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Beating Bacteria

A new methodology for identifying and prioritizing water bodies with high concentrations of fecal coliform is helping Florida address the problem of elevated bacteria levels.

By Cheryl M. Wapnick, Thomas L. Singleton, and Valerie J. Harwood

ike other states, Florida is wrestling with how to address elevated fecal bacteria levels in many of its water bodies. Although the presence of a threshold level of fecal bacteria is a common reason for designating a waterway as impaired, the sources of the "indicator bacteria" often are not well known. This uncertainty can hamper efforts to develop total maximum daily loads (TMDLs) and implement management actions aimed at addressing the impairments. (A TMDL represents the maximum amount of a given pollutant that a water body can assimilate and still meet the water body's designated uses. A water body that does not meet its designated uses, such as swimming, is defined as impaired.) However, an emerging methodology for identifying sources of fecal indicator bacteria in a given area and evaluating the relative health risks offers a unique and comprehensive approach to targeting efforts to achieve tangible, cost-effective improvements in water quality.

Known as the Decision-Support Tool, this methodology for assessing and prioritizing impaired waters is at the cutting edge of efforts to detect and address sources of fecal bacteria. Employing a weight-of-evidence approach that relies heavily on good, oldfashioned detective work with support from the latest science, the process encompasses three steps that together provide a framework for identifying, interpreting, and responding to elevated levels of fecal coliforms and other indicators observed in ambient monitoring efforts. All three steps of the methodology are combined into a Decision-Support Tool to prioritize impaired waters both at the watershed and sampling-station scales. Prioritization of the many impaired waters in a given locale helps focus source identification investigations and target appropriate restoration efforts. The Lower Hillsborough River winds through the downtown urban center of Tampa, FL. Use of the Decision-Support Tool indicated that human-related contamination is the leading cause of fecal pollution identified within this watershed.

By reducing the number of sites and narrowing the number of probable sources for investigations of the root of bacte-



Figure 1. Map of water bodies impaired for fecal coliform in Florida

rial pollution, the methodology can focus participants' efforts and reduce the costs involved. For example, instead of having to scour an entire hydrological basin for contamination sources, resource managers can target significantly smaller areas, sometimes on the order of just a few city blocks, and develop a clear picture of the source(s) of contamination and their potential risk to human health. In this way, the methodology can help limit the need for additional sampling and corrective actions, resulting in significant savings of time and money.

Florida's Bacteria Problem

In accordance with the Clean Water Act, the Florida Department of Environmental Protection (FDEP) has adopted waterquality criteria for fecal coliform bacteria to reduce human health risks from waterborne pathogens that could be present in certain water bodies. For freshwater, a three-pronged test is used to determine if a water body is impaired. The Decision-



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In recent years, FDEP has designated hundreds of water bodies as impaired because of elevated fecal coliform bacteria levels. In fact, FDEP reported in December 2008 that 345 water bodies in the state had a verified impaired status as a result of elevated levels of fecal coliform bacteria (Figure 1) and would require the development of TMDLs. Given the large number of water bodies requiring TMDLs, FDEP needed a rational method by which to prioritize basins for regulatory attention. Meanwhile, FDEP also needed a consistent vardstick to determine whether attempts by local governments to address bacterial pollution were succeeding.

To be successful, a TMDL for fecal coliform bacteria must target effective management actions. Unfortunately, the information needed for this task—that is, knowledge of the contamination sources—generally is not available due to the many nonpoint sources that may impact water quality. Moreover, measured parameters, such as indicator bacteria, do not discriminate among sources—for example, human, livestock, and wildlife and, instead, only warn that water may contain disease-causing organisms that pose a risk to public health.

Because fecal coliforms and other indicators do not always accurately predict the presence of human pathogens in surface waters, source identification is necessary to effectively determine the potential for human health risk. This is especially true in tropical and subtropical climates, such as Florida's, where fecal coliform bacteria have been found to grow naturally on vegetation (Rose et al. 2001) and persist in sediments (Anderson et al. 2005; Brownell et al. 2007). These conditions can result in false-positive findings in which the criterion is exceeded, but an elevated risk to human health is not present. On the other hand, measurement of fecal coliform levels can result in false-negative findings-for example, when pathogenic viruses and protozoa survive longer than fecal coliforms during wastewater treatment or in surface waters (Harwood et al. 2005). Because pathogens from human sources present the highest potential for human disease, identifying the type of bacterial source (human, livestock, or wildlife) also affects the evaluation of risk (Stoeckel and Harwood 2007). As a result, source identification is critical to improving water quality and protecting human health.

Given these limitations, it is evident that fecal coliform, or other indicator bacteria concentrations, should not be the sole source of information for development of a basin management action plan (BMAP) for an impaired water body. (In Florida, a BMAP constitutes a "blueprint" for restoring impaired waters by reducing pollutant loadings to meet the allowable levels established in a TMDL.) Instead, additional information regarding the source(s) of microbial contamination in surface water bodies, and the risks that those sources pose to human health, must be assessed to ensure that appropriate management actions are selected and implemented throughout the BMAP process.



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PBS&J, in collaboration with Valerie J. Harwood of the University of South Florida (USF), developed a three-pronged approach to address FDEP's needs and limitations regarding prioritization of basins for management action, indicator bacteria source identification, and tracking the effectiveness of management actions in addressing sources. The three components, when used in combination, comprise the Decision-Support Tool. The tool can be used by FDEP at the beginning of the TMDL process to evaluate all basins using a consistent set of criteria, and priorities can be identified at the level of watersheds (large scale) or individual sampling stations (small scale). This approach effectively targets likely sources of fecal contamination, helping to dramatically narrow the focus of efforts to address contamination sources.

PBS&J created the Decision-Support Tool under contract with FDEP and in collaboration with the department, USF, and local stakeholders. This effort was funded by FDEP through the TMDL program.

Building on Existing Approaches

The Decision-Support Tool borrows its logic and flow from two similar, preexisting approaches to water quality assessment: the Annapolis protocol recommended by the World Health Organization (WHO) (2003) and the phased-monitoring approach advocated by the National Research Council (NRC) (2004).

Both methods acknowledge the limitations that affect the use of existing indicators, such as fecal coliforms, *Escherichia coli*, and enterococci, and use a weight-ofevidence approach to help compensate for those limitations. Moreover, both approaches 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 help prioritize and guide management actions intended to address bacteriological water-quality impairments.

In particular, the Annapolis protocol (which is so named because it was developed at an international conference held in Annapolis, MD, in the late 1990s) utilizes the information provided by sanitary inspections and water-quality monitoring data. The two data sources are combined to provide a graded, risk-based assessment of a given water body. More recently,



the NRC conducted a review of microbial water-quality-monitoring methods in 2004 and recommended the use of a phased-monitoring approach to help reduce regulatory and resource-management uncertainties resulting from shortcomings of existing monitoring tools (NRC 2004). This approach comprises three monitoring levels: routine sampling, expanded sampling, and microbial assessment. When routine monitoring indicates a potential water quality problem, sampling is expanded to assess the risk to human health. Such efforts may be augmented by field surveys equivalent to the sanitary inspections described by the WHO (2003). Lastly, a microbial assessment is conducted to determine the sources of microbial pollution, so that health risks can be abated through management actions (NRC 2004).

The Decision-Support Tool was designed for use by the FDEP and its local partners to guide BMAP-related management actions addressing fecal coliform impairments throughout the state, and was verified and implemented in the Hillsborough River watershed. The three steps comprising the tool are described below.

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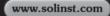
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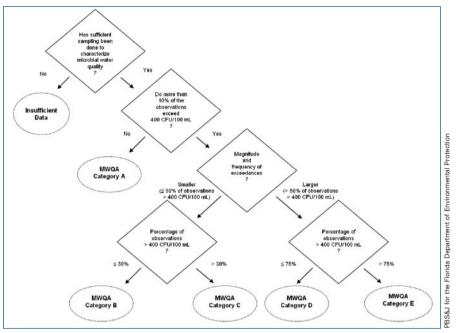


Figure 2. Decision tree for assigning monitoring locations to MWQA categories based on observed fecal coliform concentrations

Step 1: Microbial Water Quality Assessment

The first step of the Decision-Support Tool is to categorize microbial water-quality conditions within each pertinent water body based on fecal coliform concentrations observed in available monitoring data. A minimum of 30 samples per station, collected at a regular frequency over a five-year period, is recommended for this purpose.

A decision tree (Figure 2) then is used to assign a microbial water-quality assessment (MWQA) category to monitoring stations within a water body. The MWQA categories are based on the frequency with which a given site exceeds Florida's fecal coliform criterion of 400 colony-forming units (CFU)/100 mL (calculated using the binomial approach implemented by the FDEP to identify impaired waters). The categories are symbolized as letter grades (A through E). An A grade represents sites at which fewer than 10% of samples exceed the criterion, while categories B through E represent sites that exceed the criterion with increasing frequency and magnitude.

Step 2: Contaminant Source Survey

The primary purpose of the MWQA categories is to provide a tool for prioritizing monitoring locations for follow-up investigation. After step 1 of the Decision-Support Tool has been used to prioritize impaired water bodies for remediation, resource managers shift to a weight-of-evidence approach that combines background knowledge of the watershed and land-use patterns with water-quality sampling, if necessary, to document and assess the contribution of various potential sources to water bodies impaired by high levels of fecal coliform bacteria.

Pertinent information related to the watershed in question is acquired and compiled via the contaminant source survey (CSS), which is a multifaceted approach designed to identify the most probable bacterial sources. In keeping with the phased-monitoring concept recommended by the NRC (2004), the intensity of an investigation-that is, a CSS-at a given site is based on its MWOA classification. Development of a CSS includes detailed reviews of existing data, including historical sampling results and geographic information system (GIS) data. Collaboration with local stakeholders occurs in the form of intensive one-on-one interviews, as well as public workshops addressing local waterquality issues and potential sources of fecal contamination. Field reconnaissance, and, if necessary, advanced microbial source tracking (MST) (step 3), also are used. For example, sites in MWQA categories D and E are slated for more intensive investigations, including MST sampling programs, than sites exhibiting less-frequent (and lower-magnitude) exceedances of bacterial standards. The relatively large expense associated with MST analyses emphasizes

the importance of using available data as effectively as possible.

During field reconnaissance, probable sources and migration pathways are assessed, and potential sampling stations are identified. Participants also develop a sampling implementation plan. Once the study is completed and contamination sources are identified, appropriate actions for addressing the bacterial problem can be developed and initiated. Because local stakeholders participate in all aspects of the CSS, the results reflect a partnership focused on identifying the most probable sources.

Step 3: Microbial Source Tracking

As the most technically advanced component of the CSS, MST is typically conducted in those watersheds with the highest frequency and magnitude of exceedance (MWQA categories D and E). Specific MST tests for various types of human and/ or animal contributors to contamination are chosen according to a decision-tree approach. To reduce costs and time, the sampling begins by using less expensive, more basic analytic methods first, including the combined use of different indicator bacteria (for example, fecal coliforms, *E. coli*, and enterococci), followed by more costly and sophisticated methods, including source-specific assays. Using multiple MST methods increases the confidence in source identification and expands the range of potential sources that can be investigated.

MST methods are used to detect a specific gene or genes found in a microorganism that is specific to a certain host or group of hosts. Polymerase chain reaction (PCR) is frequently used to detect the target gene(s). PCR is a process in which many copies of a specific DNA sequence are synthesized from a small amount of starting material, generating enough material to be visualized or manipulated for further investigation. The target genes may be present in fecal indicator bacteria, microbial pathogens, viruses, or even host cells that are shed in feces (Stoeckel and Harwood 2007).

Working with laboratories throughout Florida, particularly at USF, PBS&J employs a "toolbox" of MST methods that currently includes non-library-based PCR tests. One of the targets is a group called the Bacteroidales, which are anaerobic fecal bacteria. The DNA of some members of this group lends itself to three separate tests for detecting whether a source is human-, ruminant-, or horse-specific. Other non-library-based PCR and qualitative PCR tests use the Enterococcus faecium esp gene, a gene for virulence factor of a bacterium found in humans, and the human polyomavirus, a nonpathogenic virus shed in urine and, therefore, commonly found in sewage. As new cost-effective MST methods, including quantitative PCR, are developed, they are added to the toolbox.

Findings from the Decision-Support Tool can be used by the FDEP or local stakeholders to determine baseline conditions before implementing corrective actions to address sources of contamination. The results also help to focus an investigation, providing a framework for interpreting and responding to bacterial exceedances. Finally, the results of the Decision-Support Tool can be easily communicated by means of a

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Contaminated water from a small tributary to the Lower Hillsborough River flows downstream, eventually entering the main channel of the river.

simple "stoplight" approach that ranks sites based on the apparent risk that they pose to human health (Table 1).

Applying the Decision-Support Tool to the Lower Hillsborough River

Working with FDEP and local stakeholders in the Hillsborough River watershed, PBS&J employed the overall methodology to help guide implementation of a BMAP for several water bodies within the area that were verified as impaired as a result of elevated fecal coliform concentrations.

Although the effort involved an examination of six sub-basins, only the Lower Hillsborough River sub-basin is discussed here. Monitoring locations along the water body—a highly urbanized waterway that experiences significant recreational use—were classified using the Decision-Support Tool. The locations received MWQA classifications on the basis of long-term monitoring data collected by the Hillsborough County Environmental Protection Commission (EPCHC) as well as more recent MST data that tested for



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markers specific to humans, ruminants, and horses. Human-specific markers were found at almost every site tested. These results often coincided with exceedances of indicator organisms. Ruminant sources, by contrast, appeared to be present but at relatively insignificant levels.

The first stage of the CSS, which was conducted before the MST testing, resulted in the identification of potential sources of fecal contamination and a thorough understanding of the characteristics of the Lower Hillsborough River. For example, several minor tributaries, spring-fed drains, and significant stormwater conveyance systems that join the main channel were identified. The most significant potential source of fecal contamination was determined to be humanassociated, including impacts from the sewer system and associated large-scale and repetitive sanitary sewer overflows, septic systems, homeless populations, and live-aboard vessels docked at marinas along the river. The potential contribution of non-human-related impacts was found to be comparatively low, including storm-



Station DHR4A, which is located on the tributary, was found to be the most impaired site during the application of the Decision-Support Tool within the basin.

water, bird populations and other wildlife, dogs, and animals housed at the local zoo. Recognition of these potential sources of



impairment and their locations played a critical role in designing the MST portion of the assessment, resulting in the use of lower-cost, more basic methods first, followed by higher-cost, more sophisticated methods. In this way, the costs and time associated with the MST were kept to a minimum.

MST results showed that human-related contamination was indeed the leading cause of fecal pollution identified within the Lower Hillsborough River. More importantly, the sampling program resulted in the ability to define the most probable sources for individual locations throughout the watershed (Figure 3). This allowed participants to effectively target additional investigation efforts-for example, infrastructure evaluations-and the required management actions to address the specific type of contamination at each particular site. Furthermore, sampling of the sediments at several sites throughout the basin showed high indicator bacteria levels, signifying periodic inputs or extended persistence of indicator bacteria. Each of these locations is suspected of being subject to recent or past wastewater leaks that may have contributed to significant reservoirs of bacteria in the sediments throughout the Lower Hillsborough River. These reservoirs have the potential to re-inoculate the water column and contribute to chronic and elevated levels of surface

Table 1. Classification Matrix

		MWQA group (based on binomial assessment of frequency of 400 CFU/100 mL fecal collform exceedances)					Exceptional Circumstances (e.g., sewer line break) °
		A (≤ 10%)	B (>10% - 30%)	C (>30% - 50%)	D (>50% - 75%)	E (>75%)	1
Contaminant source survey (CSS) assessment category (likelihood of fecal contamination posing human health risks)	1. Very Low	A1	B1	C1 ⁸	D1 ^a	E1 ^a	Immediate Action
	2. Low	A2 ^b	B2	C2	D2 ^s	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 sanitary sewer overflows that contaminate surface waters, which require immediate remedial action.

water contamination.

The frequency and severity of indicator bacteria exceedances identified during the sampling aspect of the methodology prompted additional investigations by lo-

cal stakeholders. For example, the city of Tampa, the Hillsborough County Health Department, and the EPCHC all made multiple visits to station DHR4A, which is located on a small tributary to the Lower

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Hillsborough River where human-specific markers and extremely elevated indicator bacteria counts were routinely detected. The collaborative efforts have determined that there are likely two separate sources that combine just upstream of station DHR4A. Local stakeholder efforts are currently underway to identify the exact sources of the contamination in this area.

Certain corrective actions were recommended as a result of the findings from the methodology. For example, predictive and preventative maintenance programs for wastewater infrastructure were suggested. Such programs are intended to identify problematic areas and determine the "root causes" of failures involving sewers and pump stations. Regular inspection and cleaning practices were also advised, along with efforts to address problems relating to fats, oils, and grease. To ensure greater accountability relating to private infrastructure, a comprehensive GIS database of such systems is to be developed.

In response to the apparent issues related to septic systems, it was suggested that a public education effort be launched to inform property owners about properly maintaining and repairing systems in a timely manner or connect to the sanitary sewer system. Homeowners in close proximity to surface waters would be targeted first. Professionals involved in installing and repairing septic systems would also be targeted for educational efforts.

Recommended corrective actions relating to stormwater involve efforts to remove contributing sources. In urban areas, best management practices are under evaluation. Efforts to detect and eliminate illicit discharges have been suggested as part of stormwater management plans.

Conclusion

The overall Decision-Support Tool methodology for identifying fecal bacteria sources in surface waters is designed to be flexible. In fact, individual components can be used independently, if necessary, according to the needs of a particular project. For example, a CSS can be performed independently of the other elements of the methodology. However, the Decision-Support Tool as a whole will need to be conducted later so as to provide a baseline for determining the effectiveness of subsequent corrective actions. Meanwhile, additional cost-effective technology is continually evaluated for

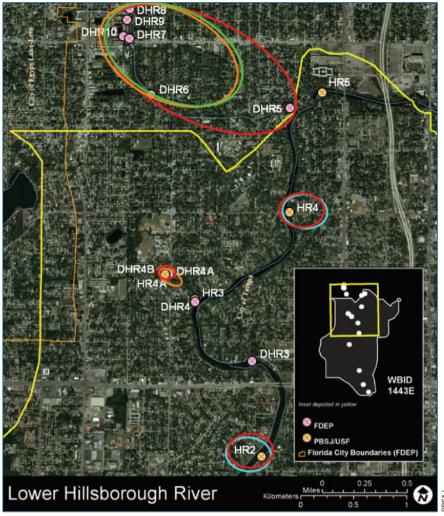


Figure 3. Map of the Lower Hillsborough River after the contaminant source survey, including identified sampling locations. The most likely sources are denoted by circles: red = sanitary sewer overflows; orange = septic systems; green = illicit discharges; and blue = stormwater (PBS&J 2008).



inclusion in the methodology.

Although the methodology was developed and tested in Florida, it can be used anywhere in the country. Using the method, water-quality managers can target efforts to identify and address bacterial sources, resulting in significant savings of time and money. By encouraging active stakeholder involvement in the process of determining sources, the approach helps to achieve consensus regarding the most likely sources of contamination, reducing the possibility of third-party challenges to implementation plans (for example, BMAPs). Stakeholder participation also facilitates decision-making, resulting in the development of restoration projects that can be expected to address the impairment as effectively as possible.

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