

Research Work Group Meeting Notes January 12, 2012 10:00 am to noon H-GAC Conference Room B, Second Floor

## Attendees

Linda Broach (TCEQ), Michael Bloom (Geosyntech), Ralph Calvino (AECOM), Richard Chapin (City of Houston, on phone), Robert Snoza (HCFCD), Steven Johnston (GBEP/TCEQ), Rachel Powers (H-GAC)

## Discussion

## Overview

The Implementation Plan was still undergoing internal review at TCEQ. TCEQ had not formally requested any changes to the document. Informally, TCEQ requested modification to the inside cover pages, which were made without changes to content.

The annual report will contain information about progress on activities identified in the Implementation Plan. The workgroup will be an important means for collecting information about implementation.

## **Review Current Research**

Rachel provided a handout listing several articles relevant to BIG research priorities. (Many thanks to Bill Hoffman for putting the list together.)

Research Priority 10.1: Evaluate the effectiveness of storm water implementation activities

- The National Stormwater BMB Database: In December 2010, the database published the "Pollutant Category Summary: Fecal Indicator Bacteria," which examined and summarized findings included within the database. The document is available on-line: <a href="http://www.bmpdatabase.org/Docs/BMP%20Database%20Bacteria%20Paper%20Dec%2">http://www.bmpdatabase.org/Docs/BMP%20Database%20Bacteria%20Paper%20Dec%2</a>
  <u>02010.pdf</u>. [In general, conclusions are that more data and analysis is needed, retention (wet) ponds and various media are probably the category of BMPs most likely to be effective. Source controls and volume reduction may be effective at reducing bacteria loads.]
- Harris County and Harris County Flood Control District are undertaking an analysis of Clean Rivers Program water quality data to identify possible correlations between bacteria levels and other water quality parameters such as total suspended solids or nutrients. A final report is not available.



- Harris County, Harris County Flood Control District, and H-GAC are conducting sampling to better describe diurnal patterns in bacteria levels.
- University of Houston Clear Lake has recently installed a wetland on campus designed to treat stormwater from the 19-acre campus. They are sampling bacteria levels of the water going into the wetland and coming out to see if the wetland effectively reduces bacteria levels. During dry weather, to maintain the wetland, they are pumping water from Horsepen Bayou. This may provide an opportunity to determine whether 'offline' treatment might be able to reduce in-stream bacteria levels.
- University of Houston Clear Lake is working on a study of nutrients. (???need more detail??)
- The City of League City received a 319 grant to develop green infrastructure within a park.
- The City of Houston is implementing an erosion control project in Memorial Park, and is hopeful that erosion control might reduce bacteria loading. The City is sampling both water and sediment to see if there are changes in bacteria levels that correlate to the project.
- Geosyntech has received a grant from the Water Environment Research Foundation to examine 'advanced' green infrastructure that responds to real-time data. For example, they are installing equipment on rainwater harvesting facilities that can query local rainfall predictions to determine release rates for the facilities, and thus maximize the effectiveness of the harvesting.

Research Priority 10.2: Further evaluate bacteria persistence and regrowth

- The handout includes a paper by Konstantinidis et all in 2011 that describes research into distinguishing gene sequences of commensal versus environmentally adapted straing of e. coli. H-GAC is developing a grant proposal to work with him to analyze samples from local waterways.
- Rachel will check with Robin Brinkmeyer to see if any of her current or recent research is related to this topic.

Research Priority 10.3: Determine appropriate indicators

- H-GAC's Clean Rivers Program will be collecting entero samples to supplement *E. coli* samples in freshwater.
- EPA has recently published draft information pertaining to recreational water quality standards

(http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/recre ation\_document\_draft.pdf) for the purpose of soliciting scientific views. Highlights include:

• A qPCR method as a rapid analytical technique for the detection of enterococci in recreational water



- EPA is introducing a new term, Statistical Threshold Value (STV), as a clarification and replacement for the term single sample maximum (SSM). In addition there are no longer recommendations for different use intensities.
- EPA is providing information on tools for assessing and managing recreational waters, such as predictive modeling and sanitary surveys.
- EPA is providing information on tools for developing alternative RWQC on a sitespecific basis, including epidemiological studies and the development of quantitative microbial risk assessment (QMRA)
- The group discussed whether it might wish to submit comments ("scientific reviews") on the EPA report. The work group felt that it would be appropriate to review the bibliography to make sure that appropriate studies have been included. ("Please include these studies in analysis, and if not, why not?") The group encourages individual stakeholders to submit comments and to let other people know that they are submitting comments. Harris County and Harris County Flood Control District are preparing comments.
- Michael Bloom, Catherine Elliott, and Linda Pechacek attended the EPA listening session last year in New Orleans pertaining to the recreational criteria process. In general, the meeting was disappointing in that EPA was proposing few changes and few specifics.

## **Identify Priorities**

The group did not recommend any priorities and felt that the current approach, which combines research by BIG stakeholders with a review of research by others, seemed appropriate.

## Potential additions to the annual report and modifications to the I-Plan

The workgroup did not wish to recommend changes to the research section.

The workgroup expressed an interest in making sure that the annual report, and possibly the implementation activity database, included a bibliography and/or information about current research efforts.

The research section of the annual report is intended to serve two functions. First, for the purpose of sharing information, it will contain a bibliography of recent research pertaining to the BIG's research priorities. Second, it will describe activities being undertaken by BIG stakeholders for the purpose of documenting efforts being undertaken.

## Wrap-up

Rachel will provide notes for the meeting.

BIG Annual Meeting: May 22, 2012.

# Adjourn



Konstantinidis et al. 2011. "Genome sequencing of environmental *Escherichia coli* expands understanding of the ecology and sequencing of the model bacteria species." Proceedings of the National Academy of Sciences.

#### www.pnas.org/cig/doi/10.1073/pnas.1015622108

*E. coli* has been considered a reliable indicator of fecal pollution because it was believed to live primarily with the gastrointestinal tract of warmblooded organisms (commensal), and could not survive for an extended period outside this environment. Evidence that some strains have adapted to other environments has accumulated in recent years. A team of researchers affiliated with several institutions has sequenced the genomes of nine strains of *E. coli* that have adapted to the environment, and that cannot be distinguished from commensal *E. coli* by standard culturebased methods such as Colilert. Knowledge of the genomes of these environmental strains will allow development of molecular assays to quantify commensal and environmental strains, and to more accurately assess the extent of fecal pollution in aquatic systems.

http://www.sciencedaily.com/releases/2011/04/110411152527.htm

Zhang et al. 2012. Development of predictive models for determining enterococci levels at Gulf Coast beaches. Water Research 46 (2012): 465-474

Zhang and colleagues developed an artificial neural network (ANN) model predicting ("nowcasting") enterococci densities at Holly Beach in Cameron County, Louisiana, and compared it to existing models developed by the USEPA ("Virtual Beach", VB). The ANN model was superior to the two VB models evaluated, in part because the VB models use 5 or 6 predictors (salinity, wind speed, wind type, tide level, rainfall, etc), while the ANN model can utilize 15 explanatory variables, allowing the model to account for more variation than the VB models. ANN is an iterative, "trainable" method completely unlike the VB models that rely on multiple regressions of linearly-transformed variables. ANN methods are used primarily in data-mining, consumer response modeling, and the like, and are not a standard piece of the environmental modeling toolbox, but can be fruitfully applied to environmental modeling as well.

Morris, et al. 2012. Enterococcus faecium of the *vanA* Genotype in Rural Drinking Water, Effluent, and the Aqueous Environment. Applied and Environmental Microbiology 72(2):596-598

Vanomycin-resistant enterococci have been reported with increasing frequency worldwide. A team of Irish researchers have demonstrated resistant strains are spreading into a variety of aqueous environments. Vanomycin-resistant enterococci were detected in hospital effluents (up to 27 percent of total enterococci), treated wastewater (up to two percent), rural water supplies (up to 6 percent) and rivers (up to 1.6 percent) of samples from fifty locations in Ireland. The authors consider this an anthropogenic disturbance of natural microbial diversity, as well as a potential human health concern.

Maraccini et al. 2012. Diurnal Variation in *Enterococcus* Species Composition in Polluted Ocean Water and a Potential Role for the Enterococcal Carotenoid in Protection against Photoinactivation. Applied and Environmental Microbiology 78(2): 305-310

Several species of enterococci are used in bacterial source tracking. The authors show that environmental and non-human species include pigmented strains that may be less sensitive to photoinactivation, resulting in diurnal variation in species composition. Because enterococci of human origin may be inactivated more rapidly, samples taken during the day may not accurately indicate the contributions of different sources to the total enterococci observed in a sample.

Rogers, et al. 2011. Decay of Bacterial Pathogens, Fecal Indicators, and Real-Time Quantitative PCR Genetic Markers in Manure-Amended Soils. Applied and Environmental Microbiology 77(17):4839-4848

Run-off from pastures, feedlots, and cropland to which manure is applied is a significant source of fecal bacteria in watersheds dominated by agriculture. Quantitative polymerase chain reaction (qPCR) techniques can be used to identify sources of fecal pollution, allowing calibration and validation of water quality models applied to watershed planning and protection. Bacteria of the *Bacteriodales* family are abundant in mammalian feces, and host-specific genetic markers have been identified. These markers are the basis of commercially-available source tracking services. Rogers et al. found that genetic markers for pigs and cattle decay more rapidly than the associated human host markers, fecal indicator bacteria, or associated pathogens. This suggests a limitation to the reliability of source tracking methods reliant upon *Bacteriodales*.

Taskin, et al. 2011. Selective Quantification of Viable Escherichia coli Bacteria in Biosolids by Quantitative PCR with Propidium Monoazide Modification. Applied and Environmental Microbiology 77(13):4829-4335

Application of polymerase chain reaction (PCR) methods to environmental investigations has been hampered by the ability of the method to amplify genetic material from dead as well as living organisms. Several means of inhibiting replication of DNA from dead organisms have been proposed. Taskin et al. report on the use of propidium monoazide (PMA), and evaluate its effectiveness in quantifying living organisms in biosolids. Their experiments show that PMA inhibits amplification of dead cells in this matrix with up to 99 percent efficiency.

Flood et al. 2011. Lack of correlation between enterococcal counts and the presence of human specific fecal markers in Mississippi creek and coastal waters. Water Research 45(2):872-878

#### Abstract

The objective of this study was to determine whether statistically valid correlations could be shown between enterococcal counts of samples from creek and coastal sites and the presence of two molecular, library-independent markers that specify human and/or sewage pollution. Four hundred ninety samples were collected between August 2007 and April 2009 to determine enterococcal counts and the presence of genetic markers for the sewage indicator organisms Methanobrevibacter smithii and Bacteroidales. The presence of human/sewage markers and enterococcal counts were higher in creek samples than coastal samples, but the higher creek levels did not statistically correlate with the either enterococcal count or the presence of the markers present in coastal samples. Furthermore, there was no correlation between enterococcal counts in coastal samples and either marker at any of the beach sites tested. The results of this investigation in Mississippi coastal waters

suggest that human/sewage markers are unlikely to correlate with enterococci counts in the nearshore environment and that enterococcal counts may be indicative of other animal or environmental sources. Additionally, a study comparing conventional gel electrophoresis with capillary electrophoresis did not convincingly establish that one method was better than the other in regard to the results obtained. The capillary method does allow reproducibility of results and the ability to analyze multiple samples in a short period of time; however, the operational expenditures exceed the cost of traditional gel electrophoresis.

Sauer, et al. 2011. Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. Water Research 45(2011):4081-4091

Abstract :

Human sewage contamination of surface waters is a major human health concern. We found urban stormwater systems that collect and convey runoff from impervious surfaces act as a conduit for sewage originating from breeches in sanitary sewer infrastructure. A total of 828 samples at 45 stormwater outfalls were collected over a four-year period and assessed by culture based methods, PCR, and quantitative PCR (qPCR) to test for traditional and alternative indicators of fecal pollution. All outfalls had the HF183 (human) Bacteroides genetic marker detected in at least one sample, suggesting sewage contamination is nearly ubiquitous in the urban environment. However, most outfalls were intermittently positive, ranging from detection in 11% -100% of the samples. Positive results did not correlate with seasonality, rainfall amounts, or days since previous rainfall. Approximately two-thirds of the outfalls had high (>5000 copy number, i.e. CN, per 100 ml) or moderate levels (1000-5000 CN per 100 ml) of the human Bacteroides genetic marker. Escherichia coli (E. coli) and enterococci levels did not correlate to human Bacteroides. A total of 66% of all outfall samples had standard fecal indicator levels above 10,000 CFU per 100 ml. A tiered assessment using this benchmark to identify high priority sites would have failed to flag 35% of the samples that had evidence of sewage contamination. In addition, high fecal indicators would have flagged 33% of samples as priority that had low or no evidence of sewage. Enteric virus levels in one outfall with high levels of the human Bacteroides genetic marker were similar to untreated

wastewater, which illustrates stormwater can serve as a pathway for pathogen contamination. The major source of fecal pollution at four of five river sites that receive stormwater discharge appeared to be from sewage sources rather than non-human sources based on the ratios of human Bacteroides to total Bacteroides spp. This study shows the feasibility and benefits of employing molecular methods to test for alternative indicators of fecal pollution to identify sewage sources and potential health risks and for prioritization of remediation efforts.

Noble, et al. 2010. Comparison of Rapid Quantitative PCR-Based and Conventional Culture-Based Methods for Enumeration of *Enterococcus* spp. and *Escherichia coli* in Recreational Waters. Applied and Environmental Microbiology 76(22):7437-7443

#### Abstract:

Recreational water quality is currently monitored using culture-based methods that require 18 to 96 h for results. Quantitative PCR (QPCR) methods that can be completed in less than 2 h have been developed, but they could yield different results than the conventional methods. We present two studies in which samples were processed simultaneously for Enterococcus spp. And Escherichia coli using two culture-based methods (EPA method 1600 and Enterolert/Colilert-18) and QPCR. The proprietary QPCR assays targeted the 23S rRNA (*Enterococcus* spp.) and uidA (E. coli) genes and were conducted using lyophilized beads containing all reagents. In the first study, the QPCR method developers processed 54 blind samples that were inoculated with sewage or pure cultures or were ambient beach samples. The second study involved 163 samples processed by water quality personnel. The correlation between results of QPCR and EPA 1600 during the first study ( $r^2$ ) was 0.69 for Enterococcus spp., which was less than that observed between the culture-based methods ( $t^2$ , 0.87). During the second study, the correlations were similar. No false positives occurred in either study when QPCR-based assays were used with blank samples. Levels of reproducibility measured through coefficients of variation were similar for results by Enterococcus QPCR and culture-based methods during both studies but were higher for *E. coli* QPCR results in the first study. Regarding the concentration at which beach management decisions are

issued in the State of California, the agreement between results of *Enterococcus* QPCR and EPA method 1600 was 88%, compared to 94% agreement between EPA method 1600 and Enterolert. The beach management decision agreement between *E. coli* QPCR and Colilert-18 was 94%. The samples showing disagreement suggested an underestimation bias for QPCR.