

**Texas AgriLife Research
Texas Water Resources Institute**

**Bacterial Source Tracking to Support the Development and Implementation of Watershed
Protection Plans for the Lampasas and Leon Rivers
FY 2010 - State General Revenue Nonpoint Source Grant Program
TSSWCB Project No. 10-51**

Quarter no. 6 From 12/1/11 Through 02/29/12.

I. Abstract

Work has continued as planned on the project, with minimal interruption. Project partners participated and attended the Bacterial Source Tracking- State of the Science Conference held February 28-29 2012, at the T-Bar M Resort & Conference Center in New Braunfels.

II. Overall Progress and Results by Task

TASK 1: Project Administration and Coordination

Subtask 1.1: *TWRI will prepare electronic quarterly progress reports (QPRs) for submission to the TSSWCB. QPRs shall document all activities performed within a quarter and shall be submitted by the 15th of March, June, September, and December. QPRs shall be posted to the project website and distributed to all project partners. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. TWRI submitted the fifth QPR for this project on December 15, 2011.

63% Complete

Subtask 1.2: *TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. Expenditures thus far have totaled \$312,503 or about 72% of total project funds have been expended.

72% Complete

Subtask 1.3: *TWRI will host coordination meetings, conference calls, or TTVN meetings with the TSSWCB, AgriLife-TP, and UTSPH-EPRC, and include as appropriate BRA, at least quarterly to discuss project activities, project schedule, communication needs, deliverables, and other requirements. TWRI will develop lists of action items needed following each project coordination meeting and distribute to project personnel. These coordination meetings may be held concurrently with TSSWCB project 06-12 or project 07-11 coordination meetings. (Start Date:*

August 2010; Completion Date: July 2012)

The following actions have been completed during this reporting period:

- A. A brief update and coordination meeting was held at the Bacterial Source Tracking- State of the Science Conference held February 28-29 1012, at the T-Bar M Resort & Conference Center in New Braunfels.

63% Complete

Subtask 1.4: *TWRI, and AgriLife-TP and UTSPH-EPRC as appropriate, will attend and participate in public meetings as appropriate in order to communicate project goals, activities, and accomplishments to affected parties. Such meetings may include, but are not limited to, Clean Rivers Program Brazos River Basin Steering Committee meetings, Clean Rivers Program Brazos River Coordinated Monitoring meetings, Lampasas River Watershed Partnership Steering Committee and Work Groups meetings, Leon River WPP Working Committee and Focus Groups meetings, and TCEQ Leon River Bacteria TMDL Advisory Group meetings. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. No activity to report this period.

63% Complete

Subtask 1.5: *TWRI, in collaboration with AgriLife-TP and UTSPH-EPRC, will develop and disseminate project informational materials, including, but not limited to, flyers, brochures, news releases, and other appropriate promotional publications. As appropriate, TWRI will include information at the project in the tx H₂O, New Waves e-letter, and AgriLife News. AgriLife-TP and BRA may solicit informational material from TWRI and UTSPH-EPRC from time to time for inclusion in Leon River and Lampasas River stakeholder newsletters and other publications, and Clean Rivers Program Basin Highlights Reports or Basin Summary Reports. All announcements, letters and publications will be provided to the TSSWCB for review and comment prior to dissemination. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. No activity to report this period.

50% Complete

Subtask 1.6: *TWRI will develop (Month 1-3), host and maintain (Months 4-24) a project website for dissemination of project materials. The project website will be linked to the project 06-12 website <http://www.brazos.org/LeonRiverWPP.asp> maintained by BRA and to the project 07-11 website <http://www.lampasasriver.org/> maintained by AgriLife-TP. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. The Leon-Lampasas BST Assessment website is currently active. It can be found at <http://leon-lampasasbst.tamu.edu/>. Since the website went online, it has been viewed by a grand total of 279 unique visitors.

- B. This quarter, the website was viewed by:
- 14 unique visitors in December 2011
 - 14 unique visitors in January 2012
 - 14 unique visitors in February 2012

80% Complete

Subtask 1.7: *TWRI will work with AgriLife-TP and UTSPH-EPRC to prepare Technical Reports on collected water quality data and BST results (one for the Leon River watershed and one for the Lampasas River watershed). A draft of these reports will be submitted to TSSWCB for review prior to finalizing the documents. These reports will be permanently housed in the TWRI online Reports Database. (Start Date: August 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. No activity to report this period.

0% Complete

TASK 2: Quality Assurance

Subtask 2.1: *TWRI, with assistance from UTSPH-EPRC and AgriLife-TP, will develop a QAPP for activities in Tasks 3 and 4 consistent with EPA Requirements for Quality Assurance Project Plans (QA/R-5) and the TSSWCB Environmental Data Quality Management Plan.*

Consistency with Title 30, Chapter 25 of the Texas Administrative Code, Environmental Testing Laboratory Accreditation and Certification, which describes Texas' approach to implementing the National Environmental Laboratory Accreditation Conference standards, shall be required.

All monitoring procedures and methods prescribed in the QAPP shall be consistent with the guidelines detailed in the TCEQ Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue (RG-415) and Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data (RG-416). (Start Date: August 2010; Completion Date: October 2010)

The following actions have been completed during this reporting period:

- A. Task Complete.

100% Complete

Subtask 2.2: *TWRI, AgriLife-TP, and UTSPH-EPRC will implement the approved QAPP. TWRI will submit revisions and necessary amendments to the QAPP as needed. (Start Date: November 2010; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. Task Complete.

100% Complete

TASK 3: Water and Fecal Sample Collection

Subtask 3.1: AgriLife-TP, with assistance from project personnel on TSSWCB projects 06-12 and 07-11 and in consultation with UTSPH-EPRC, will conduct sampling site reconnaissance at the prospective sample sites (see tables in Project Narrative for proposed sites) to determine the suitability of sample collection at these locations.

TWRI in collaboration with AgriLife-TP will submit Station Location Requests (SLOCs) to TCEQ, as needed, to obtain TCEQ station numbers for new monitoring sites. (Start Date: August 2010; Completion Date: October 2010)

The following actions have been completed during this reporting period:

- A. Task Complete.

100% Complete

Subtask 3.2: AgriLife-TP will conduct routine, ambient monitoring at 15 sites in the Leon River watershed and 15 sites in the Lampasas River watershed monthly, collecting field, flow, and bacteria parameter groups. See tables in Project Narrative for proposed sites. The QAPP, as detailed in Task 2, will precisely identify sites.

Sampling period extends over 12 months. Total number of sample events scheduled for collection through this subtask is 360.

Field parameters are pH, temperature, conductivity, and dissolved oxygen. Flow parameters are flow collected by gage, electric, mechanical or Doppler, including severity. Bacteria parameters are E. coli. (Start Date: February 2011; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. AgriLife-TP conducted 60 routine, ambient monitoring events at 15 sites in the Leon River watershed and 15 sites in the Lampasas River watershed between 1 December 2011 and 29 February, 2012. In December 2011, AgriLife-TP received and followed TCEQ Interim Guidance for Routine Surface Water Quality Monitoring During Extended Drought. Fourteen water samples were collected from Leon River Watershed sites during December 2011. Four samples were collected under no-flow conditions from standing pools. During January 2012, all Leon River Watershed sites were sampled. Five samples were collected under no-flow conditions from standing pools. Thirteen water samples were collected from Lampasas River Watershed sites during December 2011. Two sites were completely dry. During January 2012, all Lampasas River Watershed sites were sampled. Four samples were collected under no-flow conditions from standing pools. Task Complete

100% Complete

Subtask 3.3: AgriLife-TP will enumerate E. coli colonies in water samples collected through subtask 3.2 using US EPA Method 1603. Enumeration results will be recorded in hard copy and electronic format. (Start Date: February 2011; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. During this reporting period AgriLife-TP enumerated *E. coli* in 57 water samples following USEPA 1603. Results were recorded in both hard and electronic formats. Task Complete.

100% Complete

Subtask 3.4: AgriLife-TP will store Method 1603 modified mTEC plates at 4°C for shipment to UTSPH-EPRC. AgriLife-TP will coordinate the shipment of these samples with UTSPH-EPRC such that they are received in El Paso within 3 days following enumeration. (Start Date: February 2011; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. Immediately following enumeration, AgriLife-TP packed positive plates on ice and shipped to UTSPH-EPRC via overnight delivery service. Task Complete.

100% Complete

*Subtask 3.5: AgriLife-TP will collect approximately 100 known source fecal samples from the Lampasas (50) and Leon (50) Rivers watersheds. Fecal samples will be stored at 4°C and shipped to UTSPH-EPRC for *E. coli* isolation and analysis. AgriLife-TP will coordinate the shipment of these samples with UTSPH-EPRC such that they are received in El Paso within 3 days of collection. Sources of fecal samples will be selected in coordination with UTSPH-EPRC and the Leon and Lampasas Rivers watersheds coordinators. (Start Date: February 2011; Completion Date: January 2012)*

The following actions have been completed during this reporting period:

- A. *Additional known fecal samples from the Leon and Lampasas Rivers will be collected through the State Infrastructure Project, increasing the number of known source fecal samples to 100 each for the Leon and Lampasas watersheds for 200 total.*

To date 117 known-source fecal samples from the Lampasas River watershed and 87 known-source fecal samples from the Leon River watershed have been collected by AgriLife-TP and shipped to UTSPH-EPRC.

- B. During the current reporting period AgriLife-TP collected 29 known-source fecal sample within the Lampasas River watershed (1 Deer, 12 Feral Hog, 1 Raccoon, 13 Coyote, 2 Grey Fox) and 15 known-source fecal samples within the Leon River watershed (1 Opossum, 11 Feral Hog, 1 Skunk, 1 House Cat, and 1 Cottontail Rabbit). Samples were stored on ice immediately following collection and shipped to UTSPH-EPRC.
- C. The collection time for fecal samples has been extended to March 31, 2012.

90% Complete

Subtask 3.6: AgriLife-TP will collaborate with TWRI and UTSPH-EPRC to develop technical reports that present results of data collection and stream flow monitoring in each watershed.

AgriLife-TP will participate in appropriate Leon and Lampasas Rivers stakeholder meetings to present results from data collection activities. (Start Date: November 2011; Completion Date: July 2012)

The following actions have been completed during this reporting period:

- A. No activity to report this period.

0% Complete

Subtask 3.7: TWRI, with assistance from AgriLife-TP, will transfer quarterly monitoring data from activities in Task 3 to TSSWCB for inclusion in TCEQ SWQMIS. Data will be transferred in the correct format using the TCEQ file structure, along with a completed Data Summary, as described in the most recent version of TCEQ Surface Water Quality Monitoring Data Management Reference Guide. Data Correction Request Forms will be submitted to TSSWCB whenever errors are discovered in data already reported. TWRI will also provide necessary information on this monitoring regime to BRA for inclusion in the Coordinated Monitoring Schedule. (Start Date: November 2010; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. No activity to report this period.

0% Complete

TASK 4: Bacterial Source Tracking

Subtask 4.1: UTSPH-EPRC will conduct library-dependent BST on approximately 180 water samples (5 isolates per water sample) collected in the Lampasas River watershed and 180 water samples (5 isolates per water sample) from the Leon River watershed utilizing ERIC-RP (a total of approximately 1,800 E. coli isolates). Likely human and animal sources of the E. coli will be identified using the Texas E. coli BST Library. Water samples for this subtask shall be those collected by AgriLife-TP through subtask 3.2. (Start Date: November 2010; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. As of February 29, 2012, a total of 150 water samples positive for *E. coli* from the Lampasas River have been received and 1,092 isolates (up to 8 per sample) have been archived. 707 isolates (up to 5 per sample) have been prepared for ERIC and RP. 250 isolates have been fingerprinted with ERIC-PCR and 227 isolates have been RiboPrinted.
- B. As of February 29, 2012, a total of 133 water samples positive for *E. coli* from the Leon River have been received and 972 isolates (up to 8 per sample) have been archived. 631 isolates (up to 5 per sample) have been prepared for ERIC and RP. 248 isolates have been fingerprinted with ERIC-PCR and 252 isolates have been RiboPrinted.

60% Complete

Subtask 4.2: UTSPH-EPRC will isolate E. coli from 100 known source fecal samples received from AgriLife-TP (Subtask 3.5). Approximately three isolates from each fecal sample will be screened using ERIC-PCR and approximately 200 isolates will be selected for RP and inclusion in the Texas E. coli BST Library. (Start Date: November 2010; Completion Date: January 2012)

The following actions have been completed during this reporting period:

- A. As of February 29, 2012, a total of 149 of 205 known fecal samples from the Lampasas and Leon watersheds have tested positive for *E. coli* and 612 isolates (up to 5 per sample) have been archived. 386 isolates (up to 3 per sample) have been prepared for ERIC with 48 isolates fingerprinted. 30 isolates have been selected for RiboPrinting and inclusion in the library.
- B. We propose to either do 100 known source fecal samples total, with approximately 200 isolates selected as originally proposed, or to use 1 isolate each from positive source sample collected, due to time constraints. Remaining samples and isolates will be analyzed as part of the Infrastructure Project 10-50 so that data analysis for this project is not delayed.

50% Complete

Subtask 4.3: *UTSPH-EPRC will collaborate with TWRI and AgriLife-TP to develop technical reports (1 for each watershed) detailing the results of BST conducted on water samples received from both the Lampasas and Leon Rivers. UTSPH-EPRC will participate in appropriate Leon and Lampasas Rivers stakeholder meetings to present BST results. (Start Date: February 2012; Completion Date: July 2012)*

The following actions have been completed during this reporting period:

- A. No activity to report this period.

0% Complete

III. Related Issues/Current Problems and Favorable or Unusual Developments

- Water collection has been completed as of January 2012. Source sample collection has been extended through March 2012.
- An attempt to upgrade the RiboPrinter computer was not successful and only resulted in additional downtime. The system is still functional and is now running at full capacity.
- The capacity to run ERIC gels has been cut to ¼ to ½ at the new laboratory due to lack of access to a cold room. We are currently exploring all options.

IV. Projected Work for Next Quarter

- Participate in project coordination and update meetings
- Continue BST sampling and analysis