March 26, 2008

To: Stacie Greco and Robin Hallbourg, Alachua County Environmental Protection Department

From: Valerie J. Harwood, Ph.D. Valerie J Harwood

Re: Recommendations for monitoring and investigation of fecal bacterial pollution in Alachua County surface waters

Recently I reviewed the Alachua County Environmental Protection Department (ACEPD) report of a study conducted to determine the sources of fecal bacterial contamination to impaired (303d-listed) surface waters in Alachua County. The goal of ACEPD's bacterial monitoring program is to (1) ensure compliance with State guidelines for total maximum daily load (TMDL) assessment for impaired surface waters; (2) gather data to support effective TMDL implementation; and (3) protect public health by preventing contamination of surface waters with potentially dangerous bacterial levels. Below is a discussion of and recommendations for future monitoring and investigative strategies by ACEPD. These recommendations concern three fundamental questions:

- What type(s) of fecal indicator bacteria should be monitored, and what should be the frequency of monitoring?
- How many sites should be monitored, and how should they be chosen?
- Can microbial source tracking (MST) contribute to understanding the sources of fecal indicator bacteria in Gainesville-area waters, and if so, what is the most cost-effective and informative use of these tools by ACEPD?

1. The Florida Department of Environmental Protection (FDEP) stipulates that surface waters are "impaired" (fail standards) for fecal indicator bacteria (fecal coliforms) if greater than 10% of sample exceed 400 CFU/100 ml, if the one-time maximum exceeds 800 CFU/100 ml, or if the geomean exceeds 200 CFU/100 ml. Because the sampling requirements are quite stringent for the geomean calculation (at least five samples in one month), the status of most sites is determined by the 10% exceedance of 400 CFU/100 ml.

The ACEPD plan to continue to monitor sites that sporadically exceed the fecal coliform standard is appropriate; however, the best use of resources would be to reduce the number of sites sampled and to focus on strategically located sites with a known history of exceedances. These sites should be sampled at minimum every two months, or monthly if funds are available. The sampling strategy should be designed to capture conditions in rainy and dry months. Flexibility in the sampling program has proven to be valuable in our studies in Florida. Targeted sampling during extreme weather events may be useful to answer specific questions, i.e. if a wastewater treatment plant is known to deliver overflow to surface waters during high rainfall periods. If funds are available,

provisions should be made for sampling optional sites that may help track down a bacterial source, e.g. if one is sampling at the confluence of two stream branches where bacterial levels become elevated, valuable information could be provided by reconnaissance of the two creek branches and sampling at possible problem areas such as lift stations or sites with old septic systems.

The U.S. Environmental Protection Agency (EPA) recommends the use of *Escherichia coli* as a fecal indicator in freshwater, and the use of enterococci in both fresh and salt water, although the State of Florida does not require monitoring of these indicators for ambient water quality programs. The EPA's rationale for the *E. coli*/enterococci standard is that these indicators have been shown to correlate with human health risk in epidemiology studies, while fecal coliforms were not correlated. The use of a monitoring tool that is also EPA-approved may be beneficial in the future, when and if Florida adopts EPA standards for ambient water quality. There is great uncertainty as to whether this will occur, and which alternative indicator (if any) may be adopted. I would therefore recommend that a limited number of sites (those with the highest bacterial levels) be designated "intensive sites" where fecal coliform, *E. coli* and enterococci levels are monitored. At least one site with historically lower bacterial levels (a control site) should also be designated an intensive site for comparison with the highly impaired sites. In this way, ACEPD can determine the correlation of the various indicator bacteria in these sites, and will have some data in case of a decision by FDEP to accept EPA recommendations for the type of indicator bacteria used. If ACEPD follows this recommendation, it is further recommended that they use one laboratory for all tests, and that membrane filtration methods are used for all tests for consistency and ease of comparison.

Sampling of sediments can give valuable information about reservoirs of fecal indicator bacteria. Sediments shield bacteria from ultraviolet light and provide higher nutrient levels than the water column, allowing for prolonged survival and possibly some growth. When storm events or other disturbances resuspend the sediments, bacteria can be reintroduced into the water column, causing elevated levels in water samples even though recent contamination has not occurred. Although it may not be economically feasible to sample sediments at every site, sediment sampling should occur at least at the designated intensive sites.

2. The ACEPD should take steps toward development and inclusion of a decision matrix/decision tree tool to guide their investigative strategy. Such a tool would take into account the frequency of exceedance of bacterial standards, the magnitude of exceedance, the results of contaminant source surveys, and the results of advanced microbiological testing such as microbial source tracking (MST) and pathogen testing. The use of such a tool(s) would ensure application of effort and expense that is consistent and commensurate with the estimated human health risk. Such a tool is under development by FDEP and could be deployed soon in field studies. Inquire with Cheryl Wapnick or Tom Singleton of PBS&J for more information on availability of this tool.

3. The use of MST methods at sites with high levels of bacterial exceedances can be useful to rule in (or out) certain sources, such as human. Because most MST tests have

some low rate of false-positive error, it is highly recommended to use multiple tests for sources such as human, where more than one marker for the source is available. The human *Bacteroides* marker is among the most sensitive of the human source markers (detects lowest levels of contamination), but also has known cross-reactivity in dog feces. In contrast, the human polyomavirus marker and the *Methanobrevibacter smithii* marker have thus far been shown to be highly human-specific, but they are less sensitive than some other markers so more water must be sampled to gain comparable sensitivity. The *esp* human-source marker also has some cross-reactivity with dog feces, and is much less likely to be present in septic systems than it is in municipal sewage. Because library-independent MST methods are generally based on polymerase chain reaction (PCR), which is extremely sensitive, laboratory contamination must be scrupulously tested for and guarded against. The interpretation of MST testing can be therefore be challenging and should be done in close collaboration with scientific experts.

New markers for dog and bird fecal sources are available, but must be validated before use by ACEPD by testing against target (should be positive) and nontarget (should be negative) fecal material from the Gainesville area. Validation should be reviewed by ACEPD personnel who have received guidance on how to interpret such data. In general, laboratories that bid for ACEPD projects should be required to show validation of MST methods.

In the future, MST testing should be shifted away from conventional PCR methods to quantitative PCR (QPCR) methods as they become validated for field use. QPCR methods can yield information on relative loading from various sources of fecal contamination that is not possible with conventional PCR methods. Tests for fluorescent whitening agents (FWAs) using existing methodology have limited utility in Florida surface waters due to interference from naturally occurring compounds, and should be suspended until the methodology improves.