

**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. 3 From 3/1/11 Through 5/31/11.

I. Abstract

Activity for the period included continuation of the BST sampling and analysis, which includes water sampling as well as known-source fecal sampling. During the first month of sampling, getting acceptable bacteria counts plated proved difficult, but some discussion between AgriLife-TP and AgriLife-EP before the April 1st coordination meeting resolved the issue. There also remains the issue of getting the SLOC requests for the four new sites on the Lampasas River approved due to various miscommunications between agencies. No data can be submitted until approval, and much has already been collected.

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 third QPR for this project on June 15, 2011.

50% 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 invoices for the project totaling \$51,322.20 during the quarter. This includes invoices received but not yet documented in the last quarter on February 28, 2011.
- B. As of May 31, 2011, a total of \$71,272.27 or about 17% of total project funds have been expended.

17% 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 set up a project coordination meeting held in Temple on April 1. Project staff gathered to discuss current progress and issues.

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 attended the Brazos River Authority's Coordinated Monitoring meeting on April 21. Monitoring groups, parameters, and locations within the Brazos Basin were discussed. The group was made aware that AgriLife is currently monitoring *E. coli* within the Lampasas and Leon Watersheds.

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.

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 66 unique visitors.
- B. This quarter, the website was viewed by:
 - 15 unique visitors in March 2011
 - 18 unique visitors in April 2011
 - 15 unique visitors in May 2011

80% 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. No activity to report this period.

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. No activity to report this period.

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 90 routine, ambient monitoring events at 15 sites in the Leon River watershed and 15 sites in the Lampasas River watershed between 1 March and 31 May, 2011.

33% 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 90 water samples following US EPA method 1603. Results were recorded in both hard and electronic formats.

33% 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 and AgriLife-TP have developed guidelines for which plates are to be sent when multiple filter volumes of the same sample are used to get accurate plate counts. Since the least crowded plates are best for isolation, multiple plates from the same sample may be sent.
- B. Immediately following enumeration AgriLife-TP packed plates on ice and shipped via overnight delivery service to AgriLife-EP.
- C. To date, AgriLife-EP has received 57 samples from the Lampasas River and 59 samples from the Leon River.

33% 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. 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.
- B. Potential sources of concern have been identified for the Leon River watershed and additional sources of interest for the Texas *E. coli* BST library have been identified for collection targets.
- C. AgriLife-TP collected 17 known-source fecal samples within the Lampasas River watershed (4 Beef Cow, 2 Horse, 2 Deer, 5 Cliff Swallow, 2 Black Buzzard, 1 Fox, and 1 Squirrel) and 18 known-source fecal samples within the Leon River watershed (11 Beef Cow, 9 Feral Hog). Samples were stored on ice immediately following collection and shipped to AgriLife-EP.
- D. AgriLife-TP collected 8 known-source sewage samples (6 municipal wastewater, 2 septic tank) within the Lampasas River watershed and 6 known-source sewage (municipal

wastewater) 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.

- E. 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*).
- F. As of May 31, 2011, a total of 52 known source fecal samples have been collected from the Lampasas watershed of which 43 were positive for *E. coli*. To date, 116 isolates have been archived from these samples. A total of 43 known source fecal samples have been collected from the Leon watershed, of which 33 were positive for *E. coli*. To date, 95 isolates have been archived.

35% 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. As of May 31, 2011, a total of 56 water samples positive for *E. coli* from the Lampasas River have been received and 372 isolates (up to 8 per sample) have been archived. 246 isolates (up to 5 per sample) have been prepared for ERIC and RP. 133 isolates have been fingerprinted with ERIC-PCR and 6 isolates have been RiboPrinted.
- B. As of May 31, 2011, a total of 59 water samples positive for *E. coli* from the Leon River have been received and 294 isolates (up to 8 per sample) have been archived (05-11 batch is being processed). 194 isolates (up to 5 per sample) have been prepared for ERIC and RP. 36 isolates have been fingerprinted with ERIC-PCR and 115 isolates have been RiboPrinted.

15% 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. 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.
- B. As of May 31, 2011, a total of 76 known fecal samples from the Lampasas and Leon watersheds have tested positive for *E. coli* and 211 isolates (up to 5 per sample) have been archived. 137 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.

20% 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

- All cliff swallow samples collected were negative for *E. coli*.
- Quality control issues have arisen with the RiboPrinter and DuPont Qualicon consumables—a repair and maintenance visit is to be scheduled.
- AgriLife-EP has hired a part-time summer assistant to support the project.
- Strategies are being discussed to ensure sample shipments survive the summer heat.
- SLOC requests have yet to be approved, and we cannot submit any data to TCEQ until this task is completed.

IV. Projected Work for Next Quarter

- Submit the fourth quarterly progress report
- Participate in project coordination and update meetings
- Continue BST sampling and analysis
- Ensure that SLOC request approval process progresses quickly