

**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. 2 From 12/1/10 Through 2/28/11.

I. Abstract

Sampling for the project began this period, with AgriLife-TP sending both enumerated *E. coli* plates as well as known-source fecal samples to AgriLife-EP for analysis. Though some initial results came back low or even negative for bacteria, involved agencies have collaborated and made clarifications on sampling and shipping methods and the issue has since been resolved, with the latest samples displaying results. The QAPP for the project was approved in December, but an error with some of the sampling site information was discovered, so the proper amendments were made and the QAPP was re-submitted and approved in January.

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 second QPR for this project on March 15, 2011.

38% 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. TWRI received the first invoices for the project totaling \$19,950.07 during the quarter. This includes invoices received but not yet documented in the last quarter on November 31, 2010.
- B. As of November 31, 2010, a total of \$19,950.07 or about 5% of total project funds have been expended.

5% Complete

Subtask 1.3: *TWRI will host coordination meetings, conference calls, or TTVN meetings with the TSSWCB, AgriLife-TP, and AgriLife-EP, 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. TWRI hosted a conference call with the Texas AgriLife Soil and Aquatic Microbiology Laboratory, AgriLife-EP, and TSSWCB on January 11, 2011 regarding current results of ongoing BST analysis in all related TSSWCB projects, including the Leon and Lampasas BST work.

38% Complete

Subtask 1.4: *TWRI, and AgriLife-TP and AgriLife-EP 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. AgriLife-TP coordinated with Lampasas River Watershed Protection project to solicit help from stakeholders with collection of known-source fecal samples. "Collection kits" containing sterile tubes, gloves, and instructions were assembled and distributed to stakeholders at the February 18 meeting in Lampasas. AgriLife-TP conducted approximately 15 phone requests from local businesses, landowners, and stakeholders to help with known-source sample collection.

38% Complete

Subtask 1.5: *TWRI, in collaboration with AgriLife-TP and AgriLife-EP, 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 AgriLife-EP 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.

38% 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 24 unique visitors.
- B. This quarter, the website was viewed by:
- 5 unique visitors in December 2010
 - 13 unique visitors in January 2011
 - 12 unique visitors in February 2011

75% Complete

Subtask 1.7: *TWRI will work with AgriLife-TP and AgriLife-EP 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 AgriLife-EP 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. TWRI submitted the QAPP to TSSWCB for approval on December 8, 2010. Approval pages with signatures from all PIs have been received and are on file.

100% Complete

Subtask 2.2: TWRI, AgriLife-TP, and AgriLife-EP 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. TWRI amended the QAPP with updates to the water quality sampling stations and submitted the edited document to TSSWCB on January 18, 2011.

25% 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 AgriLife-EP, 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. No activity to report this period.

90% 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: November 2010; Completion Date: October 2011)

The following actions have been completed during this reporting period:

- A. AgriLife-TP conducted 30 routine, ambient monitoring at 15 sites in the Leon River watershed and 15 sites in the Lampasas River watershed during February 2011.

8.3% 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: November 2010; Completion Date: October 2011)

The following actions have been completed during this reporting period:

- A. AgriLife-TP enumerated E. coli in 30 water samples following US EPA method 1603. Results were recorded in both hard and electronic formats.

8.3% Complete

Subtask 3.4: AgriLife-TP will store Method 1603 modified mTEC plates at 4°C for shipment to AgriLife-EP. AgriLife-TP will coordinate the shipment of these samples with AgriLife-EP such that they are received in El Paso within 3 days following enumeration. (Start Date: November 2010; Completion Date: October 2011)

The following actions have been completed during this reporting period:

- A. AgriLife-EP advised AgriLife-TP on appropriate shipping and handling of samples and the labs have agreed on a sampling and shipping schedule to ensure proper processing.
- B. The COC form has been revised for clarity and ease of use; count data is also being shared between labs.
- C. AgriLife-EP has received 12 plates from the first Lampasas sampling (02-11) and 14 plates from the first Leon sampling (02-11).
- D. Immediately following enumeration AgriLife-TP packed plates on ice and shipped via overnight delivery service to AgriLife-EP.

8.3% 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 AgriLife-EP for E. coli isolation and analysis. AgriLife-TP will coordinate the shipment of these samples with AgriLife-EP such that they are received in El Paso within 3 days of collection. Sources of fecal samples will be selected in coordination with AgriLife-EP and the Leon and Lampasas Rivers watersheds coordinators. (Start Date: November 2010; Completion Date: October 2011)

The following actions have been completed during this reporting period:

- A. A survey of the Lampasas Steering Committee listing and ranking potential sources of concern was conducted and the results are being used to ensure fecal samples from those sources are collected and included in the library. Similar stakeholder input for the Leon watershed has been requested.
- B. AgriLife-EP has advised AgriLife-TP on appropriate shipping and handling of known source fecal samples, and has shared protocols for collecting wastewater and septic samples.

- C. AgriLife-TP is sending copies of all field notes to AgriLife-EP and sampling sources are being coordinated.
- D. Agrilife-EP has received 14 known source fecal/ wastewater samples from the Lampasas River watershed (13 positive for E. coli) and 17 known source fecal/wastewater samples from the Leon River watershed (8 positive for E. coli).
- E. AgriLife-TP collected 9 known-source fecal samples within the Lampasas River watershed and 2 known-source fecal samples within the Leon River watershed. Samples were stored on ice immediately following collection and shipped to AgriLife-EP.
- F. AgriLife-TP collected 5 known-source wastewater sewage samples within the Lampasas River watershed and 15 known-source wastewater sewage samples within the Leon River watershed. Samples were stored on ice immediately following collection and transported to AgriLife-TP laboratory where they were plated onto modified mTec agar using method requested by AgriLife-EP. Plated samples were shipped overnight to AgriLife-EP.
- G. AgriLife –EP has advised AgriLife-TP on filter volumes under different field conditions to ensure countable plates.

21% Complete

Subtask 3.6: AgriLife-TP will collaborate with TWRI and AgriLife-EP 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: *AgriLife-EP 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. 12 water samples (positive for E. coli) have been collected from the Lampasas River and 39 isolates (up to 8 per sample) have been archived. 30 isolates (up to 5 per sample) are being prepared for ERIC and RP.
- B. 14 water samples (positive for E. coli) have been collected from the Leon River and 93 isolates (up to 8 per sample) have been archived. 63 isolates (up to 5 per sample) are being prepared for ERIC and RP.

3% Complete

Subtask 4.2: *AgriLife-EP 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. 31 known source fecal / wastewater samples have been received with 21 samples positive for E. coli. 82 isolates (up to 5 per sample) have been archived, 52 isolates (up to 3 per sample) have been screened using ERIC-PCR, and 30 isolates have been selected as library isolates.

15% Complete

Subtask 4.3: *AgriLife-EP 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. AgriLife-EP 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

The first collection of water samples for Lampasas (02-11) gave very low or even no E. coli numbers. The volume to be filtered has been standardized to 100 mL under low or normal flow

conditions.

IV. Projected Work for Next Quarter

- Submit the third quarterly progress report
- Participate in project coordination and update meetings
- Continue BST sampling