Development of a decision-support framework to support the implementation of fecal coliform BMAPs in the Hillsborough River watershed

Draft Final Report

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Contents

1. Purpose

The purpose of this project was to develop a decision-support framework — conceptually similar to the "decision matrix" that is currently used by the Tampa Bay Estuary Program (TBEP 2006a) to assess water quality in Tampa Bay — to guide management actions that are carried out to implement the Basin Management Action Plans (BMAPs) that are currently being finalized to address fecal coliform impairments and their associated total maximum daily loads (TMDLs) in the Hillsborough River watershed.

The framework is based on technical approaches and resource management strategies recommended by the National Research Council (NRC 2000, 2004), World Health Organization (WHO 2000, 2003), European Union (EP/CEU 2006) and U.S. EPA (EPA 1986, 2007). In addition to fecal coliform levels it incorporates other relevant information, such as the presence and relative magnitudes of human fecal contamination and other potential sources of human pathogens within BMAP management areas that are currently classified as impaired due to elevated fecal coliform levels. Because the impaired portions of the Hillsborough River watershed include or discharge to water bodies which are used for public recreation and/or as sources of potable water supply, the project focuses on these two designated uses and potential exposure routes for the purpose of managing water quality conditions to help reduce human health risks posed by waterborne pathogens.

If the approach proves to be workable and cost-effective in the Hillsborough River watershed, it may be applied (with appropriate modifications and extensions to include other beneficial uses) to other impaired waters throughout the state. In addition to its primary use in the fecal coliform TMDL/BMAP process, it is also intended to provide a technical framework that will help water quality managers address other indicators of waterborne health risks in watersheds and water bodies that are used for recreational purposes and sources of potable water supply.

2. Approach

Development of the decision-support tool followed a collaborative, consensus-based process. It was done in conjunction with the Hillsborough River BMAP Working Group and Stakeholder Group, which included representatives from the Florida Department of Agriculture and Consumer Services (FDACS), Florida Department of Environmental Protection (FDEP), Florida Department of Transportation (FDOT), Southwest Florida Water Management District (SWFWMD), Tampa Bay Estuary Program (TBEP), Tampa Bay Water (TBW), Hillsborough County Health Department (Florida Department of Health), Hillsborough County City/County Planning Commission, Hillsborough County Planning and Growth Management Department, Hillsborough County Public Works Department, the Environmental Protection Commission of Hillsborough County (EPCHC), the University of South Florida, the cities of Tampa, Temple Terrace and Plant City, the U.S. Environmental Protection Agency, environmental organizations, and representatives from the private sector.

3. Background

Pursuant to the federal Clean Water Act (CWA), the State of Florida has adopted water quality criteria for fecal coliform bacteria (summarized in Appendix A) in order to reduce human health risks in cases where waterborne pathogens could potentially be present in water bodies that are used for recreation, shellfish harvesting, or potable water supply. In most cases, however, the fecal coliform bacteria that are detected in surface water samples are not human pathogens. Instead, they are indicator organisms that are used as a warning signal that water may have been contaminated by fecal material (from humans or other warm-blooded vertebrates), and may therefore contain disease-causing organisms that pose a risk to public health. The actual pathogens causing reported disease outbreaks associated with recreational waters in the U.S. during the years 1985 through 1998, and the frequency and magnitude of those outbreaks, are listed in Table 1.

Table 1. Reported disease outbreaks associated with recreational waters in the United States, 1985-1998. (Source: WHO 2003)

Etiological Agent	Number of Cases	Number of Outbreaks
Shigella spp.	1,780	20
Escherichia coli 0157:H7	234	9
Leptospira sp.	389	3
Giardia lamblia	65	4
Cryptospiridium parvum	429	3
Norwalk-like viruses	89	3
Adenovirus 3	595	1
Acute gastrointestinal infections (no causative agent identified)	1,984	21

Unfortunately, a considerable amount of research has shown that fecal coliform bacteria are not particularly accurate indicators of the presence of human pathogens in surface waters. For example, Wade et al. (2003) carried out a meta-analysis of the usefulness of fecal coliforms as predictors of the risk of gastrointestinal (GI) illness associated with recreational water use. Based on the results of 27 previously-published epidemiological studies, Wade et al. (2003) concluded:

> "This review… supports the recommended move away from the use of fecal coliform… as an indicator because there was no evidence that risk of GI illness increased at levels above the previously proposed [*i.e., pre-1986 EPA*] guideline value. In fresh water, *E. coli* was superior to enterococci at predicting illness, and the *E. coli* guideline level was supported, because exposure below presented no significant risk, whereas exposures above were associated with an elevated and statistically significant increased risk of GI illness."
>
> "We found that enteroviruses, which have been suggested as specific indicators of human contamination… were strongly associated with GI illness. They may, however, be impractical for use as water quality indicators because they are not easily cultivated in environmental samples..."
>
> "Based on the epidemiologic studies conducted to date, it is evident that no single indicator can predict illness consistently in all environments at all times, perhaps because of the wide array of pathogens that have been associated with GI illness in recreational water environments as well as natural variability in pathogen–indicator associations. For example, both bacterial and viral indicators of water quality may correlate poorly with the occurrence of protozoan parasites such as *Cryptosporidium parvum*, a leading cause of freshwater outbreaks of GI illness…"

Other issues affect the use of fecal coliforms and other indicator bacteria in tropical and subtropical climates. Rose et al. (2001), in a recent study conducted in the Tampa Bay area, provided the following summary of this issue:

> "Risks to swimmers using polluted beaches have 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… 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."

Similar points have been made, at the national and international levels, in reports issued by the National Research Council (NRC 2000, 2004), the World Health Organization (WHO 2000, 2003), the European Union (EP/CEU 2006) and the U.S. Environmental Protection Agency (EPA 2007), which have pointed out a number of limitations affecting the accuracy of fecal coliforms and other existing indicators in estimating human health risks (e.g., Appendix B). Fujioka and colleagues (1985, 1997, 1999, 2001) have reported a number of instances in tropical waters in which none of the commonly used bacterial indicators have proven adequate. Given these limitations, it is evident that fecal coliform concentrations alone should not be relied upon by FDEP or its local partners when developing BMAPs to address microbial water quality and its potential impacts on human health, or when evaluating the effectiveness of the management actions that are taken to implement those BMAPs. Additional information regarding the dominant source(s) of the fecal coliform bacteria that are detected in surface water bodies (e.g., whether the source(s) are humans, pets, livestock, birds, other wildlife, soil, aquatic sediments, or vegetation), and the risks those sources pose to human health, will need to be collected and assessed to ensure that appropriate management actions are identified, prioritized, and carried out through the BMAP process.

The shortcomings of the existing bacteriological indicators are well known within the public health and water quality management communities, and intensive work is being carried out by a number of organizations to develop a cost-effective suite of indicators that provide more accurate estimates of human health risks than reliance on fecal coliforms alone. Currently, however, no single indicator or analytical method has been found that possesses the major attributes — such as greater accuracy in estimating risk, reasonable cost, and feasibility for day-to-day use by laboratory personnel in local monitoring programs — that would be needed to justify abandoning the existing bacteriological indicators and developing new water quality criteria based on alternatives (e.g., Fujioka 1997, Wade et al. 2003, EPA 2007). Until reliable, cost-effective alternatives or supplementary measurements are found and adopted, local management programs will need to continue using the existing indicators and other available information as effectively as possible in their efforts to reduce human health risks associated with waterborne pathogens.

In the case of recreational waters, a conceptual framework for prioritizing and managing health risks from waterborne pathogens and other factors has been developed by the WHO (2000, 2003) and applied in a number of countries around the world. Some elements of that conceptual framework are shown in Figures 1 and 2. Because monitoring of indicator organisms such as fecal coliform bacteria provides only limited information regarding health risks, the WHO framework also relies on information provided by annual "sanitary inspections", which are defined as "a search for, and evaluation of, existing and potential microbiological hazards that could affect the safe use of a particular stretch of recreational water or bathing beach" (WHO 2000). Under the WHO approach—which is commonly referred to as the "Annapolis protocol" due to its initial development at an international conference held in Annapolis, Maryland in the late 1990s—the information provided by sanitary inspections and water quality monitoring data are combined to provide a graded, risk-based assessment of a given water body, as summarized in Figure 2.

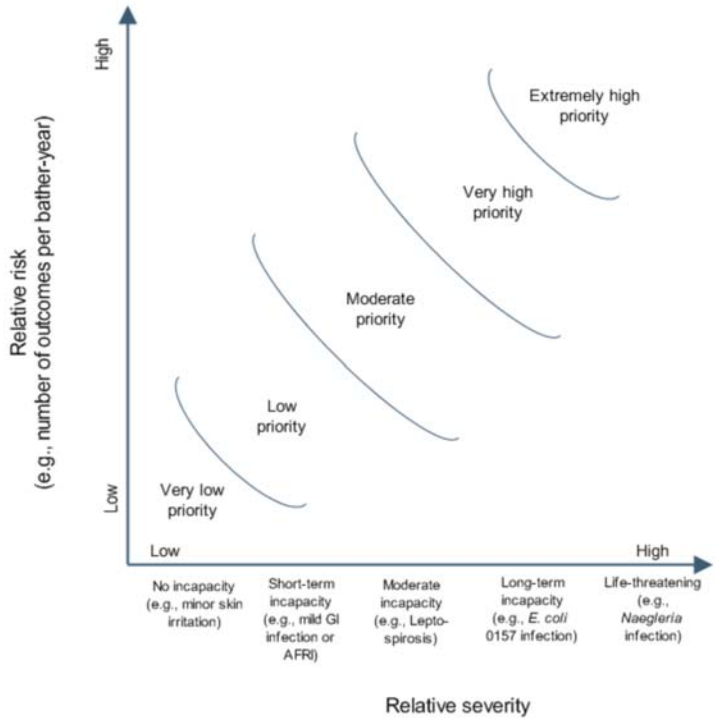


Figure 1. Schematic approach for prioritizing and managing health-related and other risks associated with recreational uses of surface water bodies. (Source: WHO 2003)

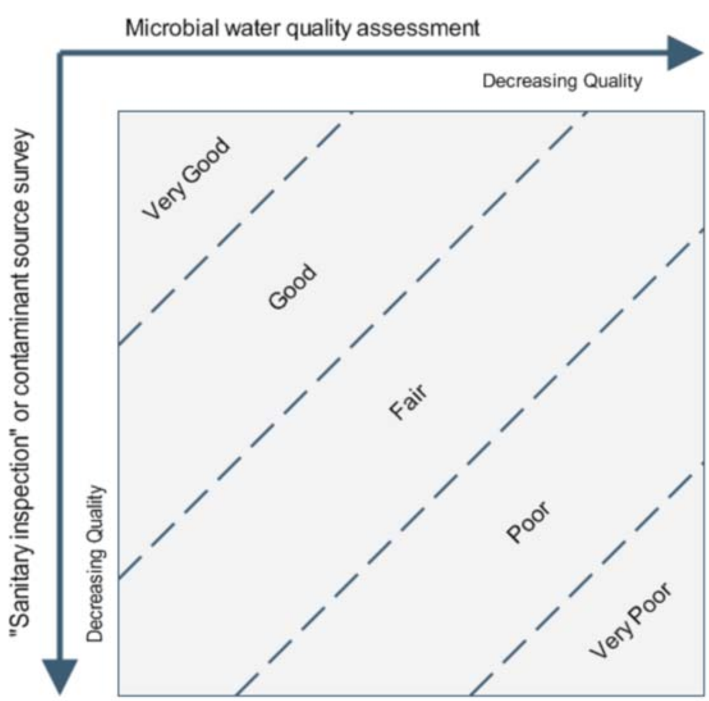


Figure 2. WHO water body classification matrix based on water quality monitoring data and sanitary inspections. (Sources: WHO 2000, 2003)

4. Recent Management and Policy Recommendations

4.1. For Recreational Waters

In March, 2007, the U.S. EPA convened a national panel of experts to address known issues, identify information gaps, and review potential approaches that could be used by EPA to develop alternative microbial water quality criteria for water bodies that are used for recreational purposes. The panel selected three potential approaches for doing so, based on methodological frameworks currently recommended by the EPA (1983, 1984, 2004, 2006), WHO (2003), and European Union (2006). The three approaches were selected because they are based on peer-reviewed epidemiological research, are currently in use at the national level in one or more countries, and meet the following benchmarks (EPA 2007):

- They are health-based and linked to health effects demonstrated in epidemiology studies;
- They are compatible with the requirements of the federal Clean Water Act; including water quality assessment for public notification at beaches in a timely manner, assessment for impaired waters listings, total maximum daily load (TMDL) development and implementation, and development of National Pollution Discharge Elimination System (NPDES) permits;
- They are scientifically defensible for application in a wide variety of geographic locations and climatic conditions, including fresh and marine waters, and temperate, subtropical, and tropical waters;
- They are sufficiently robust and flexible so that they can be configured to protect the public health of those exposed to recreational water impacted by sewage effluent, concentrated animal feed operation (CAFO) contaminated runoff, non-point sources (e.g., non-CAFO agricultural sources, urban runoff) and waters not impacted by anthropogenic sources;
- They are sufficiently robust and flexible to provide regulators the ability to protect susceptible (sensitive) subpopulations such as children and immunocompromised individuals;
- They are linked with laboratory analytical methods that are reliable, robust, and provide reproducible results; and
- They have the potential to equally protect primary contact recreation in freshwaters, marine waters, temperate, subtropical, and tropical waters, and to provide equal protection for those exposed via primary contact recreation to waters potentially impacted by effluent, urban runoff, and/or non-point source runoff.

The panel provided the following summary of the three approaches (EPA 2007):

4.1.1. World Health Organization (2003) Approach

As summarized by EPA (2007), “the WHO has been concerned with health issues associated with recreational water environments for many years and has published several influential reports that represent a well accepted view among international experts (Prüss, 1998). The WHO approach provides a basis for standard setting in light of local and regional circumstances, such as the nature and seriousness of local endemic illness, exposure patterns, and competing health risks that are not associated with recreational water exposure."

"The WHO approach is based on the perspective that recreational water quality and protection of public health are best described by a combination of sanitary inspection and microbial water quality assessments (the WHO [2003] Guidelines use enterococci as the fecal indicator of choice; see Table [2]). This approach considers possible sources of pollution in a recreational water ("sanitary inspection category" in Table [2]), as well as observed levels of fecal pollution ("microbial water quality assessment category" in Table [2]), and combines them into a five-level classification scheme for recreational water environments. To date, the classification system has been used primarily to "grade" recreational waters and to provide an assessment for regulatory compliance purposes. This approach however, also could be adapted for other CWA §304(a) applications such as NPDES permitting and TMDL development."

"The microbial water quality assessment criteria are based on a banded system, where the band divisions are equivalent to a risk of acquiring gastrointestinal (GI) illness for (A) <1 case in 100 exposures, (B) <1 case in 20 exposures, (C) <1 case in 10 exposures, and (D) >1 case in 10 exposures. The 95th percentile value was selected as an appropriate descriptor of the microbial probability density function because it is easily understood to be the probability of encountering polluted water and focuses on water quality that is likely to cause illness (i.e., greater probability of illness associated with increasing density of human sources of fecal pollution). The WHO levels of risk for the bands described above were selected based on a series of science policy decisions in consultations with numerous international experts and were intended to be reasonable for both the developed and developing world…"

Table 2. WHO classification matrix summarizing the "Annapolis protocol", which integrates microbial water quality and sanitary inspection categories. (Source: WHO 2003)

		Microbial water quality assessment category (95^{th} percentile intestinal enterococci/100 mL)				
		A ≤40	**B 41-200**	**C 201-500**	**D >500**	**Exceptional circumstances[3]**
Sanitary Inspection Category (susceptibility to fecal influence)	**Very low**	Very good	Very good	Follow up[1]	Follow up[1]	
	Low	Very good	Good	Fair	Follow up[1]	
	Moderate	Good[2]	Good	Fair	Poor	Action
	High	Good[2]	Fair[2]	Poor	Very Poor	
	Very High	Follow up[2]	Fair[2]	Poor	Very Poor	
Exceptional circumstances[3]		Action				

Notes:

1. **Implies non-sewage sources of fecal indicators, which should be verified**
2. **Indicates possible episodic contamination (e.g., rainfall- and runoff-driven), which should be investigated further, including sampling during and following storm events**
3. **"Exceptional circumstances" relate to known periods of higher risk, such as sanitary sewer overflows (SSOs) in the vicinity of recreational water bodies. Under such circumstances the matrix may not represent risk/safety accurately**

"The sanitary inspection category is intended to classify the risk of illness caused by fecal pollution in a recreational water body, although human fecal pollution will tend to drive the overall sanitary inspection category derived for an area. WHO experts believe that the three most important sources of human fecal contamination of recreational water environments for public health purposes are typically sewage, riverine discharges, and direct contamination from bathers. Sanitary inspections are required to address those sources as well as others, and inspections should take on a tiered approach, dependent on the level of perceived risk and its uncertainty. For example, if human and domestic fecal pollution is considered low based on land uses, but fecal indicator counts are relatively high, further exploration of the source(s) and their relative risks would be recommended. This higher level of examination (tier) may utilize more expensive methods and approaches and further cycles (tiers) of investigation as necessary. Based on the results of the sanitary inspections, recreational waters are ranked (from very low to very high) with respect to evidence for the degree of influence of fecal material."

4.1.2. European Union (2006) Approach

As summarized by EPA (2007), "the EU Bathing Water Directive (7/EU/EEC; dated February 15, 2006) is currently being translated by Member States for implementation (EP/CEU, 2006). The Directive establishes separate numerical microbiological criteria for fresh (inland) and marine (coastal and transitional) bathing waters for EU Member States (Table [3]). The numerical values are based on epidemiological studies reported by Kay et al. (1994) and Wiedenmann et al. (2006) — the former was used by WHO in formulating their Guidelines (Kay et al., 2004; WHO, 2003)."

Table 3. Numerical microbiological water quality assessment classification for fresh (inland) and marine (coastal and transitional) bathing waters for EU member states. (Sources: EP/CEU 2006; EPA 2007).

Indicator	Excellent	Good	Sufficient
Inland (Fresh) Waters			
Intestinal enterococci (CFU/100 mL)	200*	400*	360**
E. coli (CFU/100 mL)	500*	1,000*	900**
Coastal and Transitional (Marine) Waters			
Intestinal enterococci (CFU/100 mL)	100*	200*	200**
E. coli (CFU/100 mL)	250*	500*	500**

Notes: * = based on 95th percentile evaluation; ** = based on 90th percentile evaluation to reduce the risk of statistical anomalies when using a small data set, which also allows lower limit values for enterococci and *E. coli* densities in inland waters to be classified as sufficient versus good microbiological water quality.

4.1.3. EPA (1986) Approach

EPA (2007) provided the follow summary of the agency's earlier (1986) approach: "In the late 1970s and early 1980s, EPA conducted public health studies evaluating several organisms as possible indicators, including total and fecal coliforms, *E. coli*, and enterococci. The studies showed that enterococci and *E. coli* are the best predictors of GI illness (gastroenteritis) in sewage effluent-impacted freshwaters, while enterococci were the best predictor in sewage-impacted marine waters. Gastroenteritis describes a variety of diseases that affect the GI tract and are rarely life-threatening; self-limiting symptoms include nausea, vomiting, stomachache, diarrhea, headache, and fever. Based on these studies, EPA published a criteria document, *Ambient Water Quality Criteria for Bacteria – 1986*, recommending the use of these bacterial indicators in ambient water quality criteria values for the protection of primary contact recreation (US EPA, 1986). Table [4] summarizes the *Water Quality Standards for Coastal and Great Lakes Recreation Waters Rule* (US EPA, 2004) that requires States and Tribes to adopt the 1986 AWQC for Bacteria."

"States and Tribes generally define their designated use of 'primary contact recreation' to encompass recreational activities that could be expected to result in the ingestion of, or immersion in, water, such as swimming, water skiing, surfing, or any other recreational activity where ingestion of, or immersion in, the water is likely. EPA derived standards that implied an acceptable excess illness probability of 0.8% in swimmers exposed in freshwater and 1.9% in swimmers exposed in marine waters. EPA's 1986 bacteria criteria document indicates the illness rates are 'only approximate' and that the Agency based the 1986 values that appear in Table [4] on these approximations."

"EPA's 1986 bacteria AWQC document provides geometric mean densities as well as four different single sample maximum values (representing values below which an increasing percentage of single values are expected to fall if the geometric mean of samples from the water body is equal to the geometric mean criteria). The 1986 bacteria AWQC document categorizes the single sample maximum values based levels of beach usage as follows: 'designated bathing beach' for the 75% (most conservative) confidence level, 'moderate use for bathing' for the 82% confidence level, 'light use for bathing' for the 90% confidence level, and 'infrequent use for bathing' for the 95% confidence level. The lowest confidence level corresponds to the highest level of protection."

Table 4. Summary of EPA's 1986 recommended water quality criteria for bacteria and 2004 Rule. (Sources: EPA 2004, 2007)

		Geometric Mean	Single sample maximum allowable density (CFU/100 mL)			
Indicator	Swimming-associated gastroenteritis rate per 1,000 swimmers	Steady state geometric mean indicator density	Designated beach area	Moderate: full body contact recreation	Lightly used: full body contact recreation	Infrequently used: full body contact recreation
Freshwater						
Enterococci	8	33	61	78	107	151
E. coli	8	126	235	298	409	575
Marine Water						
Enterococci	19	35	104	158	276	501

"In the 1986 AWQC context, single sample maximum criteria are water quality assessment tools that provide a sense of when the water quality in a water body is not consistent with the AWQC. Insights based on single observations are very difficult because of the expected variability of fecal indicators. For instance, if the long-term geometric mean concentration of enterococci in the water at a marine beach is 35/100 mL and the log standard deviation is 0.4, then there is an 18% chance that the concentration of enterococci in a single sample would be over 158/100 mL. The higher the single sample maximum, the lower the probability that a single sample exceeding that value would occur as part of the normal random variability of samples (EPA, 2006)."

"Since publication of the 1986 criteria, many States have expressed concern that the current fecal indicator/illness rate relationships identified in the epidemiology studies leading up to the 1986 criteria are not appropriate or representative of all U.S. waters. For example, States have concern that the most appropriate indicator in tropical waters may be different than in temperate waters, and that appropriate levels of indicators may be different in waters where human fecal waste predominates animal waste. Other identified issues are as follows:

- Lack of clear, timely, and flexible guidance regarding use of the single sample maximum values and differing risk levels;
- No EPA-approved analytical methods for use in wastewater for the indicator bacteria;
- Lack of data to correctly assess the applicability of the 1986 bacteria criteria to flowing waters; and
- Lack of data to quantify the risk associated with contributions from nonhuman sources of fecal contamination as well as lack of flexibility to adjust the criteria for water bodies that do not receive human sources of fecal contamination."

4.2. For Potable-Supply Source Waters

Effective watershed management and source water protection are key components in the "multiple barrier" approach that is widely prescribed for the protection of drinking water supplies in the United States (AWWA 1997, EPA 1997). Because a number of the bacteriologically-impaired segments of the Hillsborough River watershed discharge to a municipal drinking water reservoir, management of pathogen loads that are contributed from those areas to the reservoir is a topic that should be addressed through the BMAP process.

A recent National Research Council (2000) review of this topic, although focused on watershed management issues that have arisen in the network of regional reservoirs that provides potable water supplies to New York City, includes a number of technical and policy-related points that appear relevant to the Hillsborough River watershed:

- The most commonly detected human pathogens associated with ingestion of water include several groups of enteric and aquatic bacteria, enteric viruses and enteric protozoa (summarized in Appendix C);
- Data collected nationally on reported waterborne disease outbreaks, from 1920 to 2000, suggest that a shift has occurred in the microorganisms responsible;
- Recognized disease outbreaks during the first half of the 20th century were caused primarily by bacterial agents – most frequently by *Salmonella typhi* and *Shigella* sp.;
- Since the 1970s, recognized outbreaks have been caused predominantly by enteric protozoa such as *Giardia* or *Cryptosporidium* (when a pathogen has been identified), or by viral agents;

- This shift may be due, in part, to the greater resistance of protozoa to chlorination;

- Most waterborne pathogens are removed or inactivated by conventional drinking water treatment processes such as coagulation and sedimentation, filtration, and disinfection;

- Enteric viruses and bacteria are particularly susceptible to disinfection by free chlorine and ozone;

- Enteric protozoa are relatively resistant to chlorine disinfection, but are typically removed by the physical processes of coagulation and flocculation followed by filtration;

- Unfortunately, these treatment processes do not guarantee complete removal of pathogens from drinking water, as evidenced by recent outbreaks of waterborne disease associated with conventionally-treated municipal water supplies;

- These disease outbreaks have generally resulted from (a) source contamination and the breakdown of one or more of the treatment barriers or (b) contamination of the distribution system; and

- Although treatment has reduced the incidence of waterborne disease in the U.S. since the 19^{th} century, waterborne pathogens continue to pose a significant threat to public health in this country;

The NRC (2000) report also highlights the following points regarding the sources and relative human health risks associated with different types of potential pathogens in drinking water reservoirs:

- Many bacterial pathogens (e.g., *Salmonella, Shigella, Vibrio*) are known to be waterborne, and may emanate from both human and nonhuman sources. However, they are at least as sensitive to disinfection with chlorine as are coliforms. Therefore, for disinfected drinking water systems, bacterial pathogens in source water are considered less significant than some other (less sensitive) microbial pathogens as potential health threats;

- There are many human enteric viruses that may be present in surface water supplies, such as rotaviruses, coxsackieviruses, and echoviruses. Human wastes (including wastewater discharges) are the most frequently documented sources of human enteric viruses in surface waters. Viruses are more sensitive to disinfection than are cysts of *Giardia* and oocysts of *Cryptosporidium*. Thus, like bacterial pathogens, the presence of viruses in water supplies that are disinfected is of comparatively less concern than the presence of protozoan pathogens;

- Two protozoa – *Giardia lamblia* (now named *Giardia intestinalis*) and *Cryptosporidium parvum* – form stages known as cysts and oocysts, respectively, that are resistant to disinfection with chlorine;

- *G. lamblia* cysts occur in human wastewater and in animal wastes, and some animals (e.g., beavers and other aquatic animals) are believed to serve as reservoirs for strains that are pathogenic to humans. Conclusive evidence that animal-derived cysts have actually caused human disease outbreaks has not yet been found. However, it is clear that aquatic animals, domestic dogs and cats, and cattle are potential sources of measurable cysts in surface waters;

- Knowledge of the life history of *Cryptosporidium* in water is less developed than that of *Giardia*, and the taxonomy and human pathogenicity of potential subspecies/strains of *C. parvum* are topics of ongoing research;

- The literature indicates that domestic animals, livestock and wildlife may serve as reservoirs of *C. parvum*, with infected calves and lambs excreting large amounts of oocysts;

- Human *C. parvum* isolates have been found to infect calves, lambs, goats, pigs, dogs, cats, mice and chickens. Isolates from calves have been found to infect humans, and there is also epidemiological evidence of transmission from cats or pigs to humans.

Based on this information it appears that, with respect to source water protection issues, the fecal coliform decision support tool for the Hillsborough River watershed should place additional emphasis on the detection and management of potential *Giardia* and *Cryptosporidium* sources and loads to the Hillsborough River Reservoir and the Tampa Bypass Canal, the two surface water bodies in the watershed that are used as sources of potable water supplies. Because bacteria and viruses continue to cause waterborne disease outbreaks in the U.S. (CDC 2004), however, sources of these pathogens should not be neglected.

5. Responding to Data Gaps and Uncertainty: A Phased Monitoring Approach

The National Research Council (NRC 2004) recently conducted a review of microbial water quality monitoring methods that summarized the shortcomings of existing indicators and explored potential solutions. Given the increased understanding of microbiology that has developed at the molecular level in recent years, the report projects that technological advances are likely to occur within the next decade or so that will provide cost-effective methods for monitoring new indicators—perhaps including pathogens themselves—and will allow managers to “rapidly and correctly identify when water used for recreational or drinking water purposes is contaminated with microorganisms that are pathogenic to humans” (NRC 2004).

Until those improved monitoring methods are developed and can be performed regularly at reasonable cost, a phased monitoring approach was recommended to help reduce the regulatory and resource management uncertainties brought about by the shortcomings of the existing monitoring tools (NRC 2004). The approach consists of three monitoring levels (routine sampling, expanded sampling, and microbial assessment), which are summarized in Figure 3.

At the first level (Level A), routine or “screening level” monitoring using existing indicators is conducted by local, state or federal water quality monitoring programs. The purpose of this monitoring is to provide an early warning of potential health risk, or a change from background conditions that could lead to unacceptable risk levels. The most important indicator attributes for use at this monitoring level are speed, low cost, logistical feasibility, applicability, and sensitivity. Because of the known drawbacks of existing indicators, significant management decisions should usually not be made based on this level of information “unless the indicator concentrations are extreme or supported by ancillary information” (NRC 2004).

In situations where routine/screening level sampling indicates a potential water quality problem, the second level of monitoring (Level B) is implemented to test for the presence of a public health risk. If this level produces evidence that a substantial risk may be present, management responses could include actions such as beach closures or boil-water orders. Level B monitoring typically includes expanded sampling with the same indicators used in Level A, to confirm the initial results and provide information on possible gradients in indicator concentrations. Ancillary information from on-the-ground surveys — which are equivalent to “sanitary inspections” in the WHO (2003) terminology and to the “contaminant source surveys” (CSS) used in FDEP’s fecal BMAP implementation process — can also be used to check for sewer system leaks, malfunctioning septic systems, illicit discharges, livestock waste entering streams, or similar potential fecal sources in upstream areas. In some situations, such as continued high levels of fecal indicators in the absence of an identifiable source, more specific indicators (including pathogens themselves) could potentially be measured at this stage. Given the currently high cost of many of these methods, however, they would presumably not be used on a routine basis (NRC 2004).

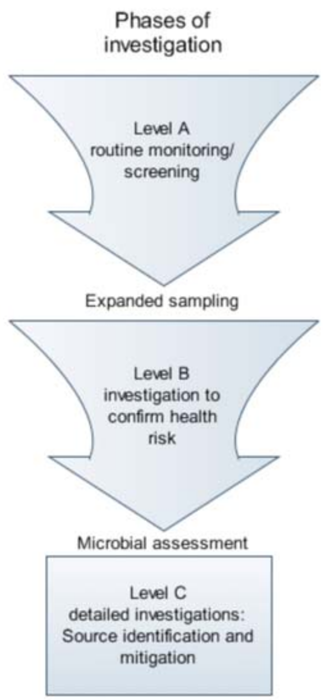


Figure 3. Phased monitoring approach recommended by the National Research Council. (Source: NRC 2004)

Because the studies carried out in Level B are intended to assess human health risk, the most important attributes for any additional indicators included at this stage are transport and survival characteristics that are similar to human pathogens, quantifiability, and effectiveness in measuring pathogen presence, viability or infectiousness (NRC 2004). Although more expensive than basic screening-level monitoring, a variety of new and emerging technologies such as quantitative polymerase chain reaction (qPCR), microarrays, and viral enumeration/detection by culture or PCR may be helpful at this level, particularly as methods are validated and refined.

The third recommended monitoring phase (Level C) adds focused studies whose objective is to “determine sources of microbial contamination so that health risk can be abated through a variety of engineering and policy solutions” (NRC 2004). In some cases expanded monitoring has been used to search for spatial gradients in indicator or pathogen concentrations. Methods based on molecular signatures have also proven helpful in some situations (NRC 2004). Important indicator attributes at this level of study include quantifiability and specificity for particular fecal sources.

The methods applied in Level A and B studies are in use today in many local environmental or health department laboratories. However, a number of the analytical techniques used in some Level B and most Level C studies have not yet been standardized and may only be available through research laboratories (NRC 2004). The responsibility for study costs may thus need to shift as the process moves forward, with parties potentially responsible for the operation of contamination sources becoming more involved in the funding process during the latter stages of Level B and much of Level C (NRC 2004).

6. A Potential Model for the fecal BMAP Implementation Process: The TBEP Water Quality "Decision Matrix"

As noted earlier, the TBEP (2006a) has developed a decision-support tool (or "decision matrix") that is applied annually to:

- track changes in water clarity and chlorophyll-*a* concentrations in Tampa Bay;
- compare annual mean water clarity and chlorophyll-*a* values to target levels that have been adopted to support the achievement of the program's seagrass restoration goals; and
- guide management responses to situations in which targets are not being achieved.

The TBEP decision matrix incorporates a number of elements that are desirable in a water quality management program, such as:

- the use of locally relevant water quality indicators and well-defined, quantitative management targets;
- the use of data from ongoing water quality monitoring programs, including annual evaluations of the data to detect exceedances of target values; and
- the adoption of a well-defined resource management process for responding to target exceedances when they occur.

It thus provides a conceptual model that could be used, with appropriate modifications, to develop a decision-support tool for addressing bacteriological impairments in the Hillsborough River watershed that would be consistent with the State's existing regulatory requirements, the "Annapolis protocol" recommended by the WHO (2000, 2003), and the phased monitoring approach recommended by the NRC (2004).

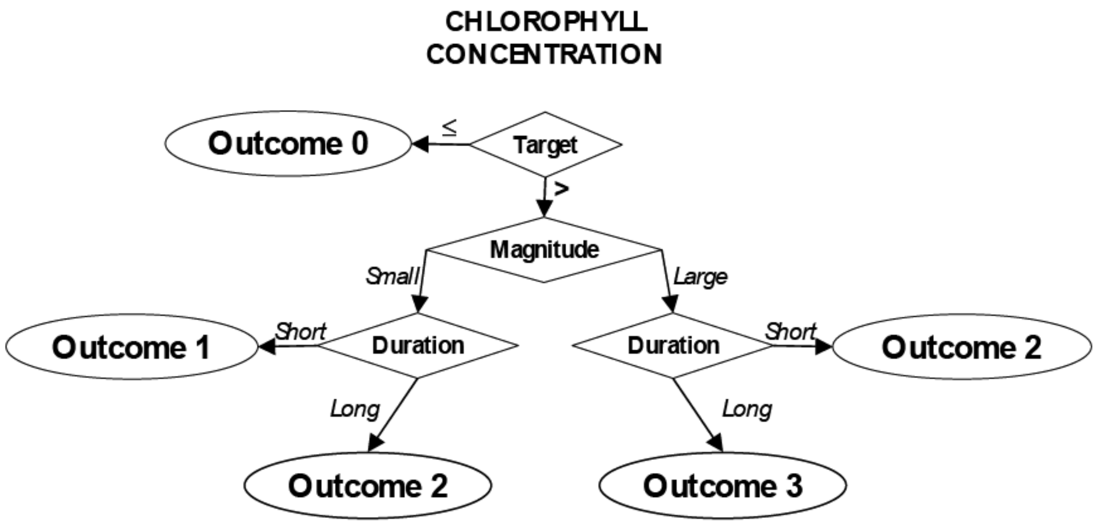
Poe et al. (2006) provide the following summary of the TBEP decision matrix approach:

> "Water quality targets have been adopted by the TBEP Management and Policy Committees for the four mainstem segments of Tampa Bay. The Tampa Bay Estuary Program has developed a tracking process to determine if water quality targets are being achieved (Janicki et al., 2000). The process to track the status of chlorophyll-*a* concentration and light attenuation involves two steps. The first step utilizes a decision framework to evaluate differences in mean annual ambient conditions from the established targets. The second step incorporates the results of the decision framework into a decision matrix leading to possible outcomes dependent upon the magnitude and duration of the events in excess of the target (Janicki et al. 2000)."
>
> "The tracking process is used not only to determine if there are differences between ambient conditions and targets, but also to determine the size of the differences and how long the conditions exist. The first step of the tracking process is presented in [Figure 4]. When mean ambient chlorophyll-*a* concentrations are less than the target, there is no cause for concern, as represented by Outcome 0 in [Figure 4]. When mean ambient chlorophyll-*a* concentrations are greater than target

values, however, the size of the difference and the duration of the difference are considered. Small differences for short time periods result in Outcome 1, while large differences for short time periods and small differences for long time periods result in Outcome 2. In the most severe condition, when large differences exist for long periods, the framework results in Outcome 3."

"The second step of the tracking process involves combining the outputs from the decision frameworks for chlorophyll-*a* concentration and light attenuation in a decision matrix to provide direction for management responses when targets are exceeded. The decision framework shown in [Figure 4] for chlorophyll-*a* concentration is the same as that for light attenuation."

"The decision matrix incorporating the outcomes for chlorophyll-*a* concentration and light attenuation is shown in [Table 5]. When outcomes for both chlorophyll-*a* concentration and light attenuation are good, as represented by Outcome 0 for both, a condition exists in which targets are being met, and so no management response is required. This condition is signified by the green cell in [Table 5]. When



conditions are intermediate, as signified by the yellow cells in [Table 5], differences from the targets exist for either or both chlorophyll-*a* concentration and light attenuation. These conditions may result in some type of management response. When conditions are problematic, such that the outcomes for the parameters fall within the red cells of [Table 5], stronger management responses may be warranted. The types of management actions resulting from the decision matrix are classified by color into three categories, shown following [Table 5]."

Figure 4. Decision framework used to identify water quality outcomes in TBEP "decision matrix" for chlorophyll-*a*. (Source: Poe et al. 2006)

Table 5. Decision matrix identifying appropriate categories of management actions in response to various outcomes of the monitoring and assessment of chlorophyll-*a* and light attenuation data.

CHLOROPHYLL	LIGHT ATTENUATION			
↓	Outcome 0	Outcome 1	Outcome 2	Outcome 3
Outcome 0	*GREEN*	*YELLOW*	*YELLOW*	*YELLOW*
Outcome 1	*YELLOW*	*YELLOW*	*YELLOW*	*RED*
Outcome 2	*YELLOW*	*YELLOW*	*RED*	*RED*
Outcome 3	*YELLOW*	*RED*	*RED*	*RED*

- **Green**: "Stay the course"; partners continue with planned projects to implement the Tampa Bay Comprehensive Conservation and Management Plan (CCMP; TBEP 2006a). Data summary and reporting occurs via the Baywide Environmental Monitoring Report (e.g., TBEP 2006b) and annual assessment and progress reports (e.g., Poe et al. 2006).

- **Yellow**: Technical advisory committee (TAC) and Management Board on caution alert; review monitoring data and loading estimates; attempt to identify causes of target exceedances; TAC report to Management Board on findings and recommended responses if needed.

- **Red**: TAC, Management and Policy Boards on alert; review and report by TAC to Management Board on recommended types of responses. Management and Policy Boards take appropriate actions to get the program back on track.

7. A Potential Framework for Addressing Fecal Coliform Impairments

An analogous decision-support framework and classification matrix can be developed to guide management actions addressing fecal coliform impairments. The Annapolis protocol recommended by the WHO (2003; summarized in Table 2), and the phased monitoring approach recommended by the NRC (2004; summarized in Figure 3), appear to offer the best means of doing so, given the well-known limitations of the existing bacterial indicators. The WHO (2003) and NRC (2004) approaches acknowledge the limitations of the existing indicators, and use a weight-of-evidence approach to help compensate for those limitations. They use two independent categories of information — bacterial indicator data to identify locations with potential fecal contamination, combined with site-specific surveys to identify and classify indicator sources on the basis of their potential human health risks — to prioritize and guide management actions.

Ways in which these approaches could be developed and applied to impaired WBIDs in the Hillsborough River watershed are described in detail in Sections 7.1 through 7.5 below. As a preliminary overview, the process involves the following steps, which are summarized in flowchart form in Figure 9:

- First, microbial water quality conditions within each WBID are categorized based on fecal coliform concentrations observed in the available monitoring data. (If existing monitoring programs do not provide sufficient data to characterize fecal coliform concentrations, additional monitoring should be conducted to ensure that adequate data will be available for future assessments);
- For characterization purposes, each monitoring station within a WBID is assigned to a microbial water quality assessment (MWQA) category. As shown in decision-tree form in Figure 8, the MWQA categories are symbolized as letter grades (A through E) reflecting how frequently the State's fecal coliform criterion of 400 CFU/100 mL is exceeded at a given site. Because sites with higher frequencies of criterion exceedances also tend to exhibit higher overall concentrations of fecal coliforms and enterococci (see Figures 5 through 7), MWQA categories A through E also represent progressively higher indicator organism concentrations and increasing levels of potential human health risk;
- Next, for sites at which more than 10% of the samples exceed the State's 400 CFU/100 mL criterion, contaminant source surveys (CSS) are carried out in order to identify the types of probable sources that could contribute to the elevated bacterial concentrations occurring at the site and to characterize their potential human health risks;
- Following the concepts outlined in the Annapolis protocol, each surveyed site is placed in a CSS category (ranging from "very low" to "very high" levels of potential risk) reflecting the types of probable bacterial sources found in the vicinity of the site and the estimated likelihood that those sources pose human health risks;
- Following the phased monitoring concept recommended by the NRC (2004), the intensity of CSS investigation that a site receives is based on its MWQA classification. That is, sites that exhibit more frequent (and higher magnitude) exceedances of the State's 400 CFU/100 mL fecal coliform criterion (e.g., sites in MWQA categories C, D or E) are subject to more intensive CSS investigations than sites exhibiting less frequent (and lower magnitude) exceedances;
- Once the MWQA and CSS analyses are completed, each site receives a two-part classification (summarized in Table 7), based on the MWQA and CSS categories into which it has been placed;
- As with the Tampa Bay water quality decision matrix discussed earlier, these classification outcomes (which are color-coded in Table 7 to indicate the estimated potential for human health risk) would be reported in an annual progress report. This report would provide policy-makers and the public with a summary of the management actions that have been implemented in the impaired WBIDs and the changes in water quality that have occurred there.

7.1. Using Bacterial Indicator Data to Develop Microbial Water Quality Assessment (MWQA) Categories

As shown in Table 2, the WHO (2003) Annapolis protocol uses observed concentrations of "intestinal enterococci" to classify locations into microbial water quality assessment (MWQA) categories. (For an explanation of the intestinal enterococcci indicator and a comparison to the enterococci and fecal coliform indicators that are used more commonly in the United States, see NRC (2004)). The particular MWQA categories developed by the WHO are not directly applicable to fresh water bodies in Florida, for two reasons:

- The epidemiological studies on which the WHO categories were based were carried out in northern temperate marine waters, primarily in the United Kingdom, and cannot be assumed *a priori* to reflect human health risks in subtropical freshwater or estuarine environments (NRC 2004); and
- The State of Florida does not have numerical criteria for the intestinal enterococci indicator, making the WHO (2003) MWQA categories difficult to integrate into Florida's existing water quality management system.

However, the conceptual approach underlying the WHO (2003) MWQA categories has been thoroughly reviewed and is technically sound (e.g., NRC 2004, EPA 2007). The approach can be applied to WBIDs in the Hillsborough River watershed (and potentially other Florida waters) by developing a comparable range of MWQA categories based on fecal coliforms, the bacterial indicators for which the State has adopted water quality criteria.

7.1.1. Florida's Fecal Coliform Criteria and the Impaired Waters Rule

The six WBIDs within the Hillsborough River watershed that are currently involved in the BMAP process are all Class III waters, whose designated uses include primary contact recreation and the provision of fish and wildlife habitat. Through its existing water quality standards (Chapter 62-302.250, F.A.C.), Florida has adopted a fecal coliform criterion for Class III waters of 400 CFU/100 mL (see Appendix A). Under the State's "impaired waters rule" (IWR; Chapter 62-303 F.A.C), water bodies are designated as impaired if more than 10% of their fecal coliform samples exceed this criterion.

Two general approaches have been evaluated in the technical literature as potential methods for defining exceedances of these types of numerical water quality criteria (e.g., Lin et al. 2000, Smith et al. 2001):

- A "raw scores" approach, in which any site at which more than 10% of the samples have fecal coliform counts greater than 400 CFU/100 mL would be designated as impaired; and
- A statistical approach, based on the binomial distribution, which asks whether the percentage of samples exceeding the 400 CFU/100 mL criterion is significantly greater than the 10% threshold.

Both approaches have positive and negative attributes, but from a statistical perspective the binomial approach has an overall advantage due to its ability to quantify and manage both "type I" and "type II" error rates.

In the TMDL/BMAP framework a type I error would occur if a water body or WBID was incorrectly designated as impaired for some water quality constituent, when it fact it met the applicable criterion and should have been designated as unimpaired. A type II error would occur if a water body or WBID was incorrectly classified as unimpaired, when it fact it failed to meet the criterion and should have been designated as impaired (Smith et al. 2001). Both types of error can potentially lead to substantial costs, by causing management resources to be allocated inefficiently and through the economic and other costs that are incurred if environmental resources and public health are not adequately protected.

The raw scores approach does not recognize the existence of type I or type II error, but in practice it tends to have a higher type I error rate and a lower type II error rate than the binomial approach at any given sample size (Smith et al. 2001). Regarding the minimum sample sizes needed for impairment designations, Smith et al. (2001) note that: "given the information routinely used in an assessment, the binomial method should replace the raw score approach when sample sizes are greater than 20. With sample sizes smaller than 20, neither the raw scores or the binomial method adequately control the error rates."

FDEP uses the binomial approach (see Chapter 62-303.420, F.A.C.) to determine whether WBIDs are impaired for fecal coliforms. This use of the binomial approach is explained in detail by Lin et al. (2000), who provide additional background information on the statistical computations involved. Using the approach, individual WBIDs are designated as impaired or unimpaired based on the total number of fecal coliform samples (*N*) collected during a defined impairment evaluation period and the number of those samples (*x*) that exceed the 400CFU/100 mL criterion. If the percentage of samples exceeding the criterion,

$$\hat{P} = 100 * x / N$$

is significantly greater than 10%, based on the binomial test applied at an alpha level of 0.10, the WBID is designated as impaired (Chapter 62-303.420, F.A.C.; Lin et al. 2000). By setting alpha (the "significance" level or expected type I error rate of the test) at 0.10, rather than at the lower 0.05 level that is traditionally used in laboratory research experiments, the expected type II error rate is reduced for a given sample size (Smith et al. 2001). The type II error rate can also be reduced, at any given alpha level, by increasing the sample size.

An application of this approach to fecal coliform data from a number of Hillsborough County water bodies is shown in Figure 5. The data used in this example were provided by the Environmental Protection Commission of Hillsborough County (EPCHC) which, since 2001, has performed monthly measurements of fecal coliforms from 39 inland (primarily fresh, but some occasionally brackish) sampling locations that are distributed throughout the county. These records, collected at a regular frequency using the same field sampling protocols and a single analytical laboratory, offer a relatively large data set that can be used to evaluate bacteriological water quality conditions and potential MWQA categories on a county-wide basis.

Figure 5 shows the relationship that exists between geometric mean fecal coliform concentrations and the proportion of samples exceeding the 400 CFU/100 mL criterion at each of these EPCHC stations during the years 2001 through 2007. Stations at which significantly more than 10% of the samples exceeded the 400 CFU/100 mL criterion (shown as closed circles in Figure 5) exhibited higher geometric mean fecal coliform levels than stations that did not significantly exceed the criterion (shown as open circles), and stations with the highest exceedance percentages also tended to have the highest geometric mean fecal coliform counts.

In addition to fecal coliform counts, the EPCHC monitoring program has also measured enterococci levels at these stations on a monthly basis since 2001. Relationships between geometric mean enterococci levels and the percentage of samples that exceeded the 400 CFU/100 mL fecal coliform criterion are also summarized, at the site level, in Figure 5.

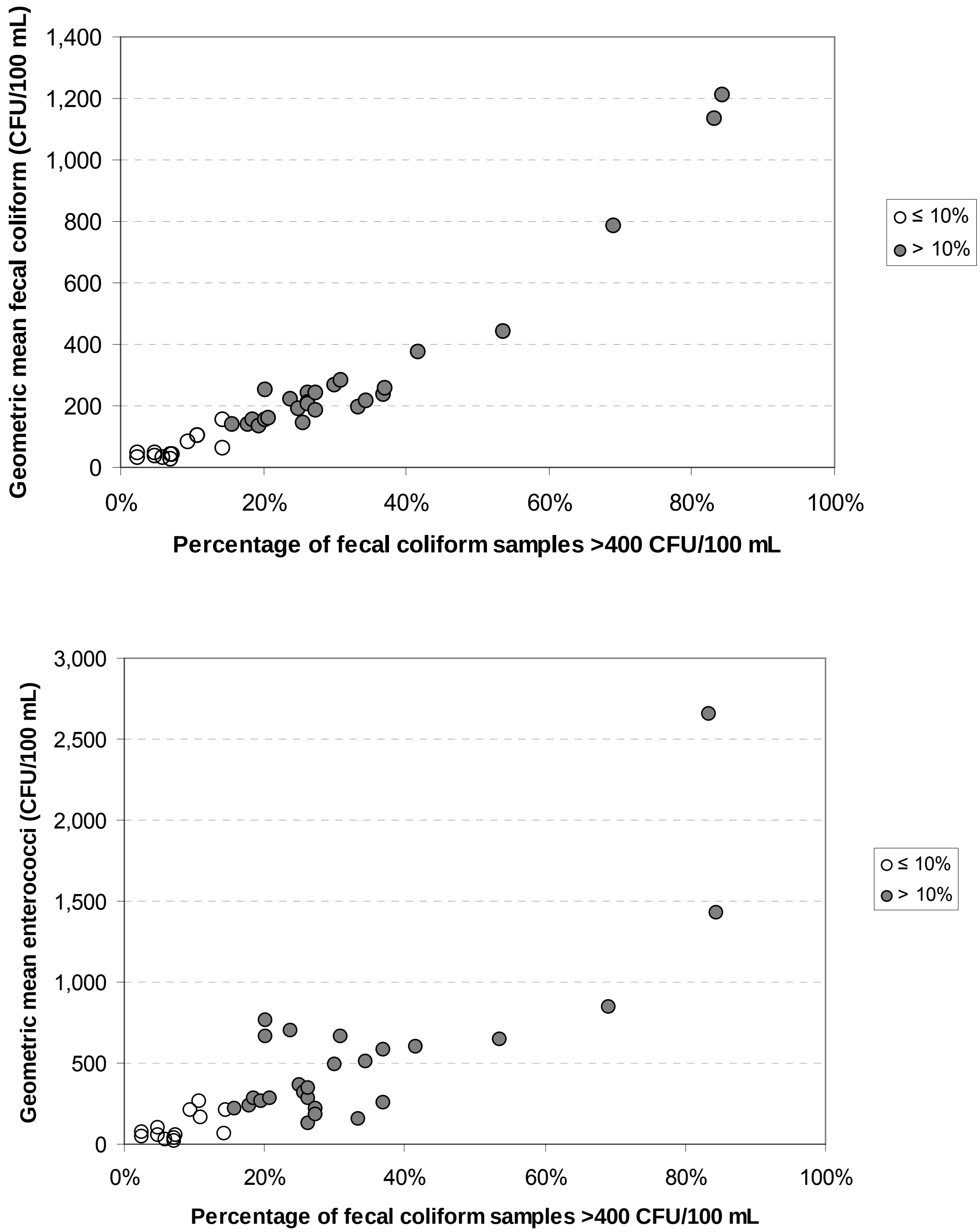


Figure 5. Relationships between geometric mean fecal coliform (upper panel) and geometric mean enterococci (lower panel) concentrations and the percentage of samples in which fecal coliform levels exceeded the 400 CFU/100 mL fecal coliform criterion (*x*-axis) at 39 stations monitored by EPCHC during the years 2001 through 2007. Stations at which the percentage of samples exceeding the criterion is significantly greater than 10%, based on the binomial test, are shown as filled circles. Each point represents one monitoring station. (Data source: EPCHC)

7.1.2. Using the 400 CFU/100 mL Fecal Coliform Criterion to Identify MWQA Categories

Based on the relationships shown in Figure 5, the percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion appears to offer a practical method for assigning monitoring locations to MWQA categories, as called for under the Annapolis protocol. Because increasing fecal coliform and enterococci concentrations have been shown to be associated with increasing (although highly variable) human health risks in a number of epidemiological studies (e.g., Prüss 1998, WHO 2003, NRC 2004), the relationships shown in Figure 5 indicate that MWQA categories based on the percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion will also reflect site-to-site variations in potential health risks.

In order to keep the MWQA scoring process as simple as possible, the *x*-axis in Figure 5 can be divided at a small number of "break points," and each sampling station can then be assigned to a MWQA category based on the exceedance frequency group into which it falls. One obvious break point is at the 10% criterion exceedance level, which is used by FDEP to classify WBIDs as impaired or unimpaired under the impaired waters rule (Chapter 62-303.420, F.A.C.). Visually (see Figures 5 through 7), additional natural break-points can be selected at exceedance frequencies of 30%, 50% and 75%, producing the five MWQA categories summarized in Table 6.

Table 6. Proposed microbial water quality assessment (MWQA) categories, based on the percentage of samples exceeding the State's 400 CFU/100 mL fecal coliform criterion. "Break points" separating the MWQA categories are at exceedance frequencies of 10%, 30%, 50% and 75%.

MWQA Category	Break Point (percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion)	Range of exceedance frequencies (percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion) included in category
A	≤ 10%	0% to 10%
B	> 10%	>10% to 30%
C	> 30%	>30% to 50%
D	> 50%	>50% to 75%
E	> 75%	>75% to 100%

For consistency with the IWR methodology, the binomial test can be carried out (at an alpha level of 0.10) to identify the EPCHC sampling locations where exceedances of the 400 CFU/100 mL criterion are significantly greater than the 10%, 30%, 50% and 75% break point levels described above. An application of this approach is shown in Figure 6, which is identical to Figure 5 but identifies the individual EPCHC stations based on their MWQA category assignments. Stations were assigned to MWQA categories based on the 10%, 30%, 50% and 75% break points, using the binomial test to identify exceedances of these break points that are statistically significant at an alpha level of 0.10.

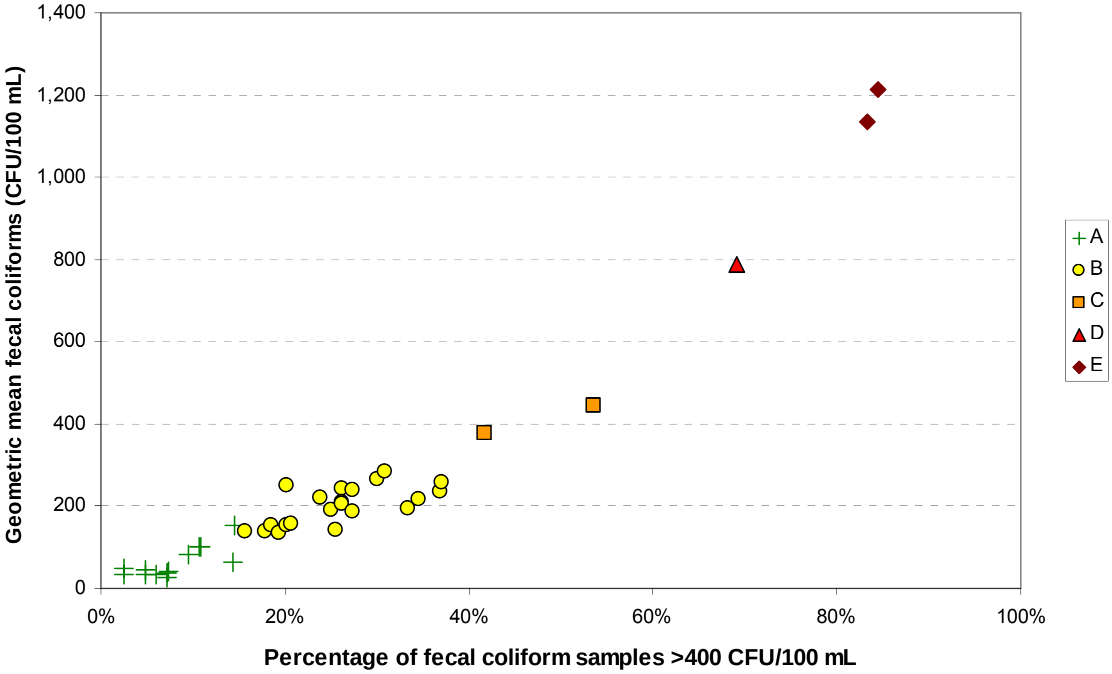
In addition to the station-by-station view shown in Figure 6, it is also helpful to pool the data (across stations) within each of the proposed MWQA categories, and consider variations in fecal coliform and enterococci concentrations that occur within and between the proposed categories. This view of the EPCHC monitoring data is shown, in box-and-whisker format, in Figure 7.

Due to the extreme variability of bacterial counts in ambient monitoring data, with values at a single station often ranging over several orders of magnitude during a multi-year sampling period (e.g., see Figures D-1 and D-2 in Appendix D), it is often difficult to plot minimum and maximum values on a single graph in a readily viewable format. This was the case with the data shown in Figure 7. As a result the 10^{th} and 90^{th} percentile values were substituted in place of the minimum and maximum values in the box-and-whisker plots shown in the figure.

Based on the concentrations of indicator bacteria observed within and between the proposed MWQA categories (Figures 6 and 7), the categories appear to provide the level of among-site discrimination of microbial water quality conditions that is needed for use in the BMAP implementation process. The primary purpose of the MWQA categories is to provide managers a tool that can be used to prioritize monitoring locations for follow-up investigations, and determine the level of effort that should be expended in those investigations, in order to evaluate and address potential human health risks. From a broader (e.g., regional) water quality management perspective, it appears that the proposed MWQA categories could also be used as a screening-level tool when prioritizing watersheds and their WBIDs for BMAP development. Watersheds or WBIDs with numerous sites in MWQA categories E and D could be given the highest priorities for BMAP development and implementation. If sources posing potential public health risks are detected in these areas, they in turn could be identified as high priorities for remedial action to reduce or eliminate the apparent risks.

The proposed approach for assigning sites to MWQA categories that is described above is summarized, in decision-tree form, in Figure 8. In addition to the five MWQA categories (A through E) described above, a sixth group — denoted as "insufficient data"— has also been included in Figure 8 to address situations in which the available data are not sufficient to allow microbial water quality conditions at a site to be evaluated. As noted earlier, Smith et al. (2001) reported that neither the raw scores nor the binomial approach appear to provide adequate protection against type I or type II errors at sample sizes smaller than 20 – 25. To minimize this concern, we would suggest that the "insufficient data" category include sites with <30 samples over the time period of interest (e.g., the previous five years).

Because this is the pilot application of the proposed MWQA categories, which have not yet been tested for their performance over time or in other geographic locations, the proposed category break-points should be viewed as provisional and subject to possible revision as additional information becomes available through the BMAP implementation process.



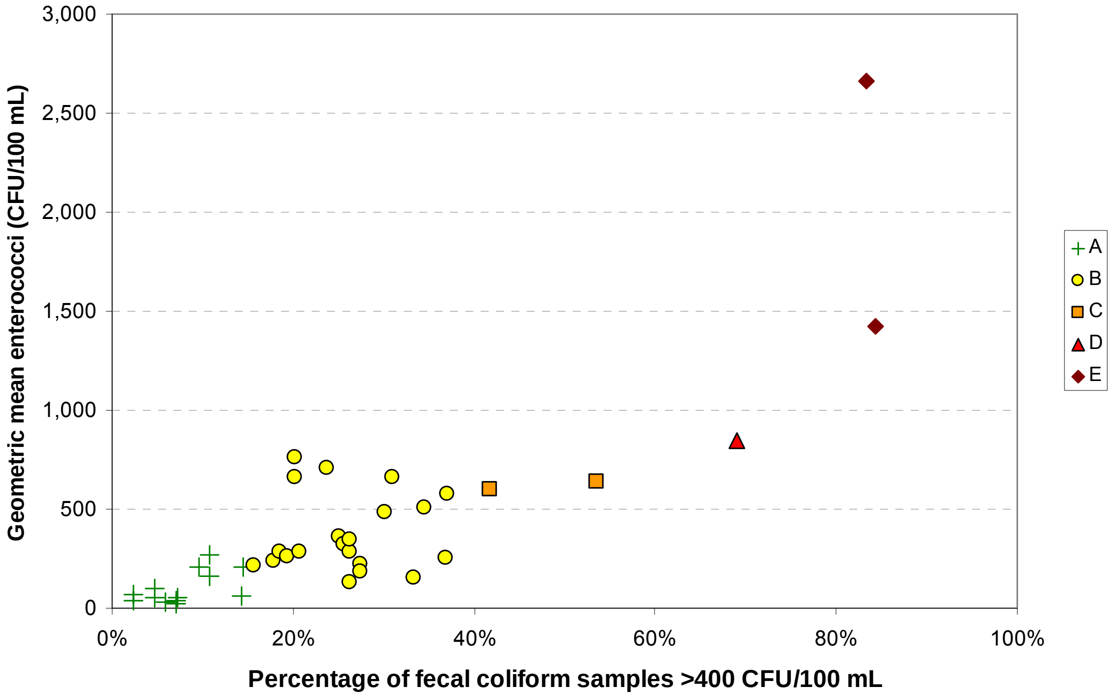
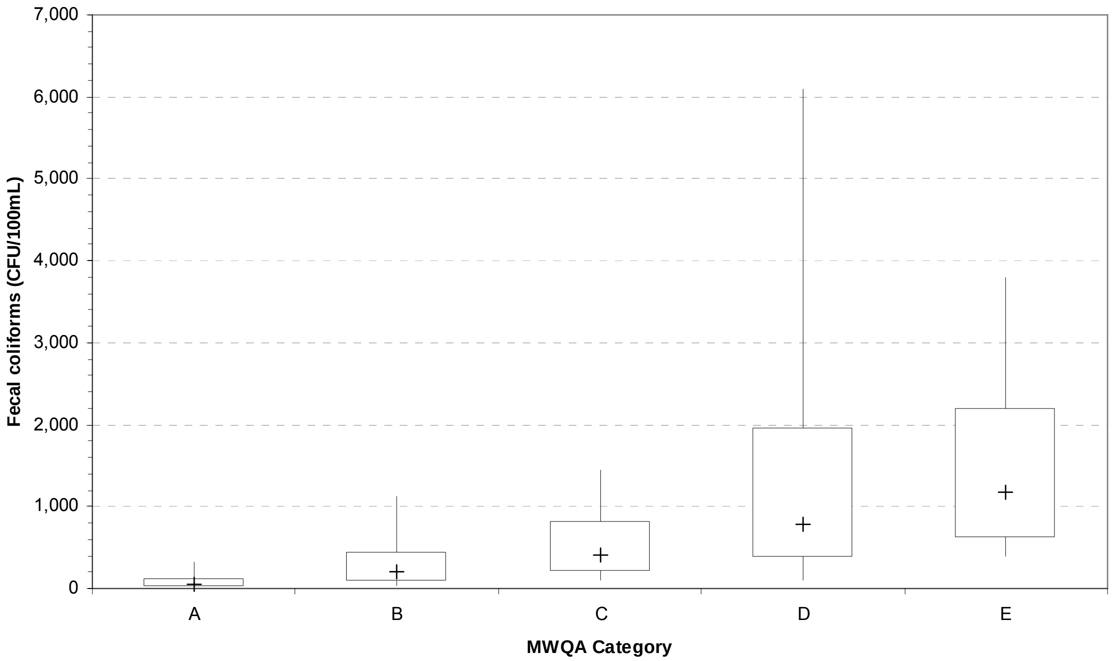


Figure 6. Geometric mean fecal coliform (upper panel) and geometric mean enterococci (lower panel) concentrations at the EPCHC monitoring stations shown in Figure 5. MWQA categories A through E were defined using the exceedance frequency break points of 10%, 30%, 50% and 75% described in Table 6. For consistency with the IWR, the binomial test was used to test the significance of break point exceedances and assign monitoring stations to MWQA categories. (Data source: EPCHC)



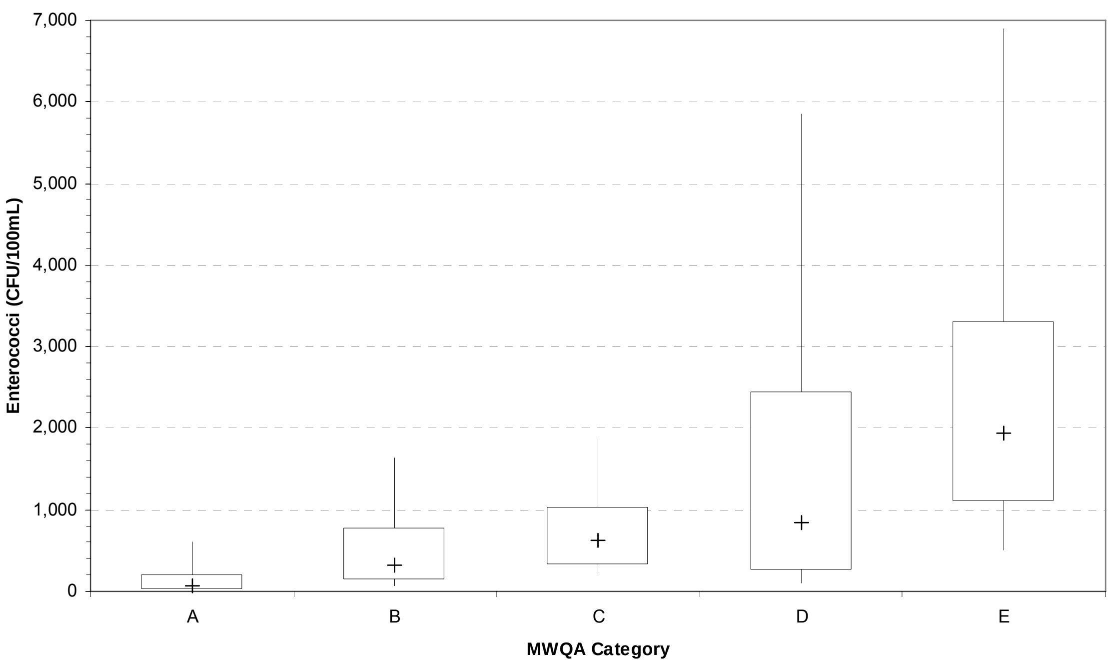


Figure 7. Box–and-whisker plots (showing geometric mean, interquartile range, and 10th and 90th percentile values) summarizing variability of fecal coliform and enterococci concentrations within and among the proposed MWQA categories listed in Table 6, based on the EPCHC monitoring stations shown in Figures 5 and 6. (Data source: EPCHC)

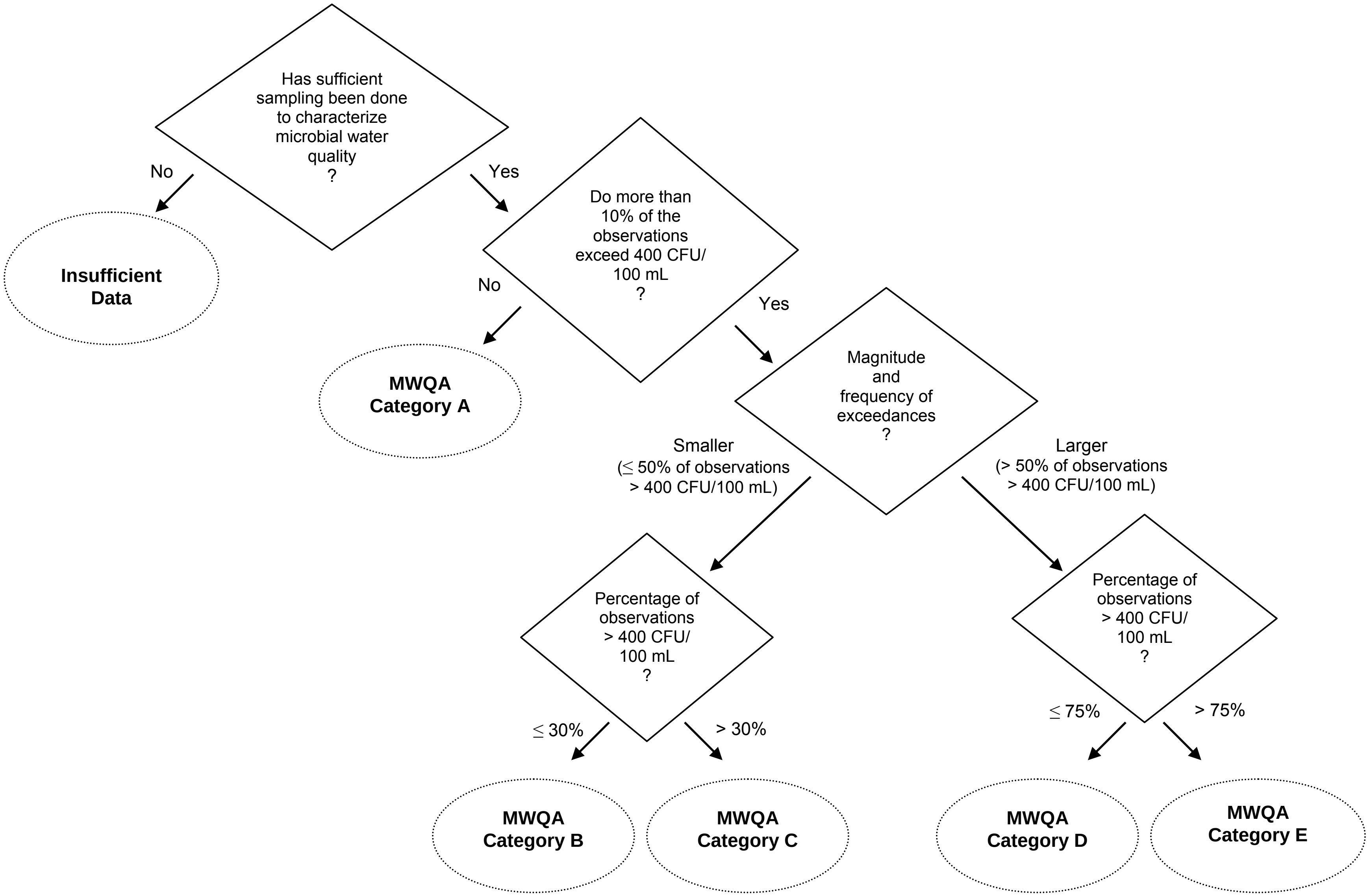


Figure 8. Decision tree for assigning monitoring locations to microbial water quality assessment (MWQA) categories based on observed fecal coliform concentrations. MWQA categories A through E are based on the percentage of samples at a given site that exceed the State's 400 CFU/100 mL fecal coliform criterion, using exceedance frequency break points of 10%, 30%, 50% and 75% as described in Section 7.1.2.

7.2. Incorporating Information from Contaminant Source Surveys and Other Sources

In addition to the bacterial indicator data considered in the previous section, another key element of the WHO (2003) Annapolis protocol and the NRC (2004) phased monitoring approach is the use of independent information — provided by contaminant source surveys (CSS) — as a cross-check for evaluating potential human health risks at monitoring locations where elevated indicator levels are observed. The additional information provided by CSS is used to identify the probable sources of the elevated indicator levels that occur at these sites, and determine if one or more of those sources fall into categories (e.g., inadequately treated sewage, livestock waste) that represent likely public health risks, or into other categories (e.g., wildlife, soils, sediments, vegetation) that appear to pose much lower risk.

Following the WHO (2003) approach, information from contaminant source surveys can be added by including a second axis in the decision-support framework, as shown in Table 7. Based on field experience from a number of areas in Florida, the project team recommends that the CSS assessment categories listed in the table be defined as follows, based on the likelihood of fecal contamination that would pose human health risks:

1. **Very Low**: No visual evidence of potential sources of human pathogens; natural environment; no or minimal anthropogenic land uses; wildlife present (any density)

2. **Low:** Low density agricultural and residential sources, including pets, livestock (without direct access to surface waters), or poultry operations; residences on septic systems

3. **Moderate**: Urban stormwater sources (including pet waste) present; well-functioning wastewater infrastructure (both sewer and septic); episodic/low volume sanitary sewer overflows (SSOs) reaching surface waters; moderate-density livestock with little direct access to surface waters; Class A residual and/or septage spreading areas may be present

4. **High**: Major stormwater outfalls present; history of failing wastewater infrastructure (central sewer or onsite systems); episodic or chronic/high volume SSOs reaching surface waters; concentrated livestock without direct access to surface waters; residual/septage spreading (Class B)

5. **Very High**: Current failing wastewater infrastructure; chronic/high volume SSOs reaching surface waters; concentrated livestock with direct access to surface waters; evidence of direct sewage inputs (e.g., confirmed illicit discharges)

For consistency with the WHO (2003) approach, an additional category ("exceptional circumstances") is also included in Table 7 to account for acute situations, such as sewer line breaks, that are known to be associated with increased health risk and would prompt immediate remedial action.

Within the classification matrix (Table 7), putative water quality conditions associated with chronic contamination sources are indicated by color, ranging from green (best water quality) to purple (poorest water quality). As a general rule, it is anticipated that the MWQA categories, based on fecal coliform measurements, and the CSS categories, based on assessments of local contaminant sources, will provide similar ratings of microbial water quality at a given site. However, because of the well-known variability of fecal coliform concentrations, their sensitivity to some environmental conditions (e.g., elevated salinity), and their frequently low correlation with human fecal contamination or other potential sources of enteric human pathogens, it is not unusual for monitoring locations to receive different water quality classifications based on their MWQA scores and initial CSS assessments (WHO 2003). In situations

Table 7. A potential classification matrix, based on the WHO (2003) Annapolis protocol approach, using a combination of fecal coliform measurements (represented by the MWQA group) and contaminant source survey (CSS) information to rank recreational sites based on the apparent likelihood of human health risk. (See text for explanation of CSS assessment categories.) (Source: Modified from WHO 2003)

		MWQA group (based on binomial assessment of frequency of 400 CFU/100 mL fecal coliform exceedances)					Exceptional Circumstances (e.g., sewer line break)[c]
		A (≤ 10%)	B (>10% - 30%)	C (>30% - 50%)	D (>50% - 75%)	E (>75%)	
Contaminant source survey (CSS) assessment category (likelihood of fecal contamination posing human health risks)	1. Very Low	A1	B1	C1[a]	D1[a]	E1[a]	**Immediate Action**
	2. Low	A2[b]	B2	C2	D2[a]	E2[a]	
	3. Moderate	A3[b]	B3	C3	D3	E3	
	4. High	A4[b]	B4[b]	C4	D4	E4	
	5. Very High	A5[b]	B5[b]	C5[b]	D5	E5	
	Exceptional Circumstances (e.g., sewer line break)[c]	**Immediate Action**					

Notes:

a) These outcomes imply that the CSS may be providing an overly optimistic rating of water quality, or the fecal coliform sources in the area may be relatively low-risk or primarily environmental (e.g., wildlife, sediments, soils, vegetation), and the cause(s) of the discrepancy should be verified.

b) These outcomes imply that the fecal coliform indicator may be providing an overly optimistic MWQA rating, or the CSS may be providing an overly negative assessment, and the cause(s) of the discrepancy should be verified.

c) As explained by WHO (2003), exceptional circumstances involve acute situations known to be associated with higher public health risks, such as sewer line breaks and other SSOs that contaminate surface waters, which require immediate remedial action.

where the fecal coliform counts are elevated due to environmental sources such as wildlife, sediments, soils or vegetation, for example, the MWQA scores may lead to substantially poorer water quality ratings than the CSS assessments. In other situations, significant fecal contaminant sources may be present but not be detected through the fecal coliform data (e.g., because the discharge is episodic), and information from the CSS may provide a more accurate assessment of the human health risks present at the site. The cells colored gray in Table 7 represent these cases. As indicated in the notes to Tables 2 and 7, management responses need to have sufficient flexibility to address these situations and managers should be prepared to conduct additional investigations in cases where the two sources of information appear to be providing conflicting information.

7.3. Recommended Management Responses to Classification Matrix Outcomes

The sets of management actions that are recommended to identify and respond to potential human health risks associated with the MWQA categories shown in Figure 8 and the classification matrix outcomes listed in Table 7 are summarized below. For each outcome, it is assumed that any sources identified during the CSS process that appear to represent significant potential public health risks will be dealt with as expeditiously as possible by local and state authorities.

For consistency with the phased monitoring approach recommended by the NRC (2004), the following levels of CSS intensity are included in these recommended management responses:

- **Phase 1 CSS** – includes basic (screening-level) analyses using available water quality data to identify patterns and trends that may be present in bacterial indicator levels, and land use information to identify potential sources in the contributing watershed. These screening-level analyses are followed by an on-the-ground survey by knowledgeable technical personnel to verify the presence of the potential sources identified through the initial analyses, identify other sources that may have been missed by the initial analyses, and assess the potential human health risks posed by all sources found in the vicinity of the site.

- **Phase 2 CSS** – includes all elements of Phase 1, plus more intensive analysis of a broader range of available information. In addition to ambient monitoring data, information from regulatory agencies regarding permitted discharges of indicator bacteria and other constituents (e.g., discharge monitoring data collected by permitees, or data collected by the agencies for permit renewal or compliance/enforcement purposes) is collated and analyzed to provide more detailed information on permitted sources. GIS and other mapping tools are used for a more detailed evaluation of factors such as land use intensity (e.g., levels of urban development or agricultural use), presence and condition of existing stormwater infrastructure, existing wastewater treatment methods (e.g., septic or central sewer), septic system failure and repair rates, reported SSOs and other leaks and spills from central sewer systems, and citizen complaints involving water quality problems, within the monitoring site's contributing watershed. If necessary, expanded spatial and temporal monitoring of indicator bacteria (and/or other water quality constituents) is also carried out to identify gradients in contamination and trace those gradients to individual sources (e.g., McDonald et al. 2006).

- **Phase 3 CSS** – includes all elements of Phases 1 and 2, plus the use of appropriate microbial source tracking (MST) tools, as necessary, to identify and characterize sources. A number of MST tools of varying cost and complexity, and their applications in identifying sources of fecal contamination, have been summarized by NRC (2004) and EPA (2005). A review of the need for validation of MST methods and strategies to accomplish this goal is presented in Stoeckel and Harwood (2007).

Before a classification matrix outcome (Table 7, Figure 8) can be calculated for a given site, sufficient monitoring data must be available to assign the site to an MWQA category. If sufficient data are not available (i.e., if the site falls into the "insufficient data" category of Figure 8), the following management actions are recommended:

- Implement a monitoring program that will provide a sufficient amount of data to characterize microbial water quality in the area of interest (e.g., collect a minimum of six bi-monthly samples per year over the next five-year period).
- Conduct a Phase 1 CSS to evaluate potential sources of human fecal contamination or enteric human pathogens in the site's contributing watershed.
- Base initial site assessment and remedial actions on CSS results, until sufficient fecal coliform data are available to assign the site to an MWQA category
- Document results in annual progress report to elected officials and the public.

Otherwise, if sufficient data are available to assign the site to an MWQA category and develop an initial classification matrix outcome (using Table 7), the following management actions are recommended:

Outcome A:

- Continue monitoring fecal coliforms.
- Continue standard permitting and compliance monitoring of potential sources; report and map all sanitary sewer overflows (SSOs) and septic system failures/repairs.
- Calculate MWQA category annually, and include result in annual progress report.
- A CSS does not appear necessary for sites in MWQA category A. However, if a CSS is conducted, and the resulting site classification is something other than A1 (i.e., A2 through A5), take follow-up action to identify and address any fecal contaminant sources that pose potential health risks.
- Document results in annual progress report.

Outcomes B1 through B5:

- Continue monitoring fecal coliforms at current, and possibly additional stations, and consider adding other indicators (enterococci and possibly *E. coli* in freshwater) if resources allow.
- Continue standard permitting and compliance monitoring of permitted sources; report and map all sanitary sewer overflows (SSOs) and septic system failures/repairs.
- Conduct a Phase 1 CSS and proceed through the classification matrix analysis (Table 7). Repeat Phase 1 CSS every 3 years.
- Take management actions to reduce fecal contamination from potential sources of human pathogens as those sources are detected and identified.
- Document results in annual progress report.

Outcomes C1 through C5:

- Continue monitoring fecal coliforms at current and additional stations, and consider adding enterococci and possibly *E. coli* in freshwater if resources allow.

- Continue standard permitting and compliance monitoring of permitted sources; report and map all sanitary sewer overflows (SSOs) and septic system failures/repairs.

- Conduct a Phase 2 CSS and proceed through the classification matrix analysis (Table 7).

- Take management actions to reduce fecal contamination from potential sources of human pathogens as those sources are detected and identified.

- Document results in annual progress report.

Outcomes D1 through D5:

- Notify media and public in areas where public health threats appear to be present.

- Take management actions to reduce fecal contamination from potential sources of human pathogens as those sources are detected and identified.

- Perform heightened compliance monitoring of permitted sources, and take corrective action as necessary; continue to report and map all sanitary sewer overflows (SSOs) and septic system failures/repairs.

- Conduct Phase 2 and, if necessary, Phase 3 CSS to identify indicator sources. Proceed through the classification matrix analysis (Table 7). Implement corrective actions for any sources detected through these investigations.

- Report actions taken and short-term results in semi-annual (twice per year) Work Group meetings.

- Continue monitoring fecal coliforms, and consider adding enterococci and possibly *E. coli* if resources allow.

- Document results in progress report.

Outcomes E1 through E5:

- Take immediate action to notify media and public in areas where public health threats appear to be present.

- Take immediate management actions to reduce fecal contamination from potential sources of human pathogens as those sources are detected and identified.

- Perform heightened compliance monitoring of permitted sources, and take corrective action as necessary; continue to report and map all sanitary sewer overflows (SSOs) and septic system failures/repairs.

- Conduct Phase 2 and, if necessary, Phase 3 CSS to identify indicator sources. Proceed through the classification matrix analysis (Table 7). Implement corrective actions for any sources detected through these investigations.

- Report actions taken and short-term results in semi-annual (twice per year) Work Group meetings.

- Continue monitoring fecal coliforms, and consider adding enterococci and possibly *E. coli* if resources allow.

- Document results in annual progress report.

These responses could be implemented by following the process summarized in Figure 9 for each monitoring location within an impaired WBID. The results (e.g., site-specific classification matrix outcomes, and the management actions taken in response to those outcomes) could be reported annually in a "citizen-friendly" format that allows status and trends in microbial water quality conditions to be tracked by non-technical readers, including interested citizens, elected officials and other policy-makers. If desired, a more technical report could also be produced on an annual or semi-annual basis for resource managers and other technical audiences.

Because potential sources of human pathogens would be addressed through the proposed process whenever they are identified, it is anticipated that microbial water quality conditions at sites impacted by such sources should improve over time as the BMAP process is implemented. Sites impacted by non-anthropogenic background sources that do not appear to pose significant public health risks (e.g., wildlife, sediments, soils, vegetation) would be identified as such through the CSS assessments, and may not require remedial management actions (e.g., Chapter 62-303.460(2) F.A.C.).

Given the large costs that could be incurred through extensive MST analyses, it is important that they be used judiciously. Recognizing this point, the management responses described above only make an explicit call for MST as part of a Phase 3 CSS. This would occur in cases where monitoring sites fall into MWQA groups D or E, indicating the potential for relatively high public health risks. During the BMAP implementation process, however, it is possible that cases may also arise in a Phase 1 or Phase 2 CSS in which MST analyses are needed to confirm the identity of suspected contaminant sources. These situations should be addressed on a case-by-case basis, with MST techniques applied when necessary to provide confirmation. A relatively low-cost approach that could be used in these cases, involving targeted sampling and three inexpensive MST methods, is described by McDonald et al. (2006).

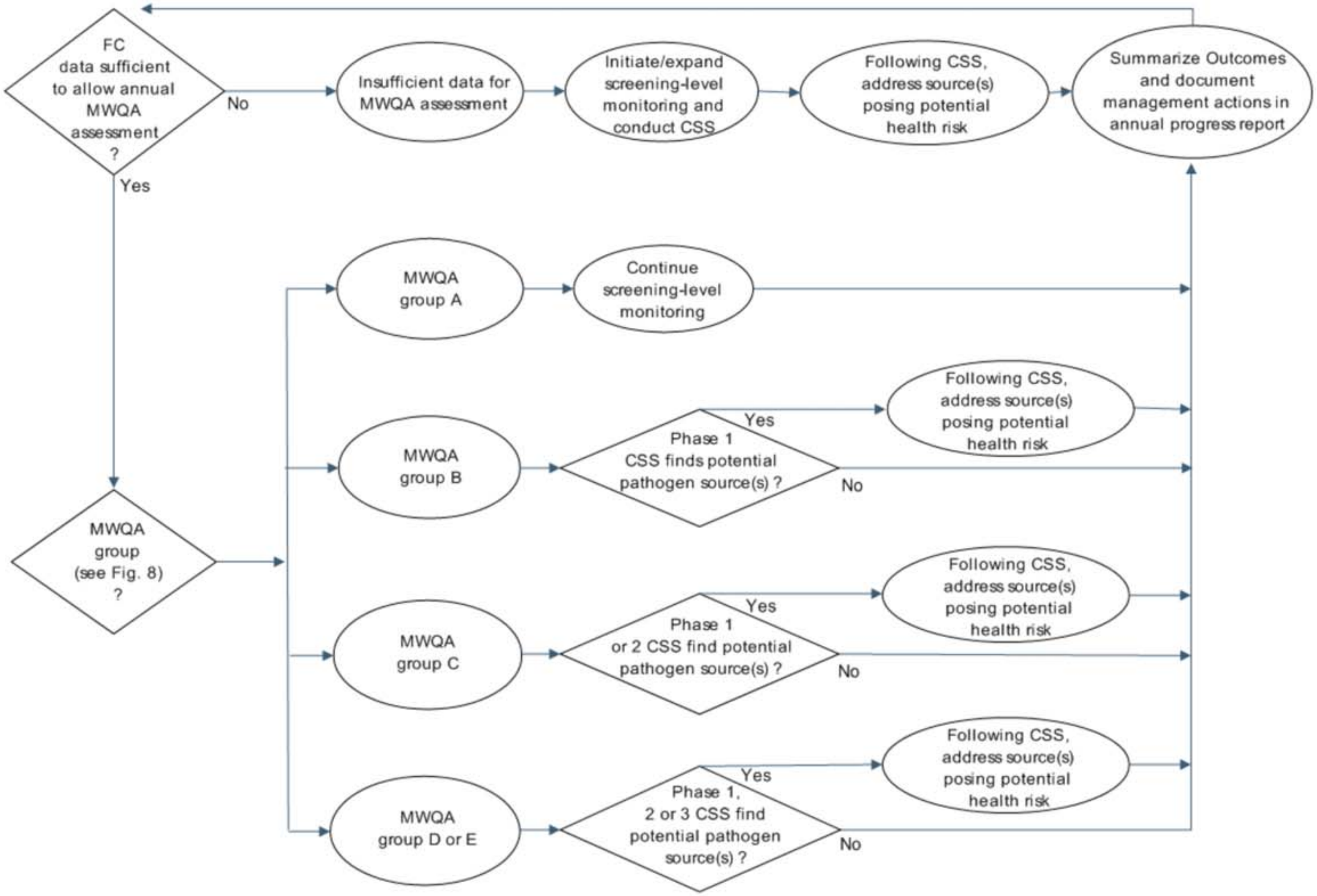


Figure 9. Potential management process for combining fecal coliform monitoring data and CSS assessment information, and for addressing and reporting fecal coliform impairment.

7.4. Applying the Classification Matrix in the Hillsborough River BMAP Areas

In order to apply the proposed approach on a pilot basis to the currently-active BMAP areas in the Hillsborough River watershed, MWQA categories (Table 6) and CSS assessments and classification matrix outcomes (Table 7) were calculated and assigned to individual EPCHC monitoring stations within each BMAP area. (CSS assessments were provided through a separate MST project, funded by FDEP, which PBS&J and USF are currently conducting in the Hillsborough River watershed.)

The six WBIDs in the Hillsborough River watershed for which BMAPs are currently being developed are:

- Blackwater Creek (WBID 1482)
- Spartman Branch (WBID 1561)
- Baker Creek (WBID 1522C)
- Flint Creek (WBID 1522A)
- New River (WBID 1442), and
- Lower Hillsborough River (WBID 1443E)

Monthly EPCHC monitoring data from the years 2001 through 2007 are available for five of these six WBIDs, the exception being New River (WBID 1442) where EPCHC monitoring was initiated only recently. MWQA categories for the nine monitoring stations located within these five WBIDS, calculated by applying the methods described in Section 7.1.2 to the EPCHC fecal coliform data, are summarized in Table 8. Within the table, monitoring sites are ranked on the basis of their geometric mean (2001 – 2007) fecal coliform counts.

None of the nine monitoring sites showed extremely high fecal coliform levels during the 2001 – 2007 period, and all sites fell within MWQA categories A (≤ 10% of samples exceeding 400 CFU/100 mL) or B (>10% and ≤ 30% of samples exceeding 400 CFU/100 mL) during the period (Table 8). Each of the monitoring stations in the lower Hillsborough River (WBID 1443E) was in MWQA category B, as were the stations on Baker Creek at Thonotosassa Road (WBID 1522C) and Flint Creek at U.S. Highway 301 (WBID 1522A). In terms of land use in the contributing watersheds, the lower Hillsborough River WBID is within a highly urbanized portion of the City of Tampa that appears to be impacted by stormwater runoff and other urban contaminant sources, including SSOs. The Baker Creek and Flint Creek WBIDs are in rural portions of unincorporated Hillsborough County and contain a mix of agricultural and low-density rural residential-associated sources. Blackwater Creek is also primarily located in rural areas of Hillsborough County, although low-, medium- and high-density population neighborhoods and associated sources are present in the upstream portion of the watershed within Polk County.

Based on the combination of MWQA categories and initial CSS assessments assigned to these stations, their classification matrix designations fell within outcomes A2, B2, B3 and B4, as summarized in Table 8. Recommended management responses for sites falling within these outcome categories were described earlier, in Section 7.3.

As an aside, water resource and public health managers should also be aware that the two Lake Thonotosassa stations (EPCHC stations 118 and 135) are located in areas that are known to be subject to frequent, high-density cyanobacterial ("blue-green algae") blooms (SWFWMD 2003). The EPCHC monitoring program does not analyze samples for cyanobacterial toxins, and it is not known if cyanotoxins occur at the sites. However, if cyanotoxins are present at the sites (e.g., during or immediately following blooms), they could potentially pose an independent (non-fecal) source of health risk during periods when they occur at sufficiently high concentrations (e.g., WHO 2003). Guidelines for human exposure to some categories of cyanotoxins have recently been recommended by the WHO (2003), and are currently being evaluated by the U.S. EPA, but to date no water quality criteria have been adopted in the U.S. for these constituents. This is an example of beneficial ancillary information that can be provided to resource managers as a result of conducting a CSS, in which a wide range of monitoring data and other information is examined to gain a better understanding of underlying water quality conditions at each evaluated site.

Returning to fecal contamination issues, it is interesting for comparative purposes to consider the sampling locations shown in Table 8 in the context of the fecal coliform and enterococci counts reported by the EPCHC for all 39 of its long-term inland stations that were monitored during the 2001 – 2007 period. Those values are summarized in Appendix D. Within this larger group of stations, five locations, all located outside the Hillsborough River watershed, would be assigned to MWQA categories C, D or E based on the frequency of exceedance of the 400 CFU/100 mL fecal coliform criterion (Appendix D). Those locations, particularly the ones falling within MWQA categories D or E, appear to be high priorities for further investigation and potential management action.

Interestingly, a Bullfrog Creek location (in the vicinity of EPCHC station 132) was identified by Rose et al. (2001), through MST analyses, as an area in which abundant biochemical indicators of human fecal contamination were detected in surface water samples. As of yet, no CSS or MST studies appear to have been done for other category C, D or E sites listed in Appendix D. The information shown in Appendix D provides an example of ways in which the classification matrix approach (Table 7) could be used, at a watershed or regional scale, to prioritize sites for further investigation and possible management action to improve water quality.

Table 8. Microbial water quality assessment (MWQA) categories, geometric mean fecal coliform and enterocci values, and initial classification matrix outcomes for stations within active BMAP areas that were monitored by the EPCHC during the years 2001 through 2007. MWQA category assignments are based on the binomial method described in the text and the decision tree shown in Figure 8. Bacterial indicator organism (fecal coliform and enterococci) values are in units of CFU/100 mL. Within the table, sites are arranged in order of increasing geometric mean fecal coliform values. (Data source: EPCHC)

Location	EPCHC Station No.	WBID No.[1]	Geometric Mean Fecal Coliform Count	Geometric Mean Enterococci Count	MWQA Category	CSS Category	Classification Matrix Outcome
L. Thonotosassa middle	135	1522A	33	42	A	2	A2[a]
L. Thonotosassa at Flint Creek	118	1522A	47	69	A	2	A2[a]
Hillsborough River at US 301	108	1482	102	159	A	2	A2[a]
Blackwater Creek at SR 39	143	1482	157	284	B	2	B2
Flint Creek at US 301	148	1522A	191	360	B	2	B2
Hillsborough River at Columbus Ave	137	1443E	195	154	B	3	B3
Hillsborough River at Rowlett Park	105	1443E	209	128	B	3	B3
Baker Creek at Thonotosassa Rd	107	1522C	216	509	B	2	B2
Hillsborough River at Sligh Ave	152	1443E	240	183	B	4	B4[a]

Notes:

a) **As indicated in Table 7, these outcomes imply that the fecal coliform indicator may be providing an overly optimistic MWQA rating, or the CSS may be providing an overly negative assessment, and the cause(s) of the discrepancy should be verified.**

7.5. Additional Questions and Issues

The proposed BMAP decision support process outlined above has been discussed with the Hillsborough River BMAP Working Group, a group that was organized by the FDEP and the Tampa Bay Estuary Program and receives technical support from FDEP contractors and technical staff in a number of local government agencies. That group has identified the following questions, issues and recommendations that its members would like to see addressed, either through the present project (if they fall within its scope) or in follow-on projects that are carried out during later phases of BMAP implementation:

1. The BMAP process should include a method for designating sites that do not meet the State's 400 CFU/100 mL fecal coliform criterion, but which are impacted only by non-anthropogenic environmental sources (e.g., wildlife, soils, aquatic sediments) that do not appear to represent significant public health risks. (A section of the impaired waters rule (Chapter 62-303.460[2], F.A.C.) notes this issue and may offer a mechanism for addressing the Working Group's concern.)

2. The process needs to consider other designated uses (e.g., Class I and II waters), in addition to the approach proposed here for Class III waters.

3. For Class III waters, it may also be helpful to include a means of prioritizing sites based on the magnitude and intensity of the recreational use they receive, in addition to the prioritization approaches proposed here based on MWQA and CSS categories.

4. An additional decision support process may need to be developed for bacteriologically-impaired Class III waters that are used as designated bathing areas. Such areas are monitored (and, when necessary, posted to discourage public use) by the Florida Department of Health and its affiliated County Health Departments.

5. The minimum number of fecal coliform samples needed to assign sites to MWQA categories when applying the decision tree (Figure 8) and classification matrix (Table 7) needs to be defined, if a working definition has not already been provided through the IWR. The project team would recommend that a minimum of 30 samples, collected at approximately bi-monthly intervals over a period of five years, be considered for this purpose.

6. Potential approaches for assigning MWQA categories at tidally-influenced monitoring sites — which exhibit freshwater salinities during some sampling events and estuarine (or marine) salinities during others — need to be considered. The very different survival times that fecal coliforms may have at these locations, depending on salinity characteristics of the site during the period immediately preceding a sampling event, complicate efforts to assign meaningful MWQA categories (or MWQA categories that are comparable to freshwater sampling stations) to these sites.

7. The impact of contaminated sediments on water quality and the associated human health risk needs to be better defined. When sediments are resuspended by storm events or other disturbances, indicator organism levels frequently become elevated; however the implications for human health are even more poorly understood in this case because the bacteria may be growing slowly in the sediments. Furthermore, it is unclear whether survival characteristics of pathogens are similar to those of the indicator bacteria in the sediments.

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Appendix A

Florida water quality criteria for fecal coliform bacteria
(62-302.250 F.A.C.)

Parameter	Units	Class I	Class II	Class III: Fresh	Class III: Marine	Class IV	Class V
(6) Bacteriological Quality (Fecal Coliform Bacteria)	Number per 100 ml (Most Probable Number (MPN) or Membrane Filter (MF))	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 5 samples taken over a 30 day period.	MPN shall not exceed a median value of 14 with not more than 10% of the samples exceeding 43, nor exceed 800 on any one day.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.		

Appendix B

The relative merits of selected indicators of sewage contamination. (Source: WHO 2000)

Indicator	Advantages	Disadvantages
Faecal streptococci/ enterococci	Marine and potentially freshwater human health indicator More persistent in water and sediments than coliforms Faecal streptococci may be cheaper than enterococci to assay	May not be valid in tropical waters due to potential growth in soils
Thermotolerant conforms	Indicator of recent faecal contamination	Possibly not suited to tropical waters due to growth in soils and waters Confounded by non-sewage sources (e.g. *Klebsiella* spp. in pulp and paper wastewaters)
E. coli	Potential freshwater human health indicator Indicator of recent faecal contamination Potential for typing *E. coli* to aid in sourcing faecal contamination Rapid identification possible if defined as β-glucuronidase-producing bacteria	Possibly not suited to tropical waters due to growth in soils and waters
Sanitary plastics	Little training of staff required and immediate assessment can be made for each bathing day Can be categorised	May reflect old sewage contamination and thus be of little health significance Subjective and prone to variable description
Preceding rainfall (12, 24, 48 or 72 h)	Simple regressions may account for 30-60% of the variation in microbial indicators for a particular beach	Each beach catchment may need to have its rainfall response assessed Response may depend on the period before the event
Sulphite-reducing clostridia[1]	Inexpensive assay with H_2S production Always in sewage impacted waters Possibly correlated with enteric viruses and parasitic protozoa	Enumeration requires anaerobic culture May also come from dog faeces May be too conservative an indicator
Somatic coliphages	Standard method well established Similar physical behaviour to human enteric viruses	Not specific to sewage May not be as persistent as human enteric viruses May grow in the environment
F-specific RNA phages	More persistent than some coliphages Standard ISO method available Host does not grow in environmental waters below 30°C	WG49 host may lose plasmid (although F-amp is more stable) Not specific to sewage Not as persistent in marine waters
Bacteroides fragilis phages	More resistant than other phages in the environment and similar to hardy human enteric viruses Appears to be specific to sewage ISO method recently published	Because numbers in sewage are lower than for other phages and most do not excrete this phage, it is of limited value for small populations Requires anaerobic culture
Faecal sterols	Coprostanol largely specific to sewage	Requires expensive gas

	Coprostanol degradation in water similar to die-off of thermotolerant coliforms A ratio of 5β: 5α stanols > 0.5 is indicative of faecal contamination; i.e. a ratio coprostanol: 5α-cholestanol of > 0.5 indicates human faecal contamination, while C_{29} 5β(24-ethylcoprostanol): 5α stanol ratio of > 0.5 indicates herbivore faeces Ratio of coprostanol: 24-ethylcoprostanol can be used to indicate the proportion of human faecal contamination, which can be further supported by ratios with faecal indicator bacteria (Leeming *et al*., 1996)	chromatography (about US$ 100 per sample) Requires up to 10 litres of sample to be filtered through a glass fibre filter to concentrate particulate stanols
Caffeine	May be specific to sewage, but unproven to date Could be developed into a dipstick assay	Yet to be proven as a reliable method
Detergents	Relatively routine methods available	May not be related to sewage (e.g. industrial pollution)
Turbidity	Simple, direct and inexpensive assay available in the field	May not be related to sewage; correlation must be shown for each site type
Cryptosporidium[2]	Required for potential zoonoses, such as *Cryptosporidium* spp., where faecal indicator bacteria may have died out, or not present	Expensive and specialised assay (e.g. Method 1622, US EPA); human/animal speciation of serotypes is not currently defined

[1] *Clostridium perfringens*

[2] Animal-sourced pathogens

Appendix C

Illnesses Caused by Microbial Agents Acquired by Ingestion of Water
(Source: NRC 2000)

Agent	Source	Incubation Period	Clinical Syndrome	Duration
Viruses:				
Astrovirus	human feces[1]	1-4 days	Acute gastroenteritis	2-3 days; occasionally 1-14 days
Enteroviruses (polioviruses, coxsackieviruses, echoviruses)	human feces	3-14 days (usually 5–10 days)	Febrile illness, respiratory illness, meningitis, herpangina, pleurodynia, conjunctivitis, myocardiopathy, diarrhea, paralytic disease, encephalitis, ataxia	Variable
Hepatitis A	human feces	15-50 days (usually 25-30 days)	Fever, malaise, jaundice, abdominal pain, anorexia, nausea	1-2 weeks to several months
Hepatitis E[2]	human feces	15-65 days (usually 35-40 days)	Fever, malaise, jaundice, abdominal pain, anorexia, nausea	1-2 weeks to several months
Norwalk-like viruses	human feces	1-2 days	Acute gastroenteritis with predominant nausea and vomiting	1-3 days
Group A rotavirus	human feces[1]	1-3 days	Acute gastroenteritis with predominant nausea and vomiting	5-7 days
Group B rotavirus[2]	human feces[1]	2-3 days	Acute gastroenteritis	
Bacteria:				
Aeromonas hydrophila	fresh water		Watery diarrhea	Average 42 days
Campylobacter jejuni	human and animal feces	3-5 days (1-7 days)	Acute gastroenteritis, possible bloody and mucoid feces	1-4 days occasionally >10 days

Appendix C

Agent	Source	Incubation Period	Clinical Syndrome	Duration
Enterohemorrhagic *E. coli* O157:H7	human and cattle feces	3-5 days	Watery, then grossly bloody diarrhea, vomiting, possible hemolytic uremic syndrome	1-12 days Average 7-10 days
Enteroinvasive *E. coli*	human feces	2-3 days	Possible dysentery with fever	1-2 weeks
Enteropathogenic *E. coli*		2-6 days	Watery to profuse watery diarrhea	1-3 weeks
Enterotoxigenic *E. coli*	human feces?	12-72 hours	Watery to profuse watery diarrhea	3-5 days
Plesiomonas shigelloides	fresh surface water, fish, crustaceans, animals	1-2 days	Bloody and mucoid diarrhea, abdominal pain, nausea, vomiting	11 days average
Salmonellae	human and animal feces	8-48 hours	Loose, watery, occasionally bloody diarrhea	3-5 days
Salmonella typhi[2]	human feces and urine	7-28 days (average 14 days)	Fever, malaise, headache, cough, nausea, vomiting, abdominal pain	Weeks to months
Shigellae	human feces	1-7 days	Possible dysentery with fever	4-7 days
Vibrio cholera O1[2]	human feces	9-72 hours	Profuse, watery diarrhea, vomiting, rapid dehydration	3-4 days
Vibrio cholera non-O1[2]	human feces	1-5 days	Watery diarrhea	3-4 days
Yersinia enterocolitica	animal feces and urine	2-7 days	Abdominal pain, mucoid, occasionally bloody diarrhea, fever	1-21 days average 9 days

Protozoa:				
Balantidium coli[2]	human and animal feces	Unknown	Abdominal pain, occasional mucoid or bloody diarrhea	Unknown
Cryptosporidium parvum	human and animal feces	1-2 weeks	Profuse, watery diarrhea	4-21 days
Entamoeba histolytica[2]	human feces	2-4 weeks	Abdominal pain, occasional mucoid or bloody diarrhea	Weeks to months
Cyclospora cayetenensis[2]	human feces	1 week average	Watery diarrhea, profound fatigue, anorexia, weight loss, bloating, abdominal cramps, nausea	Weeks if untreated
Giardia lamblia	human and animal feces	5-25 days	Abdominal pain, bloating, flatulence, loose, pale, greasy stools	1-2 weeks to months and years
Algae:				
Cyanobacteria (*Anabaena* spp., *Aphanizomenon* spp., *Microcystis* spp.) [2]	Algal blooms in water	A few hours	Toxin poisoning (blistering of mouth, gastroenteritis, pneumonia)	Variable
Helminths:				
Dracunculus medinensis[2] (Guinea worm)	larvae	8-14 months (usually 12 months)	Blister, localized arthritis of joints adjacent to site of infection	Months

[1]Animal strains of these viruses are not believed to be pathogenic for humans.
[2]Waterborne infections in the U.S. are rare or undocumented.

Appendix D

MWQA categories for other EPCHC monitoring stations

Table D-1. Criterion exceedance frequencies, microbial water quality assessment (MWQA) category assignments, and geometric mean fecal coliform and enterocci concentrations for 39 inland stations monitored by the EPCHC during the years 2001 through 2007. MWQA group assignments are based on the binomial method (see text), applied using the decision tree shown in Figure 8. (Data source: EPCHC)

Location	Sta. No.	No. Samples (N)	No. >400 CFU/ 100 mL (x)	% >400 CFU/ 100 mL ($\hat{P}$)	MWQA category	Geometric mean fecal coli. (CFU/ 100 mL)	Geometric mean enterococci (CFU/ 100 mL)
L. Thonotosassa at Flint Creek	118	82	2	2%	A	46.8	68.5
L. Thonotosassa middle	135	82	2	2%	A	32.9	41.7
Hillsborough River at Fowler Ave	106	84	4	5%	A	46.6	96.4
Little Manatee River at US 41	112	84	4	5%	A	34.6	55.1
TBC at MLK Blvd	147	84	5	6%	A	31.9	31.0
Palm River at US 41	109	84	6	7%	A	39.1	36.4
Palm River at SR 60	110	84	6	7%	A	25.4	21.8
TBC at Fowler Ave	146	82	6	7%	A	40.5	51.0
Alafia River South Prong ab confluence	116	84	8	10%	A	80.6	207.3
Alafia River North Prong ab confluence	115	84	9	11%	A	100.4	267.3
Hillsborough River at US 301	108	83	9	11%	A	101.7	159.4
Channel A at Hillsborough Ave	102	84	12	14%	A	63.2	63.1
Alafia River at US 301	153	83	12	14%	A	152.7	209.7
Alafia River South Prong at Bethlehem Rd	139	83	13	16%	B	138.8	215.7
Alafia River at Bell Shoals Rd	114	84	15	18%	B	136.4	240.7
Trout Creek at CR 581	145	65	12	18%	B	152.9	281.8
Cypress Creek at CR 581	120	72	14	19%	B	132.9	261.9
Little Manatee River at US 301	113	84	17	20%	B	250.8	664.0
Sweetwater Creek at Anderson Rd	142	84	17	20%	B	152.2	764.8
Blackwater Creek at SR 39	143	82	17	21%	B	156.9	284.2
Little Manatee River at CR 674	129	84	20	24%	B	218.5	704.0
Flint Creek at US 301	148	84	21	25%	B	191.2	359.8
Turkey Creek at Durant Rd	151	82	21	26%	B	142.0	322.6
Rocky Creek at Hillsborough Ave	103	84	22	26%	B	242.6	285.2
Hillsborough River at Rowlett Pk	105	84	22	26%	B	208.7	127.7
Rocky Creek at Waters Ave	141	84	22	26%	B	205.8	343.4
Double Branch Creek at Hillsborough Ave	101	84	23	27%	B	185.1	222.6
Hillsborough River at Sligh Ave	152	84	23	27%	B	239.8	182.8
English Creek at SR 60	154	83	25	30%	B	266.6	487.7
Little Manatee River at CR 579	140	84	26	31%	B	283.6	664.9
Hillsborough River at Columbus Ave	137	84	28	33%	B	195.5	153.7
Baker Creek at Thonotosassa Rd	107	84	29	35%	B	216.5	508.7
Bullfrog Creek at US 41	144	84	31	37%	B	233.7	254.7
Mill Creek at I-4	149	81	30	37%	B	258.0	578.7
Delaney Creek at US 41	133	84	35	42%	C	376.7	596.8
Delaney Creek at 36th Ave	138	84	45	54%	C	443.3	642.2
Sweetwater Creek at Hillsborough Ave	104	84	58	69%	D	786.4	849.9
Bullfrog Creek at Symmes Rd	132	78	65	83%	E	1,134.8	2,658.9
Turkey Creek at SR 60	111	77	65	84%	E	1,212.0	1,426.0

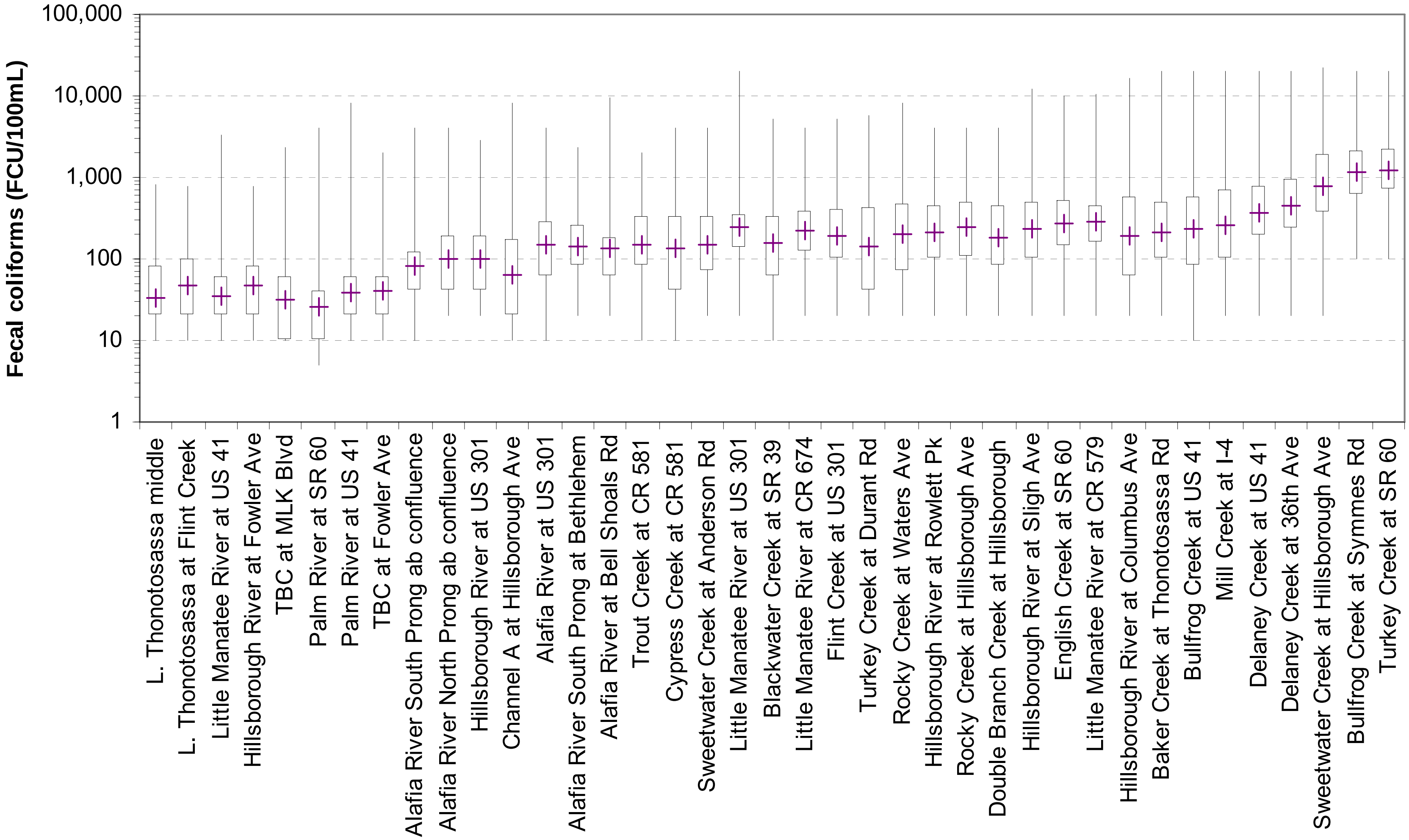

Figure D-1. Box–and-whisker plots (showing minimum, maximum, interquartile range and geometric mean) of fecal coliform counts (CFU/100 mL) observed at 39 EPCHC inland monitoring stations during the years 2001 – 2007. Stations are ranked based on the percentage of samples exceeding the State's 400 CFU/100 mL fecal coliform criterion. (Data source: EPCHC)

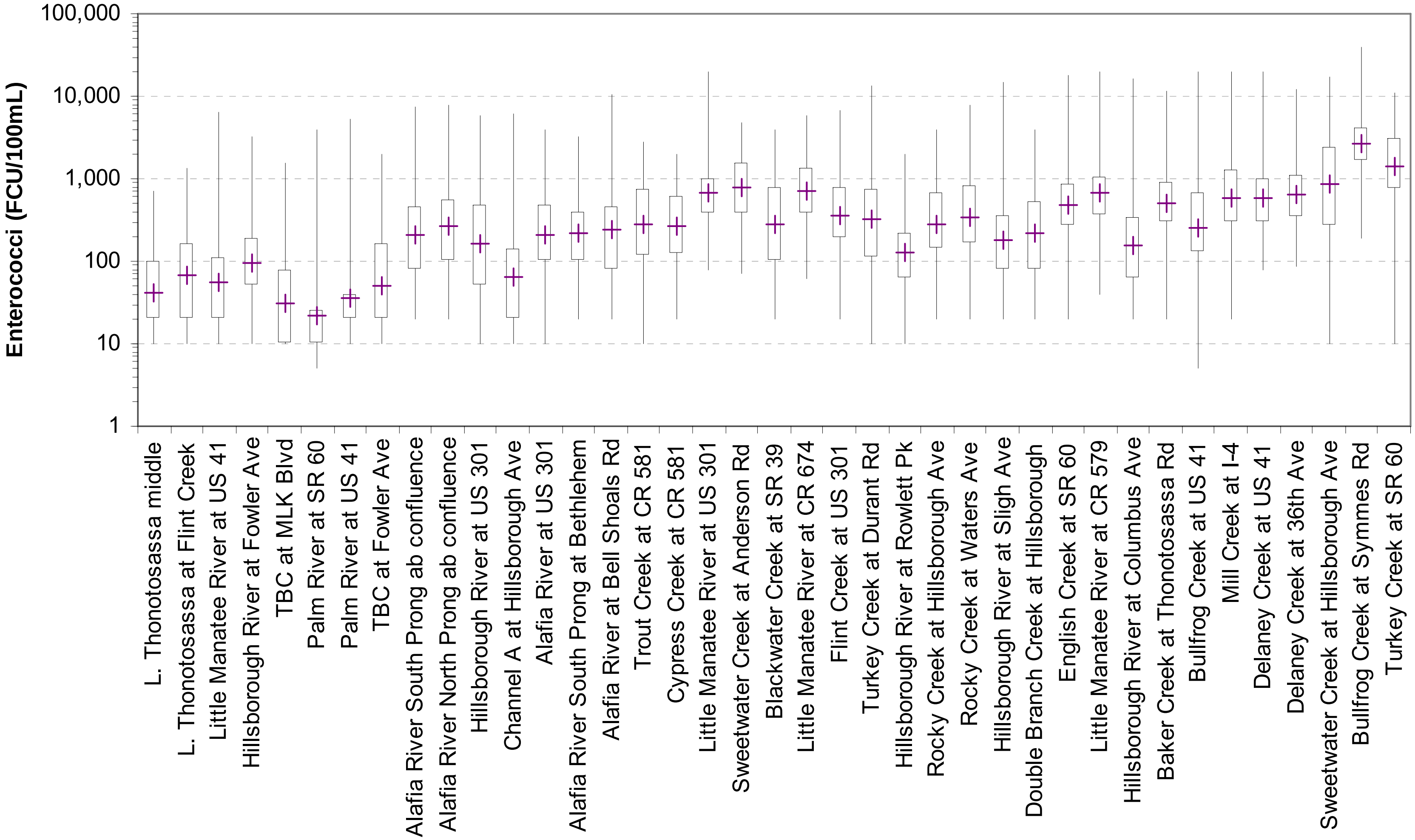

Figure D-2. Box–and-whisker plots (showing minimum, maximum, interquartile range and geometric mean) of enterococci counts (CFU/100 mL) observed at 39 EPCHC inland monitoring stations during the years 2001 – 2007. Stations are ranked based on the percentage of samples exceeding the State's 400 CFU/100 mL fecal coliform criterion. (Data source: EPCHC)