I. Abstract

This quarter, the project kicked-off with a meeting of project partners on April 9. A targeted list of needed species/watersheds for fecal sample collection was developed and distributed on April 11. The QAPP was submitted to the TSSWCB on May 10. The website was updated to include information on this newly initiated project. The Southwest Stream Restoration Conference was held on May 28-30 with the Riparian Vegetation Workshop being held on the first day of the conference. TWRI and TPWD will co-chair the program planning committee for the 2014 Southwestern Stream Restoration Conference. Finally, all correspondence materials for the barriers to BMP adoption survey have been printed and signed, and are currently being compiled and prepared for mailing next quarter.

II. Overall Progress and Results by Task

Task 1  Project Administration

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 distributed to all project partners and posted on the project website.

The following actions have been completed during this reporting period:
   a. The second quarterly progress report was submitted on June 15, 2013.

   30% 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. To date, $18,525 or 4% of project funds had been expended.
   b. Progress of Allocations to each Department is as follows:
      • TWRI has expended 4% of their funds
      • IRNR has expended 0% of their funds
      • SCSC has expended 10% of their funds
      • UTSPHEP has expended 0% of their funds

   4% Complete
Subtask 1.3  TWRI will host coordination meetings or conference calls with the TSSWCB, UTSPHEP and AgriLife SCSC, 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.

The following actions have been completed during this reporting period:
   a. On April 9, 2013, a project kick-off meeting was held to discuss the QAPP, updating SOPs, list of watersheds/species for fecal sample collection, outreach activities, and other tasks and timelines.

   **30% Complete**

Subtask 1.4  TWRI will work with AgriLife SCSC and UTSPHEP to develop a Final Report that summarizes activities completed, conclusions reached during the project, and the extent to which project goals and measures of success have been achieved.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   **0% Complete**

Task 2  Quality Assurance

Subtask 2.1  TWRI will work with UTSPHEP, AgriLife SCSC and IRNR to develop a QAPP for activities in Tasks 3 and 4 EPA Requirements for Quality Assurance Project Plans (QA/R-5) (May 2006) and the TSSWCB Environmental Data Quality Management Plan (August 2007).

The following actions have been completed during this reporting period:
   a. The QAPP was submitted to the TSSWCB on May 10, 2013 for review and approval.

   **50% Complete**

Subtask 2.2  TWRI will submit revisions and necessary amendments to the QAPPs as needed.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   **0% Complete**

Subtask 2.3  AgriLife SCSC and UTSPHEP will maintain and update, at least annually, the 7 statewide BST template-SOPs for collection of fecal samples for BST, isolation of E. coli, archival of E. coli isolates, ERIC-PCR, RP, pre-processing of water samples for Bacteroidales PCR, and Bacteroidales PCR consistent with EPA Guidance for Preparing Standard Operating Procedures (SOPs) (QA/G-6) and the TSSWCB Environmental Data Quality Management Plan so that they include the most recent advances in BST science, methodologies, markers and technologies.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   **0% Complete**
**Subtask 2.4**  AgriLife SCSC and UTSPHEP will coordinate to ensure that needed personnel training is kept on par between the groups to ensure congruity statewide.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   **0% Complete**

**Subtask 2.5**  UTSPHEP and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. UTSPHEP and AgriLife SCSC will work to ensure that methodologies and QA/QC mechanisms adopted by these other laboratories are as congruent as possible with SOPs utilized by UTSPHEP and AgriLife SCSC (subtask 2.1)

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   **0% Complete**

**Task 3  Known Source Fecal Sample Collection**

**Subtask 3.1**  TWRI will work with IRNR to collect known source fecal samples.

The following actions have been completed during this reporting period:
   a. Collection of know source fecal samples will be initiated upon approval of the QAPP.

   **0% Complete**

**Subtask 3.2**  TWRI and IRNR will work with UTSPHEP and AgriLife SCSC to develop a targeted list of needed species/watersheds for fecal sample collection and plan for their collection and delivery. This list should primarily fill gaps in the Texas E. coli BST Library identified in other TSSWCB-funded BST projects. Targeted species will include small mammals such as mice, squirrels, nutria and rabbits. In addition, samples will be collected from at least one previously studied watershed (e.g., Leon River) in order to determine the temporal stability of the Texas E. coli BST Library. Approximately 50 known source fecal samples from each of 2 watersheds (Leon and San Antonio Rivers) are budgeted for collection (total of 100 samples).

The following actions have been completed during this reporting period:
   a. A targeted list of needed species/watersheds for fecal sample collection has been developed (see attachment).

   **100% Complete**

**Subtask 3.3**  IRNR will collect fecal samples in accordance with the plan developed in Subtask 3.2 and work closely with UTSPHEP and AgriLife SCSC to coordinate delivery of the samples to the appropriate lab. IRNR will communicate with a select group of organizations, agencies and businesses in each of the 2 targeted watersheds to arrange and resolve any access concerns and gather input to improve geographic targeting of sample collection. Travel plans, scheduling and routing maps will be prepared prior to deploying the field crew. IRNR will deploy the field crew to collect known source samples from each targeted watershed. IRNR will coordinate closely with UTSPHEP and AgriLife SCSC to ensure sample
delivery adheres to established QA/QC procedures. A known source sample data set will be finalized after completion of the field work and submitted to TWRI.

The following actions have been completed during this reporting period:
  a. Collection of known source fecal samples will be initiated upon approval of the QAPP.

  0% Complete

**Task 4  Analytical Laboratory Capacity, Library Expansion and Methods Development**

**Subtask 4.1** UTSPHEP and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment. This includes securing maintenance contracts, replacement parts and expendable supplies and purchase of a new computer for the UTSPHEP RiboPrinter system.

The following actions have been completed during this reporting period:
  a. UTSPHEP and AgriLife SCSC continuously maintain BST analytical equipment and general laboratory equipment.

  30% Complete

**Subtask 4.2** UTSPHEP will retain (or hire) a Graduate Student or Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) aid in the evaluation, expansion and maintenance of the Texas E. coli BST Library, 3) evaluate library-independent methods and markers, and 4) provide support of TSSWCB project 12-10 BST to Support Adaptive Management of the Arroyo Colorado WPP.

The following actions have been completed during this reporting period:
  a. UTSPHEP has retained Joy Truesdale and Elizabeth Casarez to 1) maintain laboratory operating capacities and technical expertise, 2) aid in the evaluation, expansion and maintenance of the Texas E. coli BST Library, 3) evaluate library-independent methods and markers, and 4) provide support of TSSWCB projects. One to two graduate students will be hired once the QAPP is approved and lab work can be executed.

  90% Complete

**Subtask 4.3** AgriLife SCSC will retain (or hire) Graduate Students and/or a Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) continue BST efforts in support of TSSWCB projects 09-10 Development of a WPP for Attoyac Bayou, 11-50 Assessment of Water Quality and Watershed Planning for the Leona River and 11-51 Instream Bacteria Influences from Bird and Bat Habitation of Bridges, and 3) evaluate new poultry marker(s) for library-independent BST.

The following actions have been completed during this reporting period:
  a. AgriLife SCSC retain Emily Martin to 1) maintain laboratory operating capacities and technical expertise, 2) continue BST efforts in support of TSSWCB projects, and 3) evaluate new poultry marker(s) for library-independent BST.

  100% Complete
Subtask 4.4 UTSPHEP and AgriLife SCSC will expand the statewide E. coli BST library through the analysis of ERIC-RP data provided by AgriLife SCSC for approximately 100 E. coli known source isolates obtained from the Leona River watershed (TSSWCB Project 11-50). Additionally, UTSPHEP and AgriLife SCSC will isolate E. coli from approximately 100 known source fecal samples collected through Task 3, which should primarily fill gaps in the library identified in other TSSWCB-funded BST projects. Approximately three isolates from each fecal sample (for a total of approx. 300 isolates) will be analyzed using ERIC-PCR for inclusion in the Texas E. coli BST Library; based on the ERIC-PCR fingerprint patterns, approximately half of the isolates (150) will be further analyzed using RP for inclusion in the Texas E. coli BST Library. UTSPHEP and AgriLife SCSC will equitably split workload. AgriLife SCSC will also fingerprint (ERIC-RP) and analyze 20 known-source E. coli isolates collected as part of TSSWCB Project 11-51.

The following actions have been completed during this reporting period:
   a. Analysis will be initiated upon approval of the QAPP.

   0% Complete

Subtask 4.5 UTSPHEP and AgriLife SCSC will collaborate to evaluate the geographical and temporal stability, composition, average rates of correct classification (accuracy), diversity of source specific isolates, and further development and refinement needs of the Texas E. coli BST library.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   0% Complete

Subtask 4.6 Using known source fecal material, AgriLife SCSC and UTSPHEP will utilize the best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers. Specifically, efforts will be made on markers to 1) identify poultry litter/manure pollution, 2) evaluate the use of genetic targets based on ERIC-PCR products to differentiate human and animal derived E. coli, 3) differentiate between domestic swine and feral hogs, 4) differentiate deer from other ruminants by continued analysis of existing data on deer fecal microbial communities, and 5) evaluate the occurrence of human HF183 marker cross reactivity for all 100 known source animal samples collected under Task 3.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   0% Complete

Subtask 4.7 AgriLife SCSC and UTSPHEP will cooperate with other entities nationwide to ensure that the most up-to-date and accurate BST approaches are implemented in Texas by attending and participating in BST-related meetings, seminars and workshops, as appropriate, to learn of new and improved BST methods being employed elsewhere.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

   0% Complete
Task 5  Outreach on Bacterial Source Tracking and BMPs

Subtask 5.1  TWRI will host and maintain the http://texasbst.tamu.edu website to disseminate educational materials, project updates, science updates, notify readers about educational opportunities, and other outreach efforts to advance the science and application of BST in Texas and nationally.

The following actions have been completed during this reporting period:
   a.  TWRI continues to host and maintain the Texas BST Library website. This quarter, the website was updated to include information on this newly initiated project.
   b.  Between March 1, 2013 and May 31, 2013, there were 79 visits to the website by 74 visitors.
   c.  Since project inception in October 1, 2012, there have been 379 visits to the website by 265 visitors.

30% Complete

Subtask 5.2  TWRI, UTSPHEP, and AgriLife SCSC will periodically meet with natural resource agencies, including but not limited to USEPA-R6, TCEQ, TPWD, TDA, GLO, DSHS, and selected river authorities, to advance the general knowledge and understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the use of BST in Texas.

The following actions have been completed during this reporting period:
   a.  No activity to report this quarter.

0% Complete

Subtask 5.3  TWRI, UTSPHEP, and AgriLife SCSC will distribute the educational brochures developed through TSSWCB Project 10-50 (subtask 4.2). TWRI, UTSPHEP, and AgriLife SCSC will develop additional flyers, one-pagers, tri-folds or other appropriate printed media, as needed, that can be used to 1) discuss the appropriate application of BST in identifying fecal contamination sources, and 2) promote the analytical laboratory capability of public BST labs which the State has invested. As appropriate, TWRI will include information about BST in general, and this project specifically, in the txH2O magazine, the Conservation Matters e-mail newsletter and AgriLife Today news.

The following actions have been completed during this reporting period:
   a.  No activity to report this quarter.

0% Complete

Subtask 5.4  TWRI, UTSPHEP, and AgriLife SCSC will promote the use of and provide resources on BST by participating in conferences, workshops, seminars and other appropriate venues, including but not limited to the 2013 and 2014 TCEQ Environmental Trade Fair, WEF/WEAT events in Texas, TSCRA/TFB/TWA annual conventions and ASABE events in Texas.

The following actions have been completed during this reporting period:
   a.  No activity to report this quarter.

0% Complete
**Subtask 5.5** TWRI, UTSPHEP and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (e.g., Edrington, Brinkmeyer, Alam, Ward) about the methods and approaches recommended by the Task Force and being implemented by the State.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

**0% Complete**

**Subtask 5.6** To build on the success of the 2012 BST – State of the Science Conference, TWRI, UTSPHEP, and SCSC will evaluate the need for and timing of a follow-up conference. If the need is substantiated, TWRI, UTSPHEP and SCSC will initiate planning and logistics for a follow-up conference.

The following actions have been completed during this reporting period:
   a. No activity to report this quarter.

**0% Complete**

**Subtask 5.7** With assistance from the USDA-NASS Texas Field Office, a stratified random sampling scheme will be implemented using a target population of beef cattle producers who completed 2012 Census of Agriculture forms. The sample will be stratified according to NASS district and beef cattle herd size. USDA-NASS will provide Texas A&M Department of Soil & Crop Sciences with a list of unique identifying numbers that will be placed on all survey materials so that response/non-response can be tracked. The USDA-NASS Texas Field Office will also assist with logistics related to compiling, stuffing, and mailing survey materials that will include an introductory postcard, the first survey packet with cover letter and survey instrument, a reminder postcard, and a second survey packet with cover letter and survey instrument. This information will support assessment of barriers to BMP adoption in conjunction with TSSWCB Project #12-08.

The following actions have been completed during this reporting period:
   a. The sample is now set to be pulled in late June/early July with the first mailing (of 4) set to begin sometime in mid-August (specific mailing dates should be confirmed by mid-July). All correspondence materials (postcards, cover letters, IRB info, and survey instruments) have been printed and signed, and are currently being compiled and prepared for mailing. All four mailings are on track to be completed by the fall (likely end of September or early October).

**50% Complete**

**Subtask 5.8** In order to reduce pollutant contributions to streams, including bacteria, TWRI will coordinate a Southwestern United States Stream Restoration Conference Workshop titled: Riparian Vegetation Workshop – Putting the ‘green’ into streambank stabilization in San Antonio in 2013.

The following actions have been completed during this reporting period:
   a. The Southwest Stream Restoration Conference was held on May 28-30 at the Hyatt Regency in San Antonio, Texas. The conference was attended by more than 230 participants. The Riparian Vegetation Workshop was held on the first day of the conference (May 28 from 1:30-5:00 pm). The Workshop was very well attended with more than 50 attendees participating. The conference agenda and presentations are available at [http://southweststream.org/?page_id=21](http://southweststream.org/?page_id=21).
b. TWRI and TPWD will co-chair the program planning committee for the 2014 Southwestern Stream Restoration Conference.

100% Complete

III. Related Issues/Current Problems and Favorable of Unusual Developments
• N/A

IV. Projected Work for Next Quarter
• Conduct project coordination meeting
• Obtain final approval of QAPP
• Initiate known source fecal collection and analysis
Infrastructure Project—100 known source fecal samples to be collected, 50 from San Antonio, 50 from Leon

50 samples from San Antonio—coordinate with Laughlin/Lackland AFB study; concentrate on small mammals (we already have plenty of raccoon, opossum—not priority).

Ideally, at least 5-10 samples (one sample per individual animal) per most abundant species, and preferably from different locations in the sample collection area.

- Mice (more than 1 species?)
- Nutria
- Porcupines
- Prairie dogs
- Rabbits
- Rats
- Skunks
- Squirrels
- Others not mentioned but abundant?

50 samples from Leon River—to test stability over time—coordinate with ranchers, trappers?

Ideally ~5-10 samples per source (one sample per individual animal; multiple samples from sewage and septage sources ok), different locations

- Avian wildlife
- Cattle
- Deer
- Feral hogs
- Sewage / septage