
Site13
Site 14
Site17
Site19
Site20
ARA
Ribotype
Enterovirus
Index

Figure 31. *Enterococcus* Counts vs. IHP

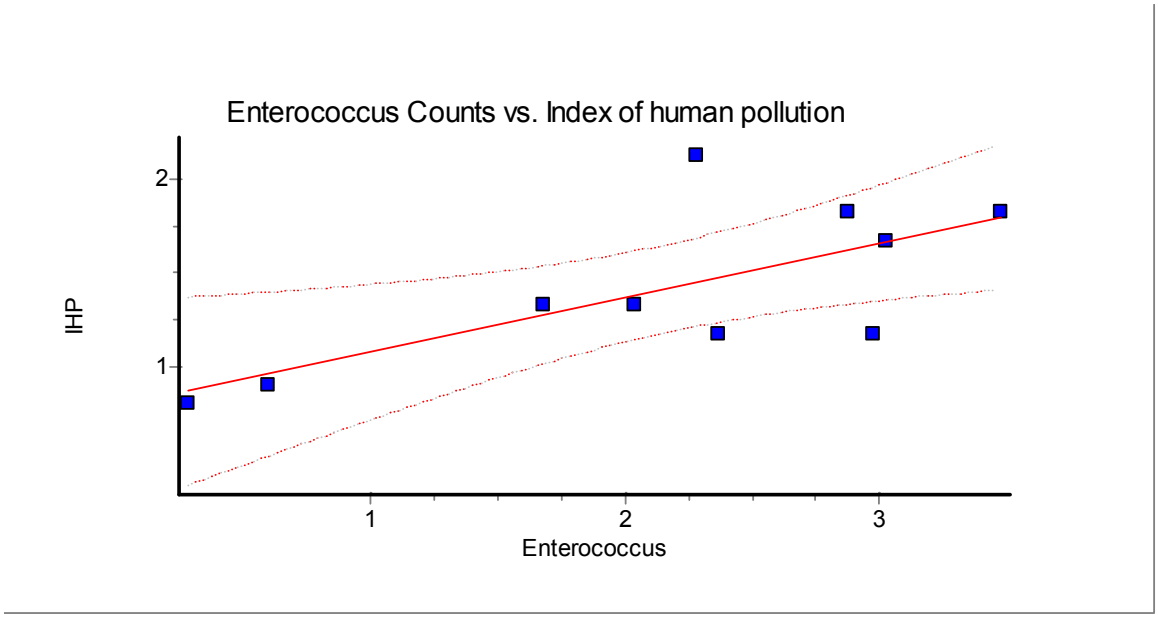


Figure 32. Fecal Coliform Counts vs. IHP

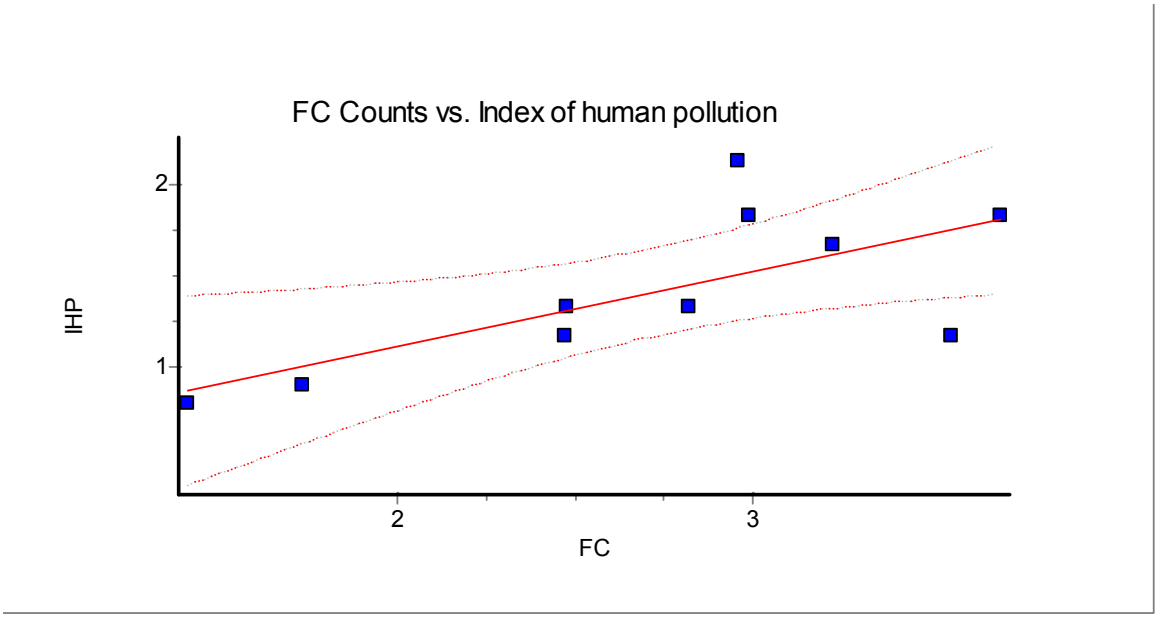


Figure 33

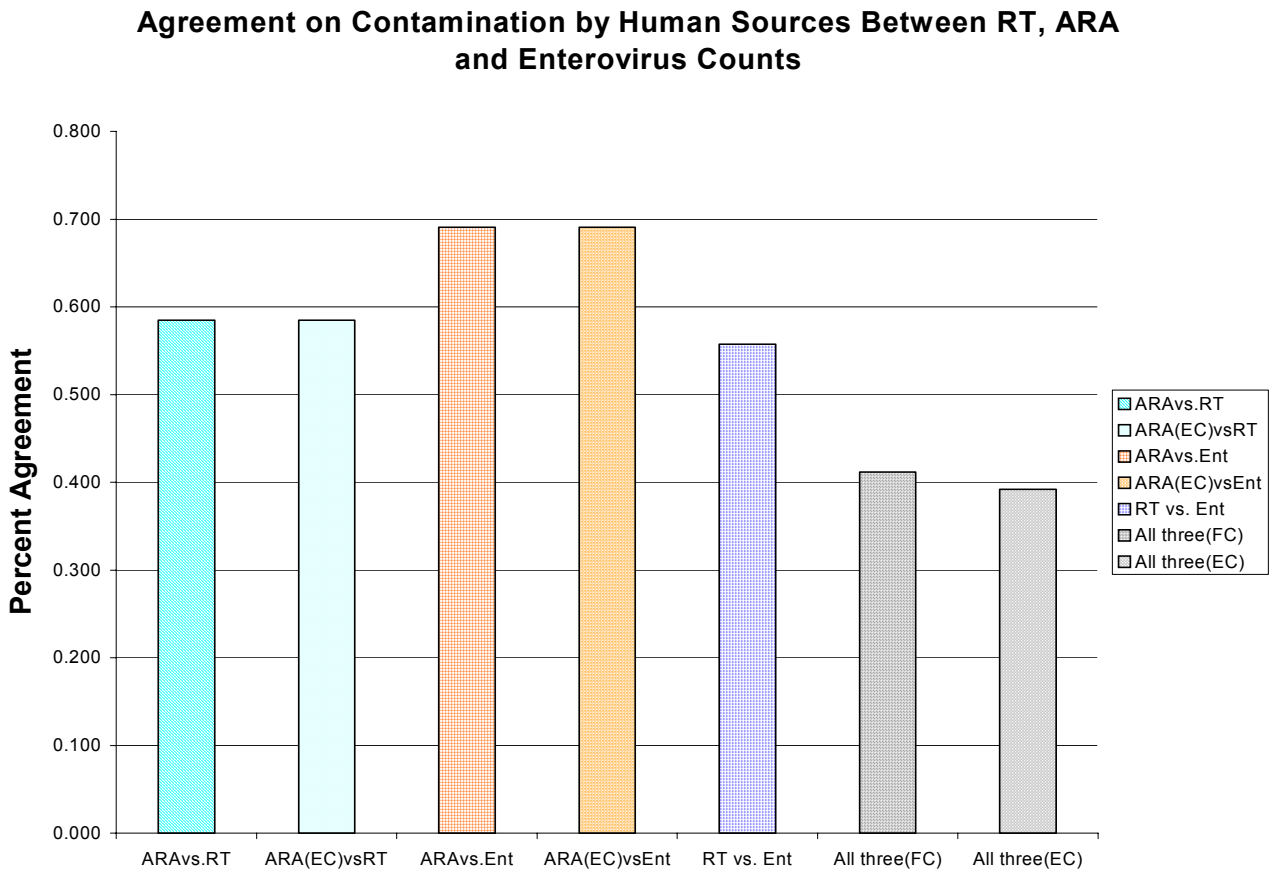
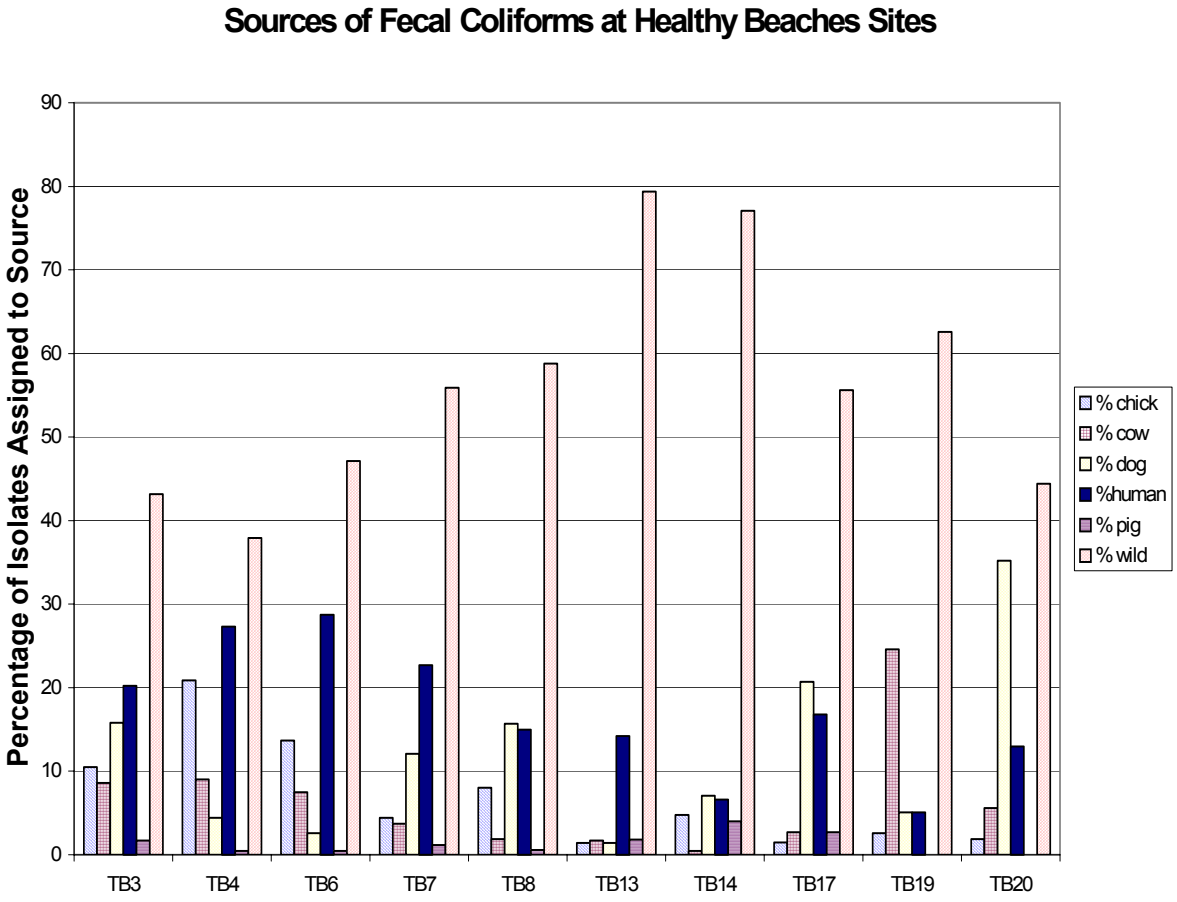

Agreement on Contamination by Human Sources Between RT, ARA and Enterovirus Counts
Percent Agreement
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
ARAvs.RT
ARA(EC)vsRT
ARAvs.Ent
ARA(EC)vsEnt
RT vs. Ent
All three(FC)
All three(EC)

Figure 34



Note: Raw data of Ribotyping Results are found in Appendix VII

F) Pathogen Monitoring

Tables 12 and 13 below show the results of the Enterovirus monitoring for the 10 in-depth sampling sites (TB3, TB4, TB6-TB8 along Bullfrog Creek, TB14 Sweetwater Creek, TB17 Allen's Creek, TB13 Courtney Campbell Causeway, TB19 John's Pass and TB20 North Beach, Ft. DeSoto). The site designation, month of sampling, and results in MPN-PFU per 100 L of water are given in Table 12. Of the 59 samples collected for virus testing, twenty-three (47%) were positive. The raw data reports for the Enterovirus analysis can be found in Appendix VIII. One or two positive results were detected each month, however, the highest number of isolations were found in September and October 1999 (with 3 and 4 sites positive out of 5, respectively), which corresponds to the indicator peak found in the rural sites during October 1999, and the September 1999 peak found in the urban and beach sites. The virus levels ranged from 1.1 to 27.1 MPN-PFU/100 L

Table 13 summarizes the positive results for each of the sampling sites. Bullfrog Creek overall shows consistent Enterovirus results, with TB3 and TB4 showing the highest percentage of positive results. The two urban sites, and the three beach sites had 1-2 positive results during the length of the study.

A Table showing the parasite data can be found in Appendix IX. Out of 20 samples collected from the in-depth sites (10 sites sampled 2 times during the study), no *Giardia* were detected during the study. Sites TB3, TB4, TB7 and TB8 along Bullfrog Creek all showed the presence of *Cryptosporidium* with results of 3.48 oocysts per 100 L of water for TB7, 7.03 oocysts/100 L for TB8, 124.4 oocysts/100 L for TB4 and 470 oocysts/100 L for TB3. Each site tested positive for *Cryptosporidium* only once during the study.

Table 12 Enterovirus Levels in Tampa Bay

Site	Date	Enterovirus MPN/100 L
TB3	Aug 99	2.3
TB4	Aug 99	7.0
TB6	Aug 99	Neg <0.95
TB7	Aug 99	Neg <1.1
TB8	Aug 99	Neg <1.2
TB13	Sept 99	Neg <1.1
TB14	Sept 99	Neg <1.2
TB17	Sept 99	8.4
TB19	Sept 99	1.1
TB20	Sept 99	2.7
TB3	Oct 99	5.1
TB4	Oct 99	7.1
TB6	Oct 99	2.4

Table 12 con't		
Site	**Date**	**Enterovirus MPN/100 L**
TB7	Oct 99	9.4
TB8	Oct 99	Neg <0.95
TB4	Dec 99	27.1
TB6	Dec 99	Neg <1.02
TB7	Dec 99	Neg <1.01
TB8	Dec 99	11.3
TB13	Feb 00	Neg <1.05
TB14	Feb 00	Neg <1.08
TB17	Feb 00	Neg <1.12
TB19	Feb 00	Neg <0.75
TB20	Feb 00	Neg
TB3	Mar 00	1.26
TB4	Mar 00	Neg <1.03
TB6	Mar 00	Neg <1.10
TB7	Mar 00	1.23
TB8	Mar 00	Neg <1.00
TB13	Apr 00	1.2
TB14	Apr 00	Neg <1.1
TB17	Apr 00	Neg <1.1
TB19	Apr 00	Neg <1.1
TB20	Apr 00	Neg <1.1
TB13	May 00	1.2
TB14	May 00	Neg <1.0
TB17	May 00	Neg <1.1
TB19	May 00	Neg <1.1
TB13	Jun 00	Neg <1.2
TB14	Jun 00	10.9
TB17	Jun 00	1.2
TB19	Jun 00	Neg <1.1
TB20	Jun 00	Neg <1.1
TB3	July 00	Neg <1.2
TB4	July 00	1.3
TB6	July 00	2.5
TB7	July 00	Neg <1.1
TB8	July 00	Neg <1.1
TB3	Aug 00	1.1
TB4	Aug 00	Neg <1.2
TB6	Aug 00	4.3
TB7	Aug 00	Neg <1.1
TB8	Aug 00	1.1
TB13	Aug 00	Neg <1.1
TB14	Aug 00	4.3

Table 12 con't		
Site	**Date**	**Enterovirus MPN/100 L**
TB17	Aug 00	Neg <1.1
TB19	Aug 00	Neg <1.2
TB20	Aug 00	Neg <1.2

Table 13 Percentage of Enterovirus Positives by Site

Site Type	Site	# of samples positive	Total samples collected
Rural	TB3 Bullfrog	4	5
	TB4 Bullfrog	4	6
	TB6 Bullfrog	3	6
	TB7 Bullfrog	2	6
	TB8 Bullfrog	2	6
Urban	TB14 Sweetwater	2	6
	TB17 Allen's	2	6
Beach	TB13 Courtney C.	2	6
	TB19 John's Pass	1	6
	TB20 Ft. Desoto	1	6

G) *Bacteroides fragilis* Phage Assay

The alternative indicator, *Bacteroides fragilis* phage, was investigated as a possible alternative viral indicator. The summary of this section of the study can be found in Appendix X. The phage was detected, but in very low concentrations, which makes the assay difficult to use. The human strain of the phage, B40-8 was detected in 6 of the 22 sampling sites, or 27% of the total sites. The phage was detected at TB4 and TB7 along Bullfrog Creek, TB1 Delaney Creek, TB12 Hillsborough River, TB14 Sweetwater Creek and TB17 Allen's Creek, and never at any of the beach sites. The high level of the animal/human strain of the phage, B56-3, found in domestic sewage indicates a human source for this phage in the environment, but when it is detected in the environment, it is difficult to trace the source.

H) Statistical Assessment

Statistical Analysis used in this report were performed using either Microsoft Excel for general calculations, or the statistical program MINITAB (Minitab, INC, State College, PA) for correlations and logistic regressions. All data were $\log_{10}$ transformed.

Correlations

Statistical correlations were used to determine if an association existed between the indicator concentrations for all samples and all sites, and to determine the strength of that association. Table 14 shows the correlation coefficients, or r values. The strongest relationship was between Fecal Coliforms and *E. coli*, which is expected due to the fact that *E.coli* makes up some percentage of the Fecal Coliform group. The second strongest link was between Coliphage and Enterococci, followed by Enterococci and Fecal Coliforms and *E.coli*. *Clostridium perfringens* showed the weakest correlation when compared with the other indicators.

Table 14
Correlations between various Indicators

Indicators against each other:	**r value**	**p value**
E.coli/FC	0.968	<0.001
Coliphage/Enterococci	0.781	<0.001
Enterococci/FC	0.650	<0.001
E.coli/Enterococci	0.625	<0.001
FC/Coliphage	0.557	<0.001
E.coli/Coliphage	0.546	<0.001
Enterococci/*C.perfringens*	0.432	<0.001
Coliphage/*C.perfringens*	0.374	<0.001
FC/*C.perfringens*	0.368	<0.001
E.coli/C.perfringens	0.367	<0.001

The indicators were compared to the presence of Enteroviruses, and in Table 15, the strongest correlation existed between Enterovirus and Enterococci, followed by Coliphage, Fecal Coliforms and *E.coli*. *Clostridium perfringens* showed no correlation to the presence of Enteroviruses. The correlations are low, with the highest only at 0.553, but this is not uncommon for environmental samples.

Table 15
Correlations between Viruses and Indicators

Indicators against entervirus:	**r value**	**p value**
Enterovirus/Enterococci	0.553	<0.001
Enterovirus/Coliphage	0.457	<0.001
Enterovirus/FC	0.442	0.001
Enterovirus/*E.coli*	0.370	0.010
Enterovirus/*C.perfringens*	0.199	0.137

Predicting the presence of Enterovirus with Indicator Results

All samples that had enterovirus results were compared to the indicator results for each individual indicator, and for combinations of indicators. The samples below the indicator guidelines suggesting safe levels were then compared with those sites that tested positive for Enteroviruses. The results are shown in Table 16a-c. The individual indicators are shown in Table 16a and combinations of indicators are shown in Table 16b. Percentages using only the beach sites for comparison are shown in Table 16c. The first row in all 3 tables is the number of samples below the guidelines out of the total sample set. The next row is the number of those samples below the guideline that also had a positive virus result, and the last row shows those samples below the guidelines that had a negative virus result. When the indicators were above the recommended guidelines, the percentage of positive Enterovirus results were 53% for fecal coliforms, 51% for Enterococci, 59% for coliphage and 50% for *Clostridium perfringens*. When the indicators were below the suggested guidelines as shown in the table, the percentage of positive Enterovirus results were 16% for Fecal Coliforms, 19% for Enterococci, 22% for Coliphage and 30% for *Clostridium perfringens*. When the indicators were combined, the percentage of positive virus results occurring when the indicators were below the guidelines lessened. The most successful combination was Fecal Coliforms and Enterococci, with only 6% of the samples under the guidelines for both showing a positive virus result. When the beach sites were analyzed separately, the same combination of Fecal Coliforms and Enterococci gave the best percentage, with 8% of the samples under the guidelines for both indicators showing a positive virus result.

Table 16a,b,c Percentage of samples positive or negative for viruses based on indicator guidelines
(comparison of sites that are under the suggested guidelines to those that are positive for enterovirus)

Table 16a

Individual	Fecal coliforms 800 cfu/100mL	Enterococci 104 cfu/100mL	Coliphage 100 cfu/100mL	*C.perfringens* 50 cfu/100mL
Below guidelines	25(**42**%)	26(**44**%)	36(**61**%)	37(**63**%)
Below gl, pos virus	4(**16**%)	5(**19**%)	8(**22**%)	11(**30**%)
Below gl, neg virus	21(**84**%)	21(**81**%)	28(**78**%)	26(**70**%)

Total samples for Enterovirus and indicators was 59, and 23 (39%) were positive

Table 16b

Combinations	Coliphage+ Enterococci	Fecal Coliforms + coliphage	Fecal Coliforms + Enterococci	*C.per* + Coliphage	*C.per* + Fecal Coliforms	*C.per* + Enterococci	Fecal Coliform + Enterococci + Coliphage
Below guidelines	24 of 59	22 of 59	16 of 59	27 of 59	18 of 59	21 of 59	16 of 59
Below gl, pos virus	4(**17**%)	2(**9**%)	1(**6**%)	5(**19**%)	2(**11**%)	2(**10**%)	1(**6%**)
Below gl, neg virus	20(**83**%)	20(**91**%)	15(**94**%)	22(**81**%)	16(**89**%)	19(**90**%)	15(**94%**)

Table 16c

Beach sites only	Fecal coliforms 800 cfu/100mL	Enterococci 104 cfu/100mL	Coliphage 100 cfu/100mL	*C.perfringens* 5 cfu/100mL	Fecal Coliforms + Enterococci + Coliphage
Below guidelines	13 of 18	16 of 18	18 of 18	16 of 18	12 of 18
Below gl, pos virus	2(**15%**)	3(**19%**)	4(**22%**)	3(**19%**)	1(**8%**)
Below gl, neg virus	11(**85%**)	13(**81%**)	14(**78%**)	13(**81%**)	11(**92%**)

When all 4 indicators are used: 14 samples were below the guidelines for all indicators, of those, 0(0%) were positive for virus, 14 (100%) were negative for virus; 13 samples had at least 1 of the four indicators above guideline, of those, 4 (31%) were pos. for virus and 9 (69%) were neg. for virus; 8 samples had at least 2 indicators above guidelines, of those, 4 (50%) were positive and 4 (50%) were neg. for virus;16 samples had 3 above guidelines, of those, 10 (63%) were pos. and 6 (37%) were neg. for virus; 8 samples had all 4 indicators above guidelines, of those, 4 (50%) were positive and 4 (50%) were negative for virus.

One way analysis of variance

Analysis of Variance, or ANOVA's , were used to determine if the sampling sites could be grouped into similar categories in regard to the indicator results. Although some variation exists, the results of the ANOVA's agree with the observation from the geometric mean and seasonal graphs. The control site (TB22), Honeymoon Island (TB16), North Beach (TB20), and John's Pass (TB19) tend to fall into one group, and Sweetwater Creek (TB14) and all sites along Bullfrog Creek (TB3, TB4, TB6, TB7 and TB8) tend to fall into another category. The other sites are scattered between these two groups.

The ANOVA's were run using the statistical software MINITAB (Minitab, Inc, PA) and GraphPad Prism (Intuitive Software for Science, CA). Both software packages gave similar results. The tables showing the individual ANOVAs and summary sections are given in the Appendix XI.

I) Climate Effects, Physical/Chemical Variables and Water Quality

Rainfall and Stream flow for Tampa Bay Watersheds

Microbial water quality was compared to climate factors (rainfall, stream flow and temperature) as well as chemical/physical variables (salinity, turbidity and pH). The rainfall and stream flow gage stations and databases used in this study are listed in Appendix XI along with the website locations of the different databases.

The Southwest Florida Water Management District monthly hydrologic reports (found on SWFWMD website) were used for the monthly rainfall averages for the entire district (covering the entire sampling area around Tampa Bay) and are shown in Table 17. The highest rainfall during the sampling schedule occurred June through September 1999 and started to peak again in June and July 2000. The late winter and spring months showed relatively little rainfall. Totaling the individual rainfall and stream flow stations throughout the watersheds (See Appendix XII) resulted in a very similar pattern to the hydrologic data supplied by SWFWMD, with the highest and lowest months agreeing in each case.

Table 17 Monthly Rainfall Averages for Tampa Bay

Month	Ave Monthly Rainfall in inches
June 1999	9.3
July 1999	5.75
August 1999	8.12
September 1999	6.13
October 1999	3.33
November 1999	1.97
December 1999	1.84
January 2000	1.59
February 2000	0.54
March 2000	1.01
April 2000	Not available
May 2000	0.42
June 2000	7.20
July 2000	8.18
August 2000	Not available

Table 18 shows the months that exhibited a peak in the traditional and alternative indicators for each individual sampling site. When the sites were divided as urban and rural, the rural sites mainly peaked in Oct 99 and Mar 00, while the urban sites were more varied, with peaks in Sept 99, Dec 99, Mar 00 and Feb 00. When looking at the monthly averages (Table 17) and comparing them to the indicator peaks (Table 18), the Oct 99 peaks occurred at the end of the rainy season (months of highest rainfall for the year).

The Mar 00 peak could not be linked to increased rain, occurring at a fairly dry time of the year. In addition, indicator peaks in Dec 99 were not linked with monthly increase in rain.

Table 18 Traditional and Alternative Indicator Peaks

Sampling site	Months that exhibited indicator peaks
TB1	Oct 99, Mar 00
TB2	Oct 99, Mar 00
TB3	Oct 99, Mar 00
TB4	Oct 99, Mar 00, Jul 00
TB5	Oct 99, Mar 00
TB6	Oct 99, Jan 00, Feb 00
TB7	Aug 99, Oct 99, Dec 99, Mar 00
TB8	Oct 99, Dec 99
TB9	Oct 99, Mar 00
TB10	Oct 99, Mar 00
TB11	Mar 00
TB12	Dec 99, Feb 00, Mar 00
TB13	Sept 99, Mar 00
TB14	Dec 99
TB15	Mar 00
TB16	Dec 99
TB17	Sept 99
TB18	Dec 99
TB19	Sept 99, Dec 99
TB20	Sept 99, Dec 99, Feb 00, Mar 00
TB21	Dec 99, Feb 00, Mar 00
TB22	None

Correlations – Indicators and Rainfall/Stream flow

Correlations between rainfall, stream flow and the traditional and alternative indicators for each individual site, as well as different groupings of sampling sites, were run using the statistical program MINITAB (Minitab,INC, State College, PA) Indicator levels were compared to rainfall using 3 day total and average values, 7 day total and average values, 10 day total and average values, and in some cases, 30 day average values. The rainfall and stream flow averages and totals used in the comparison were combined from all gage stations found within each watershed area for the rural sites. For Pinellas county, the gage stations were grouped into north and south Pinellas county, and then the sites were compared with a grouping depending on where the sampling site was located within the county. Correlations with indicator levels using rainfall and stream flow measurements from only the gage station closest to the sampling site did not correlate as well as when all gage stations in the watershed or in the surrounding areas were combined and then compared to the indicator levels.

When all the sampling sites were combined into one data set and compared with the indicator levels, no correlation between rainfall, stream flow and the indicator values was found. Using individual sampling sites and groupings of sites into rural, urban and beach sites, however, did result in the following correlations.

Fecal Coliforms and *E.coli* did not show any correlation to rainfall and stream flow except in the Alafia watershed (sites TB2 and TB5) and Joe's Creek/Cross Bayou system (TB18). In these cases, the r value for Fecal Coliforms compared to 7 day total stream flow was 0.429 (p=0.037) for the Alafia watershed (TB2 and TB5), and the r value for Fecal Coliforms compared to 3 day total rainfall was 0.721 (p=0.019) in Joe's Creek (TB18). For *E.coli*, the r value when compared to 10 day total stream flow was 0.469 (p=0.043) for the Alafia watershed, and was 0.711 (p=0.032) when compared to 3 day total rainfall in Joe's Creek.

Greater associations were found with the other indicators and rainfall/stream flow data (Tables 19-21). In Table 19, the values are given for correlations with Enterococci within the individual watersheds. The sites are listed in the grouping of rural, urban and beach sites. Each result shows the r value, p value and whether the total or average rainfall or stream flow measurement for the length of days was used in the comparison. The highest correlations for this indicator occurred in the urban site group, with sites TB12, TB15 and TB17 showing individual correlations with 7 day rainfall, with r values of 0.942 (p=<0.001), 0.932 (p=<0.001) and 0.714 (p=0.006), respectively. Site TB12 showed a correlation with 7 day stream flow, with an r value of 0.856 (p=0.002). Several smaller correlation values occurred within the watersheds of the rural group for both rainfall and stream flow. No correlations were found within the beach sites.

Table 20 shows the correlations for *C.perfringens*, in which associations with rainfall were found. For sites TB2 and TB5 (Alafia River), the r value was 0.586 (p=0.005) when the 3 day total rainfall was compared to *Clostridium* levels. The 3 day total rainfall for site TB17 Allen's Creek resulted in a correlation with an r value of 0.646 (p=0.023), and the 10 day total and average rainfall for site TB18 Joe's Creek resulted in a correlation with an r value of 0.769 (p=0.026) when compared to the level of *Clostridium.* An inverse correlation resulted for site TB11 Manatee River and for the beach site TB19 John's Pass, with r values of –0.677 (p=0.032) and –0.615 (p=0.033), respectively.

Coliphage (Table 21) were correlated with rainfall and stream flow in the watersheds within the rural grouping. The one and highest correlation at the urban site TB12 Hillsborough River had an r value of 0.895 (p=0.001) associating coliphage with rainfall.

All the sites within each of the 3 groupings (rural , urban and beach sites) were combined into a data set and the indicators were compared to the rainfall and stream flow measurements within these 3 groups. The results of the correlations are found in Table 22a-b. For the rural group, no correlations were found. In the urban data set, only rainfall correlated to Enterococci, *C. perfringens* and Coliphage levels, with r values of 0.350 (p=0.002), 0.334 (p=0.002) and –0.557 (p=0.009), respectively. The beach sites correlated with 10 day average rainfall with an r value of 0.310 (p=0.030) for Enterococci and an r value of –0.304 (p=0.036) when 7 day total rainfall was compared with Coliphage results.

Table 19 Correlations between Enterococci Levels and Rainfall/Stream flow

	Site	Rainfall (r value, p value, total or ave used)			Stream flow (r value, p value, tot or ave used)		
		3 day	7 day	10 day	3 day	7 day	10 day
Rural	TB1				0.597 Total (p=0.041)		
	TB2 & 5			0.516 Tot/Avg (p=0.014)			0.546 Tot/Avg (p=0.009)
	TB3/8		0.275 Total (p=0.027)		0.484 Tot/Avg (p=<0.001)		
	TB9 & 10	0.469 Tot/Avg (p=0.021)					
	TB11						
Urban	TB12		0.942 Tot/Avg (p=<0.001)			0.856 Tot/Avg (p=0.002)	
	TB14				0.560 Average (p=0.047)		
	TB15		0.932 Total (p=<0.001)				
	TB17		0.714 Total (p=0.006)				
	TB18						
	TB21						
Beach	TB13						
	TB16						
	TB19						
	TB20						

Table 20 Correlations between *Clostridium perfringens* Levels and Rainfall/Stream flow

	Site	**Rainfall (r value, p value and total or average used)**			**Stream flow (r value, p value, tot/av)**		
		3 day	7 day	10 day	3 day	7 day	10 day
Rural	TB1						
	TB2 & 5	0.586(p=0.005) Total					
	TB3/8						
	TB9 & 10						
	TB11		-0.677 (p=0.032) Average				
Urban	TB12						
	TB14					0.632(p=0.030) Tot/Avg	
	TB15						
	TB17	0.646(p=0.023) Total					
	TB18			0.769 (p=0.026) Tot/Avg			
	TB21						
Beach	TB13						
	TB16						
	TB19			-0.615 (p=0.033) Average			
	TB20						

Table 21 Correlations between Coliphage Levels and Rainfall/Stream flow

	Site	Rainfall (r value, p value, tot or ave used)			Stream flow (r value, p value, tot or ave used)		
		3 day	7 day	10 day	3 day	7 day	10 day
Rural	TB1	0.639(p=0.034) Total					
	TB2 & 5					0.535(p=0.010) Tot/Avg	
	TB3/8				0.280(p=0.024) Tot/Avg		
	TB9 & 10	0.503(p=0.017) Total					
	TB11						0.723(p=0.018) Tot/Avg
Urban	TB12	0.895(p=0.001) Average					
	TB14						
	TB15						
	TB17						
	TB18						
	TB21						
Beach	TB13						
	TB16						
	TB19						
	TB20						

Tables 22a,b Correlations between Site groups and Rainfall/Stream flow

Table 22a Urban Sites

	Rainfall (r value, p value and total or average values used)			**Stream flow**		
	3 day	7 day	10 day	3 day	7 day	10 day
Fecal Coliforms						
E.coli						
Enterococci		0.350 (p=0.002) Total				
C. perfringens			0.334 (p=0.005) Total			
Coliphage			-0. 557 (p=0.009) Tot/Avg			

Table 22b Beach Sites

	Rainfall (r value, p value and total or average value used)			**Stream flow**		
	3 day	7 day	10 day	3 day	7 day	10 day
Fecal Coliforms						
E.coli						
Enterococci			0.310 (p=0.030) Avg			
C. perfringens						
Coliphage		-0.304(p=0.036) Total				

Effects of Salinity, Turbidity, Temperature and pH

The correlations comparing the five indicator levels to salinity, turbidity, temperature and pH are given in Table 23a-d. The sites were combined into rural, urban, and beach sites again, as well as a group with just the Bullfrog Creek sites.

For the rural sites, salinity gave inverse correlations for all indicators except *C. perfringens*, with r values ranging from –0.241 (p=0.011) for *E.coli* to –0.422 (p=<0.001) for Enterococci. The pH correlations showed similar results to salinity. Turbidity correlated only with Fecal Coliforms , with an r value of 0.363 (p=<0.001). Temperature also correlated with all indicators except Fecal Coliforms, with r values ranging from 0.239 (p=0.011) for *C. perfringens* to 0.442 (p=<0.001) for Enterococci.

The urban sites showed inverse correlations with salinity for Enterococci, *C.perfringens* and Coliphage, with r values of –0.229 (p=0.048), -0.285 (p=0.010) and -0.306 (p=0.011), respectively. No correlations were found with turbidity, and temperature only resulted in a correlation with *C. perfringens*, with an r value of 0.311 (p=0.010). Fecal Coliforms and *E.coli* correlated inversely with pH, with r values of –0.388 (p=<0.001) and –0.412 (p=<0.001), respectively.

For the beach sites, salinity inversely correlated with Fecal Coliforms, Enterococci and *C.perfringens,* with r values of –0.341 (p=0.018), -0.616 (p=<0.001) and –0.500 (p=0.001), respectively. Turbidity correlated with Enterococci, with an r value of 0.290 (p=0.046), and no correlations were found with temperature and pH.

When Bullfrog Creek was studied as a group, salinity, temperature and pH showed little, if any, correlation with the indicator levels. Turbidity, however, showed an r value of 0.453 (p=<0.001) for Fecal Coliforms, similar results for *E.coli*, , 0.556 (p=<0.001) for Enterococci, and 0.642 (p=<0.001) for Coliphage. The Bullfrog Creek system is very shallow in most parts, and this could explain the strong relationship between turbidity and the indicator values for this creek. (This system is also a primarily freshwater system, which may contribute to this relationship as well)

Table 22 shows the correlations for all sites combined into one data set. Overall, salinity results in an inverse correlation with all indicators, ranging from –0.288 (p=<0.001) for *C.perfringens* to –0.650 (p=<0.001) for Enterococci. The correlations for pH showed a similar result to salinity, and there was no correlation with the indicators and turbidity. Temperature showed weaker inverse correlations with the indicators, ranging from –0.137 (p=0.035) for Enterococci to –0.232 (p=<0.001) for Coliphage. No correlation was found between *C.perfringens* and temperature.

Tables 23 a-d Correlations between Indicator Levels and Physical/Chemical Variables for site groupings

Rural Sites

	Salinity (r/p values)	**Turbidity**	**Temperature**	**pH**
Fecal Coliforms	-0.278 (p=0.001)	0.364 (p=<0.001)		-0.290 (p=0.001)
E.coli	-0.241 (p=0.011)		0.384 (p=<0.001)	-0.327 (p=0.001)
Enterococci	-0.422 (p=<0.001)		0.442 (p=<0.001)	-0.361 (p=<0.001)
C.perfringens			0.239 (p=0.011)	
Coliphage	-0.354 (p=<0.001)		0.432 (p=<0.001)	-0.428 (p=<0.001)

Urban Sites

	Salinity (r/p values)	**Turbidity**	**Temperature**	**pH**
Fecal Coliforms				-0.388 (p=<0.001)
E.coli				-0.412 (p=<0.001)
Enterococci	-0.285 (p=0.010)			
C.perfringens	-0.306 (p=0.011)		0.311 (p=0.010)	
Coliphage	-0.229 (p=0.048)			

Beach Sites

	Salinity (r/p values)	**Turbidity**	**Temperature**	**pH**
Fecal Coliforms	-0.341 (p=0.018)			
E.coli				
Enterococci	-0.616 (p=<0.001)	0.290 (p=0.046)		
C.perfringens	-0.500 (p=0.001)			
Coliphage				

Bullfrog Creek

	Salinity (r/p values)	**Turbidity**	**Temperature**	**pH**
Fecal Coliforms		0.453 (p=<0.001)		
E.coli		0.458 (p=0.001)		
Enterococci	-0.355 (p=0.004)	0.556 (p=<0.001)		
C.perfringens	0.290 (p=0.023)			
Coliphage		0.642 (p=<0.001)		0.275 (p=0.033)

Table 24 Correlations between Indicator Levels and Physical/Chemical Variables for entire data set

All Sites

	Salinity (r/p values)	**Turbidity**	**Temperature**	**pH**
Fecal Coliforms	-0.389 (p=<0.001)		-0.181 (p=0.005)	-0.357 (p=<0.001)
E.coli	-0.386 (p=,0.001)		-0.142 (p=0.042)	-0.392 (p=<0.001)
Enterococci	-0.650 (p=<0.001)		-0.137 (p=0.035	-0.468 (p=<0.001)
C.perfringens	-0.288 (p=<0.001)			-0.212 (p=0.002)
Coliphage	-0.649 (p=<0.001)		-0.232 (p=<0.001)	-0.402 (p=<0.001)

Viruses and Climatic/Environmental Association

Binary Logistic Regressions were used to determine the relationship between rainfall, stream flow and the presence of Enterovirus. A slightly significant logistic regression result occurred within the beach site grouping between the 7 day average rainfall values and the presence of Enterovirus, resulting in a 64.3% concordant percentage, 30.4% discordant percentage and a 5.4% tie. Salinity and Enterovirus in this same beach grouping resulted in a concordant percentage of 69.6%, 26.8% discordant percentage and a 3.6% tie. No other significant relationship was found between the climate factors used in the study, and the presence or absence of Enterovirus. The virus data set for this study is small, however, and a more intensive virus sampling regime may be needed for a more accurate statistical analysis of climate factors and their contribution to virus water quality.

Summary of Climate and Indicators

The Fall peak in fecal indicator levels corresponds to the end of the rainy season, however, the Spring peak cannot be linked to rainfall or stream flow parameters. A lag time beyond 30 days exists when rainfall is compared to the indicators, but localized peaks associated with rainfall events may still occur within individual watersheds.

Total rainfall rather than average rainfall was better than stream flow for correlations overall. For Enterococci, the 7 day total was useful, but for coliphage, the 3 day total was better perhaps because of the decreased survival in warm tropical waters. Average rainfall for beach sites was useful only when looking widely at the Bay, not for the individual sites. Enterococci compared to the 10 day rainfall was the only useful indicator at the beach sites.

Negative correlations to rainfall and stream flow suggest that in some watersheds dilution due to increased rainfall and stream flow will actually decrease the number of phage and *Clostridium*. Both coliphage and *Clostridium* were found in low numbers compared to the other indicators. Sources are more likely to be related to feces compared to coliforms and Enterococci, which might have a soil or vegetative source. And while *Clostridium* could accumulate in sediments and does survive for extended periods of time, the low concentrations make it susceptible to non-detects when fresh water increases.

IV. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The need for new approaches to study microbial water quality

Water quality is influenced by a number of factors; these include land use patterns and population growth as well as climate factors. Historically, water quality in Tampa Bay has been impacted by these same factors. Nutrients have been the focus of many of the water quality studies, yet through efforts of the Tampa Bay Estuary Program and county and city agencies as well as the private sector a plan for the reduction of nutrients has been formulated. In fact, the addition of denitrification processes at the Howard Curren facility and other efforts have lead to a reduction of nitrogen and an increase in the coverage of sea grasses, which in turn will improve the overall ecosystem and fisheries potential.

While much attention has been paid to nutrients it is only in recent years that the problem of microbiologically-contaminated waters has been highlighted. The sources of microorganisms include wastewater discharges, septic tanks, animal wastes, septage, and wildlife. The use of waters for recreational purposes (swimming, fishing, boating) as well as for drinking water supply (eg. Hillsborough river and desalination) that are contaminated can be linked to human illnesses. Total and fecal coliform bacteria (two related groups) have been used to study microbial water quality. However, these tests are inadequate. Total coliform bacteria should no longer be used in ambient water monitoring in Florida. While fecal coliform bacteria are suggested to more readily tied to fecal contamination and the sources mentioned above there are some significant problems associated with this test particularly for subtropical waters such as found in Florida.

1. The bacteria, although originally from feces, accumulate on plants and in soils in tropical and subtropical environments and grow, thus limiting their close association with contamination and health risks.
2. The bacteria are sensitive to salinity, and may die-off quickly in marine and estuarine waters.
3. The bacteria are not representative necessarily of the pathogens of concern (including human viruses) and are not adequate “indicators”.

There are some advantages to the use of the fecal coliform bacteria. There is a long historical database in most waters from sources as well as from impacted waters. It is used as a regulatory tool in storm waters and wastewaters. The test is relatively easy and quick to perform. The large database of fecal coliform bacteria in Tampa Bay has been reported in annual water quality reports and has shown that key watersheds and tributaries are more contaminated than others (eg. Bull Frog Creek area). It is also seen that there has been a deterioration of water quality over time and that climate factors, particularly rainfall and the El Niño and La Niña patterns that influence Florida, influence water quality. Yet the sources (human versus animal) and the health risks (feces versus soil, inadequate indicator of virus risks) remain unknown. Because of these limitations,

both short term and long term approaches for control of pollution and the impacts on human health remain stagnant.

The US EPA has recommended the use of an alternative bacterial indicator, the enterococci for marine recreational waters, based on a series of epidemiological studies (Pruss, 1998). The state of Hawaii has recommended the use of *Clostridium perfringens*, a spore forming bacterium for it's tropical waters. And beyond these two, there are a number of tests that are more useful in defining microbial water quality and public health risks, including bacterial source tracking, coliphage testing (a virus indicator) and direct pathogen monitoring for viruses and parasites. Studies using the newer tools will provide new insights into the microbial water contamination in Tampa Bay and provide data for recommendations for the future.

Summary of the results from the Tampa Bay Study

Twenty-two sites around the bay representing fresh water inputs and four beach sites were studied using routine indicators, alternative indicators, source tracking techniques, virus and parasite testing. As the results were analyzed it became clear that there were three distinct groupings, the rural sites (characterized by more septic tanks and agriculture), the urban sites (characterized by high density land use and storm water control) and the beach sites.

Comparison of sites

- Fecal coliform bacteria are found in a wide distribution at 100% frequency in Tampa Bay waters and the distinction of sites was often blurred because of this. The use of the alternative indicators, however, showed clearly that the rural sites were more contaminated than the urban sites, which were more contaminated than the beach sites.
- However, within each category specific sites were found to be more greatly polluted and included the Bull Frog Creek TB4 site, Sweetwater creek and Courtney Campbell Causeway Beach. These sites had increased coliphage and *Clostridium,* as well as indication of human impacts using the virus testing and bacterial source tracking.
- Viruses were detected in 15 of 29 samples in the rural sites (52%), 4 of 12 samples in the urban sites (30%) and 4 of 18 (27%) at the beach sites (page 73).
- For the 4 beach sites, *C. perfringens* was only found consistently at TB13 Courtney Campbell Causeway Beach, the most urban-located beach in the study. *C. perfringens* only occurred once at TB20 North Beach, twice at TB19 John's Pass and was never detected at TB16 Honeymoon Island. Coliphage showed a similar pattern in regard to the beach sites.

Comparison of Indicators (Correlation Results)

- The strongest relationship between indicators was found with Fecal Coliforms and *E. coli*, which is expected due to the fact that *E.coli* makes up the largest percentage of the Fecal Coliform group. The second strongest link was between Coliphage and Enterococci, followed by Enterococci and Fecal Coliforms and *E.coli*, with *Clostridium perfringens* showing the weakest correlation when compared with the other indicators.
- The *Bacteroides fragilis* phage correlated best with Enterococci and Coliphage.

Indicators and Pathogen Presence (Correlation Results)

- The strongest correlation existed between Enterovirus and Enterococci, followed by Coliphage, Fecal Coliforms and *E.coli*. *Clostridium perfringens* showed no correlation to the presence of Enterovirus.

Indicators and Guidelines

- When the indicators were above the recommended guidelines, the percentage of positive Enterovirus results were 53% for fecal coliforms, 51% for Enterococci, 59% for coliphage and 50% for *Clostridium perfringens*. When the indicators were below the suggested guidelines, suggesting that the water was safe, the percentage of positive Enterovirus results were 16% for Fecal Coliforms, 19% for Enterococci, 22% for Coliphage and 30% for *Clostridium perfringens*. The best results were obtained when multiple indicators were used.

Bacterial Source Tracking

- The most striking findings of this study was the extent to which wild animals dominate as a source of fecal coliforms and *E.coli*, in 73.6% of all samples, the majority of isolates were identified as nonhuman.
- All of the source-specific methods used in the study indicate that human pollution is significantly impacting the Bullfrog Creek Watershed.
- The consistent impact from human sources is less clear at the Pinellas county sites, although there were days when "spikes" of human isolates dominated the sites.
- The percentage of isolates identified as human by ARA was significantly correlated with enterovirus counts, but the percentage of isolates identified as human by ribotyping was not significantly correlated with enterovirus counts. This discrepancy points to the need for including the fingerprints of more isolates from known, local sources in the respective databases.

Summary of Seasonal Occurrences

- For sites located in rural and suburban areas, *C.perfringens* and coliphage occur primarily in the winter and early spring months, whereas highly developed urban areas show these indicators occurred throughout the year. The exception to this is the Bullfrog Creek system, which showed indicator levels similar to that of urban sites.

- Fecal coliforms and *E.coli* levels may peak without a corresponding peak in the other indicators.

- Most rural and suburban sites show a seasonal increase in indicator levels during the winter and early spring months, most urban sites are fairly consistent throughout the year.

Summary of Climate and Indicators

- The Fall peak in indicator levels corresponds to the end of the rainy season.
- The Spring peak was not linked to monthly rainfall or stream flow.
- Total rainfall rather than average rainfall was better than stream flow for correlations overall.
- For Enterococci, the 7 day total was useful, but for coliphage, the 3 day total was better perhaps because of the decreased survival in warm tropical and subtropical waters.
- Average rainfall for beach sites was useful only when looking widely at the Bay, not for the individual sites. Enterococci compared to the 10 day rainfall was the only useful indicator at the beach sites.

Recommendations

What indicators are appropriate for Tampa Bay?

- The use of two indicators, the fecal coliform bacteria and enterococci , is warranted on a routine basis based on the risk and contamination level assessment of this study. *E.coli* is of little added value even in waters that are primarily fresh.
- Source tracking using multiple antibiotic resistance for fecal coliform bacteria should be included and a large catalog and repository for Tampa Bay should be built and supported.
- Coliphage should be added as a third indicator in areas with fresh water inputs during the study of storm events on water quality.
- *Clostridium* may be useful during one-time sanitary surveys.
- *Bacteriodes* will be useful in studying wastewater facilities (disinfected wastewater) and septic tank inputs into common warm marine waters.

The continued use of fecal coliform bacteria is supported but only with the addition of enterococci, as well as characterization of the types of fecal coliform bacteria found using the source tracking techniques. Coliphage as a third indicator should be added during specialized surveys. This approach will be useful in demonstrating risk, seasonal variability and sources, and the data can be used to make both short-term and long-term management decisions on the watershed.

What levels are appropriate for Tampa Bay?

- The 104 CFU single sample level and geometric mean of 35 CFU associated with Enterococci is partially supported by this study for the fresh water tributaries. However, the 200 and 800 CFU for the fecal coliform bacteria are not and may be too stringent. A set of values for the fecal coliform bacteria can not be supported at this time.
- A greater database is needed at the contrasting beaches to make recommendations for beach water quality monitoring and levels.

Is pathogen monitoring warranted?

- Viruses have been the group of pathogens which have shown the most value in marine waters as a benchmark to compare to the indicators representing human health risks.
- Risk assessment models suggest that the likelihood of becoming ill is 1/1000 to 1/10,000 if ingesting water at the levels recorded on the beaches from a single swimming event. In order to further define this risk, virus testing is warranted, as a part of any particular beach study.
- Enteroviruses were found in Tampa Bay sites in 39% (23 out of 59 samples) of the samples tested, but at 100% of the sites tested. In other words, at least one positive result occurred at every site tested at some time during the study.

What other information is needed to move into Phase II Healthy Beaches?

- A more detailed study directly on the beaches is needed.
- Specifically working with a transport model, a temporal and spatial study is needed, this can be accomplished using indicators. The current data set could be used to support an initial study, however more data are needed on the beaches.

The Healthy Beaches Phase II Proposal is included in Appendix XIII.

Are the data and recommendations for Tampa Bay useful for a State-wide program?

- Yes, state, local and private agencies involved in water quality studies (wastewater, stromwater, septage etc), should move immediately to monitoring for both enterococci and fecal coliform bacteria as well as contributing to a state-wide database on the characterization of "source-tracking" isolates.

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Healthy Beaches Tampa Bay

Microbiological Monitoring of Water Quality Conditions and Public Health Impacts

Executive Summary

1999-2000

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Executive Summary – Healthy Beaches Tampa Bay

I. Introduction

Clean beaches and the recreational activities associated with them form the backbone of the tourist industry in the Tampa Bay region. Risks to swimmers using polluted beaches has been a major issue associated with the setting of ambient water quality standards and discharge limits to recreational sites. Prevention of disease associated with recreational waters depends on the use of appropriate fecal indicators. Suitable indicators should mirror the source and fate of common human fecal pathogens, in other words, they should come from the same general source as pathogens and die off at a similar rate when exposed to environmental variables such as salinity, temperature and sunlight. However, the finding that the most widely used fecal contamination indicator, fecal coliforms, and more specifically *E. coli*, grow naturally on vegetation in warm climates clearly brings into question whether these or other indicators developed for temperate climates are applicable in Florida and other southeastern areas. (Fujioka et al, 1999) In addition, total and fecal coliform bacterial indicators have not been able to consistently indicate the persistence of pathogens, especially viruses, in surface waters. F-specific RNA coliphage, enterococci and *Clostridium perfringens* have been suggested as alternative indicators of fecal contamination and public health risks.

In order to ascertain the validity of these proposed indicators of fecal pollution, this study examined traditional and alternative pollution indicators, as well as the presence of pathogenic viruses, and their association with environmental variables (salinity, rainfall, stream flow) in fresh and marine water systems of the Tampa Bay area. From this and other available information, recommendations could be make as to the applicability of these indicators. The final goal of this project was to form the baseline for other studies and help to develop a long-term strategy for addressing or enhancing Florida water quality.

II. Goals of Healthy Beaches Tampa Bay and Sampling Strategy

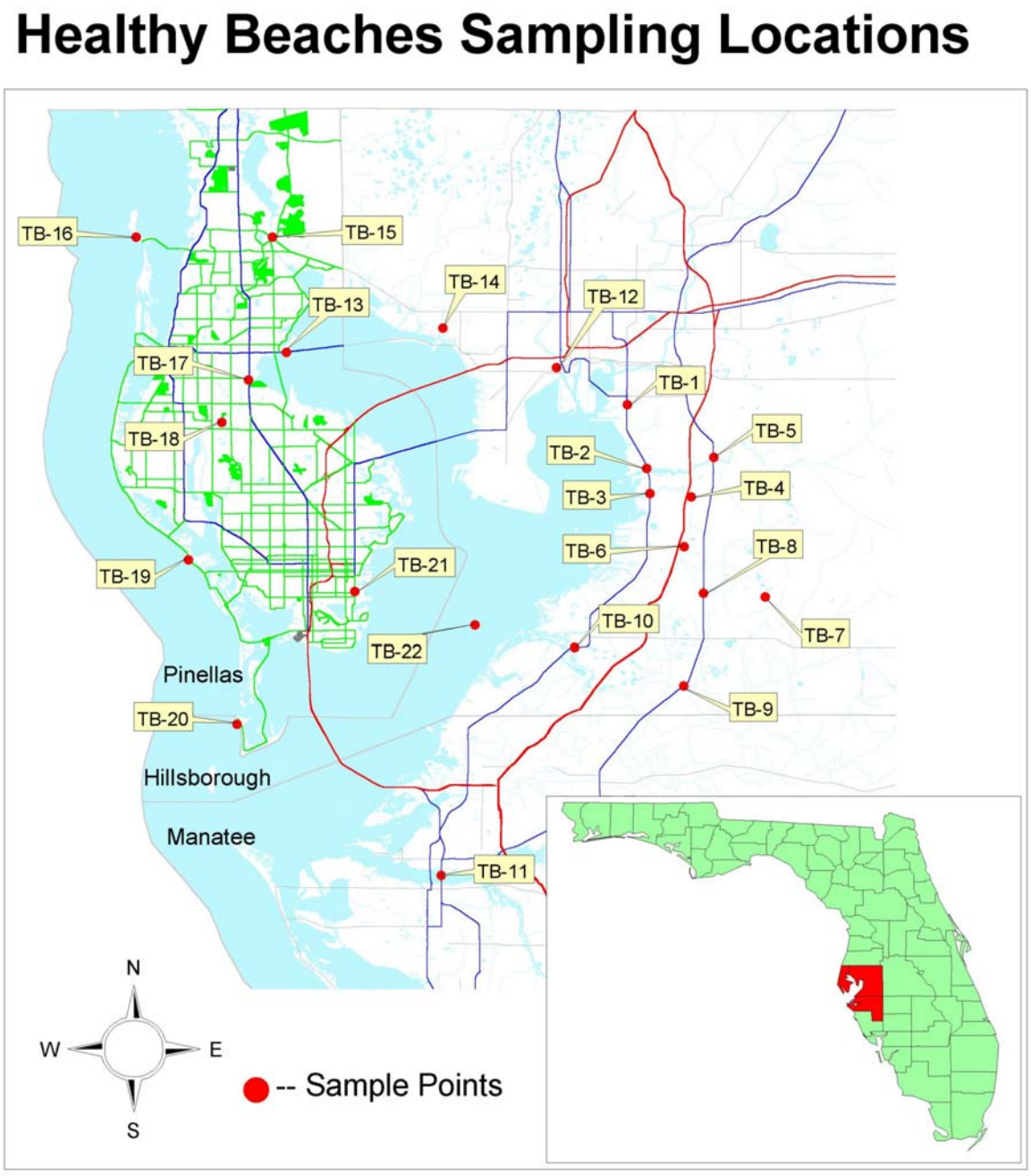
The goals of this study were:

1) To determine appropriate indicators for microbiological water quality for recreational sites in area beaches and for Tampa Bay.
2) To determine the occurrence of pathogens along with indicators in Tampa Bay watersheds and area beaches, their associated sources (animal vs human), public health risks and potential for management.

Twenty-two sites were chosen in Tampa Bay for this study with the assistance of an advisory council. Figure 1 shows their location along Tampa Bay. Four beach sites were chosen to represent several different beach types, including urban (TB13 Courtney Campbell Causeway beach), heavy boat use (TB19 John's Pass), recreational site in rural area (TB20 North Beach, Ft. Desoto) and pristine unpopulated beach (TB16 Honeymoon Island). The Alafia watershed was represented by sites TB2 and TB5, the Little Manatee by sites TB9 and TB10, the Manatee watershed by site TB11 and the Hillsborough

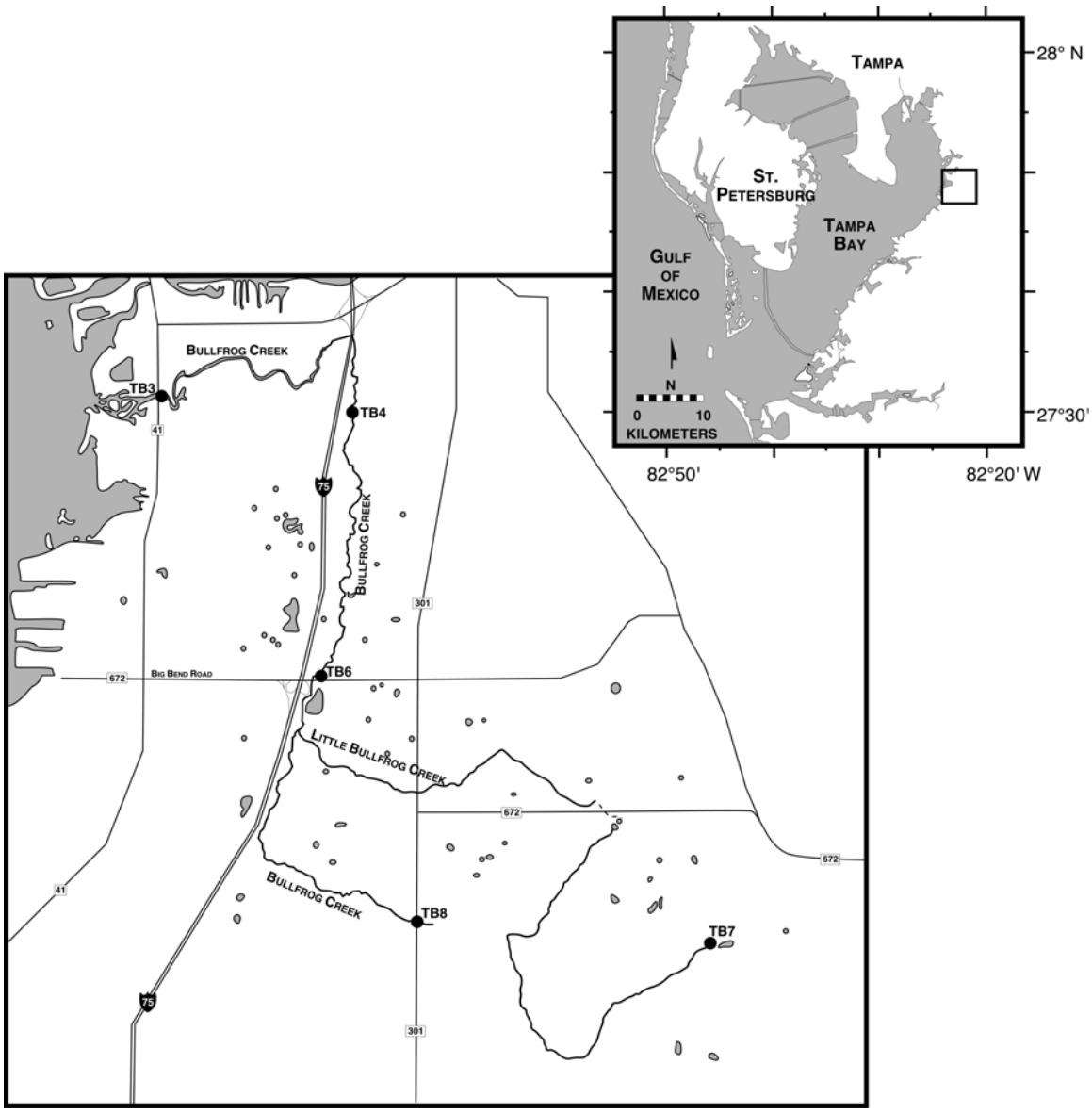
watershed by site TB12. The Bullfrog Creek sub-basin was chosen for in-depth monitoring due to the history of heavy pollution in the system, and included sites TB3, TB4, TB6, TB7 and TB8 (See Figure 2). The Delaney Creek sub-basin was represented by site TB1. The remaining sites were located in Pinellas county, which cannot be divided into distinct watersheds, but is rather several non-continuous creek and wetland systems. These sites included TB14 Sweetwater Creek, TB15 Lake Tarpon Canal, TB17 Allen's Creek, TB18 Joe's Creek/Cross Bayou and TB21 Salt Creek.

Figure 1
Tampa Bay Sampling Sites



Graphic produced by Pinellas County Health Department - Environmental Engineering Division

Figure 2
Bullfrog Creek Sampling Sites in Detail



Sampling extended from June 1999 to August 2000, and each site was sampled for traditional and alternative fecal indicators, which included Fecal Coliforms, *E.coli*, Enterococci, *Clostridium perfringens* and Coliphage. Physical parameters were measured at the time of sampling as well, and included temperature, pH, turbidity and salinity. Out of the 22 total sites, 10 were chosen for in-depth testing (including antibiotic resistance analysis, ribotyping of *E. coli* isolates and *Bacteroides fragilis* phage assay for differentiating animal and human contamination, and human pathogenic enteroviruses). These sites were monitored 6 times throughout the study. The sites chosen for in-depth study in Hillsborough County included all sites along Bullfrog Creek: TB3, TB4, TB6, TB7, TB8. In Pinellas County, the sites included TB13 Courtney Campbell Causeway, TB14 Sweetwater Creek, TB17 Allen's Creek, TB19 John's Pass Beach and TB20 North Beach, Ft. DeSoto. Twenty parasite (*Cryptosporidium* and *Giardia*) samples were collected and analyzed for the 10 in-depth sites as well, one set every 6 months during the study.

The following table (Table 1) gives the fecal indicator guidelines and levels used for the comparison of the data in this study. For the individual sampling results, the single sample guideline was used for Fecal Coliforms and Enterococci. No single sample guidelines are given for *E.coli, Clostridium perfringens* and Coliphage. In these cases, the geometric mean guideline was used. For the site to site comparisons, the geometric mean of all the results obtained throughout the study were used and compared to the geometric mean guidelines given.

Table 1 Indicator Guidelines used in this study

Fecal Coliforms	EPA and the state of Florida recommended guidelines for a single sample of 800 cfu/100 mL, for a geometric mean, 200 cfu/100 mL
E.coli	EPA recommended guideline for a geometric mean sample 126 cfu/100 mL
Enterococci	EPA recommended guidelines for a single sample of 104 cfu/100 mL, for a geometric mean , 33-35 cfu/100 mL for marine and fresh water respectively.
C. perfringens	Guidelines used by state of Hawaii based on research by Dr. Roger Fujioka et al at the University of Hawaii of 50 cfu/100 mL for fresh and brackish water and 5 cfu/100 mL for marine waters.
Coliphage	Level used - 100 pfu/100 mL based on previous research by Dr. Joan Rose, USF

III. Material and Methods

Samples were collected using sterile 1 L plastic bottles and placed on ice for transportation to the lab. Samples were processed within 8 hours of collection. For each bacterial indicator, volumes of the water sample were analyzed using membrane filtration. The filters were then placed on the appropriate media for each individual bacterial indicator assay. Coliphage were enumerated using the standard overlay technique according to the Standard Methods for Examination of Water and Wastewater, APHA, 1989. Culturable Enteroviruses were detected by cell culture methods, (Standard Methods for Examination of Water and Wastewater, 1989), and Protozoan analysis was carried out using filtration and immunofluorescence microscopy techniques (Proposed ICR Protozoan Method for Detecting *Giardia* cysts and *Cryptosporidium* oocysts in Water by Fluorescent Antibody Technique, Standard Methods for the Examination of Water and Wastewater, 18th ed. Supplement).

For Antibiotic Resistance Analysis (ARA), Fecal coliform isolates were picked from filters incubated with mFC medium (see Fecal Coliforms). The antibiotic resistance pattern of each isolate was compared isolates from known sources (cattle, wild animals, human, etc.) using discriminant analysis. The molecular ribotyping of *E.coli* isolates was accomplished by the method of Parveen *et al* (1997).

IV. Results and Discussion

A) Indicators

As the results were analyzed it became clear that there were three distinct groupings, the rural sites (characterized by more septic tanks and agriculture), the urban sites (characterized by high density land use and storm water control) and the beach sites. The rural sites included Delaney Creek (TB1), the Alafia River (TB2 and TB5), the Bullfrog Creek system (TB3, TB4, T6, TB7 and TB8), the Little Manatee River (TB9 and TB10) and the Manatee River (TB11). The urban sites included the Hillsborough River (TB12), Sweetwater Creek (TB14), Tarpon Lake Canal (TB15), Allen's Creek (TB17), Joe's Creek/Cross Bayou (TB18) , and Salt Creek (TB21). The four beach sites were the Courtney Campbell Causeway Beach (TB13), Honeymoon Island (TB16), John's Pass (TB19) and North Beach at Ft. Desoto (TB20).

In the rural site grouping, site TB4 Bullfrog Creek consistently had high levels of indicators except for *C. perfringens.* Sites TB6 and TB7 along Bullfrog Creek generally had high levels of Fecal Coliforms, *E.coli*, Enterococci and Coliphage as well. Site TB5 Alafia River showed moderate levels of indicators, and sites TB2, TB8, TB9, TB10 and TB11 showed less contamination. Site TB1 Delaney Creek had high levels of *E.coli,* Enterococci and Coliphage, but low levels of Fecal Coliforms and *C. perfringens.* Site TB3 Bullfrog Creek had the highest levels detected for *C.perfringens*.

For the urban site grouping, site TB14 Sweetwater Creek had the highest levels of indicators except for *C. perfringens*. Site TB17 Allen's Creek showed moderate levels of indicators, and sites TB15, TB12, TB18 and TB21 showed slightly less contamination. Sites TB17 Allen's Creek and TB18 Joe's Creek had the highest levels detected for *C. perfringens*.

For the beach sites, TB13 Courtney Campbell Causeway beach had the highest levels of indicators followed by TB20 Ft. Desoto and TB16 Honeymoon Island. *Clostridium perfringens* was only found consistently at TB13 Courtney Campbell Causeway Beach, the most urban-located beach in the study. *Clostridium perfringens* only occurred once at TB20 North Beach, twice at TB19 John's Pass and was never detected at TB16 Honeymoon Island. Coliphage showed a similar pattern in regard to the beach sites. The control site, TB22, had indicator levels below all guidelines for the entire length of the study.

For sites exceeding the suggested geometric guidelines, the two consistently high sites were TB4 Bullfrog Creek and TB14 Sweetwater Creek. The remaining sites along Bullfrog Creek (TB3, TB6, TB7 and TB8) were next among the highest sites when comparing indicator levels. Sites TB16 Honeymoon Island, TB19 John's Pass and TB20 Ft. Desoto were among the lowest sites when comparing geometric means of indicator levels.

Among most of the sites, a peak in indicator values occurred in September and October of 1999, and again in March of 2000. Overall, however, most rural sites show a stronger seasonal increase in indicator levels during the winter and early spring months while most urban sites were fairly consistent throughout the year. When looking at the seasonal graphs for each site, those located in rural areas show *C. perfringens* and coliphage occurring primarily in the winter and early spring months, whereas highly developed urban areas show these indicators occurring throughout the year. The exception to this is the Bullfrog Creek system, which shows indicator levels similar to that of urban sites. In addition, Fecal coliforms and *E.coli* levels were shown to peak without a corresponding peak in the other indicators.

When using statistical correlation, the strongest relationship between indicators was found with Fecal Coliforms and *E. coli*, which is expected due to the fact that *E.coli* makes up the largest percentage of the Fecal Coliform group. The second strongest link was between Coliphage and Enterococci, followed by Enterococci and Fecal Coliforms and *E.coli*, with *Clostridium perfringens* showing the weakest correlation when compared with the other indicators. The *Bacteroides fragilis* phage correlated best with Enterococci and Coliphage.

B) Pathogens

The 10 in-depth sites were monitored for the presence of Enteroviruses (a group of human viruses found in feces which include Poliovirus, Coxsackieviruses and Echoviruses). The highest number of virus isolations occurred in September and October 1999 (with 3 and 4 sites positive out of 5, respectively), which corresponds to the indicator peak found in the rural sites during October 1999, and the September 1999 peak found in the urban and beach sites. The virus levels ranged from 1.1 to 27.1 MPN-PFU/100 L. Bullfrog Creek overall showed consistent Enterovirus results, with TB3 and TB4 showing the highest percentage of positive results. The two urban sites and the three beach sites had 1-2 positive results during the length of the study.

For the Protozoan parasites, 20 samples were collected from the in-depth sites (10 sites sampled 2 times during the study). No *Giardia* were detected during the study. Sites

TB3, TB4, TB7 and TB8 along Bullfrog Creek all showed the presence of *Cryptosporidium* with results of 3.48 oocysts per 100 L of water for TB7, 7.03 oocysts/100 L for TB8, 124.4 oocysts/100 L for TB4 and 470 oocysts/100 L for TB3. Each site tested positive for *Cryptosporidium* only once during the study. (See Table 2)

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Table 2 Percentage of Enterovirus and Parasite Positives by Site

		Viruses	**Parasites**
Site Type	**Site**	+ virus out of total samples collected	+ *Crypto* out of total samples collected
Rural	TB3 Bullfrog	4 of 5	1 of 2
	TB4 Bullfrog	4 of 6	1 of 2
	TB6 Bullfrog	3 of 6	0 of 2
	TB7 Bullfrog	2 of 6	1 of 2
	TB8 Bullfrog	2 of 6	1 of 2
Urban	TB14 Sweetwater	2 of 6	0 of 2
	TB17 Allen's	2 of 6	0 of 2
Beach	TB13 Courtney C.	2 of 6	0 of 2
	TB19 John's Pass	1 of 6	0 of 2
	TB20 Ft. DeSoto	1 of 6	0 of 2

C) Predicting pathogen presence (Enterovirus) with Indicators

The indicators were compared to the presence of Enteroviruses using statistical correlations. The strongest correlation existed between Enterovirus and Enterococci, with an r value of 0.553 ($p=<0.001$), followed by Coliphage (r value=0.457, $p=<0.001$), Fecal Coliforms (r value=0.442, p=0.001) and *E.coli* (r value=0.370, p=0.010). *Clostridium perfringens* showed no correlation to the presence of Enteroviruses. These correlations are low, with the highest r value only at 0.553, but this is not uncommon for environmental samples.

The presence or absence of enteroviruses was compared against the suggested guidelines for the indicators included in this study (See Table 1) for all samples with both enterovirus and indicator data. When the indicators were below the suggested guidelines, suggesting that the water was safe, the percentage of positive Enterovirus results were 16% for Fecal Coliforms, 19% for Enterococci, 22% for Coliphage and 30% for *Clostridium perfringens*. The percentages improved when multiple indicators were used. Combining Fecal Coliforms and Enterococci or Fecal Coliforms and Coliphage reduce that percentage to 6% and 9%, respectively.

D) Fecal Coliform Source Tracking

The most striking finding of this study was the extent to which wild animals dominate as a source of fecal coliforms and *E.coli*, in 73.6% of all samples, the majority of isolates were identified as nonhuman. All of the source-specific methods used in the study indicate that human pollution is significantly impacting the Bullfrog Creek Watershed. The consistent impact from human sources is less clear at the Pinellas county sites,

although there were days when "spikes" of human isolates dominated the sites. The percentage of isolates identified as human by antibiotic resistance analysis was significantly correlated with enterovirus counts, but the percentage of isolates identified as human by ribotyping was not significantly correlated with enterovirus counts. This discrepancy points to the need for including the fingerprints of more isolates from known, local sources in the respective databases.

E) Climate and Indicators

The Fall peak in fecal indicator levels corresponded to the end of the rainy season, however, the Spring peak could not be linked to rainfall or stream flow parameters. A lag time beyond 30 days existed when rainfall was compared to the indicators, but localized peaks associated with rainfall events may still occur within individual watersheds.

Total rainfall rather than average rainfall was better than stream flow for predicting indicator level peaks overall. For Enterococci, the 7 day total rainfall value was useful, but for coliphage, the 3 day total was better perhaps because of the decreased survival of this indicator in warm tropical waters. Average rainfall for beach sites was useful only when looking widely at the Bay, not for the individual sites. Enterococci compared to the 10 day total rainfall value was the only useful indicator correlation at the beach sites.

Negative correlations to rainfall and stream flow suggest that in some watersheds dilution due to increased rainfall and stream flow will actually decrease the number of phage and *Clostridium*. Both coliphage and *Clostridium* were found in low numbers compared to the other indicators. Sources are more likely to be related to feces compared to coliforms and Enterococci, which might have a soil or vegetative source. And while *Clostridium* could accumulate in sediments and does survive for extended periods of time, the low concentrations make it susceptible to non-detects when fresh water increases.

Binary Logistic Regressions were used to determine the relationship between rainfall, stream flow and the presence of Enterovirus. A slightly significant logistic regression result occurred within the beach site grouping between the 7 day average rainfall values and the presence of Enterovirus, resulting in a 64.3% concordant percentage, 30.4% discordant percentage and a 5.4% tie. Salinity and Enterovirus in this same beach grouping resulted in a concordant percentage of 69.6%, 26.8% discordant percentage and a 3.6% tie. No other significant relationship was found between the climate factors used in the study, and the presence or absence of Enterovirus. The virus data set for this study is small, however, and a more intensive virus sampling regime may be needed for a more accurate statistical analysis of climate factors and their contribution to virus water quality on the beaches.

V. Recommendations

What indicators are appropriate for Tampa Bay?

- The use of two indicators, both the fecal coliform bacteria and enterococci on a routine basis is warranted based on the results of this study. *E.coli* appears to be of little added value in either marine or fresh waters.
- Source tracking using multiple antibiotic resistance for fecal coliform bacteria should be included and a large catalog and repository for Tampa Bay should be built and supported.
- Coliphage should be added as a third indicator in areas with fresh water inputs during the study of storm events on water quality.
- *Clostridium perfringens* and *Bacteroides* phage, while indicative of fecal pollution, only have limited added value as alternative indicators.
- *Clostridium* may be useful during one-time sanitary surveys.
- *Bacteriodes* will be useful in studying wastewater facilities (disinfected wastewater) and septic tank inputs into common warm marine waters.
- Biological Source Tracking is a very useful tool, and a large database for Tampa Bay should be built and supported.

The continued use of fecal coliform bacteria is supported but only with the addition of enterococci, as well as characterization of the types of fecal coliform bacteria found using the source tracking techniques. Coliphage as a third indicator should be added during specialized surveys. This approach will be useful in demonstrating risk, seasonal variability, sources and the data can be used to make both short-term and long-term management decisions on the watershed.

What levels are appropriate for Tampa Bay?

- The 104 CFU single sample level and geometric mean of 35 CFU associated with Enterococci is partially supported by this study for the fresh water tributaries. However the 200 and 800 CFU for the fecal coliform bacteria are not and may be too stringent. A set of values for the fecal coliform bacteria can not be supported at this time.
- A greater database is needed at the contrasting beaches to make recommendations for beach water quality monitoring and levels.

Is pathogen monitoring warranted?

- Viruses have been the group of pathogens which have shown the most value in marine waters as a benchmark to compare to the indicators representing human health risks.
- Risk assessment models suggest that the likelihood of becoming ill is 1/1000 to 1/10,000 if ingesting water at the levels recorded on the beaches from a single swimming event. In order to further define this risk, virus testing is warranted, as a part of any particular beach study.

- Enteroviruses were found in Tampa Bay sites in 39% (23 out of 59 samples) of the samples tested, but at 100% of the sites tested. In other words, at least one positive result occurred at every site tested at some time during the study.

What other information is needed to move into Phase II Healthy Beaches?
- A more detailed study directly on the beaches is needed.
- Specifically working with a transport model, a temporal and spatial study is needed, this can be accomplished using indicators. The current data set could be used to support an initial study, however more data are needed on the beaches.

Are the data and recommendations for Tampa Bay useful for a State-wide program?
- Yes, state, local and private agencies involved in water quality studies (wastewater, stromwater, septage etc), should move immediately to monitoring for both enterococci and fecal coliform bacteria as well as contributing to a state-wide database on the characterization of "source-tracking" isolates. Virus testing should be built into specialized studies.

Perspective and Future Directions: Healthy Beaches Phase II and beyond

Because most pathogens are host-specific, the goal of this study has been to access the risk of human disease by measuring pollutants of human origin. However, a great deal of additional work remains in order to protect public health and enhance the environment, including:

- Modeling of conditions that determine pollution events to provide ways to predict, avoid and mitigate. Healthy Beaches Phase II has been proposed to address modeling and risk assessment. (Proposal is included in Appendix XII)
- Development of technology and methods such as biosensors to enable the rapid measurement of indicators or actual pathogens.
- Better understanding and response to waterborne diseases not necessarily of human origin, such as those that cause wound infections, animal parasites such as *Giardia* and *Cryptosporidium*, organisms from animal waste (e.g. *E.coli* 0157:H7), and natural organisms such as *Vibrio vulnificus* and harmful algal blooms.
- Development of a comprehensive database of Enterococci and Fecal Coliforms for use in biological source tracking, and development of methods to quickly perform the analysis locally.
- Increase efforts to eliminate or reduce identified causes of pollution, such as septic tanks, leaking sewer collections systems, failing lift stations, provision of sanitary facilities at beaches, and selected sources of animal pollution.
- Develop statutes, guidelines, methods and education programs so that the public will be aware of risks and take action accordingly as it is not possible to obtain a natural environment that is entirely risk free.
- Undertake a risk assessment investigation specific to warm climates areas, including epidemiological methods, to quantify the relationship between exposure to various concentrations of pathogens and the associated risk of acquiring disease.

Appendix I

DETERMINING THE EFFECTS OF EL NINO-SOUTHERN OSCILLATION ON COASTAL WATER QUALITY

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Abstract

The importance of El Niño-Southern Oscillation on regional-scale climate variability is well recognized; however the associated effects on local weather patterns are poorly understood. Little work has addressed the ancillary impacts of climate variability at the community level, which require analysis at a local scale. In coastal communities water quality and public health effects are of particular interest. Here we describe the historical influence of ENSO on coastal water quality in Tampa Bay, Florida (USA) as a test case. Using approximate randomized statistics, we show significant ENSO influences on water quality, particularly during winter months, with significantly greater fecal pollution levels during strong El Niño winters and significantly lower levels during strong La Niña winters as compared to neutral conditions. Significant patterns were also noted for El Niño and La Niña falls. The success of the analyses in this test case demonstrates the feasibility of assessing local effects associated with large-scale climate variability in any area and highlight the possibility of using ENSO forecasts to predict periods of poor coastal water quality in urban regions.

Introduction

The link between climate and health was recognized as early as the 16th century (Rees 1996). Without knowledge of disease-causing agents, many believed that strange weather patterns caused a variety of health problems, resulting in the adage, "under the weather" (Rees 1996). While it has long been long realized that "bad airs" do not cause disease, in recent years there has been increased scientific recognition that climate change and variability contribute to the distribution, growth and survival of certain pathogenic microorganisms and, therefore, impact public health (Colwell 1996; Checkley et al. 2000; NOAA 1999). Both local weather patterns and climate variability play a role in the dispersion of pathogenic microorganisms. Events such as extreme rainfall and floods often overburden water treatment facilities and onsite disposal systems and increase storm water run-off; all of which may result in the introduction of high levels of enteric pathogens to nearby surface waters and wells. Interannual climate variability due to El Niño-Southern Oscillation (ENSO)

and other phenomena also affects water quality and public health both directly and indirectly by resulting in poor sanitation due to floods (Gueri et al. 1986) or promoting favorable conditions for growth/survival of certain pathogens, i.e. *Vibrio cholerae* (Colwell 1996). Also, given the importance of non-point sources of pollution in the United States and elsewhere, heavy or prolonged rains may contribute to pollutant loading, including pathogenic microorganisms, from urban and agricultural run-off and on-site sewage disposal (O'Shea and Field 1992; Paul et al. 1997). Exposure to the public during these events occurs from the contamination of drinking water, recreational water and shellfish (Rose 1997).

Short-term predictive models forecast ENSO events with varying success rates and research has effectively demonstrated a strong relationship between regional precipitation patterns and ENSO (Ropelewki and Halpert 1986; Schmidt et al. in review). In addition to the importance and utility of regional-scale models, an understanding of anomalies in local weather patterns is an important and immediate concern. However, there has been little work to demonstrate statistically significant weather patterns, or determine the ancillary effects of these anomalies related to ENSO at the local scale. This contribution builds upon previous research by the authors on the topic of ENSO influence on local variability in seasonal rainfall and river discharge in Florida. Using an approximate randomized difference of means test, Schmidt et al. (in review) demonstrated significant seasonal responses of rainfall and streamflow to El Niño and La Niña conditions in south central Florida. The study also found significant seasonal variability in rainfall within the state of Florida, with distinct patterns noted particularly between the panhandle and southernmost Florida.

We hypothesize that a statistical relationship may exist between ENSO and water quality given reported relationships between ENSO, precipitation and river discharge (Sun and Furbish 1997; Zorn and Waylen 1997) and the subsequent relationship between water quality and both rainfall and discharge (Barbé and Francis 1995). Using historical data, we analyzed the relationship between microbiological water quality and ENSO in south central Florida, a region that is known to experience anomalous precipitation and river flow associated with ENSO phases (Schmidt et al. in review). The strength of these relationships and seasonal changes were evaluated with analyses of continuous and categorical data. Here we demonstrate a simple approach to define the role of particular modes of climate variability (i.e., ENSO) on coastal water quality by focusing on temporal and spatial scales that are important to public health decisions at local levels using Tampa Bay, FL (USA) as a test case.

Materials and Methods

Description of Study Site

Historical changes in water quality and their relationship to ENSO were assessed in Tampa Bay, FL. Tampa Bay is the second largest Gulf Coast estuary and the largest estuary in Florida. The entire watershed contains 35,500 km^2, which are drained by 31 major basins (SWFWMD 1998). We studied seven drainage basins (Fig. 1), which are qualitatively described in terms of major land-use patterns and other sources of pollution in Table 1. Currently, within the entire Tampa Bay watershed 56% of the land is developed; 40% of the built-up areas are urban and 16% include agricultural and pasture lands (SWFWMD 1998).

El Niño-Southern Oscillation Indices

The ENSO state was measured using the Climate Prediction Center's Niño Region 3.4 monthly Sea Surface Temperature anomaly (SSTA) indices, which are based on recorded temperatures from 5 ^{o}N to 5 ^{o}S and 170 ^{o}W to 120 ^{o}W in the equatorial Pacific Ocean. Seasons from 1974-1998 were classified as extreme or neutral. Seasons were defined as follows: winter included January, February and March; spring included April, May and June; summer included July, August and September; and fall included October, November and

December. ENSO extreme seasons were defined to occur when the five-month running average, centered on the season, of the Niño Region 3.4 SSTA exceeded ± 0.7 ^{o}C. Neutral ENSO seasons were defined to occur when the five-month running average, centered on the season, fell between ± 0.4 ^{o}C (Tbl. 2). These thresholds excluded questionable ENSO events while providing an adequate number of cases for analyses for all ENSO phase seasons for all seasons except extreme La Niña spring. Where data were available there was often a strong signal noted in extreme La Niña springs. Therefore, for those cases where insufficient data existed for extreme La Niña, all La Niña springs, including weak (-0.40 to -0.69 ^{o}C) episodes, were tested.

Monthly water quality data (1974–1998) were obtained for Tampa Bay and its tributaries from the Hillsborough County Environmental Protection Commission (HEPC). Water quality was quantitatively assessed using concentrations of fecal coliform bacteria (colony forming units (CFU)/100 ml) at 29 stations. Concentrations for each sample were transformed by the equation, $\log_{10}$ (CFU/100 ml) + 1, to obtain a normal distribution. When concentrations were below detectable limits (<1 - <100 CFU/100 ml), a value of zero was used for statistical analyses. Samples were collected only once per month from each station. Consequently, stations were combined in individual watersheds to mitigate small-scale events that might dominate a local area during the monthly sampling but would not be indicative of an entire drainage basin. In all, seven drainage basins were examined and included an average of 4.1 stations per watershed. A maximum of 7 and a minimum of 2 stations were used per drainage basin.

Approximate randomized statistics were used in all analyses. These computer-intensive tests generate the probability distribution of the test statistic by recomputing it for many (>100) artificially constructed data sets and can be used to assess significance under minimal assumptions. The observations that are tested do not need to meet the normal distribution criteria of conventional parametric statistics; likewise they need not constitute a random sample. In addition to avoiding the assumptions required of parametric statistics, approximate randomized tests maximize the ability to discriminate between hypotheses because the sampling distribution is known (Noreen 1989).

In order to perform correlation analyses against Niño Region 3.4 SSTA, anomalies in fecal coliform levels were obtained by subtracting the basin-averaged mean monthly value (over the data record) from individual basin-averaged monthly values. Significance of the Pearson correlation coefficients was determined by comparing the r-value of the observed correlation to that of the distribution of the correlation under the null hypothesis. This distribution was generated by randomly shuffling the SSTA values against fecal coliform anomaly values and recalculating the r-value 10,000 times. Correlations were run against the entire data record, with a lag of zero to three months between monthly Niño Region 3.4 SSTA and monthly water quality anomalies to allow the detection of any delayed responses.

The correlation test provides information regarding the significance of the relationship between ENSO and fecal coliform levels but does not reveal details concerning the relative importance of particular seasons. Therefore, the differences in mean fecal coliform concentrations between extreme El Niño, neutral and extreme La Niña events for each season were analyzed using an approximate randomized difference of means test (Noreen 1989). Log-transformed basin-averaged seasonal fecal coliform concentrations for extreme El Niño and La Niña events were tested against the seasonal fecal coliform levels for neutral periods. The observed difference in means was compared to the distribution of the randomly generated difference under the null hypothesis. As in the correlation analysis, recalculating the difference in means 10,000 times generated the distribution. Given the high level of noise inherent in the fecal coliform data set and the need to average over time and space, results were considered to be statistically significant at an α level of 0.10 rather than 0.05.

Results

Correlation Analysis

Work by Schmidt et al. (in review) indicates that both precipitation and streamflow in south central Florida are significantly related to ENSO. Given that water quality often deteriorates during periods of high precipitation and river discharge, we hypothesized that a direct relationship may exist between ENSO state and water quality. Fecal coliform bacteria were used as a proxy for water quality, as they are the most commonly used indicator of poor water quality due to fecal pollution and potential health risks world-wide. Correlation analyses were used to provide an initial assessment of whether any relationship existed between Niño 3.4 SSTA (ENSO state) and changes in fecal coliform levels (water quality) in Tampa Bay.

Analysis of monthly anomalies in fecal coliform levels with monthly Niño 3.4 SSTA revealed a significant and positive correlation in five of the seven watersheds examined over the 25-year period of record. In general, even for significant correlations, coefficients were low ($r = 0.088$ to 0.23). However, these values were similar to those obtained for comparisons between Niño 3.4 SSTA and both precipitation and streamflow in Florida (Schmidt, N. unpublished data). The majority of basins with strong correlations were located in the eastern portion of the Tampa Bay watershed. In this region, land use includes broad areas devoted to agriculture and pasture. Furthermore, there is substantial land application of sewage sludge in some areas (SWFWMD 1998; Tbl. 1). Land use appears to be an important factor in relating changes in water quality to the strength of ENSO.

Seasonal Analysis

The importance of the ENSO phenomenon to variability in factors such as rainfall and streamflow in Florida, and elsewhere, varies with season (Schmidt et al. in review). Therefore, given the significant relationship for most of the studied watersheds between fecal coliform levels and Niño 3.4 SSTA, we expanded our examination to assess the seasonal differences in fecal coliform levels between extreme ENSO phases (El Niño and La Niña; Tbl. 2) and neutral conditions to better define the relationship between ENSO state and water quality. Basin-averaged fecal coliform values were compared between seasons and ENSO phase.

Winter. Fecal coliform levels were compared between neutral winters and both extreme El Niño and extreme La Niña winters (Fig. 2). For extreme El Niño winters, there was an overall increase in fecal coliform levels as compared to neutral. With the exception of Delaney Creek, where the percent deviation was –20.4 ($P = 0.087$), the deviations in fecal coliform levels from neutral ranged between 7.6% and 18.7%. However, only at Rocky and Sweetwater Creeks were the fecal coliform levels significantly greater than neutral values ($P < 0.10$). During extreme La Niña winters, there was an overall decrease in fecal coliform levels as compared to neutral winters. With the exception of Rocky Creek, where the percent deviation from neutral was 36.8 ($P = 0.034$), the deviations in fecal coliform levels from neutral ranged between –16.2% and –45.5%. Fecal coliform levels at Bullfrog Creek, Delaney Creek, Hillsborough River, and Sweetwater Creek were significantly below neutral values ($P < 0.05$). Fecal coliform levels at Little Manatee River were significantly lower than neutral at $P < 0.10$. Although Alafia River fecal coliform values showed a negative deviation during extreme La Niña winters, the difference from neutral was not significant.

Spring. For extreme El Niño springs, the average fecal coliform levels were lower than those found during neutral springs (Fig. 2). Percent deviations from neutral ranged between –4.6 and –20.1; however, differences were not significant in any watershed. For the one case of an extreme La Niña spring, fecal coliform levels in all basins were significantly below neutral values ($P < 0.10$). Deviations from neutral ranged from -0.9% to -46.0%. Given the lack of data during extreme La Niña spring events, deviations from neutral were also examined for all five spring La Niña events. For the more general La Niña springs cases, levels in all basins were lower than during neutral spring (-2.1% to -47.4% deviation). However, the differences were not significantly different from neutral values.

Summer. Fecal coliform levels during extreme El Niño summers showed a varied response with neutral values (Fig. 2). None of the fecal coliform concentrations were significantly different than levels found in neutral summers and percent deviations ranged from –13.7 to 11.8. For extreme La Niña summers, percent deviations were consistently negative (-0.1% to -45.1%). However, the differences from neutral were not significant.

Fall. Fecal coliform concentrations during extreme El Niño falls were generally greater than that found during neutral periods (Fig. 2). Although fecal coliform values at Bullfrog Creek, Delaney Creek and Little Manatee River were less than that found during neutral fall, the differences were not significant. The remaining stations all showed greater than neutral fecal coliform concentrations, with deviations between 8.2% and 25.3%. The difference from neutral was only significant for Hillsborough River (25.3% deviation) and Rocky Creek (22.3% deviation). Patterns during extreme La Niña falls showed both positive and negative deviations from neutral. The only drainage with significant differences from neutral was the Little Manatee River, where levels were 17.5% below neutral values.

Discussion

Extreme weather conditions including droughts and floods can dramatically affect communities at many levels. Direct effects may include crop damage, property damage, destruction of homes and loss of life. However, even moderate changes in climate can affect water resources in both quantity and quality and thus indirectly affect public health. Although research has demonstrated regional-scale climate variability during ENSO phases, particularly relating to changes in temperature and precipitation (Ropelewski and Halpert 1986; Livezey et al. 1997; Gershunov and Barnett 1998; Livezey and Smith 1999), it is at the local level where economic and public health impacts are felt and where decisions regarding public policy must be made. Consequently, there is a need to better predict and understand the effects of climate variability at the local scale. To our knowledge this is the first study to examine the ancillary or indirect effects of ENSO on fecal pollution in coastal waters as it relates to recognized changes in precipitation and streamflow.

The extreme El Niño conditions observed in 1997 and early 1998 spurred investigations into the effects of climate variability on human health (NOAA 1999). During this time, the role of rainfall and streamflow in the introduction and transport of indicators of fecal pollution and human enteroviruses to coastal waters was demonstrated in southwest Florida (Weiskel et al. 1996). Higher than average precipitation and subsequent river discharge were found in the winter months (1997 – 1998) along with lower water temperatures. Those patterns, which are typical of El Niño winters, provided a mechanism for transport of enteric contaminants by run-off and discharge into coastal waters and increased survival due to lower salinity and temperature (Barbé and Francis 1995; Wyer et al. 1995; Weiskel et al. 19996; Sun and Furbish 1997).

Throughout Florida and in the Tampa Bay area, tremendous population growth in the last 20 years has been accompanied by an increased volume of wastewater discharged to coastal waters. Furthermore, non-point sources (from septic systems and stormwater run-off) constitute a major cause of coastal pollution. Factors such as agricultural lands, land

application of sewage sludge, septic systems and wildlife contribute to high levels of coastal fecal pollution when transport mechanisms, such as high precipitation and river discharge, are in operation. The consequences of such pollution include closures of recreational and shellfish propagating waters and potential exposure to human pathogens (Lipp and Rose 1997).

The predictable, or "normal," seasonal nature of rainfall in Florida has lead to specific water management strategies. The majority of rainfall in southern and central Florida occurs in the summer months, while spring and fall are relatively dry. In the southern part of the state winter storms account for less than 15% of the average annual precipitation (Nese and Grenci 1996). Similar to the larger-scale patterns noted for the southeastern United States (Ropelewski and Halpert 1986) precipitation and consequently streamflow along the south central Gulf coast of Florida are strongly related to ENSO (Schmidt et al. in review). The seasonal ENSO effects results in precipitation patterns that are superimposed upon the normal seasonal trends. Significant correlations between ENSO (using Niño 3.4 SSTA) and fecal coliform levels at the majority of basins analyzed demonstrate that Tampa Bay water quality also may be broadly linked to the ENSO state via teleconnections that results in anomalous precipitation and river discharge. Significantly increased fecal pollution, relative to neutral conditions, was most dramatic for extreme El Niño winter months. ENSO development tends to peak in the winter; consequently the strongest weather patterns are also noted during that time (Ropelewski and Halpert 1986). Significant increases in wintertime precipitation and discharge in Florida during extreme El Niño events (Zorn and Waylen 1997; Schmidt et al. in review) may exacerbate conditions and result in greater than average levels of indicator organisms or introduction of enteric pathogens (Lipp et al. in review). Conversely, below average precipitation and discharge (Schmidt et al. in review) lead to depressed fecal coliform levels during extreme La Niña winters. A significant ENSO signal was also often noted in the fall, and the signal was generally variable, although not significant, in the spring. The ENSO signal was ambiguous in the summer. In general, these observations follow patterns noted for both precipitation and river discharge in south central Florida (Schmidt et al. in review) (Fig. 2). This type of information will be useful in proactively tailoring water quality monitoring and control programs in the Tampa Bay region.

Conclusion

In this historical assessment, changes in water quality (using fecal coliform bacteria) in Tampa Bay, FL were shown to vary with ENSO phases. We have previously shown a significant relationship between ENSO and precipitation and discharge (Schmidt et al. in review) and between water quality and rainfall and discharge in Florida (Lipp et al. in review), and now report that a direct association between fecal pollution and ENSO can be measured. Despite an inherently noisy data set, significant trends between fecal coliform levels and Niño 3.4 SSTA were noted for the majority of the Tampa Bay watersheds we examined. This study provides a baseline to initiate the development of water quality "forecast" models, ultimately using factors such as ENSO and other climatic variables combined with land-use characteristics to predict periods of poor water quality. This will further the development of public policies for monitoring, assessing and managing important bays and coastlines for recreation, industry and fisheries.

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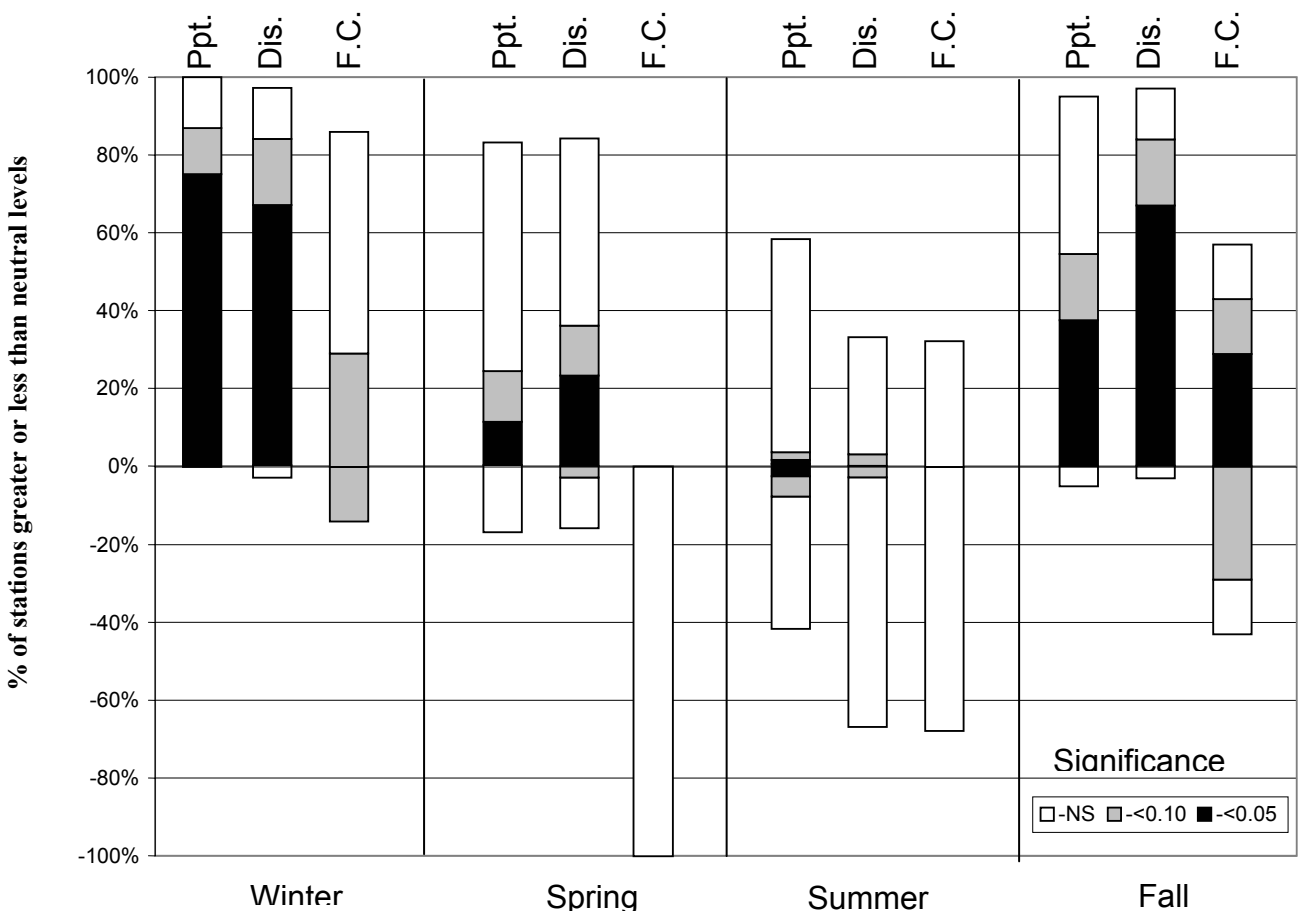
Table 2. Classification of ENSO events between seasons, based on period of record available for water quality (1974 – 1998).

Season	Extreme El Niño	Neutral	Extreme La Niña
Winter J F M	1983, 1987, 1992, 1995, 1998	1979, 1981, 1982, 1990, 1991, 1994, 1997	1974, 1976, 1985, 1989
Spring A M J	1982, 1983, 1987, 1992, 1993, 1997	1976, 1977, 1978, 1979, 1980, 1981, 1984, 1986, 1990, 1994, 1995, 1996, 1998	1988 (1974, 1975, 1985, 1989)[a]
Summer J A S	1982, 1987, 1991, 1997	1978, 1979, 1980, 1981, 1983, 1984, 1985, 1989, 1990, 1992, 1995, 1996	1975, 1988, 1998
Fall O N D	1976, 1977, 1982, 1986, 1987, 1991, 1994, 1997	1978, 1980, 1981, 1985, 1989, 1992, 1996	1975, 1984, 1988, 1995, 1998

[a] weak La Niña events (Niño 3.4 SSTA -0.4 – -0.69) are included in parentheses

Figure 1. Map of Tampa Bay watersheds. Filled circles represent river gage stations, open circles represent water quality stations. BC: Booker Creek, RC: Rocky Creek, SC: Sweetwater Creek, CC: Cypress Creek, HR: Hillborough River, DC: Delaney Creek, AR: Alafia River, BFC: Bullfrog Creek, LMR: Little Manatee River and MR: Manatee River.

Figure 2. Chart shows the percentage of stations with fecal coliform (FC), precipitation (PPT) or discharge (DIS.) values that were significantly greater than or less than that of neutral seasons for extreme El Niño (A) and extreme La Niña (B) events. One full bar represents 100% of stations: no color – difference was not significant, gray – difference was significant at $P<0.10$, black – difference significant at $P<0.05$. For spring, all La Niña events were used as there was only one extreme La Niña event within the period of record. Significance results are from Schmidt et al. (in review) and included statewide precipitation stations and streamflow for south central Florida (Charlotte Harbor and Tampa Bay) for 1950 to 1998.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Winter
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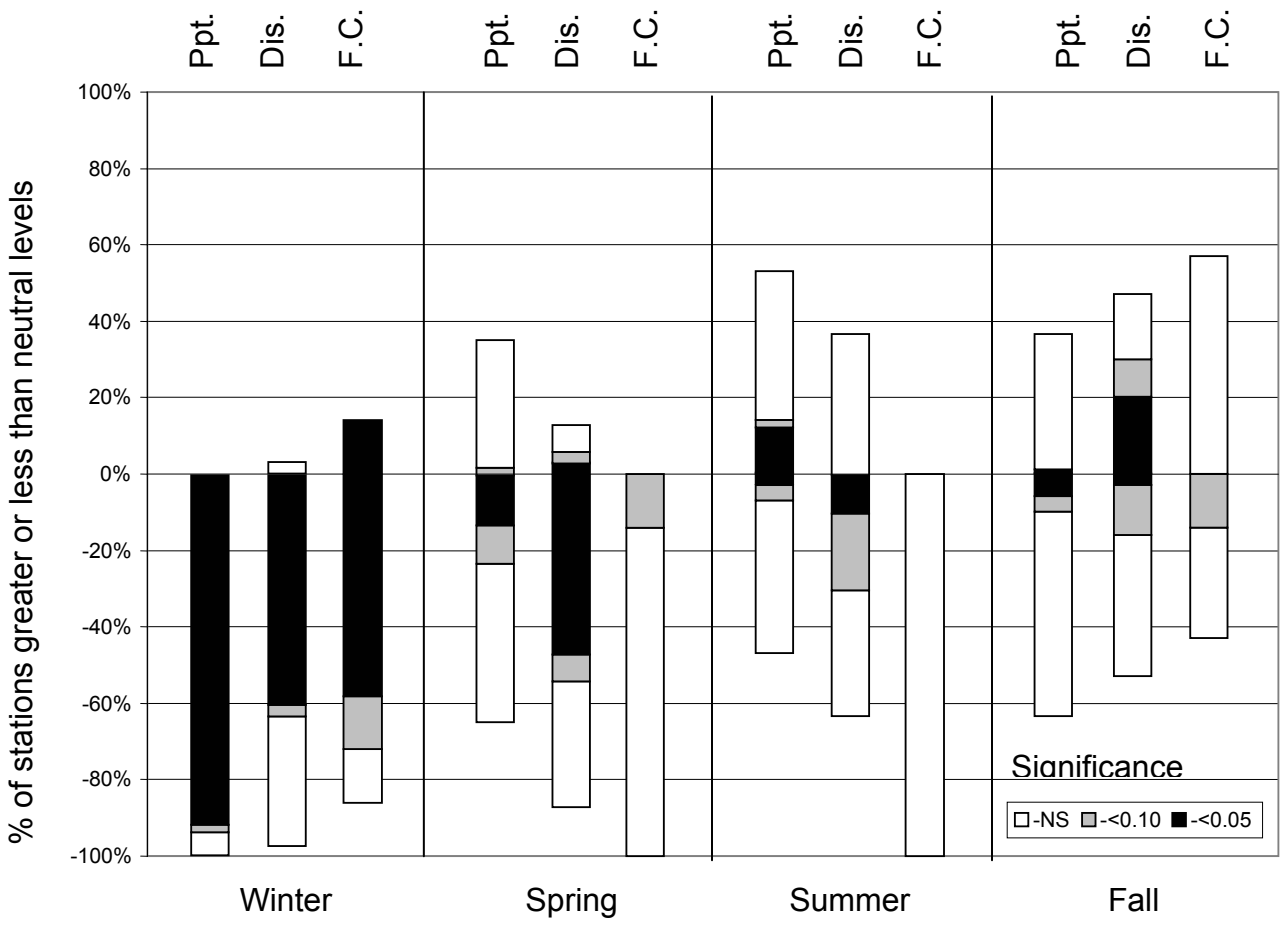
Summer
Fall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% of stations greater or less than neutral levels
Significance
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-<0.10
-<0.05
Winter
Spring
Summer
Fall

Table 1. Description of Tampa Bay drainage basins.

Basin	Drainage Area (acres)	Major Land Use	Notes
Hillsborough River	431,742	Agriculture (32%; north & ctl); Urban & Industrial (25%; south)	Springs in upper and lower reaches (1 each), dam and drinking water reservoir
Alafia River	270,000	Agriculture (68%; south); Phosphate Mining (east); Urban & Industrial (north & west)	2 springs; >91% of watershed is developed or altered
Bullfrog Creek	25,758	Agriculture (50%; east), Urban & Residential (12%; west)	
Rocky Creek	30,008	Urban (41%; south), Agriculture (north)	Several lakes up to 93 acres
Sweet Water Creek	23,896	Urban (69%)	2 large lakes (191 and 283 acres)
Delaney Creek	15,161	Urban (55%)	
Little Manatee River	135,046	Forest (38%), Agriculture (84%)	

OSDS On-site Sewage Disposal Systems [2] WWTP Wastewater Treatment Plant

Figure 1

Appendix II

Site Descriptions for Tampa Bay Healthy Beaches 1999-2000

Hillsborough County:

TB1 Delaney Creek – U.S. 41 just after 36th street (coming from the north), bridge access only, EPC station 133. Small bridge next to Adams Auto Parts on left hand side of road.

TB2 Alafia River – U.S. 41 just before Gibsonton, access from Williams Park dock on right side of road traveling south, upstream of fertilizer plant/industrial area, EPC station.

TB3 Bullfrog Creek - #5 – continue south on U.S. 41 to next bridge, access from Anderson's RV park on left side of road, between Beach Ave and Symmes Road. EPC site 144.

TB4 Bullfrog Creek - #4 – turn left at the next blinking yellow traffic light just south of TB3 site and head east on Symmes Road, small dip in road just past I-75. Path to water level from north west corner, EPC station 132.

TB5 Alafia River – continue east on Symmes Road and turn left onto S.R. 301/43, next big bridge.

TB6 Bullfrog Creek - #3 – go back south on 301, west (right) on Big Bend Road (traffic light) to service road just before interstate. (East Bay), continue west on service road to bridge just before I-75, easy path to water on southwest corner leading under 301 bridge, this is a USGS gauging station and just downstream of cattle/agricultural land. You can also get to service road via Big Bend to Lincoln, then left onto service road.

TB7 Bullfrog Creek - #1 –take Big Bend Road back to 301 and turn right (south), then east (left) on C.R.672 (go past correctional institute), Look for large American Flag at Goodson Farms on right side of road, take a right on McGrady Road (south) to end. Mr. Lambert – property owner, site is just upstream of planned stormwater retention/treatment pond, around 1600 acres.

TB8 Bullfrog Creek - #2 –back track to 301 and continue south (left turn from 672), look for small bridge ("Big Bullfrog Creek")between Sumner and Bill Tucker Road, main channel flow is under the bridge..

TB9 Little Manatee River – continue south on S. R. 301 from TB8,just south of Bonita and Saffold Roads, and south of Sun City, on south west side (right side of road) look for canoe sign, dirt road leading under bridge, EPC station 113.

Site Descriptions for Tampa Bay Healthy Beaches 1999-2000, con't

TB10 Little Manatee River – back track north on 301, turn left (west) on 674 (Sun City Center Blvd) and follow west to U.S. 41 and turn left (south). Paradise Mobile Home Park on right just before bridge, easy access from fragment of old bridge. EPC station 112.

TB11 Manatee River - continue south on U.S. 41 to Palmetto/Bradenten, (do not follow signs to business U.S.41), small access road on right side before Hernando-DeSoto bridge, access road goes under bridge and comes out on northbound U.S.41.

TB12 Hillborough River – On West Kennedy, left side of road just before large white bridge leading to downtown, (Cresent Place North or University Drive) , access to seawall.

Pinellas County:

TB13 Courtney Campbell Causeway –Pinellas County side, south beach. (Gulf to Bay/SR60).

TB14 Sweetwater Creek –on Memorial Hwy, Creek is just past Waltham Ave, small turn off to right just before bridge, seawall on other side of creek near office bldg. EPC station 104

TB15 Tarpon Lake Bypass Canal – Continue west on 576 Memorial Hwy from TB14, turn left (northwest) on 580 (Hillsborough Ave), this turns into 584 (Tampa Rd), continue past Curlew, there should be a large bridge going over canal. Pull into shopping center parking lot (Shoppes of Boot Ranch, next to Blockbuster Video) just before bridge.

TB16 Honeymoon Island – Return to 586(Tampa Road) and go back to Curlew. Turn right onto Curlew and follow road, dead-ends at state park (King Duncan Road). Take main park road to end; no swimming beach, high bird population. .

TB17 Allan's Creek –U.S. 19 South just after Belleair Rd intersection, Orange Blossom Grove parking lot, northwest corner.

TB18 Joe's Creek/Cross Bayou – Continue south on U.S.19 to 694, turn right onto Park Blvd, then left onto U.S.1 or Park/Starkey, first large bridge, pull off just past bridge, seawall in southwest corner.

Site Descriptions for Tampa Bay Healthy Beaches 1999-2000, con't

TB19 John's Pass Beach – Continue south on Park Street to ALT 19 (Bay Pines Blvd/Tyrone/595) and turn right (west), stay to left to 666 to Madeira Beach. Turn left on 699 (Gulf Blvd). 1st major bridge will be John's Pass, small public beach on south west side of bridge, outside of island.

TB20 North Beach, Ft. DeSoto Park – continue south on 699 to 682 (Pinellas Bayway) and turn left, turn right onto 679 to Fort DeSoto Park, turn right at ranger station and sample from north beach near lifeguard station.

TB21– stop at 4th Street south near USF coming back to university, between 17th and 18th Ave S., small white bridge on west side of road, good water movement.

TB22 Control Site – Middle of Tampa Bay, midwater, EPC station 16.

Appendix III

GIS Locations of Tampa Bay Sampling Sites

Location	Location Name	Lat	Long	Lat	Min	Sec	Long Calc
TB-1	Delaney Creek	27-54-55	82-24-06	82.00	24.00	6.00	-82.4017
TB-2	Alafia River Mouth	27-51-36	82-23-05	82.00	23.00	5.00	-82.3847
TB-3	Bullfrog Creek Mouth	27-50-17	82-22-54	82.00	22.00	54.00	-82.3817
TB-4	Bullfrog Creek at Symmes	27-50-07	82-20-46	82.00	20.00	46.00	-82.3461
TB-5	Alafia River at US 301	27-52-10	82-19-35	82.00	19.00	35.00	-82.3264
TB-6	Bullfrog Creek at Big Bend	27-47-31	82-21-07	82.00	21.00	7.00	-82.3519
TB-7	Little Bullfrog Creek	27-44-54	82-16-54	82.00	16.00	54.00	-82.2817
TB-8	Big Bullfrog Creek	27-45-05	82-20-07	82.00	20.00	7.00	-82.3353
TB-9	Little Manatee River at US 301	27-40-17	82-21-09	82.00	21.00	9.00	-82.3525
TB-10	Little Manatee River	27-42-16	82-26-50	82.00	26.00	50.00	-82.4472
TB-11	Manatee River	27-30-24	82-33-48	82.00	33.00	48.00	-82.5633
TB-12	Hillsborough River	27-56-52	82-27-47	82.00	27.00	47.00	-82.4631
TB-13	Courtney Campbell Causeway	27-57-39	82-41-52	82.00	41.00	52.00	-82.6978
TB-14	Sweetwater Creek	27-58-55	82-33-43	82.00	33.00	43.00	-82.5619
TB-15	Lake Tarpon Outfall Canal	28-03-40	82-42-36	82.00	42.00	36.00	-82.7100
TB-16	Honeymoon Island Beach	28-03-40	82-49-43	82.00	49.00	43.00	-82.8286
TB-17	Allens Creek	27-56-13	82-43-51	82.00	43.00	51.00	-82.7308
TB-18	Joe's Creek	27-54-00	82-45-15	82.00	45.00	15.00	-82.7542
TB-19	John's Pass Beach	27-46-50	82-46-59	82.00	46.00	59.00	-82.7831
TB-20	Fort Desoto North Beach	27-38-17	82-44-27	82.00	44.00	27.00	-82.7408
TB-21	Salt Creek	27-45-10	82-38-19	82.00	38.00	19.00	-82.6386
TB-22	Control Site	27-58-16	82-40-25	82.00	40.00	25.20	-82.6737

Appendix IV

Watershed Descriptions

Manatee

Area: 364.48 square miles, perimeter: 117.11 miles
Habitat: Forest Riparian, agricultural/urban riparian
Cities: Sarasota/Bradenton, Palmetto, Ellenton
Rivers and streams: 3
Lakes: 120
Total number of watershed acres: 2223.8
River and stream miles: 561.6 total river miles, 372.6 perennial river miles
Surficial aquifer system: 325 square miles of unconsolidated sand and gravel aquifers in this watershed
Physiographic province: Gulf Coastal Lowlands, Desoto Plain and Polk Upland
Materials: In the coastal areas, undifferentiated sandsss and shells occur near surface. Phosphatic, sandy carbonates subcrop further inland along the Manatee River. An isolated occurrence of phosphatic sandy clays lies in the center of the watershed.
Age: Oligocene to Holocene (30 mya to present)
Mineral Resources: shell, sand, limestone and phosphate

Little Manatee

Area: 211.01 square miles , perimeter: 92.7 miles Flow station is at TB10
Habitat: Forest Riparian, agricultural/urban riparian
Cities: Ruskin, Sun City Center, Wimauma, Gulf City
Rivers and streams: 1
Lakes: 75
Total number of watershed acres: 5142.7
River and stream miles: 235.7 total river miles, 218 perennial river miles
Surficial aquifer system: 144 square miles of unconsolidated sand and gravel aquifer in this watershed
Physiographic province: Gulf Coastal Lowlands and Polk Upland
Materials: In the coastal areas, undifferentiated sands and shells occur at or near surface. Phosphatic, sandy clays occurs toward the center of this watershed, especially along the Little Manatee River. The east half of the area is covered by up to 50 feet of ?
Age: Miocene to Holocene (14 mya to present)
Mineral Resources: phosphate, shell, quartz sand

Alafia

Area: 421.67 square miles , perimeter: 103.77 miles Flow station upstream from TB5
Habitat: Forest Riparian, agricultural/urban riparian
Cities: Brandon, Riverview, Gibsonton, Plant City
Rivers and streams: 5
Lakes: 370
Total number of watershed acres: 11643.2
River and stream miles: 302.4 total river miles, 298 perennial river miles
Surficial aquifer system: 346 square miles of unconsolidated sand and gravel aquifer in this watershed
Physiographic province: Polk Uplands and Gulf Coastal Lowlands. Several springs discharge ground water into the Alafia River.
Materials: Undifferentiated sands in this area range in thickness from less than five feet to more than 30 feet and contain variable amounts of clay and trace amounts of phosphate. These sands cover sandy carbonates along the Alafia river. In other areas, near-surf?
Age: Oligocene to Holocene (30 mya to present)
Mineral Resources: phosphate, quartz sand

Hillsborough

Area: 658.32 square miles , perimeter: 149.96 miles Flow station upstream from TB12
Habitat: Forest Riparian, agricultural/urban riparian
Cities: Tampa area, Temple Terrace
Rivers and streams: 5
Lakes: 327
Total number of watershed acres: 6571
River and stream miles: 480.5 total river miles, 279.4 perennial river miles
Surficial aquifer system: 371 square miles with no principal aquifer, 281 sq miles with Florida aquifer system (carbonate-rock aquifers) and 7 sq miles of surficial aquifer system of unconsolidated sand and gravel aquifers
Physiographic province: Gulf Coastal Lowlands, Zephyrhills Gap, Polk Upland, Lakeland Ridge, Western Valley
Materials: Undifferentiated sands cover much of the area, with limestones along the Hillsborough River and to the northeast along the Hills/Pasco county line. In eastern Hills county, near-surface sediments include phosphate clays.
Age: Oligocene to Holocene (30 mya to present)
Mineral Resources: phosphate and limestone
Hillsborough County pop 940,484 as of July 1999

Pinellas County Watershed

Pinellas County is not divided into distinct watersheds, but is rather a pennisula with a series of creeks, wetlands and inter-coastal waterways with non-continuous waterflow. The population as of July 1999 was 878,499. The county covers 264 square miles, and although it is the 2nd smallest county in Florida, it is the most densely developed and populated.

All watershed information was obtained from the Environmental Protection Agency's watershed website: http://www.epa.gov/surf3/states/FL/ and the Pinellas County website: http://www.co.pinellas.fl.us/

Appendix V

Raw Data Sheets – Physical and Chemical Measurements

Tampa Bay Healthy Beaches Project 1999-2000								
					(ntu)	(Degrees C)	(ppt)	
	Station	**Date**	**Time**	**pH**	**Turbidity**	**Temperature**	**Salinity**	**Flow/Tide Information**
Jun-99	TB1	06-Jun	8:58	7.31	1.00	26.5	5	slow
	TB2	06-Jun	9:22	8.13	2.00	28.0	24	no tide
	TB3	06-Jun	9:45	7.66	4.00	26.0	19	low tide
	TB4	06-Jun	10:04	7.42	1.75	24.5	0.5	
	TB5	06-Jun	10:26	7.50	2.75	26.5	5	
	TB6	06-Jun	11:00	7.40	1.50	25.0	0.5	
	TB7	06-Jun	11:19	6.97	0.50	24.5	0	
	TB8	06-Jun	11:37	6.82	0.50	24.0	0.5	
	TB9	06-Jun	11:56	6.79	2.00	25.0	0	
	TB10	06-Jun	12:45	7.63	1.50	27.5	19	outgoing
	TB11	06-Jun	1:15	8.28	3.50	28.0	29	outgoing
	TB12	22-Jun	7:45	7.53	1.50	28.0	14	high tide
	TB13	22-Jun	8:35	7.90	4.00	28.0	28	slack tide
	TB14	22-Jun	8:55	7.83	8.50	26.0	0	slack tide
	TB15	22-Jun	9:30	7.61	1.25	29.0	0	slack tide
	TB16	22-Jun	10:00	8.00	5.00	29.0	33	slack tide
	TB17	22-Jun	10:40	7.39	2.50	29.0	21	slack tide
	TB18	22-Jun	11:11	7.53	2.00	30.0	10	outgoing high tide
	TB19	22-Jun	12:00	8.11	4.75	31.0	36	incoming high tide
	TB20	22-Jun	12:50	8.16	3.75	32.0	35	low tide
	TB21	22-Jun	13:30	7.80	3.50	32.0	24	outgoing high tide
	TB22	22-Jun	9:40	8.10	1.50	28.4	31.2	incoming tide
Jul-99	TB1	07-Jul	9:30	6.95	7.50	28.0	0	outgoing
	TB2	07-Jul	9:50	7.54	2.00	29.0	15	outgoing
	TB3	07-Jul	10:20	7.44	2.00	29.0	16	flowing in
	TB4	07-Jul	10:45	7.61	3.00	26.0	2	flowing in
	TB5	07-Jul	11:00	7.34	1.00	28.0	0	flowing out
	TB6	07-Jul	11:20	7.35	1.00	26.0	0	flowing out
	TB7	07-Jul	11:45	6.75	0.50	26.0	2	flowing out
	TB8	07-Jul	12:05	6.82	0.50	28.0	0	still
	TB9	07-Jul	12:25	6.80	1.50	27.0	0	still
	TB10	07-Jul	13:30	6.79	1.50	32.0	5	still
	TB11	07-Jul	14:05	8.09	2.00	32.0	15	flowing out
	TB12	20-Jul	6:41	7.39	0.5	30	8	flowing in
	TB13	20-Jul	9:01	7.9	3.25	31	20	flowing in
	TB14	20-Jul	7:21	7.35	3	29.5	7	flowing in
	TB15	20-Jul	7:45	7.77	2	30	0	flowing out
	TB16	20-Jul	8:17	8.05	3	29	32	flowing in
	TB17	20-Jul	9:16	7.65	2	30	20	flowing in
	TB18	20-Jul	9:46	7.59	1.5	32	11	flowing in
	TB19	20-Jul	10:04	8.1	1	31	31	
	TB20	20-Jul	10:45	8.33	3	32	32	
	TB21	20-Jul	11:25	7.9	4.5	32	24	
	TB22	20-Jul	9:40			31	29.4	incoming tide

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Aug-99	TB1	10-Aug	9:35	7.26	3	28	0	high flow
	TB2	10-Aug	9:55	7.63	2.5	29	12	incoming-high tide
	TB3	04-Aug	8:05	7.6	3.5	30	21	
	TB4	04-Aug	7:40	7.4	1.25	27	0	
	TB5	10-Aug	10:20	7.57	5	27	0	high tide
	TB6	04-Aug	10:08	7.2	1.25	27	0	
	TB7	04-Aug	8:56	7.3	1	26.5	0	
	TB8	04-Aug	7:30	6.9	0.5	28	5	
	TB9	10-Aug	10:45	7.05	4.5	25	0	high water
	TB10	10-Aug	11:10	7.4	1.5	30	19	high tide-incoming
	TB11	10-Aug	11:35	7.8	3	31	17	high tide-incoming
	TB12	24-Aug	6:43	7.36	1.5	28	3	
	TB13	24-Aug	9:06	8.2	9.5	27	20	high tide
	TB14	24-Aug	7:25	7.45	6.5	28	2	
	TB15	24-Aug	7:54	7.42	1.25	29	0	
	TB16	24-Aug	8:29	8.12	20	28	33	high tide-strong surf
	TB17	24-Aug	9:24	7.45	2	27	12	slack tide
	TB18	24-Aug	9:55		4.25	27.5	2	slack tide-health warning
	TB19	24-Aug	10:14	8.2	10.25	29.5	34	high tide
	TB20	24-Aug	11:00	8.23	3.5	31	34	
	TB21	24-Aug	11:40	7.67	2	29	16	
	TB22	24-Aug	9:17	8.16	1.25	29.1	28.3	incoming tide
Sep-99	TB1	07-Sep	9:15	7.35	4	26	0	raining
	TB2	07-Sep	9:30	7.82	2.5	29	18	raining,high incoming tide
	TB3	07-Sep	9:45	7.46	3.5	27	10	raining,high incoming tide
	TB4	07-Sep	9:55	7.65	5.5	25	0	raining
	TB5	07-Sep	10:10	7.5	2.5	27	0	high tide
	TB6	07-Sep	10:20	7.13	3.75	25	0	high flow
	TB7	07-Sep	10:40	6.9	2	24.5	0	high flow
	TB8	07-Sep	10:50	7.1	1	25	0	high flow
	TB9	07-Sep	11:05	7.13	2.5	24.5	0	high flow
	TB10	07-Sep	11:50	7.3	1.5	29	16	high/incoming
	TB11	07-Sep	12:15	7.8	1.5	30	18	high tide
	TB12	23-Sep	7:55	7.49	1	27	15	
	TB13	23-Sep	9:30	7.89	6	27	22	
	TB14	23-Sep	8:00	7.54	3	27	2	
	TB15	23-Sep	8:35	7.75	2	26	0	
	TB16	23-Sep	9:15	8.01	10	24	33	
	TB17	23-Sep	10:30	7.51	5	27	18	
	TB18	23-Sep	10:05	7.63	4	26	14	
	TB19	23-Sep		7.9	3.5	26	34	
	TB20	23-Sep		8.1	4	25	32	
	TB21	23-Sep	10:45	7.7	2	26	18	
	TB22	28-Sep	9:32			27.1	27.1	outgoing

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Oct-99	TB1	05-Oct	9:20	7.3	7	25	0	raining
	TB2	05-Oct	9:35	7.4	3	26	8	raining,outgoing
	TB3	05-Oct	10:00	7.1	15	25	0	high flow,raining
	TB4	05-Oct	8:35	7	20	25	0	high flow,raining
	TB5	05-Oct	9:50	7.4	3.5	25	0	raining,outgoing
	TB6	05-Oct	11:15	7.1	10	24	1	
	TB7	05-Oct	10:00	6.6	15	24	1	
	TB8	05-Oct	8:40	7.6	20	24	0	
	TB9	05-Oct	10:20	7.1	8	25	0	raining,high water
	TB10	05-Oct	11:05	7.2	2.5	27	10	raining
	TB11	05-Oct	11:35	7.5	2.5	28	24	raining,outgoing
	TB12	26-Oct	7:05	7.42	1.5	21	0	
	TB13	26-Oct	9:25	8	5.5	20	22	very low tide
	TB14	26-Oct	7:38	7.5	5.5	21	8	
	TB15	26-Oct	8:10	7.8	1	22	2	
	TB16	26-Oct	8:48	7.8	25	19	30	very low tide
	TB17	26-Oct	9:38	7.7	5	21	20	low tide
	TB18	26-Oct	10:06	7.5	4	21	14	low tide
	TB19	26-Oct	10:26	7.9	25	21	34	low tide
	TB20	26-Oct	11:06	8.1	10	21	36	low tide
	TB21	26-Oct	11:43	7.7	5	22	20	low tide
	TB22	26-Oct	9:54		3.5	22.2	26.2	outgoing
Nov-99	TB1	09-Nov	9:30	6.6	2.2	19	0	
	TB2	09-Nov	9:45	7.4	2.4	21	10	low tide
	TB3	09-Nov	9:55	7.4	2.25	22	8	low tide
	TB4	09-Nov	10:06	7.5	3	18.5	0	low water
	TB5	09-Nov	10:17	7.7	2	22	2	low water
	TB6	09-Nov	10:36	7.8	2.5	20	0	low water
	TB7	09-Nov	10:46	7.1	0.8	19	0	
	TB8	09-Nov	11:01	6.9	0.33	19	0	
	TB9	09-Nov	11:15	7.1	1.2	19	0	low water
	TB10	09-Nov	10:45	6.9	1.5	22	10	low tide
	TB11	09-Nov	12:10	7.8	5.9	22	24	windy
	TB12	30-Nov	7:09	8.4	1.25	20	20	incoming tide
	TB13	30-Nov	9:45	8.3	14	20	24	low tide
	TB14	30-Nov	7:37	8.5	2.75	18	0	outgoing
	TB15	30-Nov	8:07	8.1	1.3	21	0	high tide
	TB16	30-Nov	8:45	7.9	4.5	18	30	low tide
	TB17	30-Nov	10:06	8.1	3.4	19	23	low tide
	TB18	30-Nov	10:35	7.8	4.1	21	22	low tide
	TB19	30-Nov	10:55	8	7.5	21	35	mid tide
	TB20	30-Nov	11:30	8.1	4.9	21	36	low tide
	TB21	30-Nov	12:10	7.7	4.75	21	26	very low tide
	TB22	30-Nov	9:30			20.5	28.8	outgoing

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Dec-99	TB1	15-Dec		7.3	4.1			
	TB2	15-Dec		7.6	3.6			
	TB3	15-Dec		7.6	1.75			low tide
	TB4	15-Dec		7.6	1.75			
	TB5	15-Dec	8:23	7.5	1.25	20	5	
	TB6	15-Dec	9:00	7.7	1.5	18	0	
	TB7	15-Dec	10:00	7.3	0.6	19	0	
	TB8	15-Dec	11:15	7	0.4	20	0	
	TB9	15-Dec	12:10	7.3	1.4	20	0	
	TB10	15-Dec	13:00	7.6	1.1	24	11	
	TB11	15-Dec	13:35	8	0.9	23	30	
	TB12	15-Dec	7:47	7.6	0.6	20	17	flowing in
	TB13	15-Dec	10:00	7.8	4.4	20	25	low tide
	TB14	15-Dec	8:18	7.7	2.4	19	6	flowing in
	TB15	15-Dec	8:44	7.7	1.25	20	2	flowing out
	TB16	15-Dec	9:22	8.1	11	19	34	low tide
	TB17	15-Dec	10:16	7.7	2.4	20	23	low tide
	TB18	15-Dec	10:36	7.5	2	22	21	flowing in
	TB19	15-Dec	10:54	8	7.4	20	35	
	TB20	15-Dec	11:33	8.2	3	22	35	
	TB21	15-Dec	12:05	7.5	2.6	21	25	
	TB22	08-Dec	9:30			18.3	29.2	incoming tide
Jan-00	TB1	25-Jan	9:25		1.8	15	0	
	TB2	25-Jan	9:40		4.2	17	16	
	TB3	25-Jan	9:50		7.6	16	5	
	TB4	25-Jan	10:05		4.3	14	0	
	TB5	25-Jan	10:20		11	17	2	
	TB6	25-Jan	10:35		2.7	14	0	
	TB7	25-Jan	10:55		1.3	14	0	
	TB8	25-Jan	11:10		0.3	15	0	
	TB9	25-Jan	11:25		1.1	16	0	
	TB10	25-Jan	12:20		1	17	14	
	TB11	25-Jan	12:45		6.5	17	30	
	TB12							
	TB13							
	TB14							
	TB15							
	TB16							
	TB17							
	TB18							
	TB19							
	TB20							
	TB21							
	TB22	25-Jan	9:27		5.8	16.3	30	outgoing

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Feb-00	TB1	15-Feb	9:44	7.1	1.9	20	2	
	TB2	15-Feb	9:57	7.9	2.1	20	15	
	TB3	15-Feb	10:07	7.8	6	19	12	
	TB4	15-Feb	10:18	8	3.9	18	2	
	TB5	15-Feb	10:33	7.9	2.6	20	5	
	TB6	15-Feb	10:48	8.1	3.6	19	0	
	TB7	15-Feb	11:13	7.3	1.5	19	1	
	TB8	15-Feb	11:25	7.3	0.4	20	1	
	TB9	15-Feb	11:37	7.6	0.7	19	1	
	TB10	15-Feb	12:45	7.5	1.5	21	16	
	TB11	15-Feb	1:12	7.9	1.1	23	30	
	TB12	22-Feb	8:10	7.6	1.4		24	
	TB13	22-Feb	9:30	7.9	19	19	26	outgoing low
	TB14	22-Feb	8:30	7.5	6.5	18	8	outgoing low
	TB15	22-Feb	9:24	7.8	1.1		0	
	TB16	22-Feb	9:52	8	5.4		33	
	TB17	22-Feb	10:30	7.5	3	19	16	outgoing low
	TB18	22-Feb	11:37	7.7	1.8	21	18	
	TB19	22-Feb	7:50	7.9	4	18	34	
	TB20	22-Feb	9:50	8.1	3.4	18.5	36	
	TB21	22-Feb	10:50	7.5	4.3	17	26	
	TB22	22-Feb	9:34			18.8	29.1	outgoing
Mar-00	TB1	21-Mar		7.4	2.5		10	
	TB2	21-Mar		7.4	2.4		22	
	TB3	21-Mar		7.3	1.5	23	12	
	TB4	21-Mar		7.5	0.9		0	
	TB5	21-Mar	8:45	7.4	2.3	24	5	
	TB6	21-Mar	9:20	7.6	0.7	20.5	1	
	TB7	21-Mar	11:00	6.8	1.1	20	1	
	TB8	21-Mar	11:30	6.9	0.25	23	0	
	TB9	21-Mar	1:20	7.1	2	22	1	
	TB10	21-Mar	1:50	7.4	2.4	24	23	
	TB11	21-Mar	2:20	7.9	2.2	25	32	
	TB12	21-Mar	8:31	7.3	3.1	23	26	
	TB13	21-Mar	10:35	7.4	7.5	26	28	
	TB14	21-Mar	9:00	7.4	1.9	23	15	
	TB15	21-Mar	9:30	7.9	1.1	23	2	
	TB16	21-Mar	9:55	8	21	22	36	
	TB17	21-Mar	10:50	7.6	4	25	26	
	TB18	21-Mar	11:10	7.4	1.9	25	26	
	TB19	21-Mar	3:45	7.9	7.6	24	36	
	TB20	21-Mar	1:51	7.9	10	27	35	
	TB21	21-Mar	3:00	7.6	1.7	25.5	30	
	TB22	21-Mar	9:25	7.8	4	22.4	30.7	incoming

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Apr-00	TB1	12-Apr	8:45	7.4	2.2	23	13	
	TB2	12-Apr	9:00	8	2	22	27	
	TB3	12-Apr	9:10	7.7	3	21	23	
	TB4	12-Apr	9:15	8.2	0.7	19	0	
	TB5	12-Apr	9:30	8.4	11	21	7	
	TB6	12-Apr	9:50	8.6	0.5	20	0	
	TB7	12-Apr	10:10	7.6	2.5	19	0	
	TB8	12-Apr	10:25	7.5	0.4	20	0	
	TB9	12-Apr	10:40	7.9	0.6	19	0	
	TB10	12-Apr	11:00	7.7	1.4	22	24	
	TB11	12-Apr	11:30	8	1.4	23	33	
	TB12							
	TB13	25-Feb	10:00	7.9	35	26.5	30	
	TB14	25-Feb	8	7.5	2.3	26.5	27	
	TB15							
	TB16							
	TB17	25-Apr	11:30	6	4.2	27	25	
	TB18							
	TB19	25-Apr	8:30	8	12	20	36	high tide
	TB20	25-Apr	10:30	8.1	5	25	36	low tide
	TB21	12-Apr	12:00	8	1.6	23	31	
	TB22	25-Apr	10:00	7.8		24.4	32.3	
May-00	TB1	10-May	10:35	7.2		26	15	
	TB2	10-May	11:00	8		27	30	
	TB3							
	TB4							
	TB5	10-May	11:20	8.3		26	0	
	TB6							
	TB7							
	TB8							
	TB9	10-May	12:00	8.1		24	0	
	TB10	10-May	12:20	7.6		26.5	28	
	TB11							
	TB12	10-May	7:00	7.8		27	30	
	TB13	30-May	9:15	7.6	7	28	31	
	TB14	30-May	8:20	7.2	5.5	27.5	19	
	TB15	10-May	7:45	8.7		27	0	
	TB16	10-May	8:15	8.2		26	36	
	TB17	30-May	10:15	7.4	3.5	29.5	30	
	TB18	10-May	9:15	7.5		27	34	
	TB19	30-May	8:30	8	3	78	38	
	TB20	30-May	10:15	8	4	90	36	
	TB21	10-May	1:10	7.8		27	32	
	TB22	23-May	14:55			27.7	33.6	incoming strong

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Jun-00	TB1							
	TB2							
	TB3							
	TB4							
	TB5							
	TB6							
	TB7							
	TB8							
	TB9							
	TB10							
	TB11							
	TB12							
	TB13	19-Jun	10:00	7.9	4.3	30	30	
	TB14	19-Jun	9:00	6.9	5	27	20	
	TB15							
	TB16							
	TB17	19-Jun	11:15	7.4	4	30	29	
	TB18							
	TB19	19-Jun		7.7	2.5	32	38	
	TB20	19-Jun		7.8	7	30	36	
	TB21							
	TB22							
Jul-00	TB1							
	TB2							
	TB3	11-Jul	9:15	7.9	4.25	28	20	
	TB4	11-Jul	11:00	7.5	1.5	30	0	
	TB5							
	TB6	11-Jun	10:25	6.4	0.45		0	
	TB7	11-Jun	9:30	6.2	0.5		0	
	TB8	11-Jun	8:25	6.2	2.5	27	0	
	TB9							
	TB10							
	TB11							
	TB12							
	TB13							
	TB14							
	TB15							
	TB16							
	TB17							
	TB18							
	TB19							
	TB20							
	TB21							
	TB22							

	Station	Date	Time	pH	Turbidity	Temperature	Salinity	Flow/Tide Information
Aug-00	TB1							
	TB2							
	TB3	01-Aug	9:45	6.6	3	27	5	low tide
	TB4	01-Aug	9:00	6.7	4.5	27	0	low tide
	TB5							
	TB6	01-Aug	8:50	6.7	1.8	26.5	0	
	TB7	01-Aug	9:55	6.5	0.4	26	0	
	TB8	01-Aug	11:00	6.2	0.4	26	0	
	TB9							
	TB10							
	TB11							
	TB12							
	TB13	08-Aug	9:30	8.1	4.5	30.5	28	
	TB14	08-Aug	8:20	7.2	5.8	28	3	
	TB15							
	TB16							
	TB17	08-Aug	10:20	7.7	3.3	32	25	
	TB18							
	TB19	08-Aug	9:00	7.9	4	31	36	
	TB20	08-Aug	10:00	7.7	3.2	32	36	
	TB21							
	TB22							

Appendix VI

Results of Traditional and Alternative Indicator Monitoring

Table 1

Fecal Coliform Results

Fecal Coliform results are given for each sampling site for all months of the study, and are reported in colony forming units (CFU) per 100 mL of water. The figures in **bold** show the months that the suggested guideline of 800 cfu/100mL for a single sample was exceeded.
TB1: Fecal Coliforms (FC) ranged from 55 to 24,450 cfu/100mL, with 2 months (Oct 99 and Mar 00) of out 12 exceeding the guideline.
TB2: FC ranged from <10 to 7415 cfu/100mL, with only 1 month (Mar 00) exceeding the guideline.
TB3: FC ranged from 100 to 16,350 cfu/100mL, with 4 months (Aug 99, Oct 99, Nov 99 and Mar 00) exceeding the guideline.
TB4: FC ranged from 550 to 174,900 cfu/100mL, with all months **except** Jun 99, Aug 99 and Apr 00 exceeding the guideline.
TB5: FC ranged from 35 to 5300 cfu/100mL, with 5 months (Jun 99, Sep 99, Oct 99, Dec 99 and Mar 00) exceeding the guideline.
TB6: FC ranged from 300 to 110,200 cfu/100 mL, with all months **except** Jun 99, Jul 99, Mar 00 and Aug 00 exceeding the guideline.
TB7: FC ranged from 12 to 13,850 cfu/100 mL, with 5 months (Aug 99, Sep 99, Oct 99, Dec 99 and Mar 000 exceeding the guideline.
TB8: FC ranged from 90 to 6050 cfu/100 mL, with 2 months (Oct 99 and Dec 99) exceeding the guideline.
TB9: FC ranged from 100 to 10,250 cfu/100 mL, with 3 months (Aug 99, Oct 99 and Mar 00) exceeding the guideline.
TB10: FC ranged from 10 to 4140 cfu/100 mL, with 2 months (Oct 99 and Mar 00) exceeding the guideline.
TB11: FC ranged from <10 to 3890 cfu/100 mL, with only one month (Mar 00) exceeding the guideline.
TB12: FC ranged from <10 to 3400 cfu/100 mL, with 4 months (Aug 99, Dec 99, Feb 99 and Mar 00) exceeding the guideline.
TB13: FC ranged from 15 to 26,900 cfu/100 mL, with 4 months (Sep 99, Dec 99, Feb 00 and Mar 00) exceeding the guideline.
TB14: FC ranged from 80 to 33,150 cfu/100 mL, with all months **except** Nov 99 and Apr 00 exceeding the guideline.
TB15: FC ranged from 35 to 40,000 cfu/100 mL, with only Mar 00 exceeding the guideline.

TB16: FC ranged from <4 to 4745 cfu/100 mL, with only Dec 99 exceeding the guideline.
TB17: FC ranged from <10 to 23,700 cfu/100 mL, with 7 months (Aug 99, Sep 99, Dec 99, Feb 00, Apr 00, Jun 00 and Aug 00) exceeding the guidelines.
TB18: FC ranged from <10 to 3115 cfu/100 mL, with 2 months (Aug 99 and Dec 99) exceeding the guideline.
Table 1 con't
TB19: FC ranged from <10 to 13,240 cfu/100 mL, with 2 months (Sep 99 and Dec 99) exceeding the guideline.
TB20: FC ranged from <4 to 10,900 cfu/100 mL, with 4 months (Sep 99, Dec 99, Feb 00 and Mar 00) exceeding the guideline.
TB21: FC ranged from <10 to 6100 cfu/100 mL, with 3 months (Sep 99, Feb 00 and Mar 00) exceeding the guideline.
TB22: FC ranged from <1 to 4, with no months exceeding the guideline.

The months of Mar 00 and Dec 99 were the worst in terms of the most sites exceeding the FC guideline, with 15 and 13 sites respectively out of 22. Sep and Oct 99 follow closely behind with 10 sites out of 22 for both months.

				Reported in CFU/100ml											
Table 1 Fecal Coliform Results															
Station	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99	Jan-00	Feb-00	Mar-00	Apr-00	May-00	Jun-00	Jul-00	Aug-00
TB1	160	335	440	225	9050	600	215	400	540	24450	55	65			
TB2	<10	52	265	15	555	190	140	90	125	7415		<10			
TB3	590	435	3350	440	16350	3500	380	660	420	9600	100			150	
TB4	605	980	800	2600	174900	3950	16450	6000	3950	28650	550			50500	5000
TB5	1350	175	485	810	5300	440	1230	700	580	2925	650	35			
TB6	435	580	4100	1210	110200	1750	5050	1080	2150	800	1100			1200	300
TB7	175	315	8450	1890	8300	500	13850	430	680	17200	450			490	12
TB8	90	130	90	105	1035	200	6050	650	370	140	200			430	320
TB9	256.5	280	1500	185	10250	160	495	180	100	4200	200	315			
TB10	110	270	500	52	985	40	32	20	130	4140	10	16			
TB11	<10	20	76	56	200	15	4	145	10	3890	10				
TB12	575	240	940	460	55	15	3050		3400	3200		<10			
TB13	50	370	270	9550	40	75	900		1100	26900	200	210	15		60
TB14	4250	1000	6200	12600	1850	170	33150		10500	5650	80	15100	22500		>3000
TB15	335	175	350	320	250	123	100		35	40000		185			
TB16	<10	16	390	2	56	<4	4745		10	5		10			
TB17	610	310	950	23700	160	<10	1690		5700	325	2700	75	5685		2000
TB18	185	110	850	330	280	<10	3115		70	50		20			
TB19	15	26	34	13240	80	10	3055		700	70	<10	<10	20		
TB20	<10	<4	166	10900	<10	<4	4495		2550	2340	<10	<10	12		2
TB21	<10	190	325	1050	180	25	5340		6100	4810	190	<10			
TB22	<10	4	<1	<4	<4	<2		<4	<4		<2	<2			

Table 2

E.coli Results

E.coli (EC) results are given for each sampling site for all months of the study, and are reported in colony forming units (CFU) per 100 mL of water. The figures in bold show the months that the suggested guideline of 126 cfu/100mL was exceeded
TB1: EC ranged from 50 to 24,450 cfu/100 mL, with all months **except** Dec 99 and Apr 00 exceeding the guideline.
TB2: EC ranged from 0.5 to 7415 cfu/100 mL, with 4 months (Aug 99, Oct 99, Nov 99 and Mar 00) exceeding the guideline.
TB3: EC ranged from 75 to 16,350 cfu/100 mL, with all months **except** Dec 99, Apr 00 and Jul 00) exceeding the guideline.
TB4: EC ranged from 50 to 174,900 cfu/100 mL, with all months **except** Dec 99 exceeding the guideline.
TB5: EC ranged from 85 to 5300 cfu/100 mL, with all months **except** Jul 99 exceeding the guideline.
TB6: EC ranged from 350 to 110,200 cfu/100 mL, with all months exceeding the guideline.
TB7: EC ranged from 175 to 17,200 cfu/100 mL, with all months exceeding the guideline.
TB8: EC ranged from 70 to 900 cfu/100 mL, with all months **except** Jun 99, Jul 99, Aug 99 and Mar 00 exceeding the guideline.
TB9: EC ranged from 45 to 10,250 cfu/100 mL, with 6 months (Jun 99, Jul 99, Aug 99, Oct 99, Mar 00 and Apr 00) exceeding the guideline.
TB10: EC ranged from 10 to 4140 cfu/100 mL, with 3 months (Jul 99, Oct 99 and Mar 00) exceeding the guideline.
TB11: EC ranged from <10 to 3890 cfu/100 mL, with only Mar 00 exceeding the guideline.
TB12: EC ranged from 15 to 3200 cfu/100 mL, with all months **except** Oct 99 and Nov 99 exceeding the guideline.
TB13: EC ranged from 10 to 26,900 cfu/100 mL, with 6 months (Jul 99, Sep 99, Feb 00, Mar 00, Apr 00 and May 00) exceeding the guideline.
TB14: EC ranged from 80 to 15,100 cfu/100 mL, with all months **except** Apr 00 exceeding the guideline.
TB15: EC ranged from 45 to 235 cfu/100 mL, with 2 months (Jun 99 and Aug 99) exceeding the guideline.
TB16: EC ranged from <4 to 4745 cfu/100 mL, with 2 months (Aug 99 and Dec 99) exceeding the guideline.
TB17: EC ranged from <10 to 23,700 cfu/100 mL, with all months **except** Nov 99 and May 00 exceeding the guideline.
TB18: EC ranged from <10 to 3115 cfu/100 mL, with 3 months (Jun 99, Aug 99 and Dec 99) exceeding the guideline.
TB19: EC ranged from <10 to 13,240 cfu/100 mL, with 3 months (Sep 99, Dec 99 and Feb 00) exceeding the guideline.

TB20: EC ranged from <4 to 10,900 cfu/100 mL, with 4 months (Sep 99, Dec 99, Feb 00 and Mar 00) exceeding the guideline.
TB21: EC ranged from <10 to 5340 cfu/100 mL, with 6 months (Jul 99, Aug 99, Sep 99, Dec 99, Feb 00 and Mar 00) exceeding the guideline.
TB22: EC results were negative for all months.

				Reported in CFU/100ml										
Table 2 *Ecoli* Results														
Station	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99	Jan-00	Feb-00	Mar-00	Apr-00	May-00	Jun-00	Jul-00
TB1	160	335	440		9050	550	50	200	350	24450	300			
TB2	0.5	30	265		200	190	50	80	75	7415	0			
TB3	150	435	2150		16350	1500	75	650	190	9600	100			100
TB4	605	500	520		174900	3950	50	1900	1400	28650	450			50500
TB5	1200	85	485		5300	440	900	550	210	2925	400			
TB6	435	580	2860		110200	1750	350	800	800	700	750			600
TB7	175	200	8450		8300	300		250	680	17200	400			210
TB8	90	70	70		900	200		310	220	95	200			170
TB9	256	200	750		10250	100	45	120	100	4200	200			
TB10	110	270	30		650	40	25	20	45	4140	10			
TB11	<10	8	28		55	15	4	55	5	3890	10			
TB12	500	180	310	190	50	15			1700	3200				
TB13	35	305	80	9500	30	60			800	26900	200	135	10	
TB14	425	900	2300	12600	1100	170			1350	1650	80	15100	6000	
TB15	150	45	235	85	125	123	90		35					
TB16	<10	12	205	2	48	<4	4745		10	5				
TB17	610	240	390	23700	140	<10	1690		1850	140	650	75	225	
TB18	170	80	500	125	105	<10	3115		60	40				
TB19	15	20	24	13240	70	10	3055		310	40	<10	<10	12	
TB20	<10	<4	122	10900	<10	<4	4495		1850	2340	<10	<10	4	
TB21	<10	130	195	650	90	25	5340		2250	4810	130			
TB22	<10	<4	<1	<4	<4				<4		<2			

Table 3

Enterococci Results

Enterococci results are given for each sampling site for all months of the study, and are reported in colony forming units (CFU) per 100 mL of water. The figures in **bold** show the months that the suggested guideline of 104 cfu/100mL for a single sampling was exceeded.
TB1: Enterococci (ETC) ranged from 40 to 12,300 cfu/100 mL, with all months **except** Jun 99, Mar 00 and May 00 exceeding the guideline.
TB2: ETC ranged from <4 to 496 cfu/100 mL, with 2 months (Aug 99 and Oct 99) exceeding the guideline.
TB3: ETC ranged from 10 to 17,200 cfu/100 mL, with 5 months (Sep 99, Oct 99, Nov 99, Jan 00 and Feb 00) exceeding the guideline.
TB4: ETC ranged from 134 to 135,650 cfu/100 mL, with all months exceeding the guideline.
TB5: ETC ranged from 2 to 6350 cfu/100 mL, with all months **except** Feb 00, Mar 00, Apr 00 and May 00) exceeding the guideline.
TB6: ETC ranged from 68 to 43,000 cfu/100 mL, with all months **except** Mar 00 and Apr 00 exceeding the guideline.
TB7: ETC ranged from 44 to 31650 cfu/100 mL, with all months **except** Jun 99 exceeding the guideline.
TB8: ETC ranged from 42 to 17,850 cfu/100 mL, with 8 months (Jul 99, Aug 99, Sep 99, Oct 99, Dec 99, Jan 00, Jul 00 and Aug 00) exceeding the guideline.
TB9: ETC ranged from 20 to 17,200 cfu/100 mL, with 7 months (Jul 99, Aug 99, Sep 99, Oct 99, Nov 99, Dec 99 and Jan 00) exceeding the guideline.
TB10: ETC ranged from 10 to 2905 cfu/100 mL, with 3 months (Jul 99, Oct 99 and May 00) exceeding the guideline.
TB11: ETC ranged from <4 to 102 cfu/100 mL, with no months exceeding the guideline.
TB12: ETC ranged from 2 to 585 cfu/100 mL, with 4 months (Jun 99, Jul 99, Aug 99 and Sep 99) exceeding the guideline.
TB13: ETC ranged from <10 to 600 cfu/100 mL, with 5 months (Jul 99, Aug 99, Nov 99, Feb 00 and May 00) exceeding the guideline.
TB14: ETC ranged from 5 to 35,000 cfu/100 mL, with all months **except** Feb 00 and Apr 00 exceeding the guideline.
TB15: ETC ranged from 8 to 236 cfu/100 mL, with 2 months (Jun 99 and Aug 99) exceeding the guideline.
TB16: ETC ranged from <2 to 557 cfu/100 mL, with only Aug 99 exceeding the guideline.
TB17: ETC ranged from 14 to 720 cfu/100 mL, with 8 months (Jun 99, Jul 99, Aug 99, Sep 99, Nov 99, Dec 99, Jun 00 and Aug 00) exceeding the guideline.
TB18: ETC ranged from 8 to 124 cfu/100 mL, with only Aug 99 exceeding the guideline.
TB19: ETC ranged from <4 to 28 cfu/100 mL, with no month exceeding the guideline.
TB20: ETC ranged from <2 to 77 cfu/100 mL, with no month exceeding the guideline.

TB21: ETC ranged from <4 to 1270 cfu/100 mL, with 2 months (Jul 99 and Sep 99) exceeding the guideline.
TB22: ETC ranged from <2 to 1 cfu/100 mL, with no months exceeding the guideline.

The months of Aug 99 and Jul 99 were the worst in terms of the most sites exceeding the Enterococci guideline, with 15 and 13 sites respectively out of 22. Sep, Oct and Nov 99 follow closely behind with 12, 11 and 10 sites out of 22 respectively.

				Reported in CFU/100 ml														
Table 3 Enterococci Results																		
Station	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99	Jan-00	Feb-00	Mar-00	Apr-00	May-00	Jun-00	Jul-00	Aug-00			
TB1	42	354	476	765	12300	2450	340	710	550	85	380	40						
TB2	<4	4	164	6	496	35	40	46	34	4	8	<4						
TB3	86	70	90	368	17200	300	90	280	200	10	82			40	2105			
TB4	530	985	2030	4520	136650	3050	8100	12550	4700	134	480			14050	2000	1451992		
TB5	1795	185	2615	1550	6350	250	275	510	80	2	94	20						
TB6	152	440	585	300	41700	1000	2000	9050	43000	68	78			720	1500			
TB7	44	580	7620	5450	31650	2200	580	530	420	310	110			425	160	4169583		
TB8	50	366	115	612	17850	50	360	525	90	82	42			200	370			
TB9	76	202	4340	1110	17200	170	130	120	30	20	935	50						
TB10	32	128	10	36	2905	30	10	24	36	90	20	335						
TB11	<10	4	32	24	102	4	2	18	<4		6	4						
TB12	585	120	310	300	65	96	70		12	12		2						
TB13	72	335	125	44	6	146	32		600	6	45	142	<10		50			
TB14	5980	500	1390	980	1850	1550	3100		100	108	5	224	24350		35000			
TB15	210	84	236	62	40	12	20		8	12		16						
TB16	<4	4	557	4	2	<4	<2		6	<4		4						
TB17	450	145	510	720	45	118	190		22	30	40	14	116		258			
TB18	30	12	124	95	78	86	66		8	10		8						
TB19	<4	<4	28	2	2	4	18		6	2	<4	2	5		1			
TB20	<4	<4	77	4	2	1	<2		<2	<4	<4	<4	1		<2			
TB21	<4	146	50	1270	50	36	28		4	10	6	<4						
TB22	<4	<1	0.5	<2	<1	<2		1	<2	1	<2							

Table 4

Clostridium perfringens Results

Clostridium perfringens results are given for each sampling site for all months of the study, and are reported in colony forming units (CFU) per 100 mL of water. The figures in **bold** show the months that the level exceeded 50 cfu/100mL, which is recommended by Fujioka et al, 1985, in Hawaiian waters. The underlined figures show the months that the saline water sites exceeded 5 cfu/100 mL, which has been proposed as the guideline for marine waters. (Fujioka, University of Hawaii, personal communication)
TB1: *Clostridium perfringens* (CP) ranged from <4 to 32, with no months exceeding the guideline.
TB2: CP ranged from <4 to 14 cfu/100 mL, with 3 months (Aug 99, Dec 99 and Jan 00) exceeding the marine water guideline.
TB3: CP ranged from <4 to 50 cfu/100 mL, with 8 months (Jun 99, Aug 99, Sep 99, Nov 99, Feb 00, Mar 00, Jul 00 and Aug 00) exceeding the marine water guideline.
TB4: CP ranged from <4 to 160 cfu/100 mL, with 3 months (Aug 99, Mar 00 and Aug 00) exceeding the fresh water guideline.
TB5: CP ranged from <4 to 46 cfu/100 mL, with no months exceeding the guideline.
TB6: CP ranged from <4 to 46 cfu/100 mL, with no months exceeding the guideline.
TB7: CP ranged from <4 to 32 cfu/100 mL, with no months exceeding the guideline.
TB8: CP ranged from <4 to 16 cfu/100 mL, with no months exceeding the guideline.
TB9: CP ranged from <4 to 16 cfu/100 mL, with no months exceeding the guideline.
TB10: CP ranged from <4 to 14 cfu/100 mL, with 2 months (Feb 00 and Mar 00) exceeding the marine water guideline.
TB11: CP ranged from <4 to 6 cfu/100 mL, with only Nov 99 exceeding the marine water guideline.
TB12: CP ranged from <4 to 22 cfu/100 mL, with only Aug 99 exceeding the marine water guideline.
TB13: CP ranged from <4 to 52 cfu/100 mL, with 4 months (Jul 99, Aug 99, Feb 00 and Apr 00) exceeding the marine water guideline.
TB14: CP ranged from <4 to 148 cfu/100 mL, with 2 months (Jun 99 and Aug 99) exceeding the guideline.
TB15: CP ranged from <4 to 34 cfu/100 mL, with no months exceeding the guideline.
TB16: CP was below the detection limit for all months of the study.
TB17: CP ranged from < 2 to 26 cfu/100 mL, with 5 months (Jun 99, Jul 99, Aug 99, Sep 99 and Mar 00) exceeding the marine water guideline.
TB18: CP ranged from <2 to 30 cfu/100 mL, with 4 months (Jun 99, Aug 99, Sep 99, Feb 00) exceeding the guideline.
TB19: CP ranged from <2 to 4 cfu/100 mL, with no months exceeding the guideline.
TB20: CP ranged from <2 to 10 cfu/100 mL, with only Dec 99 exceeding the marine water guideline.
TB21: CP ranged from <2 to 18 cfu/100 mL, with only Aug 99 exceeding the marine water guideline.
TB22: CP was below the detection limit for all months of the study.

				Reported in CFU/100ml											
Table 4 *Clostridum perfringens* Results															
Station	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99	Jan-00	Feb-00	Mar-00	Apr-00	May-00	Jun-00	Jul-00	Aug-00
TB1	<10	<4	7	15	8	12	<4	<4	6	32	6				
TB2	<10	<4	8	5	4	4	14	8	2	<4	<4				
TB3	20	<4	20	14	2	50	<4		44	28	8			26	38
TB4	<10	<4	62	9	<4	30	<4	<4	8	98	14			44	160
TB5	<10	<4	12	9	2	26	<4	<4	8	46	2				
TB6	<10	<4	46	10	<4	18	<4	22	26	12	<4			28	<4
TB7	15	<4	12	2	2	6	2	4	20	32	<4			8	<4
TB8	<10	<4	16	8	<4	10	4	6	4	<4	2			12	<4
TB9	<10	<4	16	6	<4	10	<4	<4	<4	4	2				
TB10	<10	<4	<4	<4	2	2	<4	2	14	12	<4				
TB11	<10	<4	1	2	2	6	<4	<4	2	<4	<4				
TB12	4	4	22	<4	<2		4	<4	<4	2					
TB13	<4	18	12	2	<2		2		52	<4	44	2	<4		<4
TB14	148	20	54	2	4		<4		<4	8	<4	6	40		18
TB15	34	5	8	4	<2		2		14	<4					
TB16	<4	<4	<4	<4	<2		<4		<4	<4					
TB17	12	26	20	14	<2		<4		<4	12	4	<4	2		4
TB18	18	5	30	10	<2		<4		26	<4					
TB19	<4	<4	<4	<4	<2		4		<4	<4	2	<4	<4		<4
TB20	<4	<4	<4	<4	<2		10		<4	<4	<4	<4	<4		<4
TB21	<4	<4	18	<4	<2		4		<4	<4	<4				
TB22	<4	<4	<4	<4	<2				<4	<4	<4				

Table 5

Coliphage Results

Coliphage results are given for each sampling site for all months of the study, and are reported in plaque forming units (PFU) per 100 mL of water. The figures in **bold** show the months that the level of 100 cfu/100mL for a single sampling was exceeded. (This guideline has been suggested by Lipp et al, ????)
TB1: Coliphage ranged from 50 to 7560 pfu/100 mL, with all months **except** Mar 00 exceeding the guideline.
TB2: Coliphage ranged from <10 to 140 pfu/100 mL, with only Nov 99 exceeding the guideline.
TB3: Coliphage ranged from 20 to 1850 pfu/100 mL, with all months **except** Jun 99, Aug 99, Sep 99, Apr 00 and Jul 00 exceeding the guideline.
TB4: Coliphage ranged from 110 to 28,180 pfu/100 mL, with all months exceeding the guideline.
TB5: Coliphage ranged from <10 to 980 pfu/100 mL, with 4 months (Sep 99, Oct 99, Dec 99 and Jan 00) exceeding the guideline.
TB6: Coliphage ranged from 90 to 22,920 pfu/100 mL, with all months **except** Jun 99 exceeding the guideline.
TB7: Coliphage ranged from <10 to 3680 pfu/100 mL, with all months **except** Jun 99, Jul 99, Aug 99, Jul 00 and Aug 00 exceeding the guideline.
TB8: Coliphage ranged from <10 to 1680 pfu/100 mL, with 3 months (Sep 99, Oct 99 and Jan 00) exceeding the guideline.
TB9: Coliphage ranged from 30 to 1470 pfu/100 mL, with all months **except** Nov 99, Mar 00 and Apr 00 exceeding the guideline.
TB10: Coliphage ranged from <10 to 1080 pfu/100 mL, with only Oct 99 exceeding the guideline.
TB11: Coliphage ranged from <10 to 20 pfu/100 mL, with no months exceeding the guideline.
TB12: Coliphage ranged from <10 to 260 pfu/100 mL, with only Aug 99 exceeding the guideline.
TB13: Coliphage ranged from <10 to 20 pfu/100 mL, with no months exceeding the guideline.
TB14: Coliphage ranged from 70 to 2650 pfu/100 mL, with all months **except** Jul 99, Feb 00 and Apr 00 exceeding the guideline.
TB15: Coliphage ranged from <10 to 30 pfu/100 mL, with no months exceeding the guideline.
TB16: Coliphage results were below the detection limit for all months of the study.
TB17: Coliphage ranged from <10 to 120 pfu/100 mL, with only Aug 99 exceeding the guideline.
TB18: Coliphage ranged from <10 to 220 pfu/100 mL, with 2 months (Aug 99 and Sep 99) exceeding the guideline.
TB19: Coliphage ranged from <10 to 20 pfu/100 mL, with no months exceeding the guideline.

TB20: Coliphage ranged from <10 to 20 pfu/100 mL, with no months exceeding the guideline.
TB21: Coliphage ranged from <10 to 230 pfu/100 mL, with only Sep 99 exceeding the guideline.
TB22: Coliphage results were below the detection limit for all months of the study.

The months of Sep 99 and Oct 99 were the worst in terms of the most sites exceeding the Coliphage guideline, with 10 sites out of 22 for both months. Aug 99, Dec 99 and Jan 00 follow closely behind with 8 sites out of 22 for all three months.

			Reported in PFU/100ml												
Table 5 Coliphage Results															
Station	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99	Jan-00	Feb-00	Mar-00	Apr-00	May-00	Jun-00	Jul-00	Aug-00
TB1	320	110	180	1400	7560	160	580	720	2030	50	380				
TB2	<10	10	50	10	90	140	20	10	90	<10	<10				
TB3	30	150	20	20	1850	700	450	1050	610	310	90			80	556
TB4	1850	110	530	1800	28180	600	670	600	870	590	960			930	486
TB5	<10	50	100	350	980	40	450	200	40	20	30				
TB6	90	330	520	630	22920	430	880	710	630	200	310			420	112.5
TB7	40	50	60	340	3680	200	110	320	380	170	160			<10	33
TB8	50	20	<10	110	1680	20	70	120	40	30	<10			50	25
TB9	1470	110	370	410	1420	80	250	350	150	30	90				
TB10	80	50	10	10	1080	10	<10	20	<10	<10	<10				
TB11	<10	10	10	20	10	10	<10	10	<10	<10	<10				
TB12	70	30	260	60	20	30	80	<10	50	<10					
TB13	10	<10	<10	<10	<10	10	<10		10	<10	20	<10	<10		<10
TB14	1400	80	540	240	460	470	990		70	330	90	2650	520		
TB15	30	<10	10	10	20	30	<10		<10	10					
TB16	<10	<10	<10	<10	<10	<10	<10		<10	<10					
TB17	<10	10	120	90	20	10	50		10	60	<10	10	30		50
TB18	<10	30	220	190	90	80	30		40	10					
TB19	<10	<10	<10	10	<10	<10	10		20	<10	<10	<10	<10		<10
TB20	<10	<10	<10	<10	<10	<10	<10		<10	<10	<10	<10	<10		20
TB21	20	<10	20	230	10	10	20		10	<10	10				
TB22	<10	<10	<10	<10	<10	<10			<10	<10	<10				

Some of the appendixes are not in electronic format. Copies will be provided upon request from the Rose lab.

Appendix X

Bacteroides fragilis, an Alternative Indicator

In recent years, *Bacteriodes fragilis* bacteriophage has been evaluated as a possible alternative indicator of sewage pollution. The host, *Bacteriodes fragilis*, is an anaerobic bacterium found in the normal intestinal flora of humans and mammals. It is not considered pathogenic, however it is an opportunistic pathogen and is the most common anaerobic isolate in human wound infections. The morphology of *Bacteriodes fragilis* bacteria is non-motile, gram negative rods with rounded ends measuring 0.5 to 0.8 microns in diameter to 1.5 to 9 microns long. The colony appears gray and opaque when grown on nutrient agar.

The phage of *B.fragilis* belongs to the bacteriophage group Siphoviridae, and contains double-stranded DNA. These somatic phages have icosahedral heads, exhibit flexible tails, and are often observed in star-shaped clusters when viewed under an electron microscope. (25, 53, 54) According to Lasobras et al (1997), bacteriophage with flexible tails are often the most resistant to environmental stress.(53) *B. fragilis* phage is only found in 10-13% of human feces studied, so their numbers tend to be low in the environment.(25, 28) The bacteriophage and their host bacterium are strict anaerobes and do not replicate in the environment. *B. fragilis* bacteriophage are more resistant chlorination that *S. faecalis*, *E. coli* and some enteroviruses.(8, 26, 37) They also display a positive correlation with the levels of enteroviruses and rotaviruses (Jofre et al, 1989).

Grabow and Jofre (1995) in South Africa found the B40-8, or HSP40 (host ATCC 51477) *B. fragilis* phage strain in 13% of humans tested, but not in animals or birds. Environmental numbers can be highly varied depending on the source water. Lucena et al (1996)(64) found 7 per100 ml to 5.3 x 10^3 per 100 ml in polluted river water, 1.2 x 10^2 per 100 grams in sediment and 2-3 per100 ml in ground water. Araujo et al (1997)(66) found waters receiving recent sewage imput showed levels of 2.3 x 10^3 per 100 ml, waters receiving intermediate sewage imput showed levels of 2.3 x 10^1 per 100 ml, and waters with persistently low levels of pollution averaged 1.67 per 100 ml and was only detected in 21.9% of the samples tested.

B. fragilis phage showed no replication in the environment and demonstrated decay rates similar to human enteric viruses, coliphages and poliovirus. (25, 67) Their persistence in seawater is similar to Hepatitis A virus.(8, 68) *B.fragilis* phage was also show to be highly resistant to disinfection. A study by Bosch et al in 1989 showed that in tapwater carrying 2-3 mg/l residual chlorine levels with 15 minutes exposure time, *B. fragilis* phage were more resistant than poliovirus type 1, coliphage f2, *E. coli*, enterococci and simian rotavirus. The phage also showed higher resistance in sewage containing 20-24 mg/l of residual chlorine with a contact time of 15 minutes. F2 coliphage showed the best resistance to UV radiation, with *B. fragilis* phage showing similar decay rates to poliovirus and rotavirus. (28) Chung and Sobsey (1993) showed that *B.fragilis* survived better than enterovirus in lab conditions at 5 and 25^0 C. (68) Jofre et al (1995) found *B.fragilis* phage to be more resistant to treatment processes than either somatic or F-specific coliphages, or other bacterial indicators. (69, 70)

Bacteria and Phage Strains

The *B. fragilis* bacteriophage B56-3 (host: RYC2056) and B40-8, or HSP40 (host: ATCC51477) and their corresponding host bacteria were kindly provided by Javier Mindez and Dr. Juan Jofre at the University of Barcelona.

B. fragilis BPRMA Media and Reagents

All reagents and media used in the detection of *B. fragilis* were made according to the International Standards ISO protocol CD 10705-4 (ISO/TC 147/SC 4WG 11 N36), 1998.

B. fragilis Overlay Protocol

The overlay protocol used in this study was as stated in the ISO protocol CD 10705-4 (ISO/TC 147/SC 4WG 11 N36), 1998. To a melted tube containing 2.5 ml of prepared *B. fragilis* soft agar, 1 ml of a log phase host culture and 1 ml of the water sample at room temperature was added. After gently mixing to avoid bubble formation, the sample was poured onto the surface of a BPRMA plate and swirled to distribute the agar equally. The agar was allowed to solidify and then the plate was inverted and incubated for 18 to 24 hours at 37^{o}C anaerobically in a GasPak jar. Plaques appearing on the bacterial lawn were considered *B. fragilis* bacteriophage. If replicates of 10 are used for each sample, the limit of detection for this assay is 10 PFU/100ml.

B. *fragilis* Bacteriophage Enrichment Assay

One hundred ml of pre-filtered seawater (0.45 μm Millipore PVDF filter) was added to 100 ml of double-strength BPRMA broth in a sterile 250 ml glass flask with a screw-top lid. Both were prewarmed to at least room temperature. Thirty ml of a log-phase culture of either RYC2056 or ATCC 51477 (HSP40) *B. fragilis* host bacterium was added, and the flask was filled to the top with regular strength BPRMA broth to provide an anaerobic environment. A positive and negative control flask were used for each host. Both controls used 100 ml of a NaCl and peptone dilution buffer as the sample, and 1μl of a phage stock culture dilution was added to the positive control flask. All flasks were incubated at 37^{0}C for 48 hours. A 1 ml aliquot was taken from each flask and placed in a 1.5 ml sterile μ–fuge tube with 0.4 ml of chloroform and vortexed. The tubes were spun in the micro-centrifuge at 3000 rpm for 5 minutes. To a tube containing 2.5 ml of melted BPRMA soft agar, 1 ml of log phase host culture was added and overlayed on BPRMA agar plates. For each sample, 1 μl was dotted in triplicate on the surface of the appropriate host lawn without disturbing the surface top agar. The plates were allowed to solidify and were inverted and incubated overnight at 37^{0}C for 24 hours in a GasPak anaerobic jar. Samples were considered positive for the presence of the bacteriophage if clearing was noted on the lawn. All samples positive for the enrichment assay were repeated using 10 ml of pre-filtered sample and the standard overlay procedure.

Environmental Survey

One of the objectives of this project was to determine if the *Bacteroides fragilis* phage assay would be applicable as an alternative indicator in our geographic location. Marine water samples were analyzed for the presence of two *B. fragilis* phages, the human strain B40-8 (host ATCC 51477) and the animal/human strain B56-3 (host RYC2056). During the first 2 months of the sampling schedule, all sites were analyzed for the *Bacteroides fragilis* human strain B40-8 (host ATCC 51477 (HSP40)) and the animal/human strain B56-3 (host RYC2056) using the standard overlay procedure (see Material and Methods setion). Phage levels were consistently below the detection limit

of 10 PFU per 100ml for all sampling sites. Each site was then evaluated for the remaining 3 months of the survey using the presence/absence *B. fragilis* enrichment assay (see Material and Methods section). Tables 1 and 2 show the results of the overlays and enrichments for both strains of the *B. fragilis* bacteriophage for all sampling sites. For the *B. fragilis* human strain B40-8, 56 total enrichments were performed during the months of August, September and October. Seven samples, or 12.5% of the total, tested positive for the presence of the phage strain. Only one site, TB17 Allen's Creek, tested positive for two of the three sampling dates. The *B. fragilis* animal/human phage strain B56-3 was used for a total of 57 enrichments during the months of August, September and October. Twenty eight samples, or 49% of the total, tested positive for the presence of the phage strain. Three sites, TB4 and 6 along Bullfrog Creek and TB14 Sweetwater Creek tested positive during all three months of the study.

All positive enrichment samples were repeated using the standard overlay procedure (See Material and Methods section). Results were consistently below the detection limit of the assay. Positive enrichment results can be considered to be <10 PFU/100ml for all sample sites.

Table 1
B.fragilis phage B40-8 (host ATCC 51477) Human Strain

Site	Jun	July	Aug	Sept	Oct
TB1	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	**Positive**	Negative
TB2	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	Negative
TB3	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB4	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	**Positive**
TB5	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative
TB6	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB7	<20 pfu/100ml	<20 pfu/100ml	Negative	**Positive**	Negative
TB8	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB9	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	Negative
TB10	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	Negative
TB11	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	Negative
TB12	<20 pfu/100ml	<20 pfu/100ml	**Positive**	Negative	Negative
TB13	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB14	<20 pfu/100ml	<20 pfu/100ml	Negative	**Positive**	Negative
TB15	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB16	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB17	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	Negative
TB18	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB19	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB20	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	**Negative**
TB21	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB22	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative

Results of <20 pfu/100ml are from the standard overlay procedure, Positive and Negative results were obtained using the enrichment assay.

Table 2

B. fragilis phage B56-3 (host RYC2056) Animal/Human Strain

Site	Jun	July	Aug	Sept	Oct
TB1	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	**Positive**	Negative
TB2	<20 pfu/100ml	<20 pfu/100ml	**20 pfu/100ml**	N/A	Positive
TB3	<20 pfu/100ml	<20 pfu/100ml	Negative	**Positive**	**Positive**
TB4	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	**Positive**
TB5	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**
TB6	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	**Positive**
TB7	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB8	<20 pfu/100ml	<20 pfu/100ml	Negative	**Positive**	**Positive**
TB9	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	**Positive**
TB10	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	**Positive**
TB11	<20 pfu/100ml	<20 pfu/100ml	<20 pfu/100ml	N/A	Negative
TB12	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	Negative
TB13	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB14	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	**Positive**
TB15	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB16	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB17	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	Negative
TB18	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	Negative
TB19	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB20	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative
TB21	<20 pfu/100ml	<20 pfu/100ml	**Positive**	**Positive**	Negative
TB22	<20 pfu/100ml	<20 pfu/100ml	Negative	Negative	Negative

Results of <20 pfu/100ml are from the standard overlay procedure, Positive and Negative results were obtained using the enrichment assay.

Discussion and Conclusions

Studies have been undertaken in Europe and South Africa to determine if the *Bacteroides fragilis* bacteriophage could be detected in ambient water and used as an alternative indicator of fecal contamination Most of the studies determining environmental levels of *B.fragilis* phage have been conducted in Spain. (previous studies for *B. fragilis* phage levels are summarized in Table 3) Results for the animal/human phage strain B56-3 have generally not been included, as most studies concentrated on detecting only the B40-8 human strain. These studies describe heavily impacted or polluted waters as those directly receiving untreated sewage. The methods used were either the standard overlay method, or the presence/absence assay in an MPN format. The results exhibit a great deal of variation depending on the pollution level of the sampling sites; phages were found to range from 10^1 to 10^4 PFU per 100 ml. Waters that were considered low impact, or those not receiving urban sewage and runoff, did consistently fall below the detection limit of the assay for the human strain B40-8.

The first of this project's objectives was to determine if the human strain B40-8 and the animal/human strain B56-3 *B. fragilis* phages could be detected in ambient waters in the Tampa Bay region. Using the standard overlay method (detection limit of <10 PFU/100ml), the phages were not detected at any of the sampling sites. All positive

results were obtained using the presence /absence procedure. We detected the human strain (B40-8) at 27% of the sampling sites; TB1 Delaney Creek, TB4 Bullfrog Creek, TB7 Bullfrog Creek, TB12 Hillsborough River, TB14 Sweetwater Creek and TB17 Allen's Creek. More than 50% of the Delaney Creek watershed is urban development. Bullfrog Creek watershed includes 50% agricultural use, 12% urban and residential. The Hillsborough River has 32% agricultural use, 25% urban and industrial impacts. Sweetwater Creek has high urban development at 69% of the total watershed. (Lipp et al, 1999, submitted to Hydrobiologia)

The animal/human strain B56-3 was detected at 63% of the sites. The negative sites included TB7, 15, 13, 11, 19, 16, 20 and 22. Not enough human enterovirus data has been collected from the Tampa Bay sampling sites to determine if the presence of the *B. fragilis* phage correlates to the presence of enteroviruses.

Table 3
Summary of *B. fragilis* phage environmental studies

Reported in PFU/100ml

Year	Area	Water type	Phage B40-8	Other Indicators	Results
1988 (a)	Spain	polluted river	Ave 4.8 x 10^4		
		polluted seawater	Ave 7.3 x 10^2		
		groundwater	Not detected		
1989 (b)	Spain	intermediate water	0-43		
				coliphage	9.06 x 10^2
				fecal coliforms	~10^3
				enterococci	~10^2
				C.perfringens	~10^2
		low impact waters	Not detected		
				fecal coliforms	73-2.4 x 10^2
				enterococci	~10^2
				C.perfringens	Not detected
1996 (c)	Spain	polluted river	7-5.3 x 10^3		
		groundwater	2-3		
1997 (d)	France	polluted river	>/= 1.6 x 10^3		
1997 (e)	Spain	polluted river		Somatic coliphage	~10^3 - 10^6
				f-specific coliphage	~10^3- 10^5
			~10^1- 10^4		
		intermediate water		Somatic coliphage	~10^2- 10^5
				f-specific coliphage	<10– 3.6x 10^3
			<10-1.5x 10^3		
		low impact water		Somatic coliphage	<10 - 680
				f-specific coliphage	<10 - 400
			<10 - 270		

(a)(79)
(b)(62)
(c)(64)
(d)(60)
(e)(80)

Bacteroides fragilis phage strain B56-3 (host RYC2056) has been isolated from 28% of human fecal samples tested, 31% of pigs tested and 29% of poultry. It has never been

isolated from cattle, sheep or horse fecal samples. The levels found were 6.7 x 10^2 PFU per gram of feces in pigs and 1.3 x 10^2 PFU per gram of feces in poultry. (58, 82) The human strain B40-8 (host ATCC 51477) has been isolated from 10-13% of human fecal samples tested, but not from any animal fecal sample. (domestic animals, primates and seabirds). (26, 27, 55, 58) In comparison, somatic coliphage was detected in 54 % of human isolates tested, 56% of domestic animals, 53-57% of primates and 60% of seabirds. F-specific coliphage was found in 26% of humans, 90% of domestic animals, 63-76% of primates and 20% of seabirds fecal samples tested. (26) All of the fecal studies used samples obtained from Spain, Great Britain and South Africa.

The second objective of this project was to determine the local levels of the B40-8 and B56-3 phage strains in domestic sewage in order to identify possible sources to the environment. Table 4 shows summary of previous sewage studies done in Europe and South Africa. Puig et al, 1999, utilized 114 strains of *B. fragilis* host (including B40-8) to detect bacteriophage in sewage. Sixty-six of these strains detected phage in human sewage, however, the numbers were highly variable between strains. Host RYC2056 (B56-3) detected the overall highest number of phage in sewage.(55) Jofre et al, 1989, found that the ratio of *B. fragilis* phage to enterovirus in sediments was similar to the ratio found in sewage, showing that the fate of both may be similar in the environment. (83) Levels of B40-8 were highly variable and ranged from 7 PFU to 10^5 PFU per 100 ml.

Table 4

Summary of sewage indicator studies

Reported in PFU/100ml

Year	Area	Sample type	Phage B56-3	Phage B40-8	Indicator	Results
1987 (a)	Spain	sewage influent		7 – 1.1x 10^5		
1988 (b)	Spain	sewage influent		8.9 x 10^4		
1989 (c)	Spain	sewage influent		5.3 x 10^3		
					coliphage	1.2 x 10^6
					fecal coliforms	~10^7
					enterococci	~10^6
					C. perfringens	~10^5
1996 (d)	France	sewage influent		>=4.4x 10^4		
1999 (e)	Spain*	sewage influent		32-190		
			82-440			
					E.coli	1400-7600
					fecal coliforms	~10^3 - 10^5
		slaughter house waste		0-7.5		
			2.9–2.4x10^2			
					E.coli	~10^2-10^5
					fecal coliforms	~10^4-10^6

* average levels for samples from Netherlands, Ireland, Germany, Austria, Portugal, Germany, Sweden, France and South Africa

(a)(27) (b)(79) (c)(62) (d)(60) (e)(55)

The level of *B. fragilis* phage in domestic sewage was much lower in this region compared to the European studies. Phage strain B40-8 detected in Tampa Bay wastewater treatment plants ranged from 66.7 to 350 PFU per 100 ml, and was found in 100% of the sewage influent samples tested. Phage B56-3 levels in the 1999 study by Puig et al ranged from 82 to 440 in sewage and 2.9 to 240 in slaughter house waste. Our study found much higher levels in sewage samples, with results ranging from 1.19×10^4 to 1.11×10^5 PFU per 100 ml, and was detected in 100% of sewage samples tested.

Samples from all 3 plants gave consistent coliphage numbers of around 10^5 PFU per 100 ml, fecal coliforms numbers of 10^6 CFU per 100 ml, enterococci levels of 10^5 CFU per 100 ml and *C. perfringens* levels of 10^4 CFU per 100 ml. Each indicator consistently averaged one log lower than studies done in Spain. The low numbers of the human strain B40-8 in Florida sewage may explain the low numbers in ambient water in the geographic location studied. The B56-3 phage strain found in domestic sewage should only be coming from human waste, however, the environmental sources from pigs and poultry would make it difficult to trace the source when it is found in the environment. Seabirds may be a consideration in Tampa Bay as a possible environmental source, however, no studies were found determining the presence of phage B56-3 in seabird fecal samples.

Positive enrichment for the B56-3 *B. fragilis* phage occurred in 2 of the 3 treatment plant effluent samples on 3 occasions. During the first event, fecal coliforms were 1 CFU per 100 ml, enterococci was negative, *C.perfringens* was 24 CFU per 100 ml and coliphage was 10^3 PFU per 100 ml. The second event showed fecal coliform levels of 6 CFU per 100 ml, enterococci was negative, *C. perfringens* was 8 CFU per 100ml and coliphage was 10^2 PFU per 100 ml. The last event had fecal coliform levels of 1 CFU per 100 ml, both enterococci and *C. perfringens* were negative, and coliphage was 30 PFU per 100 ml. The human strain B40-8 was not detected in any of the effluent samples analyzed, but given the low numbers in the influent, that may be expected. The sewage study further highlights the fact that the indicator bacteria are not suitable models for the fate and transport of human viruses. *Clostridium perfringens* shows to be the more resistant to treatment than the other indicator bacteria. In each case of a positive enrichment result for B56-3 in the effluent, coliphage was also detected. All effluent samples showing negative enrichment results for *B. fragilis* phage were negative for coliphage.

Table 5 shows a summary of the indicator levels found in the treatment plant survey, and the average removal rate for each. Coliphage and the *B. fragilis* phage B56-3 showed similar removal rates following chlorination. Removal rates for the human strain B40-8 are difficult to compare because of the low numbers found in the influent.

Table 5
Average indicator levels from treatment plant survey

Indicator	Influent*	Effluent*	Removal rate
Fecal Coliforms	2.7×10^6	1.6	5 to 6 log removal
Enterococci	3.1×10^5	0	5 log removal
C. perfringens	2.6×10^4	6.7	3 to 4 log removal
Coliphage	1.85×10^5	190	2 to 4 log removal
B56-3phage	4.46×10^4	3 of 6 positive (<10)	2 to 4 log removal
B40-8 phage	194	Negative	2 log removal

*reported in PFU or CFU per 100 ml

Final conclusions:
1) *Bacteroides fragilis* phage strains B40-8 and B56-3 were found in the environment in our geographic location, but in very low numbers. This differs from the findings in Europe, but most of the polluted sites sampled in their studies were directly receiving untreated domestic sewage.
2) The sewage survey showed the B56-3 phage strain was found in greater abundance than the human strain B40-8. Both phage strains were consistently found in domestic sewage in the Tampa Bay area.

This still does not completely answer the "animal vs human sources for fecal contamination" question. While the animal/human strain B56-3 does have environmental sources, the high levels found in the domestic sewage in the Tampa Bay region may indicate that humans are a significant source in this area. The presence of the human strain B40-8 associated with human enterovirus data will determine if this can be a useful indicator tool.

Future areas of interest regarding the *Bacteroides fragilis* phage assay in the Tampa Bay region should include investigation into septic tank systems as a possible source to the environment, and analysis of fecal samples could determine if the local seabird population is contributing to the phage B56-3 found in the ambient water.

Appendix XI

ANOVA's or analysis of variance is used to determine if a collection of data points can be grouped into similar categories. The following ANOVA's were used to analysis the sampling sites of the study for each individual indicator to determine high, intermediate and low indicator sites. The ANOVA table and resulting groups are given for each, and a summary table appears at the end of the appendix.

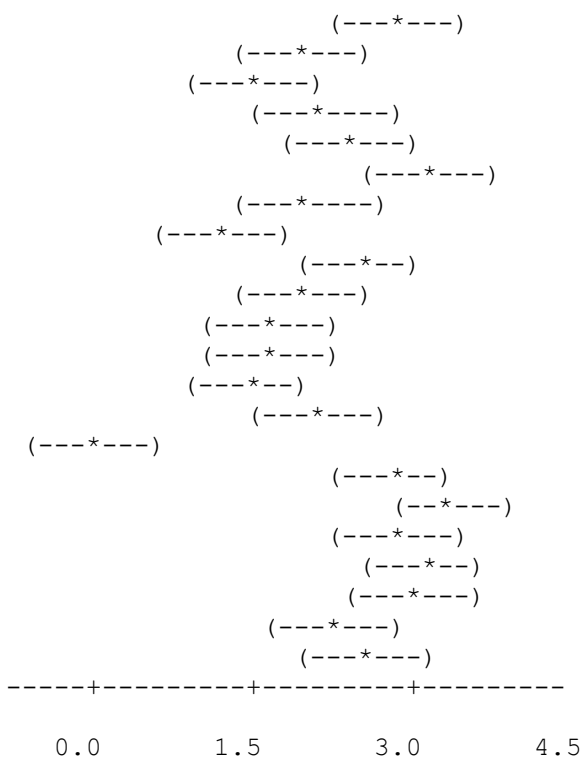
```
Analysis of Variance for Fecal Coliforms
Source     DF        SS        MS        F        P
Site       21   163.052     7.764     8.63    0.000
Error     237   213.199     0.900
Total     258   376.252
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+-
-
TB1        12    2.6745    0.7775                    (---*--)
TB10       12    2.0297    0.7812                 (---*--)
TB11       11    1.5462    0.9528              (--*---)
TB12       10    2.4253    1.1458                   (---*---)
TB13       13    2.4790    0.9344                    (---*--)
TB14       12    3.5630    0.8214                            (---*--)
TB15       10    2.4749    0.8059                    (--*---)
TB16       10    1.2593    1.1535           (---*---)
TB17       13    2.8234    1.0946                      (---*--)
TB18       10    2.0588    0.9530                 (---*---)
TB19       12    1.7296    1.2475              (---*--)
TB2        12    1.7697    1.1246              (---*--)
TB20       13    1.4062    1.6660            (--*---)
TB21       11    2.2695    1.3445                 (---*---)
TB22       10    0.0699    0.2210   (--*---)
TB3        12    2.9608    0.6951                       (---*--)
TB4        13    3.7019    0.7763                            (---*--)
TB5        12    2.8231    0.5553                      (---*--)
TB6        13    3.2276    0.6468                         (---*--)
TB7        13    2.9901    0.8875                       (---*--)
TB8        13    2.4721    0.5146                    (--*---)
TB9        12    2.6589    0.6201                     (---*--)
                                    ----+---------+---------+---------+-
-
Pooled StDev =   0.9485              0.0       1.5       3.0       4.5


Low
TB22

Intermediate
TB10, 11, 16, 18, 19, 2, 20

Intermediate hi
TB1, 12, 13, 15, 17, 21, 5, 8

High
TB14, 3, 4, 6, 7
```

Analysis of Variance for E.coli L

Source	DF	SS	MS	F	P
Site	21	131.448	6.259	6.73	0.000
Error	206	191.581	0.930		
Total	227	323.029			

Level	N	Mean	StDev
TB1	10	2.7809	0.8110
TB10	10	1.9500	0.7976
TB11	10	1.4543	0.9674
TB12	9	2.1532	1.0704
TB13	11	2.3651	1.0459
TB14	11	3.1396	0.7147
TB15	8	1.9823	0.2683
TB16	9	1.2323	1.1841
TB17	12	2.5031	1.0346
TB18	9	1.9843	0.9346
TB19	12	1.6370	1.2322
TB2	11	1.6403	1.1883
TB20	12	1.4263	1.7087
TB21	11	2.1342	1.2870
TB22	10	0.0000	0.0000
TB3	11	2.7789	0.8120
TB4	11	3.3681	1.0490
TB5	10	2.8533	0.5251
TB6	11	3.0934	0.6946
TB7	10	2.9502	0.7628
TB8	10	2.2275	0.3399
TB9	10	2.5566	0.7554

Pooled StDev = 0.9644



Low
TB22

Intermediate lo
TB16, 20

Intermediate
TB10, 11, 12, 15, 18, 19, 2, 21

Intermediate hi
TB13, 17, 8

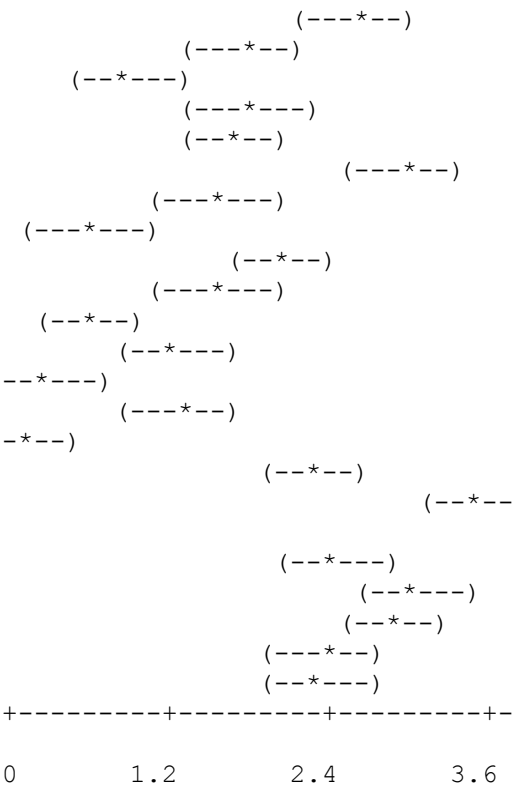
High
TB1, 14, 3, 4, 5, 6, 7, 9

Analysis of Variance for Enterococci

Source	DF	SS	MS	F	P
Site	21	226.352	10.779	19.85	0.000
Error	241	130.849	0.543		
Total	262	357.201			

Level	N	Mean	StDev
TB1	12	2.6233	0.6990
TB10	12	1.7507	0.6882
TB11	11	0.8752	0.6268
TB12	10	1.8185	0.7213
TB13	13	1.6788	0.7551
TB14	13	2.9735	1.0262
TB15	10	1.5775	0.5133
TB16	10	0.6166	0.8266
TB17	13	2.0406	0.5432
TB18	10	1.5298	0.4706
TB19	13	0.5962	0.4273
TB2	12	1.2080	0.8247
TB20	13	0.2823	0.5355
TB21	11	1.3002	0.9194
TB22	11	0.0707	0.1254
TB3	13	2.2790	0.7996
TB4	13	3.4786	0.7728
TB5	12	2.4308	0.9410
TB6	13	3.0276	0.9138
TB7	13	2.8744	0.7938
TB8	13	2.3651	0.6977
TB9	12	2.3369	0.8737

Pooled StDev = 0.7368



Low
TB16, 19, 20

Intermediate lo
TB2 and 21

Intermediate
TB10, 11, 12, 15, 17, 18

Intermediate hi
TB1, 3, 5, 7, 8 and 19

High
TB14, 4, 6

Analysis of Variance for C.perfringens

Source	DF	SS	MS	F	P
Site	21	23.743	1.131	4.00	0.000
Error	211	59.569	0.282		
Total	232	83.311			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
TB1	10	0.7384	0.5476	(----*-----)
TB10	10	0.3721	0.4621	(----*-----)
TB11	10	0.2577	0.3025	(----*-----)
TB12	9	0.4373	0.4778	(-----*-----)
TB13	12	0.6001	0.6704	(----*----)
TB14	12	0.9252	0.7264	(----*----)
TB15	8	0.7036	0.5398	(-----*-----)
TB16	8	0.0000	0.0000	(-----*-----)
TB17	12	0.6694	0.5633	(----*----)
TB18	8	0.7526	0.6619	(------*-----)
TB19	12	0.0980	0.2337	(----*----)
TB2	11	0.5216	0.4496	(-----*----)
TB20	12	0.0868	0.3006	(----*----)
TB21	9	0.2197	0.4595	(-----*----)
TB22	9	0.0000	0.0000	(-----*-----)
TB3	11	1.1907	0.5283	(----*----)
TB4	12	1.0231	0.8411	(----*----)
TB5	10	0.7126	0.6122	(-----*----)
TB6	12	0.7801	0.7068	(----*----)
TB7	13	0.6991	0.5154	(----*---)
TB8	13	0.5431	0.4860	(----*----)
TB9	10	0.4293	0.4928	(----*-----)
Pooled StDev =		0.5313		0.00 0.60 1.20

Low
TB10, 11, 12, 16, 19, 20, 21, 22, 9

Intermediate
TB1, 13, 15, 17, 18, 2, 5, 6, 7 ,8

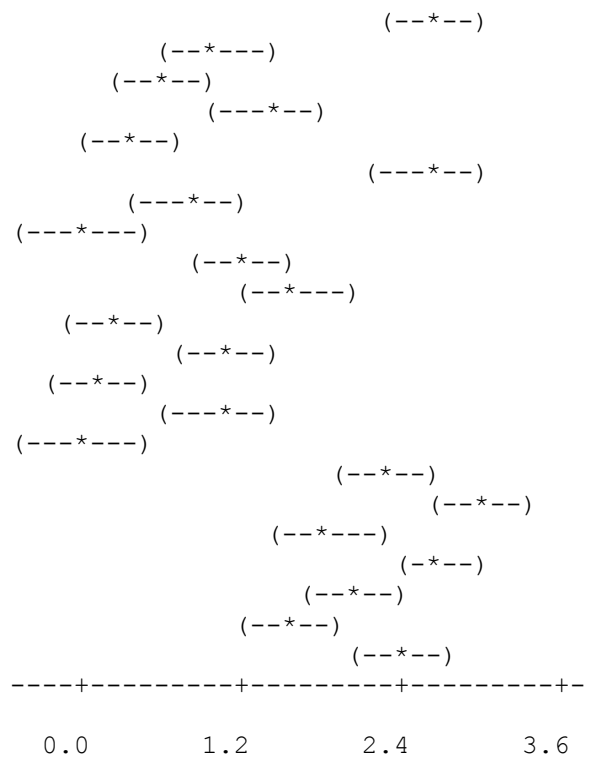
High
TB14, 3, 4

Analysis of Variance for Coliphage

Source	DF	SS	MS	F	P
Site	21	219.395	10.447	25.14	0.000
Error	229	95.164	0.416		
Total	250	314.559			

Level	N	Mean	StDev
TB1	11	2.6600	0.6223
TB10	11	1.0088	0.9783
TB11	11	0.5936	0.5740
TB12	10	1.3974	0.7945
TB13	13	0.3420	0.5386
TB14	12	2.5811	0.4930
TB15	9	0.8255	0.6440
TB16	9	0.0000	0.0000
TB17	13	1.2478	0.6623
TB18	9	1.5700	0.7185
TB19	13	0.2619	0.5021
TB2	11	1.1110	0.8145
TB20	13	0.1017	0.3667
TB21	10	1.0496	0.6793
TB22	9	0.0000	0.0000
TB3	13	2.3074	0.6584
TB4	13	2.9599	0.5391
TB5	11	1.8405	0.8139
TB6	13	2.6971	0.5822
TB7	13	2.0471	0.8162
TB8	13	1.5218	0.8367
TB9	11	2.3665	0.5172

Pooled StDev = 0.6446



Low
TB11, 13, 16, 19, 20, 22

Intermediate lo
TB10, 15, 17, 2, 21

Intermediate
TB12, 18, 8

Intermediate hi
TB3, 5, 7, 9

High
TB1, 14, 4, 6

Summary for ANOVA Indicator Results

Indicator and Sites:

Low
Fecal Coliforms:TB22
E.coli: TB22
Enterococci:TB16, 19, 20
C.perfringens:TB10, 11, 12, 16, 19, 20, 21, 22, 9
Coliphage:TB11, 13, 16, 19, 20, 22

Intermediate lo
Fecal Coliforms:TB10, 11, 16, 18, 19, 2, 20
E.coli: TB16, 20
Enterococci:TB2, 21
C.perfringens:none
Coliphage:TB10, 15, 17, 2, 21

Intermediate
Fecal Coliforms:none
*E.coli:*TB10, 11, 12, 15, 18, 19, 2, 21
Enterococci:TB10, 11, 12, 15, 17, 18
C.perfringens:TB1, 13, 15, 17, 18, 2, 5, 6, 7, 8
Coliphage:TB2, 18, 8

Intermediate hi
Fecal Coliforms:TB1, 12, 13, 15, 17, 21, 5, 8
E.coli: TB13, 17, 8
Enterococci:TB1, 3, 5, 7, 8, 19
C.perfringens:none
Coliphage:TB3, 5, 7, 9

High
Fecal Coliforms:TB14, 3, 4, 6, 7
*E.coli:*TB1, 14, 3, 4, 5, 6, 7, 9
Enterococci:TB14, 4, 6
C.perfringens:TB14, 3, 4
Coliphage:TB1, 14, 4, 6

Appendix XII

Rainfall and Streamflow Databases

There are several major databases available for rainfall and streamflow data.
1) The Southwest Florida Water Management District:
http://www.swfwmd.state.fl.us/data/rain/raindata.htm
2) Florida Environmental Protection Agency: http://www.epa.gov/surf3/states/FL/
3) National Climatic Data Center:
http://www.ncdc.noaa.gov/ol/climate/stationlocator.html
4) The U.S. Geological Survey: http://www.usgs.gov
When compiling climate data, close attention was paid to the gage station identifier numbers because several of the databases shared gage station information. Rainfall was given in inches of rain per day, and the stream flow was reported as cubic feet per second. The following stations were used in this study:
Rural Sites
Rainfall and Stream flow station USGS 02301750, Delaney Creek
Rainfall stations SWFWMD 252, 31, 428, 94, 107 and 27 Alafia Watershed
Rainfall and Stream flow station USGS 02301500 Alafia River
Stream flow stations USGS 02301000 and 02301300 Alafia River
Rainfall station SWFWMD 27 Bullfrog Creek
Rainfall and Stream flow station USGS 0230070 Bullfrog Creek
Rainfall stations SWFWMD 179, 388 and 429 Little Manatee Watershed
Rainfall and Stream flow station USGS 02300500 Little Manatee River
Stream flow station USGS 02300100 Little Manatee River
Rainfall stations SWFWMD 473, 357, 284, 14 and 214 Manatee Watershed
Rainfall and Stream flow station USGS 02299950 Manatee River
Rainfall station NCDC Bradenton, Manatee Watershed
Stream flow station USGS 02300032 Manatee River

Urban sites
Rainfall stations SWFWMD 135, 125, 353, 21, 407, 69, 391, 385, 4, 259, 444, 281, 292, 396, 56, 499, 376, 497, 245, 196, 64, 215, 395 and 498 Hillsborough Watershed
Rainfall and Stream flow station USGS 02303330 Hillsborough River
Stream flow station USGS 02303000 Hillsborough River
Rainfall stations SWFWMD 410 and 299 Sweetwater Creek
Rainfall station NCDC Tampa International Airport, Sweetwater Creek Sub-basin
Stream flow stations USGS 02306500 and 02306647 Sweetwater Creek
Rainfall station NCDC Tarpon Springs
Rainfall stations SWFWMD 53 and 298 Tarpon Lake
Rainfall stations SWFWMD 311, 97, 305 and 90 Lower Pinellas County
Rainfall stations SWFWMD 15, 411, 435, 53, 382, 393, 177 and 298 Upper Pinellas Co.
Rainfall station SWFWMD 305 Salt Creek
Urban sites, con't
Rainfall station NCDC Albert Whitted, Salt Creek sub-basin

Beach sites
Rainfall station SWFWMD 435 Honeymoon Island
Rainfall station SWFWMD 311 North Beach, Ft. Desoto