



Brazos River Authority

Bacterial Source Tracking Scope of Work

Presented by Tiffany Morgan

Principal Investigator

- Developed by Dr. George Di Giovanni
 - Specializes in Environmental Microbiology
 - El Paso Agricultural Research & Extension Center of the Texas Agricultural Experiment Station (EP AREC)
- Performed analysis for Lake Waco/Belton Lake Bacterial Source Tracking Study
- Brazos River Authority Environmental Field Staff will collect field samples

Project Description

- Identification of fecal pollution sources impacting Lake Granbury
 - Human
 - Livestock
 - Wildlife
- Library Independent Analyses
 - *Bacteroidales*
 - Optical Brighteners
- Library Dependent Analyses
 - Ribotyping
 - ERIC-PCR
- Follows recommendations of Bacterial Total Maximum Daily Load Task Force

Quality Assurance

- Quality Assurance Project Plan will be developed and approved by TCEQ
- No BST method to date can provide 100% accuracy
- Rates of Correct Classification (RCC)
 - Domestic Sewage – 83%
 - Livestock – 72%
 - Wildlife – 73%
 - Subdividing the categories further results in a reduction in the RCC values for the categories

Library Dependent Methods

- Will rely on Texas *E. coli* Source Library
- Will collect 25 – 50 library samples to contribute to the library
- Quantifiable
- DNA and RNA – molecules that enable living organisms to reproduce their complex equipment from one generation to the next
 - DNA – double-stranded molecule capable of replicating and determining the inherited structure of a cell's protein
 - RNA – single-stranded molecule involved in protein synthesis
- DNA → RNA → Proteins

ERIC - PCR

- Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR)
- Generates a DNA banding pattern or fingerprint
- Different strains of *E. coli* have different numbers and location of ERIC elements and therefore have different fingerprints

Ribotyping

- Uses ribosomal RNA (rRNA) rather than DNA
- Creates finger print similar to a barcode
- Different strains will have different fingerprints

Library Independent Methods

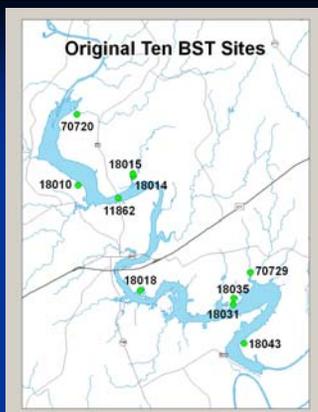
- Do not rely on DNA library
- Qualitative not quantitative
- *Bacteroidales* has host specific genetic markers (human, horse, pig, and ruminant)
- Optical brighteners – man-made chemicals found in laundry detergent

Anticipated Results

- Library Dependent Methods
 - Statistically significant identification of pollution source load into Lake Granbury as a whole
 - If possible, statistically significant identification of pollution source load per site
- Library Independent Methods
 - Corroborate library dependent methods
 - Provide confirmation of presence of domestic sewage and some animal pollution sources

Sampling

- Five sites for 6 months (selected by Authority and EP AREC)
 - *Bacteroidales*
 - ERIC-PCR
 - Ribotyping
- Known source samples – 25 to 50
- Optical Brighteners – performed twice



Site List

Site Number	Site Name/Location	Impairment
70270	Long Creek	Insufficient Data
18010	Oak Trail Shores	Borderline
18014	Sky Harbor	Borderline
18015	Sky Harbor	Borderline
11862	Brazos at FM 51	Unimpaired
18018	Waters Edge	Unimpaired
18035	Port Ridglea East	Borderline
18031	Port Ridglea East	Borderline
70729	Walnut Creek	Insufficient Data
18043	Blue Water Shores	Unimpaired

Resolution

BE IT RESOLVED by the Lake Granbury Watershed Protection Plan Stakeholders Group that the Bacterial Source Tracking Scope of Work as presented and discussed on February 13, 2007 be implemented by the Brazos River Authority upon contract execution between the Brazos River Authority and El Paso Agricultural Research & Extension Center and Texas Commission on Environmental Quality approval of the Quality Assurance Project Plan.