I. Abstract

Work this quarter consisted of the creation of historic Load Duration Curves (LDCs) that were presented to stakeholders on February 4, 2010 during a public stakeholder meeting. The LDCs are intended to represent the historic bacteria loading to the waterbodies and give estimated load reductions needed based on the current water quality standards. Also, SAML received and archived 96 samples from Ana-Lab and performed *Bacteroidales* PCR for universal, human, ruminant, swine, and horse markers on 48 samples. TWRI submitted the third QPR on 3-15-10. Work next quarter will focus on the continuation of archiving the collected samples and the performance of *Bacteroidales* PCR as well as continued modeling.

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 December, March, June and September. QPRs shall be posted to the project website and provided to all project partners. *(Start Date: Month 1; Completion Date: Month 24)*

The following actions have been completed during this reporting period:

a. The Third quarterly report was submitted to TSSWCB on March 15, 2010.

25% 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: Month 1; Completion Date: Month 24)*

The following actions have been completed during this reporting period:

a. As of November 30, 2009, a total of $3,357.00 or about 2% of total project funds have been expended.

2% Complete
Subtask 1.3: TWRI will host, and BAEN and SAML will participate in, coordination meetings or conference calls with TSSWCB, and any project partners as appropriate, at least quarterly to discuss project activities, project schedule, communication needs, deliverables and other requirements. Coordination with TSSWCB project 09-54, Assessment of Contact Recreation Use Impairments and Watershed Planning for Big Cypress Creek and Tributaries (Hart and Tankersley Creek), will be especially critical to achieve project goals. (Start Date: Month 1; Completion Date: Month 24)

The following actions have been completed during this reporting period:
  a. SAML participated in a meeting with NETMWD, Ana-Lab, TWRI, and TSSWCB on February 4, 2010 to discuss project coordination.

  25% Complete

Subtask 1.4: In order to communicate project goals, activities, results and accomplishments to affected parties, TWRI, BAEN, and SAML will participate in public stakeholder meetings as needed. At a minimum, public stakeholder meetings shall consist of an organizational/kick-off meeting (month 3), a source survey design meeting (month 4), a meeting presenting results from initial LDCs and the GIS inventory (month 6), Texas Watershed Steward Program workshop (month 9), two project update meetings (months 12 and 18), a meeting presenting data analysis results (month 21), and a meeting presenting final technical reports (month 24). (Start Date: Month 1; Completion Date: Month 24)

The following actions have been completed during this reporting period:
  a. On the 4th of February, a public stakeholder meeting was held in Mount Pleasant where historic Load Duration Curves, GIS inventory and Land Use Analysis, and water quality sampling activities were presented.

  25% Complete

Subtask 1.5: TWRI will develop and disseminate educational materials to watershed stakeholders, including, but not limited to, flyers, brochures, letters, and news releases. BAEN, SAML, and NETMWD (through TSSWCB project 09-54), shall contribute content matter for educational materials as appropriate. (Start Date: Month 1; Completion Date: Month 24)

The following actions have been completed during this reporting period:
  a. Prior to the stakeholder meeting, a meeting announcement was mailed and emailed to stakeholders
  b. Presentations at the public stakeholder meeting distributed

  25% Complete

Subtask 1.6: TWRI will develop (Months 1-3), host and maintain (Months 4-24) an internet webpage for the dissemination of project information. BAEN, SAML, and NETMWD (through TSSWCB project 09-54), shall contribute content matter for the webpage as appropriate. (Start Date: Month 1; Completion Date: Month 24)
The following actions have been completed during this reporting period:
   a. The Water Quality Education Modeling Support and Bacterial Source Tracking For Big Cypress Creek Bacteria Assessment website went online during the first quarter of the project. It can be found at [http://bcc.tamu.edu/](http://bcc.tamu.edu/). Since the website went online it has been viewed by a grand total of 186 unique visitors.
   b. This quarter, the website was viewed by:
      - 34 unique visitors in December 2009
      - 47 unique visitors in January 2010
      - 48 unique visitors in February 2010

80% Complete

**TASK 2: Quality Assurance**

Subtask 2.1: TWRI will develop a QAPP for activities in Tasks 3 and 4 consistent with EPA Requirements for Quality Assurance Project Plans (QA/R-5) (May 2006) and the TSSWCB Environmental Data Quality Management Plan (August 2007). (Start Date: Month 1; Completion Date: Month 2)

The following actions have been completed during this reporting period:
   a. This task is complete.

100% Complete

Subtask 2.2: TWRI will submit revisions and necessary amendments to the QAPP as needed. (Start Date: Month 3; Completion Date: Month 24)

The following actions have been completed during this reporting period:
   a. A letter was sent to the TSSWCB stating that the QAPP will be updated during the next annual update.

15% Complete

**TASK 3: Bacterial Source Tracking**

Subtask 3.1: SAML will conduct library-independent BST on 50-100 water samples per segment utilizing the Bacteroidales PCR genetic test for human, ruminant, horse, and swine markers. The number of samples may be adjusted depending on the size of each watershed in the study area and the complexity of sources as identified in the source survey (Subtask 3.4). Budgeted number of samples is 75 from each of Hart and Tankersley Creeks and 100 from Big Cypress Creek (main stem) for a total of 250. Specific genetic markers for various animal sources are continually being developed by the scientific community and as new markers are identified, they should be included in this analysis, as the budget allows. Water samples for this subtask shall be a subset of those collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)
The following actions have been completed during this reporting period:

a. Received and archived 96 samples from Ana-Lab. Performed *Bacteroidales* PCR for universal, human, ruminant, swine, and horse markers on 48 samples.

19% Complete

Subtask 3.2: SAML will conduct limited library-dependent BST and analyze *E. coli* isolates from 50-100 water samples (1 isolate per water sample) from across the study area utilizing the ERIC-PCR and RiboPrinting combination method. Budgeted number of samples is 100. This will serve to 1) confirm that the sources of *E. coli* and *Bacteroidales* are comparable and 2) assess the spatial and temporal adequacy of the Texas Known Source Library. Water samples for this subtask shall be a subset of those collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)

The following actions have been completed during this reporting period:

a. Received plates for 16 samples from Ana-Lab, but were unable to isolate and archive *E. coli*.

15% Complete

Subtask 3.3: SAML will add up to 30 known source fecal samples (1-2 isolates per fecal sample) to the Texas Known Source Library. Fecal samples will be added to the BST library utilizing the ERIC-PCR and RiboPrinting combination method. Samples for this subtask shall be collected by NETMWD through TSSWCB project 09-54. (Start Date: Month 3; Completion Date: Month 21)

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Subtask 3.4: SAML will assist NETMWD in designing a watershed source survey (also known as a sanitary survey), to be conducted through TSSWCB project 09-54, that better characterizes possible sources of bacteria loadings in the study area. Results from the source survey will be used by SAML to make appropriate adjustments to the BST sampling design and assess the adequacy of the Texas Known Source Library. (Start Date: Month 1; Completion Date: Month 15)

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Subtask 3.5: BAEN will conduct watershed modeling for the study area (Task 4). SAML will work with BAEN to 1) integrate BST results into the model, to the extent possible, and 2) address and reconcile discrepancies between BST and modeling results. (Start Date: Month 7; Completion Date: Month 21)
The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Task 4: Data Analysis and Watershed Modeling

Subtask 4.1: BAEN will cooperate with NETMWD to develop a comprehensive GIS inventory for the study area through TSSWCB project 09-54. Data will be collected by NETMWD and should include the most recent information available on land use, elevation, soils, stream networks, reservoirs, roads, public parks, municipalities and satellite imagery or aerial photography. Locations of SWQM stations, USGS gages, public access points to the waterbodies, floodwater-retarding structures, wetlands, TPDES permittees (including WWTFs, CAFOs and MS4s), and subdivisions should also be included. Sites permitted for land application of sewage sludge and septage should be included. Locations of possible bacteria sources, identified in the source survey, should be incorporated. The cumulative impact of TSSWCB-certified WQMPs on the management of agricultural and silvicultural lands should be documented. BAEN will be responsible for incorporating these data into a GIS, creating maps for stakeholder meetings and utilizing this information for SELECT modeling. (Start Date: Month 1; Completion Date: Month 3)

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Subtask 4.2: TSSWCB, in coordination with the SSL, will provide BAEN a current land use classification for the study area through TSSWCB project 08-52, Classification of Current Land Use/Land Cover for Certain Watersheds Where TMDLs or WPPs Are In Development. (Start Date: Month 1; Completion Date: Month 3)

The following actions have been completed during this reporting period:

a. This task is complete.

100% Complete

Subtask 4.3: In order to apply knowledge gained through TSSWCB project 07-06, Fate and Transport of E. coli in Rural Texas Landscapes and Streams, BAEN will assist NETMWD in designing a watershed source survey, to be conducted through TSSWCB project 09-54, that better characterizes the possible sources of bacteria loadings in the study area. (Start Date: Month 1; Completion Date: Month 4)

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete
Subtask 4.4: BAEN, with assistance from NETMWD through TSSWCB project 09-54, will conduct a LDC analysis of all historic and existing water quality monitoring data from the study area. LDCs will be developed for one critical index site per segment (0404, 0404B, 0404C). LDCs shall be consistent with 1) EPA’s An Approach for Using Load Duration Curves in the Development of TMDLs, 2) EPA’s Options for Expressing Daily Loads in TMDLs, and 3) EPA’s Development of Duration-Curve Based Methods for Quantifying Variability and Change in Watershed Hydrology and Water Quality. LDC development will be completed using available USGS flow gage data and SWAT generated flow data as reported in the Lake O’ the Pines TMDL. (Start Date: Month 3; Completion Date: Month 6)

The following actions have been completed during this reporting period:

a. Flow and water quality data was processed to develop preliminary LDCs for Big Cypress, Tankersley and Hart Creek.

25% Complete

Subtask 4.5: Using water quality monitoring data collected by NETMWD through TSSWCB project 09-54, BAEN, with assistance from NETMWD, will refine LDCs developed in subtask 4.4. LDCs will be used to determine bacteria load reductions needed to achieve water quality standards. (Start Date: Month 7; Completion Date: Month 21)

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Subtask 4.6: BAEN, with assistance from NETMWD through TSSWCB project 09-54, will conduct watershed modeling for the study area. Utilizing information from the GIS inventory (Subtask 4.1), the source survey (Subtask 4.3), and water quality monitoring (TSSWCB project 09-54), and in combination with LDCs from Subtasks 4.4-4.5, BAEN will develop a spatially explicit or mass balance model, such as SELECT, for each of the three segments in the study area. The model will be conducted on the entire Big Cypress Creek watershed; SELECT output subsets highlighting the individual impacts of Hart and Tankersley Creeks will also be provided. Modeling will be used to estimate loadings from various sources and to identify critical loading areas within the watersheds. (Start Date: Month 7; Completion Date: Month 21)

The following actions have been completed during this reporting period:

a. Data layers needed for SELECT were assembled and initial SELECT runs were done for Big Cypress, Tankersley and Hart Creek Maps were generated for each creek after running SELECT.

25% Complete

III. Related Issues/Current Problems and Favorable of Unusual Developments
• It appears that the *Bacteroidales* samples previously processed by Ana-Lab using the incorrect lysis buffer may be acceptable for analyses. Preliminary results from these analyses will be forwarded to TWRI and TSSWCB in a separate email.
• Communications with Ana-Lab have been difficult

IV. Projected Work for Next Quarter

• Submit fourth quarterly report
• Participate in coordination and stakeholder meetings
• Revision of QAPP
• Continue BST sample collection and processing