I. Abstract

Work this quarter has focused on wrapping up the majority of project tasks. All sample analysis was completed this quarter as was data processing and analysis. Work on reports continued this quarter but was not completed due to several delays in sample processing and analysis was experienced as a result of equipment breakdowns, this in turn delayed reporting as well. Information dissemination was also completed this quarter with the publication and deliver of the BST Conference Proceedings and BST informational brochures. All expenditures to the project were made and final invoicing has been completed. Just over $4,000 remained in project accounts when spending was completed.

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. TWRI submitted the 8th Quarter, Year 2 report to TSSWCB on 9/14/2012.

100% 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. Expenditures and encumbrances thus far have totaled $435,112 or roughly 99% of total project funding.
   b. All expenditures have been made.
   c. Task Complete.

100% Complete

Subtask 1.3: TWRI will host coordination meetings, conference calls, or TTVN meetings with the TSSWCB, UTSPH-EPRC, 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. TWRI has conferred with the project team repeatedly this quarter ensuring that final expenditures were made and final deliverables were achieved.
   b. Task Complete.

   100% Complete

Subtask 1.4: TWRI will develop (Months 1-3), host and maintain (Months 4-24) a website (e.g., http://bst.tamu.edu) that will be used as a means 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. This quarter, the website http://texasbst.tamu.edu/ was viewed by 136 unique visitors with 56% of them being new visitors to the site.
   b. Task Complete.

   100% Complete

Subtask 1.5: TWRI will work with AgriLife SCSC and UTSPH-EPRC to prepare Technical Reports as required by project Tasks into published technical reports. These reports will be permanently housed in the TWRI online Reports Database.

The following actions have been completed during this reporting period:
   a. TWRI is awaiting draft language on the BST final reports from AgriLife SCSC and UTSPH-EPRC.
   b. Initial report development has begun and will be added to when subsequent material is received.

   30% Complete

TASK 2: Support and maintain BST analytical infrastructure

Subtask 2.1: UTSPH-EPRC and AgriLife SCSC will ensure needed operational and maintenance support for current BST analytical equipment (i.e., RiboPrinter) and general laboratory equipment is executed. This includes securing maintenance contracts, replacement parts and expendable supplies.

The following actions have been completed during this reporting period:
   a. Machine repairs are completed.

   100% Complete

Subtask 2.2: UTSPH-EPRC will hire a Postdoctoral Research Associate/Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Leon River, Lampasas River, and Buck Creek watersheds (projects 10-51, 06-11, 06-07).

The following actions have been completed during this reporting period:
   a. Task Complete.

   100% Complete
Subtask 2.3: AgriLife SCSC will hire a Postdoctoral Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Little Brazos River tributaries, Big Cypress Creek, and Attoyac Bayou watersheds (projects 09-52, 09-55, 09-10).

The following actions have been completed during this reporting period:

a. Task Complete.

100% Complete

TASK 3: Quality Assurance

Subtask 3.1: AgriLife SCSC and UTSPH-EPRC will maintain and update (at least annually) statewide BST SOPs for ERIC-PCR, RP 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. TSSWCB review of SOPs is still pending.

b. Task Complete.

100% Complete

Subtask 3.2: AgriLife SCSC and UTSPH-EPRC 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. AgriLife SCSC and UTSPH-EPRC staff maintain regular communication to ensure consistency between entities.

b. Task Complete.

100% Complete

Subtask 3.3: AgriLife SCSC and UTSPH-EPRC will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, and others to develop strategies for 1) reconciling BST and modeling results (SELECT, SWAT, etc.) and 2) using BST in model development, calibration, and validation.

The following actions have been completed during this reporting period:

a. A meeting was held July 3rd consisting of BAEN, USDA-ARS, NRCS WRAT, SCSC, UTSPH-EPRC, TAIER, TWRI, and TSSWCB personnel to discuss a way to reconcile BST and modeling results as well as use BST data to calibrate and validate models.

b. The development of a delivery ratio was deemed most appropriate.

c. Task Complete.

100% Complete

Subtask 3.4: UTSPH-EPRC and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. AgriLife EP 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 AgriLife EP and AgriLife SCSC (subtask 3.1).

The following actions have been completed during this reporting period:
   a. Attempts to contact private labs have been largely futile. Numerous personnel changes have taken place at various labs across the country.
   b. Efforts will continue in this area as opportunities present themselves.
   c. These efforts will be detailed in the final report.
   d. Task Complete.

50% Complete

Subtask 3.5: UTSPH-EPRC and AgriLife SCSC will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, USGS, TIAER, and selected river authorities to define appropriate ambient water sampling protocols to provide desired statistical confidence with BST findings. The level of sampling adequate for statistical characterization of sources and determination of environmental conditions influencing source contributions (often wet versus dry weather conditions) will be described.

The following actions have been completed during this reporting period:
   a. The most appropriate monitoring regimes were found to be cost prohibitive, as such, case by case sampling regimes development is the best path forward.
   b. This will be described in the final report.
   c. Task Complete.

100% Complete

TASK 4: Promotion of BST

Subtask 4.1: AgriLife SCSC with assistance from TWRI will develop a publication that describes the extent of BST work conducted to date in Texas. This report should compare and contrast methodologies and results. This publication should build on the discussion in the Task Force Report.

The following actions have been completed during this reporting period:
   a. Relevant publications, presentations, and technical reports have been reviewed and a draft report has been written. The final publication will be completed early in the Fall of 2012.

80% Complete

Subtask 4.2: TWRI will develop flyers, one-pagers, tri-folds or other appropriate printed media that can be used to 1) promote the general use of BST consistent with the Task Force Report, 2) discuss the appropriate application of BST in identifying fecal contamination sources, and 3) promote the analytical lab capability of public BST labs which the state has invested. Printed media should be appropriately developed for each target audience including, but not limited to, 1) state and federal agencies with water quality responsibilities and jurisdictions (TCEQ, TPWD, DSHS, GLO, USEPA (Region 6 and GOMP), USGS, NOAA), 2) political subdivisions of state government (municipal, county), including TML and TAC, that may be involved in watershed planning processes focused on abatement of bacterial contamination, 3) livestock producer organizations such as TFB, TSCRA, ICA, TAD, TCFA, TSGRA, TPPA, TPF, TWA, 4) private and public water quality labs, and 5) the general public. As appropriate, TWRI will include information about BST in general, and this project specifically, in the tx H2O, New Waves e-letter, AgriLife News.
The following actions have been completed during this reporting period:

a. Informational materials developed were mailed out to a total of 423 recipients this quarter.
   
b. Task Complete.

100% Complete

Subtask 4.3: TWRI, UTSPH-EPRC, and AgriLife SCSC will promote BST by making presentations at conferences, workshops, seminars and other appropriate venues such as WEF/WEAT, TSCRA/TFB/TWA Annual Conventions, ASABE, TCEQ Environmental Trade Fair.

The following actions have been completed during this reporting period:

   
b. Task Complete.

100% Complete

Subtask 4.4: TWRI, UTSPH-EPRC and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (Edrington, Brinkmeyer, Alam) 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. Efforts to contact these researchers continued, but with limited success. Initial contact with Dr. Sanghoon Kang (new Aquatic Microbial Ecologist at Baylor) was made and he later participated in the BST conference call.
   
b. A BST Investigator Update conference call was held July 24, 2012 with participants from AgriLife SCSC, UTSPH-EPRC, Baylor, TWRI, USEPA Region 6 and TSSWCB personnel.
   
c. Task Complete.

100% Complete

TASK 5: Texas E. coli BST Library expansion, PCR marker development/refinement and E. coli isolate selection method evaluation

Subtask 5.1: UTSPH-EPRC and AgriLife SCSC will isolate E. coli from known source fecal samples. Known source fecal samples should primarily fill gaps identified in other TSSWCB-funded BST projects, as well as, archived known sources identified through subtask 5.5, and gaps identified through subtask 6.3. Approximately three isolates from each fecal sample 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 will be further analyzed using RP for inclusion in the Texas E. coli BST Library. UTSPH-EPRC and AgriLife SCSC will equitably split workload.

The following actions have been completed during this reporting period:

a. Sample analysis for the Lampasas & Leon River as well as Attoyac Bayou project was completed this quarter.
   
b. Final report will contain information on current library development.
Subtask 5.2: UTSPH-EPRC and AgriLife SCSC will quantify species-specific bacteria production (E. coli and Enterococcus) in feces and measure the variability of this production. While bacteria content of feces has been reported in literature for some species and has been summarized in some reports used in Texas bacteria projects, often this information has been limited to fecal coliform. Known source fecal samples from subtask 5.1 should be used.

The following actions have been completed during this reporting period:
  a. Task Complete.

100% Complete

Subtask 5.3: Utilizing known source fecal material, AgriLife SCSC and UTSPH-EPRC will utilize the best available bacterial indicators to further develop and refine species-specific bacteria markers for Bacteroidales PCR. Specifically, efforts will be made on markers to 1) differentiate between ruminants (primarily cattle and deer), 2) identify poultry, and 3) differentiate between domestic swine and feral hogs.

The following actions have been completed during this reporting period:
  a. UTSPH-EPRC has been reviewing PCR and high resolution melt analysis data from domestic swine and feral hogs, and from dairy cattle and dairy lagoon samples for further analysis. Selected ERIC-PCR amplicons were evaluated by DNA sequence analysis to evaluate the possibility of identifying host-specific markers. Additional research tasks in this area were included in the FY13-14 Infrastructure proposal.

100% Complete

Subtask 5.4: AgriLife SCSC and UTSPH-EPRC will coordinate to conduct comparison studies to evaluate differences in E. coli isolate selection using isolation methods EPA 1603, EPA 1604 and the IDEXX methods. Six water samples will be processed using the EPA 1603 and 1604 methods and the IDEXX method. Ten E. coli isolates per sample per enumeration/isolation (total of 180 isolates) will be analyzed using the ERIC-RP. The suitability of utilizing E. coli isolates processed using methods other than EPA 1603 in BST will be determined.

The following actions have been completed during this reporting period:
  a. Task Complete.

100% Complete

Subtask 5.5: UTSPH-EPRC and AgriLife SCSC will work to quantify the extent (quantity) and nature (method) of known source library samples that have been collected by out-of-state labs (Harwood @ University of South Florida, Source Molecular Corp., IEH Laboratories & Consulting Group, Ellender @ University of Southern Mississippi) and other in-state labs (Brinkmeyer @ TAMU-Galveston) for Texas BST projects. To the extent practical and appropriate, UTSPH-EPRC and AgriLife SCSC will work to incorporate known source library samples from these 3rd party labs into the Texas E. coli BST Library. UTSPH-EPRC and AgriLife SCSC and will work with AgriLife BAEN to incorporate known source fecal samples collected through TSSWCB project 07-06 into the Texas E. coli BST Library.

The following actions have been completed during this reporting period:
  a. Task Complete.
100% Complete

Subtask 5.6: TWRI, UTSPH-EPRC and AgriLife SCSC will collaborate to 1) expand and update on the list of BST-related R&D activities identified by the Task Force, and 2) prioritize the updated list of BST-related R&D activities.

The following actions have been completed during this reporting period:
  a. Priorities discussed this quarter. Will be summarized in final report.

90% Complete

TASK 6: BST workshop and state of the science conference delivery and development

Subtask 6.1: AgriLife SCSC and UTSPH-EPRC 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 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:

95% Complete

Subtask 6.2: TWRI, AgriLife SCSC and UTSPH-EPRC will cooperate to establish a Conference Planning Team that serves as the advisory committee for planning a statewide BST workshop (subtask 6.4) and agency specific meetings. Planning activities will include setting meeting agendas, providing information for the development of meeting materials and identifying invited speakers for the statewide BST workshop.

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

100% Complete

Subtask 6.3: TWRI will work to coordinate BST meetings with targeted agencies (TCEQ, TDA, TPWD, GLO, DSHS, USEPA, and selected river authorities). The intent of these meetings is further the understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the validity of BST in general, and methods and the Texas E. coli BST Library particularly.

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

90% Complete

Subtask 6.4: TWRI will coordinate the planning of a statewide BST workshop to be held in Austin in summer/winter 2011/2012. The purpose of this workshop will be to 1) highlight the extent of BST work that has been and is being conducted in the state, 2) discuss the scientific advances and improvements in the application of BST, and 3) identify research needs to further the science of BST. TWRI will handle all meeting logistics, speaker invitations, meeting materials preparation and advertising/promotion of the
meeting.

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

100% Complete

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

   Due to several equipment breakdowns, sample processing and analysis was delayed resulting in final reporting delays.

IV. Projected Work for Next Quarter
   i. Completion of report on BST work conducted in Texas to date
   ii. Completion of final report