Problem/Need Statement

As of January 2006, 197 water bodies in Texas were impaired because they did not meet bacteria criteria established by the state to protect contact recreation use (freshwater and saltwater) and/or oyster water use. The freshwater contact recreation use criterion used to determine impairment includes both a geometric mean for indicator bacteria, *Escherichia coli* (*E. coli*), of 126 colonies per 100 mL and a single sample maximum of 394 colonies per 100 mL.

A bacteria TMDL task-force was formed in the State of Texas to evaluate the current TMDLL development process, address current weaknesses in the process and to develop a roadmap for further scientific research needed to reduce uncertainty in watershed modeling. There are several recommendations provided in the task-force document (Draft Four, 06/04/2007) to "scientifically" address the current "uncertainties" in bacteria TMDL development and implementation. (http://twri.tamu.edu/bacteriatmdl/). The objectives of this proposal are formulated based on several key recommendations compiled by task force experts. The key recommendations include identifying and characterizing *E. coli* sources and monitoring fate and transport of *E. coli* in impaired watersheds and streams. This project proposes a "holistic plan" for TMDL development that includes identification, characterization, and quantification of *E. coli* sources in impaired watersheds and monitoring the fate and transport of *E. coli* in these impaired watersheds. The outputs of this project will help to decrease uncertainties in *E. coli* load estimation from various sources and simulation of fate and transport processes of *E. coli* in watersheds and streams. The overall outcome of this project will help in developing scientifically sound TMDLs.

The first bacteria TMDL task-force recommendation that this project will focus on is conducting a sanitary survey of the watershed. Inventory sanitary surveys identify various potential *E. coli* sources in an impaired watershed are indispensable in TMDL development. In the majority of rural and agricultural stream-impairments due to bacteria, specifically *E. coli*, the specific sources and accurate quantities from each source have not been accurately determined. This lack of information makes managing the impaired waterway difficult and expensive. Currently, the most widely used approach to determine the source of *E. coli* is Bacteria Source Tracking (BST). This approach is good in determining the source of the impairment, but not in determining the load produced by specific sources. Moreover, BST methods are very labor intensive and expensive. Contrarily, inventory sanitary surveys identify various potential *E. coli* sources in impaired watersheds, are simpler and less expensive. *E. coli* load estimation tools such as Spatially Explicit Load Enrichment Calculation Tool (SELECT) estimates *E. coli* loads from various sources using literature values. *E. coli* content of feces has been reported in literature for certain domestic and wildlife species and has been summarized in some reports used in TMDL development. However, this information has not been the focus of the reported research and therefore has not undergone extensive peer review. Consequently, there is a high level of uncertainty in identifying *E. coli* loads and sources for use in watershed modeling and *E. coli* load estimation tools. This project will conduct inventory sanitary surveys to identify potential *E. coli* sources in the chosen watershed. Then, *E. coli* in various identified animal sources in the inventory survey will be characterized and quantified. Accurate identification, characterization, and quantification of *E. coli* sources in the impaired watershed will improve the predictions of SELECT, which is currently developed and successfully applied in TMDL development for an impaired watershed in the State of Texas.

The Bacteria TMDL task-force also emphasized the need for additional studies that focus on developing a better understanding of fecal bacteria fate and transport processes. Currently,

knowledge of these processes is limited at best and contributes to significant uncertainties in the modeling of these processes. Fate and transport of *E. coli* in rural and agricultural landscapes is largely dependent on various environmental factors and management practices. Dominant environmental factors that affect *E. coli* transport in landscapes (e.g., waste source, soil type, temperature, rainfall, moisture content, nutrient status, etc) and persistence, re-growth, and survival in landscapes need to be identified. Re-growth of *E. coli* in landscapes due to favorable environmental conditions (e.g., rainfall after dry weather conditions) is one of the major fate processes that influence *E. coli* concentrations in streams. The influence of different environmental variables on growth kinetics of *E. coli* and re-growth also need to be thoroughly studied and demonstrated to strengthen watershed models that simulate *E. coli* fate and transport in landscapes. The kinetic parameters obtained from this monitoring task will be used to validate and improve fate and transport models used in TMDL development and implementation plans. Growth kinetics, survival rates, and re-growth are critical factors to accurately model the fate and transport of bacteria in watersheds and, as identified by the Task Force, current understanding of these processes is limited.

Re-suspension of *E. coli* in streams (e.g. scouring of stream bed sediments due to high flows) is one of the major fate processes that influence stream impairment. Unfortunately this process is not well studied or understood. The effect of rainfall and runoff on survival and growth of *E. coli* in streams and stream-bed sediments and subsequent re-suspension of *E. coli* in streams need to be quantified to properly assess the impairment of a stream. Parameters obtained from the stream-monitoring study will be used to improve in-stream hydrodynamic processes modeled by fate and transport models. Information gleaned from this evaluation can and most likely will have a significant impact on the types of BMPs recommended to alleviate bacterial contamination issues in agricultural watersheds.

Identifying, characterizing, and quantifying *E. coli* loads resulting from various sources are critical tasks in TMDL development for any impaired watershed. Monitoring and assessing the fate and transport processes of *E. coli* in landscapes and streams and monitoring the effects of environmental factors on fate and transport processes are required to develop and validate watershed models that utilize process-based fate and transport subroutines. Effective communication of findings is a crucial task that will not be overlooked in this project. Concise, easy to read publications and brochures will be developed that will inform readers about the findings of the study. This project will combine these vital aspects of TMDL development and demonstrate that they are effective tools for enhancing the understanding of these complicated processes.

General Project Description

The first portion of this monitoring and assessment project (Tasks 3, 4, & 5) will involve conducting sanitary surveys in the selected creek twice a year (winter and summer) to identify various dominant and relevant *E. coli* sources (cattle, poultry, deer, feral hogs, etc). The developed sanitary surveys will be updated with inputs from local experts (e.g. wildlife experts and enthusiasts, stakeholders, county agents, farmers, citizens). Waste streams will be quantified for identified sources in the watershed by utilizing collected fecal production numbers (Task 4) and assumed population densities from sanitary surveys (Task 3) (for example if “dairy cows” are a source, excreted manure, flushed manure, separated solids, lagoon wastewater, composted dairy manure, etc will be waste streams). *E. coli* numbers resulting from characterized waste streams (*five maximum*) for all *dominant* identified sources (*five maximum*) will be quantified. Random and representative samples of identified waste streams and fecal material of identified species

will be collected. Four sub-samples will be collected for each waste stream/fecal material and a composite sample will be made on-site. Each composite sample will be transferred to a 1 L bottle. All sample bottles will be stored in a cooler at 5°C and transported to the laboratory for *E. coli* analysis. Each composite sample collected will be extracted with de-ionized water and filtered through a filter paper for *E.coli* incubation and enumeration. Samples of each identified waste stream and fecal material will be collected separately in 1 gallon containers and brought back to the laboratory for conducting the Phase II study.

This demonstration project also addresses another major concern stated by the Bacteria TMDL task force. This involves monitoring the survival, growth, re-growth, and die-off of *E. coli* in water under different environmental conditions. Currently, there is a significant knowledge gap about the fate of *E. coli* from various sources under different environmental conditions. Composite samples from each waste stream and species will be subjected to different temperatures (0°C, 10° C, 25° C, and 50°C), moisture conditions (0%, 5%, 25%, 50%, and 75% dry-basis), and pH (acidic, neutral, and alkaline). Growth and die-off at above mentioned environmental conditions will be monitored. Once optimum conditions are identified, re-growth will be monitored by bringing back the environmental conditions for the above mentioned scenarios to optimum conditions.

E. coli survival, growth, re-growth, and die-off in stream sediments (Task 6) and re-suspension of *E. coli* in streams are also poorly understood. In many cases, the re-suspension of *E. coli* has been suspected as a significant source of *E. coli* measured in the stream. Physical disturbances from humans, animals, waterfowl, fish, and large rainfall events etc. can significantly increase the amount of suspended sediments in the stream and thus may have a significant impact on the amount of *E. coli* suspended in the water column. Automatic water sample collection systems will be installed at two locations along the impaired stream in the impaired watershed. Data will be collected under baseflow, highflow, rainfall events and while sediment has been mechanical agitated. Analysis will result in information about survival, growth, re-growth and die off under all conditions. Sediment samples will be collected from 24 random locations along the instrumented stream and three sub-samples will be used to make one composite sample. Sediment samples will be brought to the laboratory to monitor survival, growth, re-growth, and die-off of *E. coli* under different temperatures (0°C, 10°C, 25°C, and 35°C), nutrient conditions (three different concentrations of organic carbon), pH (acidic, neutral, and alkaline), light intensity (three different light intensities), and chlorination (three residual chlorine concentration levels). Re-suspension of *E. coli* in streams will be monitored during four rainfall events two each during winter and summer and will be reconciled with runoff samples using re-suspension data collected from mechanical sediment disturbance. Water samples will be collected for those rainfall events using the automatic sample collection systems. Mechanical re-suspension will be made by disturbing the stream bed-sediments four times; two each during winter and summer; and grab samples of water will be taken at six different locations along the stream. All water samples will be stored in coolers at 5°C and transported to the laboratory for *E. coli* analysis.

Findings from this study will be presented in a series of publications. Results from each component of the project will be published in the form of easy to read brochures or fact sheets that will clearly present information in a way that the reader can easily understand what is being presented. A technical report will be developed at the conclusion of the project and will present complete findings from the monitoring and assessment phases of the project. Additionally, all materials related to the project will be posted on a website that will be developed specifically for the project. The website will also contain a brief description of the project, the need for the project, provide contact information for all parties involved, goals and objectives and any project updates that may occur. Findings from all Tasks and specifically, Task 6, will support the

development and implementation of a WPP (TSSWCB 319 project 06-12) and TMDLs in the Leon River watershed.