I. Abstract
Work this quarter focused on continued data collection and analysis. A third round of soil sampling was conducted and again produced very few usable E. coli isolates. Small mammal trapping was conducted again and produced another 13 samples bringing our current total to 25, or 50% of the project goal. Runoff sampling did not occur this quarter as no runoff producing rain events occurred. Camera trapping continued to document the species present at the watersheds. Flow chamber experiments were undertaken with the second trial being completed this quarter. Sample processing for later BST analysis continued as samples were received. Lastly, preliminary results of soil BST work were presented at the 2014 American Water Resources Association annual conference in Tysons Corner, VA this coming November. Approximately 20 people attended the presentation.

II. Overall Progress and Results by Task

**TASK 1: Project Administration, Public Notification and Engagement**

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 will be distributed to all Project Partners.

The following actions have been completed during this reporting period:
A. Submitted Year 2, Quarter 2 report to TSSWCB on 12.11.2014.

75% Complete

Subtask 1.2: TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly.

The following actions have been completed during this reporting period:
A. As of 11/30/2014, a total of $151,390, or about 44% of project funding had been spent. An additional $91,888 is currently encumbered.

55% Complete
Subtask 1.3: TWRI will host coordination meetings or conference calls as needed with Project Partners 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.

The following actions have been completed during this reporting period:
A. TWRI PM has maintained contact with project team and is ensuring that project activities and progress remain on track.

75% Complete

Subtask 1.4: TWRI, with assistance from other project partners will develop a final project report.

The following actions have been completed during this reporting period:
A. No activity to report at this time.

0% Complete

TASK 2: Quality Assurance

Subtask 2.1: TWRI, with assistance from other project partners, will develop a QAPP for activities in Tasks 3 and 4 consistent with the most recent versions of EPA Requirements for Quality Assurance Project Plans (QA/R-5) and the TSSWCB Environmental Data Quality Management Plan. 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). [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 (NELAC) standards, shall be required where applicable.]

The following actions have been completed during this reporting period:
A. The QAPP for the project was approved on November 5, 2013 by TSSWCB.

100% Complete

Subtask 2.2: TWRI will implement the approved QAPP. TWRI will submit revisions and necessary amendments to the QAPP as needed.

The following actions have been completed during this reporting period:
A. The annual update for the QAPP was submitted to TSSWCB on 9.15.2014. Comments were received and addressed and signatures are currently being obtained. No major changes were included in the QAPP.

75% Complete
TASK 3: Simulated Instream *E. coli* Growth and Persistence Assessment

Subtask 3.1: *TWRI and BAEN* will establish laboratory-scale flow chambers to simulate stream environments in a controlled setting.

The following actions have been completed during this reporting period:
A. Task complete.

100% Complete

Subtask 3.2: *TWRI and BAEN* will populate flow chambers with known volumes of various types of water from the Carters Creek watershed.

The following actions have been completed during this reporting period:
A. Trial #2 was completed this quarter and Trials 3 & 4 are scheduled for December.

60% Complete

Subtask 3.3: *TWRI and BAEN* will conduct replicated monitoring and analysis over time to simulate instream conditions and assess temporal variations in *E. coli* growth and persistence in response to water quality changes (DO, DOC, pH, ammonium, nitrate, phosphorus, and turbidity). With the exception of DOC, these parameters will be monitored continuously using automated instrumentation. Water will be monitored by sampling the approximate midpoint of the flowing water column. Ten water samples will be collected from each flow chamber per trial at defined time intervals. Simulations in triplicate; one control and two treatments. Treatment will consist of stream water spiked with prescribed nutrient amendments to evaluate *E. coli* response.

The following actions have been completed during this reporting period:
A. Trial #2 was completed this quarter and Trials 3 & 4 are scheduled for December.

60% Complete

Subtask 3.4: *TWRI and BAEN* will evaluate bacterial population dynamics in response to varying levels of nutrients and will simultaneously monitor DO, pH, and turbidity. Using this information, relevant rates of growth and die-off will be calculated and a descriptive summary of findings and results for use in the project final report and other published materials will be produced.

The following actions have been completed during this reporting period:
A. No activity to report at this time.

0% Complete

TASK 4: *E. coli* Source Assessment of Varying LU/LC Types

Subtask 4.1: *IRNR* will establish camera trap arrays on each LU/LC type and operate them for two week intervals in summer and winter to document species presence and establish species indices.
The following actions have been completed during this reporting period:
A. Camera trapping continued this quarter and will continue through the end of the project.

75% Complete

Subtask 4.2: Using survey data collected in Subtask 4.1, TWRI/BAEN will develop estimated fecal loading rates from known source fecal samples collected from identified species (Subtask 4.3) contributing E. coli in each LU/LC type. Estimated fecal production rates for each species will be utilized in this estimation.

The following actions have been completed during this reporting period:
A. No activity to report at this time.

0% Complete

Subtask 4.3: IRNR/USDA-ARS as appropriate will collect identified sources of fecal material at each selected location and transfer samples to SAML where E. coli colonies will be isolated from these known sources. Identified E. coli will be DNA fingerprinted and included in the Texas E. coli BST Library. 50 known source samples is the target number of sources to collect; 2 E. coli isolates will be analyzed by ERIC-RP per known source sample. Additionally, an E. coli density per gram of feces will be determined for each processed sample.

The following actions have been completed during this reporting period:
A. A second round of small mammal sampling was complete this quarter with an additional 13 samples produced. This brings the total up to 25, or 50% of the project goal.
B. Plans are in place to trap mesomammals early next quarter and birds will be targeted in early 2015.

55% Complete

Subtask 4.4: USDA-ARS will collect 20 surface water runoff samples from each of 3 designated catchments (un-grazed rangeland, cropland, managed hay pasture) at the USDA-ARS Grassland Research Center in Riesel. 60 runoff samples will be remitted to SAML for E. coli BST analysis. Five E. coli isolate will be analyzed by ERIC-RP per sample.

The following actions have been completed during this reporting period:
A. No additional samples were collected this quarter as no runoff occurred.

75% Complete

Subtask 4.5: USDA-ARS will collect 25 soil samples from each of 3 designated catchments (un-grazed rangeland, cropland, managed hay pasture) at the USDA-ARS Grassland Research Center in Riesel. 75 soil samples will be remitted to SAML for E. coli enumeration and BST analysis. Four E. coli isolate will be analyzed by ERIC-RP per sample.

The following actions have been completed during this reporting period:
A. A third round of soil sampling was carried out early this quarter; however, of the 39 samples collected, only 9 produced E. coli and numbers from those samples were extremely low.
B. A decent amount of E. coli isolates were produced from these samples enabling 212 E. coli isolates to be produced thus far. This is 85% of the project goal.

**85% Complete**

Subtask 4.6: Using soil and water samples received, SAML will process samples using EPA Method 1603. E. coli colonies will be isolated from each sample processed and subjected to BST analysis.

The following actions have been completed during this reporting period:
A. Samples received to date have been processed and archived for later BST analysis.

**75% Complete**

Subtask 4.7: SAML will compare E. coli isolates extracted from soil and water samples collected at these sites to E. coli strains isolated and typed in Subtask 4.3 and other included in the Texas E. coli BST Library using the ERIC-RP methodology.

The following actions have been completed during this reporting period:
A. No new activity to report this quarter. Remaining isolates will be screened toward the end of the project.

**25% Complete**

Subtask 4.8: SAML will develop descriptive write up of BST technical approach and its results for inclusion in the project final report.

The following actions have been completed during this reporting period:
A. No activity to report at this time.

**0% Complete**

**Task 5: Education and Outreach**

Subtask 5.1: Project personnel will deliver project findings as appropriate at regional, state and national meetings to convey project findings to interested audiences and expand the utilization of project results.

The following actions have been completed during this reporting period:
A. Preliminary findings on the soil BST work were presented at the 2014 American Water Resources Association conference held in Tysons Corner, VA on November 3 – 6, 2014. Approximately 20 people attended the presentation.

**75% Complete**

Subtask 5.2: TWRI will maintain a program website to house information, findings and progress as well
as serve as an avenue to expand education and outreach to larger audiences.

The following actions have been completed during this reporting period:
A. The project website continued to be updated this quarter.

75% Complete

III. Projected Work for Next Quarter

1. Continue *E. coli* source survey work at Riesel to document potential sources of *E. coli* on research plots and collect known source fecal samples.
2. Continue flow chamber experiments to determine *E. coli* response to nutrient amendments.
3. Collect runoff samples from plots in Riesel when rainfall runoff occurs.
4. Begin to draft project findings into a draft final report.
5. Host project team meeting to discuss project progress.