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Identify and Characterize Nonpoint Source Bacteria Pollution to Support Implementation of Bacteria TMDLs in the Oso Bay Watershed

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Identify and Characterize Nonpoint Source Bacteria Pollution to Support Implementation of Bacteria TMDLs in the Oso Bay Watershed

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Executive Summary

Oso Creek (Segment 2485A) has been identified on the Texas Water Quality Inventory and 303(d) List since 2002 as impaired due to excessive bacteria. Results of a modeling study of bacteria loading for Oso Creek, submitted to Texas Commission on Environmental Quality (TCEQ) for use in the TMDL (Total Maximum Daily Load) process, showed that loading occurs throughout the length of the creek, including the upper reaches and that there is “dry day” loading in addition to wet weather runoff and inflows. Modeling efforts demonstrated that the removal of the relatively small dry day loading could nearly achieve the geometric mean water quality standards in the creek; however, modeling work was unable to discern the source of the “dry day” loading. Even with removal of dry day loading some reduction of run-off loading is also needed to achieve water quality objectives. While there are several identified inflows downstream (stormwater, etc.) carrying runoff, the upper sections of the creek run through primarily rural agricultural row crop fields with no obvious sources of fecal bacteria. The creek is effluent driven, receiving water from the Robstown WWTP (wastewater treatment plant). The WWTP is permitted by TCEQ and is in compliance with effluent limits in the discharge permit. However, sampling of the creek showed elevated enterococci levels and loading is occurring in the upstream sections. A recently conducted study, (TSSWCB Project 02-13; USGS 2008) which includes limited bacterial sampling of agricultural land runoff, has indicated elevated levels of enterococci in this runoff.

Thus, the previous studies to support the TMDL (monitoring data and modeling) provided information on the levels of enterococci in the creek and bacteria loading for the TMDL, but have not answered the key questions needed to finalize the TMDL or develop the Implementation Plan (I-Plan): what and where are the source(s) of the bacteria? In order for effective planning by stakeholders, the questions of where the bacteria are originating from in the upper portion of the creek needs to be answered.

The goal of this project was to determine the nonpoint sources of enterococci in the upper sections of the creek and the potential animal sources contributing to the loading. In order to answer key questions which have arisen during the initial phase of the TMDL, a sampling plan was developed based on the project goals with input from multiple stakeholder groups and state agencies.

The first year of sampling provided a synoptic dataset of the study area to generate information on potential sources of enterococci and allowed optimization of sampling

resources for follow-up sampling and laboratory experiments by focusing on those areas identified as having high enterococci concentrations. Samples were collected from a variety of potential sources including sediments, soils, sub-surface, surface and groundwater to evaluate contributions from these sources. Based on these results additional sampling was conducted in years 2 and 3. Additionally, focused small-scale laboratory experiments were conducted to examine survival of enterococci under different conditions once soil was identified as a potential contributing source of enterococci to the creek.

Animal sources of enterococci in the upper Oso Creek were determined using a bacteria source tracking toolbox approach. Data from two library-dependent BST techniques – Carbon Source Utilization (CSU) and Antibiotic Resistance Profiling (ARP) of isolates was combined to form a composite library for comparison with creek enterococci isolate profiles to categorize these isolates into animal source groups using discriminant analysis, and *esp* gene analysis was used to detect human source contamination in creek water samples.

Based on results of multiple sampling events during year 1, groundwater and sub-surface seepage were not identified as constituting major contributions of enterococci to the creek and sampling was reduced (groundwater) or discontinued (sub-surface seepage) in year 2 for these matrices. Sediments from the creek contained enterococci, especially following rainfall, but the enterococci appeared not to persist in this matrix as numbers dropped to very low levels during dry months, suggesting deposition in sediments during wet weather loading, but gradual die off over time. This sampling continued through year 2. Soil samples collected during year 1 from a variety of fields under different crop covers were found to contain viable enterococci, particularly during wet fall conditions and dry winter months. Sampling was continued through year 2, with lower numbers found during the hot dry summer months.

Historic TCEQ surface water quality monitoring stations were sampled quarterly throughout the project to maintain a record of enterococci levels in the upper creek. Levels of enterococci continued to routinely exceed U.S. EPA recommended single sample criteria for the group indicating that the loading identified in earlier studies continued through the period of this study.

Small-scale laboratory experiments were conducted to assess survival of *Enterococcus mundtii,* commonly found in both soil and creek water, in Oso Creek watershed soil at different temperatures and moisture conditions. Due to a lack of enterococci in the soil, autoclaving was not necessary. *E. mundtii* survived throughout a 90 day period in wet or dry soil at 10^{o}C, but died off or at least were not culturable within 14 days at 40^{o}C. At 25^{o}C the bacteria persisted in dry soil, but died off over time in wet soil. These results support findings from the field that enterococci can persist in dry cold winter soils and could be a source in runoff into the creek, but that during hot summer months the levels are reduced and may be undetectable.

Bacteria source tracking by both genotypic and phenotypic methods demonstrated that the main sources of enterococci contamination in the upper Oso Creek are non-human. Less than 10% isolates were classified as human and the *esp* gene was detected infrequently. The majority of the creek isolates were classified as wildlife (non-avian) and bird, and the results suggest wildlife (avian and non-avian) and livestock both contribute to the contamination. Additional studies are needed to further elucidate where and when these sources reach the creek and potential pathogens that may be carried into the creek with the contamination.

The results of the study suggest that the bulk of bacterial loading into the creek is directly attributed to surface water contributions with sediment playing a minor role as a reservoir for enterococci at some portions of the creek and soil (carrying either indigenous bacteria/animal feces) as a potential source during wet weather. The sources of the enterococci appear to be largely wildlife (non-avian) and bird, with some contribution from livestock and with wildlife sources increasing, in terms of loading, during runoff driven events (wet weather).

In terms of mitigation, the finding that non-human sources are the major contributors to contamination and that wildlife and bird sources may be significant, suggest control and reduction of enterococci levels will be difficult to achieve in Oso Creek. Contributions from agricultural activity can be reduced with cooperation from farmers using best management practices (BMPs) for livestock and erosion/sediment control.

Overall, this project provided critical information for understanding the bacteria loading in the Oso Creek watershed to aid in the development of the creek's TMDL and I-Plan. The findings will also be of use in developing approaches for study of other similarly impaired watersheds.

Introduction

Oso Creek (Segment 2485A) has been identified on the *Texas Water Quality Inventory and 303(d)* List since 2002 as impaired due to elevated levels of bacteria. Results of a modeling study of bacteria loading for Oso Creek (Hay and Mott, 2005, 2006) showed that loading occurs throughout the length of the creek and that there is "dry day" loading in addition to wet weather runoff and inflows. Modeling efforts demonstrated that the removal of the dry day loading could nearly achieve the geometric mean water quality standards in the creek, bringing the water body into compliance. The identity of the source of this "dry day" loading could not be discerned. Even with removal of dry day loading some reduction of run-off loading is needed to achieve water quality objectives.

Historical data gathered from projects in the Oso watershed over the past ten years has been primarily focused on water quality monitoring and development of bacteria loading models. Surface water samples collected during monitoring studies were analyzed for enterococci, a bacterial indicator organism currently recommended for use in both marine and freshwaters by the United States Environmental Protection Agency (US EPA). Enterococci - various species of *Enterococcus* - are normally associated with the lower intestinal tract of mammals and birds, making their presence in surface waters a reliable indicator of fecal pollution. An alternative indicator, *E. coli*, is also recommended by the USEPA for use in freshwater, but is not recommended for use in marine waters (USEPA, 1986). As Oso Creek is classified as having a portion that is freshwater and a portion that is tidal, and it flows into a marine bay, enterococci have been selected for the series of recent studies as the indicator to evaluate water quality throughout the Oso Creek watershed.

Oso Creek is effluent driven, receiving outflow water from the Robstown WWTP. The WWTP is permitted by the TCEQ and is in compliance with effluent limits in the discharge permit. While there are several identified inflows downstream (stormwater, etc.) carrying runoff (Hay and Mott 2005, 2006, Mott and Hay, 2009) , the upper sections of the creek run through primarily rural agricultural row crop fields with no obvious sources of fecal bacteria. However, sampling of the creek has revealed consistently elevated enterococci levels and loading in the upstream sections. A recently conducted study (TSSWCB Project 02-13: USGS 2008), which included limited bacterial sampling of Oso Creek watershed showed that agricultural land runoff contained elevated levels of enterococci.

Thus, while the previous monitoring and modeling studies to support the TMDL provided information on the levels of enterococci in the creek and bacteria loading for the TMDL, they did not answer the key questions needed to finalize the TMDL or develop the Implementation (I)-Plan: what and where are the source(s) of the bacteria? Effective planning by stake-holders requires identification of the sources of the enterococci in the upper portion of the creek.

In this study, to answer key questions that have arisen during the initial phase of the TMDL – what are the nonpoint sources of enterococci in the upper sections of the creek and what are the animal sources contributing to the loading? - A variety of environmental samples, including soil, sediment, surface water and groundwater, were analyzed for presence and concentrations of enterococci. Once this environmental sampling identified sources of loading into the upper region of the creek an additional laboratory study was performed to examine the ability of *Enterococcus sp*., to survive and persist in the relevant matrices. To answer the question of whether the enterococci sources were human or animal, additional analyses using multiple bacteria source tracking techniques were conducted.

Bacteria source tracking (BST) is a tool used to elucidate the animal source or sources of bacteria in the environment. Different techniques included under the umbrella of bacteria source tracking, include genotypic (based on genetic information) and phenotypic (based on physical expressions of a characteristic) tests. These techniques vary in the level of discrimination to which sources can be distinguished (i.e. animal vs. human; livestock vs. human; cow vs. horse vs. human). The source tracking method selected for a study depends on a number of factors including specificity of source identification needed, cost, time of analysis, and level of experience required for analysis.

Antibiotic resistance analysis (ARA) is a phenotypic method that uses the patterns of antibiotic resistance produced by bacterial isolates from different animals to differentiate between sources. This method utilizes a library of known source isolates with which to compare unknown source isolates. ARA is cost-effective, with moderate analytical time involved and minimal training required. It can also provide a reasonable level of discrimination between source groups in a complex watershed. Although ARA was one of the earlier BST methods developed, numerous recent studies have utilized this technique to successfully determine sources of fecal contamination (Sayah et al., 2005; Anderson et al., 2006; Ebdon & Taylor, 2006; Casarez et al., 2007; Dickerson et al., 2007; Kaneene et al., 2007; Mott and Smith, 2008; Mott, Lehman & Smith, 2008; Smith et al., 2010).

Carbon source utilization (CSU is another phenotypic method that creates profiles; however, in this technique the profiles are based on bacterial utilization of different carbon sources. Like ARA, CSU is a cost-effective method that requires little training. CSU has been used in several studies in the U.S. and abroad (Hagedorn et al., 2003; Wallis & Taylor, 2004; Ahmed et al., 2005; Ahmed & Katouli, 2008; Moussa & Massengale, 2008; Graves et al., 2009).

An alternative approach in BST is to determine whether a particular source (e.g. human) is contributing to contamination. Analysis for the presence of the enterococcal surface protein gene (*esp*) is a rapid genotypic method used to specifically detect human source contamination. The analytical method is a PCR-based detection of the gene for esp, which is considered to be a virulence factor of *Enterococcus faecium* associated with the human intestinal tract (Scott et al., 2005). This method has been used in a number

of recent bacterial source tracking studies (McDonald et al., 2006; Brownell et al., 2007; Ahmed et al., 2008; Korajkic et al. 2009; Abdelzaher et al. 2010; Mott et al., 2010).

As the field of bacterial source tracking has evolved in recent years, there has been an increasing recognition of the importance of incorporating multiple methods into studies to increase accuracy of results (Stewart et al. 2003, Stoeckel and Harwood, 2007). A TSSWCB-TCEQ interagency task force recommended this toolbox approach for Texas studies. (Jones et al., 2009) Although using more methods may increase the cost of the study, the expense is offset by the increased confidence in the results.

This study used an analytical toolbox approach to detect sources contributing to fecal contamination in the Oso watershed. A combination of antibiotic resistance analysis, carbon source utilization and *esp* gene analysis was used to increase confidence in the data and identify the major contributors to contamination of the upper section of Oso Creek.

Study Goals and Objectives

Project goal:

To determine the nonpoint sources of enterococci in the upper sections of the creek and the animal sources contributing to the loading.

Objectives:

(1) Enterococci levels in the upper section of Oso Creek will be explained by identification of nonpoint sources of fecal contamination
(2) Enterococci levels in the upper sections of the creek, sediments and subsurface waters will be quantified
(3) Enterococci isolated from the creek under dry and wet conditions will be categorized by source type (human/non human, etc.)
(4) Additional data on enterococci levels in the creek will be collected

The project focused on the Oso Creek watershed to answer key questions that have arisen during the initial phase of the TMDL – what are the nonpoint sources of enterococci in the upper sections of the creek and what are the animal sources contributing to the loading? Nonpoint sources were identified by sampling a number of different potential contributing sources. Animal sources were determined using two library-dependent BST techniques – Carbon Source Utilization (CSU) and Antibiotic Resistance Profiling (ARP) of isolates. Additionally, human source contamination was assessed separately using a subset of approximately100 isolates which were analyzed to detect the *esp* gene (a library-independent BST technique using a human-specific source molecular marker) to provide added confidence in the BST results. This

information will also be of use in determining sources of enterococci in other similar watersheds (e.g., contributions of sediment and agricultural runoff).

Sampling Plan

In the development of previous Oso Creek studies involved in TMDL development, the decision was made to use enterococci as the fecal indicator group as explained in the introduction. Thus, all samples in this study were analyzed for this group.

Prior to the start of the project, a sampling strategy was developed to elucidate the contributions of possible nonpoint sources of fecal bacteria (enterococci) with consultation and input from entities including the TCEQ, Coastal Bend Bays and Estuaries Program (CBBEP), the U.S. Geological Survey (USGS), Texas Parks and Wildlife Department (TPWD), the Nueces River Authority, the Texas A&M AgriLife Research Center at Corpus Christi (AgriLife Research), and local stakeholders (e.g., Cities of Corpus Christi and Robstown, local farmers, developers, discharge permit holders, homeowners). At a stakeholder meeting in January 2008, the plan was presented and input solicited. The sampling plan was then finalized and incorporated into the approved project Quality Assurance Project Plan (QAPP).

The sampling plan included laboratory analysis for enterococci from field samples and laboratory experiment samples over the duration of the project. Field samples were collected from a variety of potential sources including agricultural soil from representative in-field locations, sub-surface and groundwater, and in-creek sediment and water at multiple stations along the upper section of the creek. The first year of sampling formed a synoptic dataset of the study area that provided information on the potential sources of enterococci. This allowed for optimization of sampling resources for follow-up sampling and small-scale laboratory experiments by focusing on those areas identified as having high enterococci concentrations. Existing TCEQ stations were sampled quarterly throughout the study to maintain a record of bacteria levels at those sites (18499, 18500, 18501). Sampling of groundwater to identify any role of this source in bacterial loading to the creek was accomplished using ten wells constructed and maintained at a number of locations in the watershed through another project (funded by CBBEP), and these were sampled at multiple depths, seasonally, under both dry and wet weather conditions. Appropriate fields under different crop cover were identified for sampling and access was facilitated by the United States Department of Agriculture Natural Resource Conservation Service (USDA-NRCS). Composite samples from both well-drained and poorly-drained areas of the fields were analyzed. Sediment and sub-surface samples were collected at TCEQ stations in the upper creek.

To determine animal sources of contamination, BST was conducted. While Texas BST work has focused on *E. coli* (as it is the indicator for freshwater bodies), for coastal (marine) waters where the indicator is enterococci, it is more appropriate to use this group in TMDL studies. Enterococci have been used in previous studies in other states for BST work and can provide at least equivalent (and sometimes better) discrimination between sources.

Land use and sanitary survey information collated by the Principal Investigators (PIs) under an approved QAPP for a previous TCEQ project (2005) and comments from multiple Oso Creek/Oso Bay TMDL Stakeholder meetings including one held January 28, 2008 with the TSSWCB were used to determine potential source animals and appropriate locations for fecal collection. A variety of animal feces were identified as potential sources of enterococci into the creek. This information was used in developing a library of isolates from feces of potential animal sources. Enterococci isolates were characterized using the Microlog™ Microbial Identification System (Biolog, 1994), which provides a species level identification and a CSU profile for each isolate. Speciation provides some information about sources as certain species are associated with specific animals. The existing Texas Known Source Library does not contain known source isolates of enterococci; however, a small library exists at Texas A&M University-Corpus Christi (TAMU-CC) from a previous special study approved and funded by the Texas General Land Office (TGLO) (Mott et al., 2003). These isolates were incorporated into this project in order to develop as comprehensive a library as possible. ARPs were also developed for each isolate to provide a composite data set with the CSU. ARA and CSU profiles of known and creek source isolates were compared using Discriminant Analysis to categorize creek isolates into animal sources. A subset of samples was also analyzed using a library-independent method for detection of the *esp* gene, which is a marker for human source enterococci. This provided an additional level of confidence in the data.

Study Area

Figure 1 shows the Oso Creek and Bay watershed. This study focused on the upper section of Oso Creek including the West Oso Creek tributary.

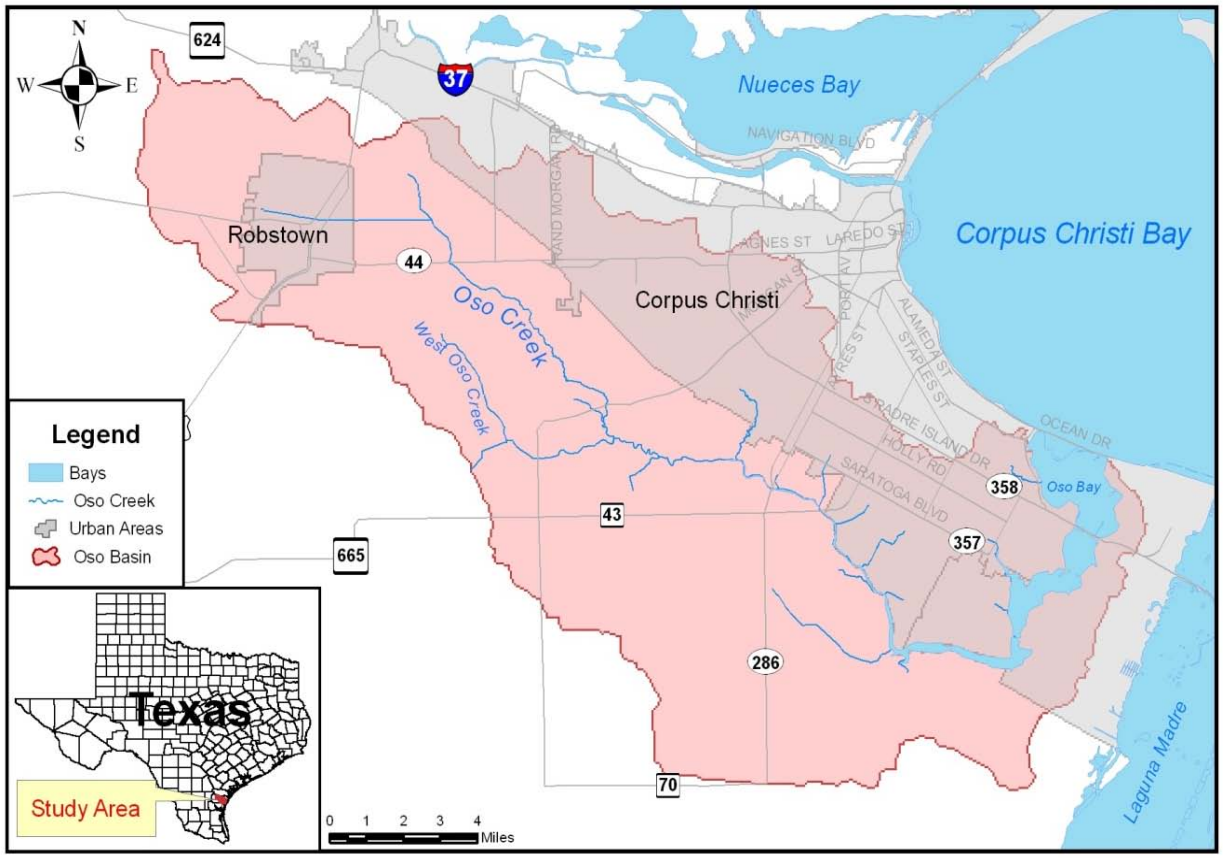

Figure 1. Oso watershed study area.

Land use information for the watershed was obtained from the 1992 National Land Cover Dataset (Table 1), and permitted discharge information was taken from the TCEQ permit database (Table 2). The Oso Bay/Creek watershed was assessed using aerial maps to examine land use and accessibility for sampling. Field surveys were conducted in 2005 and GPS coordinates of each potential sampling site were taken. Follow-up surveys were conducted in 2008 to confirm locations of animals such as livestock. Figure 2 shows the major discharges and locations of animals identified during sanitary surveys of the watershed.

Table 1. Land use in the Oso Bay/Oso Creek Watershed (Hay and Mott, 2005). *Source – USEPA/USGS 1992 National Land Cover Dataset.

Land Use Types	Percent
Planted/Cultivated	67.8
Urban Development	13.8
Grasslands	5.2
Water	4.5
Shrubland	3.8
Wetlands	2.8
Forested Upland	2.0
Barren	0.2

Table 2. Permitted dischargers in the Oso Bay/Oso Creek watershed (Hay and Mott, 2005).

Permitted Discharger	TCEQ Permit No.	Permitted Daily Avg. Flow (MGD)
American Electric and Power Barney Davis Power Station (1)	01490-000	540
City of Corpus Christi Oso WWTP (2)	10401-004	16.2
City of Corpus Christi Greenwood WWTP (3)	10401-003	8.0
Texas A&M University CBI La Coss Facility (4)	03646-000	5.04
City of Robstown (5)	10261-001	2.4
Equistar Chemical LP Corpus Christi Plant (6)	02075-003	2.0
Tennessee Pipeline Construction Co. Cuddihy Airfield WWTP (7)	14228-001	0.06
Corpus Christi Peoples Baptist Church Roloff WWTP (8)	11134-001	0.02
Texas A&M University Agriculture Research Ext. (9)	11345-001	0.0015
City of Corpus Christi Storm Water (10)	04200-000	NA

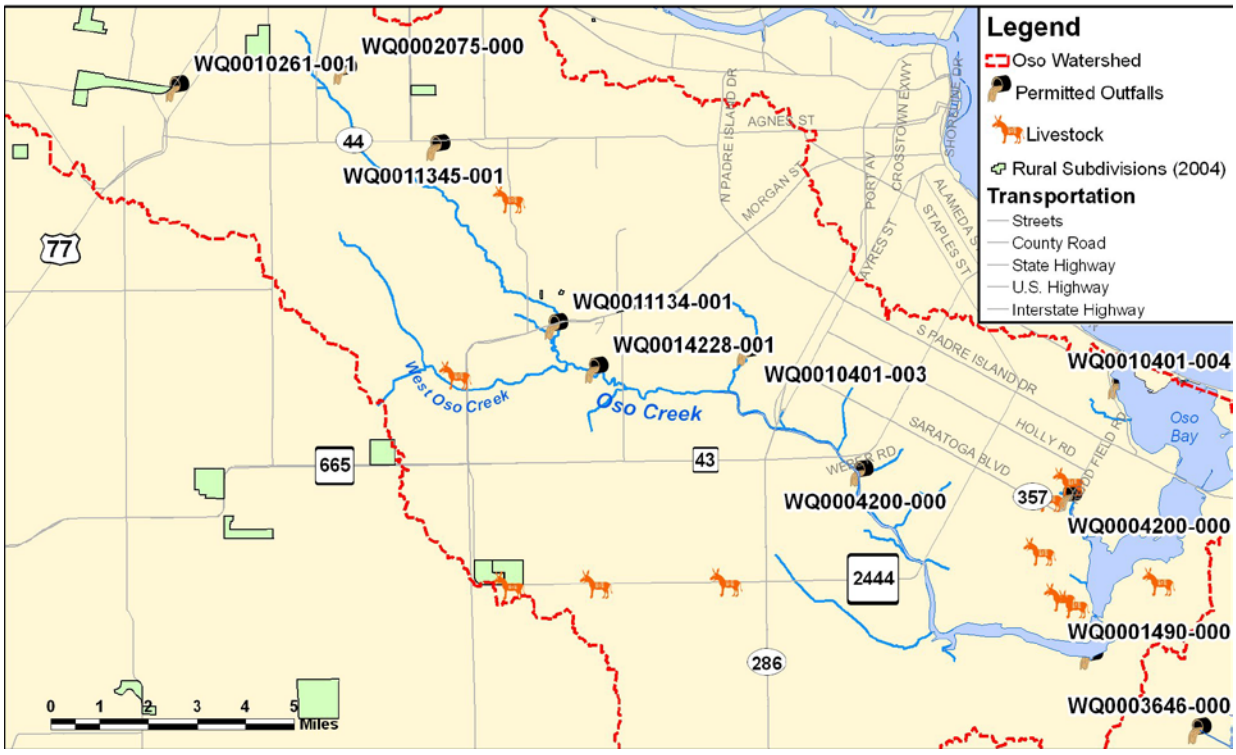

Figure 2. Map developed from sanitary survey, showing permitted discharges and potential sources of bacteria loading into Oso Bay/Oso Creek.

Methods

All work in this report followed the QAPP developed and approved for the project. Laboratory analyses followed standard methods as described and referenced in the QAPP and were conducted in a National Environmental Laboratory Accreditation Program (NELAP) certified laboratory at TAMU-CC, following all quality system requirements described in the QAPP. Laboratory Data Reports for sampling events with enterococci enumerations are maintained at TAMU-CC. Field sample collection and documentation was also completed in accordance with the approved QAPP.

Field Sampling Methods

Field samples were collected from a variety of potential non-point sources in the upper section of the Oso watershed: surface water, groundwater, sediment, seepage, and soil, to enumerate enterococci. Additionally, fecal samples from local sources were collected to isolate enterococci for bacterial source tracking analysis. Larger soil samples were collected from field site SO02 in the months of June, July, and August 2011 for use in small-scale laboratory experiments.

Field sampling procedures followed *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) for collection of water samples and measurement of field parameters. The field parameters: dissolved oxygen, water temperature, conductivity and pH were measured with a calibrated YSI or Hydrolab $H_20^{®}$ water quality multi-probe instrument in the field. Other parameters were analyzed in the laboratory. Additional procedures for field sampling are outlined below to reflect specific requirements for sampling under this project and/or provide additional clarification.

All field sampling activities were documented on field data sheets. Flow work sheets, multi-probe calibration records, and records of bacteria analyses are part of the field data record. For all visits, station ID (if applicable), location, sampling time, sampling date, sampling depth, preservatives added to samples and sample collector's name/signature were recorded. Values for all measured field parameters were also recorded. Detailed observational data were noted including, as appropriate: water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, missing parameters (items that were to have been sampled that day, but were not), days since last significant rainfall, and flow severity.

Precipitation data for the area reported on the National Oceanic and Atmospheric Administration (NOAA) National Weather Service (NWS) site and from the Corpus Christi International Airport reported through the Southern Regional Climate Center (SRCC), sponsored by Louisiana State University (LSU) (for water, soil, and sediment collection activities), NOAA, and the National Climate Data Center (NCDC) (for fecal sample collection activities) were used to determine amounts and dates of rainfall in the watershed, needed for scheduling sampling events following rainfall and for inclusion in field data sheets
(http://www.ncdc.noaa.gov/oa/ncdc.html , http://www.srcc.lsu.edu/stations/index.php?action=metadata&network_station_id=412015)

Flow data from USGS gage station (Gage #08211520) on FM 763 was referred to for flow information for the creek during the study, in addition to measurements made during field collections events.

Surface Water

Surface water samples were collected from three historic TCEQ sampling stations in the upper section of the creek, designated by station ID: 18499, 18500, and 18501 (Table 3, Figure 3).

Table 3. Historic TCEQ water quality sampling stations on Oso Creek and Oso Bay.

Station ID /TCEQ ID	Description	LAT	LON
18499	Oso Creek at SH44	27.783250	-97.592430
18501	West Oso Creek at FM 665	27.709360	-97.554220
18500	Oso Creek at FM 665	27.729470	-97.523570
13029	Oso Creek at FB 763	27.711111	-97.501663
20559	Ditch on US 77 near Robstown WWTP	27.800060	-97.646530

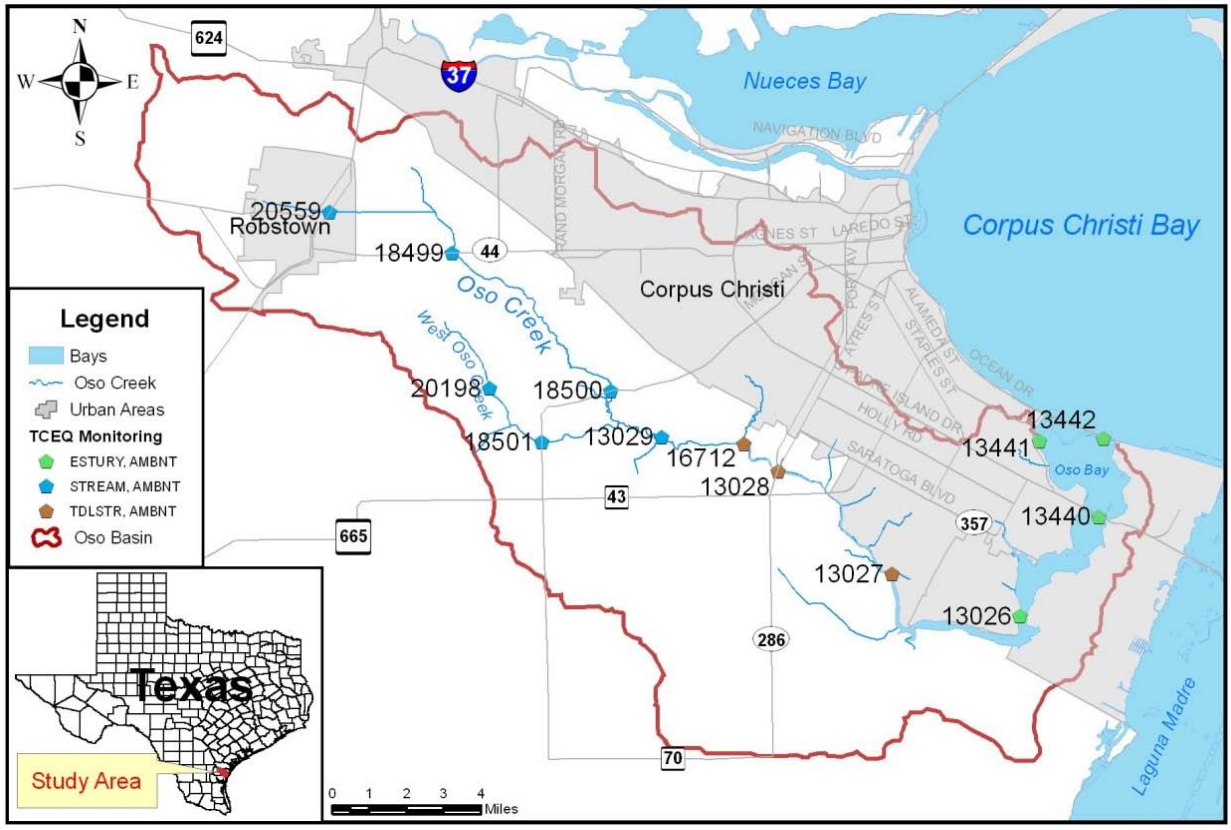

Figure 3. Historic TCEQ water quality sampling stations on Oso Creek and Oso Bay.

Sampling was conducted quarterly from Year 1 through Year 3 to generate a total of ten sampling events. Two water samples were collected at each sampling site using labeled one liter screw-cap polypropylene bottles which were cleaned and autoclaved prior to each use. Water sampling followed procedures described in *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Field water quality parameters (specific flow rate, specific conductance, dissolved oxygen, pH, salinity, water temperature) were measured at the time of surface water sample collection following guidelines outlined in *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Field data was also collected for the following parameters: air temperature, wind intensity, wind direction, weather, water color, water odor and rainfall. Water samples were collected in-stream by wading into the stream to reach the center of the flow. Flow measurements were taken first when possible, to delay collection of bacteria and water samples that have limited holding times. If flow measurements were taken first, multi-probe instruments were not deployed in the same area and water sample collections were taken in an undisturbed area. Flow measurement methods and flow estimation procedures followed guidelines stated in *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Flow measurements were collected mechanically. Two exceptions were no flow at a stream site and only isolated pools remaining in the stream bed, and dry stream bed containing no water. Site(s) that were dry or with limited pooled water were noted on the field data sheet and not sampled. Samples were collected on a routine schedule, regardless of prior rainfall capturing dry and runoff-influenced events at their natural frequency.

Groundwater

Water samples were collected from ten groundwater wells (Figure 4) established in pairs (one shallow—drilled to water table, and one deep—drilled to water table plus approximately ten feet depending on subsurface stratigraphy). Samples were collected in duplicate from each well. Wells were located near historic TCEQ sampling stations (18501, 18500, 18499 and 13029) and near agricultural nonpoint source sites used in other AgriLife Research Projects (TSSWCB projects 02-13 and 07-07). Groundwater samples were collected according to the procedures set forth in *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) with the following additions. Prior to sample collection, at least three casing volumes (length of water column times the well diameter) of water were removed from the well using a bailer (either new disposable or dedicated for use in only a specific well). Casing volume calculations are detailed in the QAPP for the project. Field data was also collected for the following parameters: air temperature, wind intensity, wind direction, weather, water color, water odor and rainfall. Samples were collected quarterly in Year 1, with an additional sampling event collected after rainfall. Based on enterococci results from year 1, sampling frequency was reduced to two sampling events in year 2.

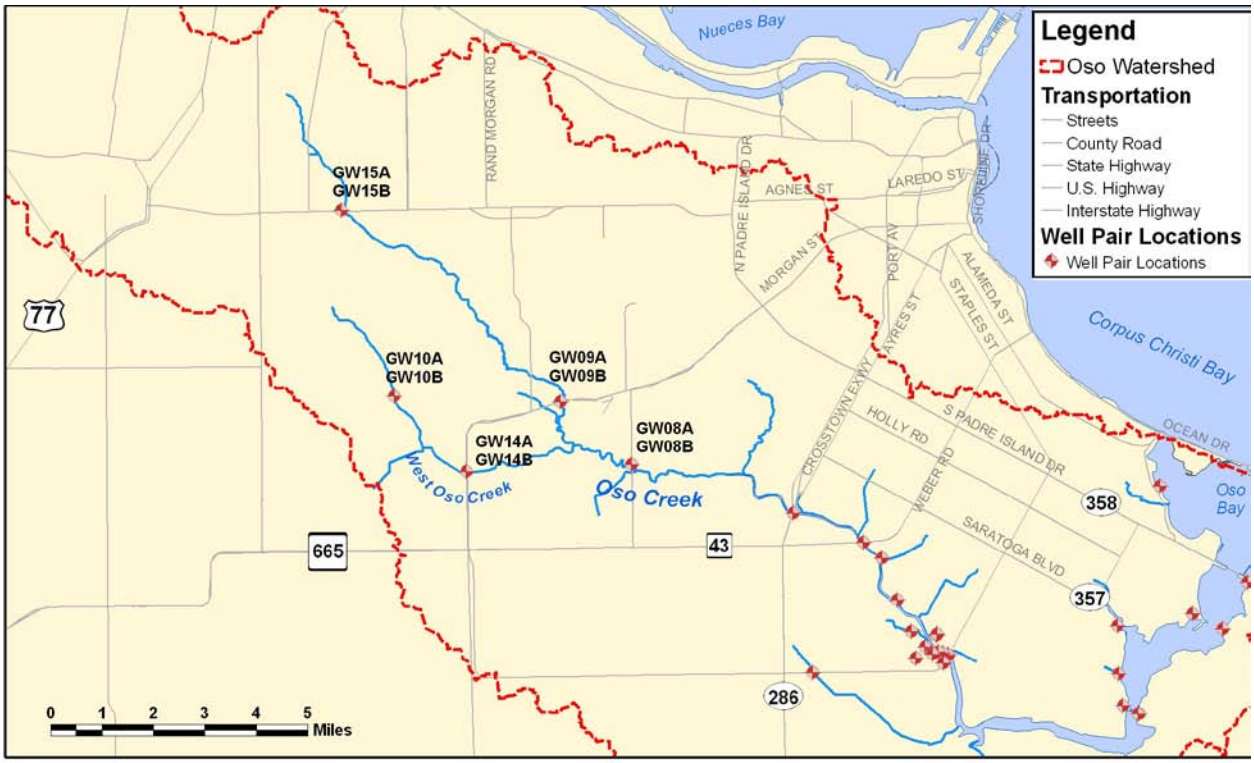

Figure 4. Monitoring well locations in the upper Oso Creek watershed.

Water and Sediment

Surface water and sediment samples were collected immediately upstream from three historic monitoring sites (18501, 18500, 18499); immediately upstream from the USGS gage station (gage #08211520) on Merritt Road (20198), where high concentrations have been measured in previous and on-going agricultural NPS studies (TSSWCB projects 02-13 and 07-07); and one location immediately upstream from a targeted monitoring site S7 (TCEQ station 20599, established during a recent TMDL study downstream of the Robstown WWTF (Hay and Mott, 2005)), which has some historic data collected after rain events (Figure 3). Sampling was conducted quarterly for two years. Site 20198 was dry and not sampled throughout Year 1, but sediment sampling was conducted during Year 2, regardless of water level at the site. Two water and two sediment samples were taken at each site following procedures in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Field parameters were documented on field data sheets. Sediment samples were collected from the shoreline when the sediment was covered with water using sterile 6 inch PVC corers with a PVC cap on each side. Water samples were transported in labeled one liter screw-cap polypropylene bottles, sediment samples in sterilized corers and then held/transported in sterile whirlpak bags.

Sub-surface Seepage

Groundwater seepage into the creek was sampled at three of the five locations where samples for water and sediments were taken, immediately upstream of 18499, 18500 and 18501. Samples were collected in duplicate at each site. Seepage meters were used to collect the samples following a procedure modified from Lee (1977). The meters were constructed as described in the QAPP, built from the bottom 15cm of a 5-gallon plastic bucket, with 50-150cm tubing (depending on water depth) and a collapsible wall triple laminate bag. The meters were located in a relatively soft area of the stream bed and pushed into the bed by hand. Tubing was attached and filled with ambient water and a collapsible collection bag containing 100mL of de-ionized water was then attached, with time of attachment noted on the field data sheet. After a minimum of 24 hours and maximum of 4 days, the collection bag was removed, capped and transported to the lab for analysis. Seepage sampling occurred following rainfall for four separate events, with only one sample per site for the first two events. Based on Year 1 results this sampling was not continued.

Soil

Soil samples were collected from five fields, selected on the basis of differing crop cover, location in watershed, drainage variation within the field, proximity to the creek and access (Figure 5, Table 4). Selection of the five locations was made in consultation with the Nueces Soil and Water Conservation District #357, local USDA NRCS field staff, TSSWCB Regional Office personnel, and Texas AgriLife Extension Service county agents. Due to drought conditions, crops planted in year 1 were atypical, and cotton was planted only in a few fields. However, in year 2, cotton was planted in all the fields sampled from year 1 with the exception of one field under pasture throughout the period of the study. Soil samples were collected according to the procedures in the Soil Science Society of America's *Methods of Soil Analysis Part 2 – Microbiological and Biochemical Properties* (1994). Two composite samples were collected at each location for each sampling event, one from a well-drained higher elevation area and one from a poorly drained portion of the field. Samples were taken from the top six inches of soil from up to five closely spaced (1-2 meters) sub-sites. Cores containing the samples were transported on ice within Whirl-Pak® bags. The subsamples for a site were combined into a single composite sample in a large Whirl-Pak® bag. Samples for Year 1 were collected during five (5) sampling events: three (3) wet (following rainfall) and two (2) dry events. Four additional events were collected during Year 2: three (3) dry and one (1) wet event to follow up from the initial Year 1 results.

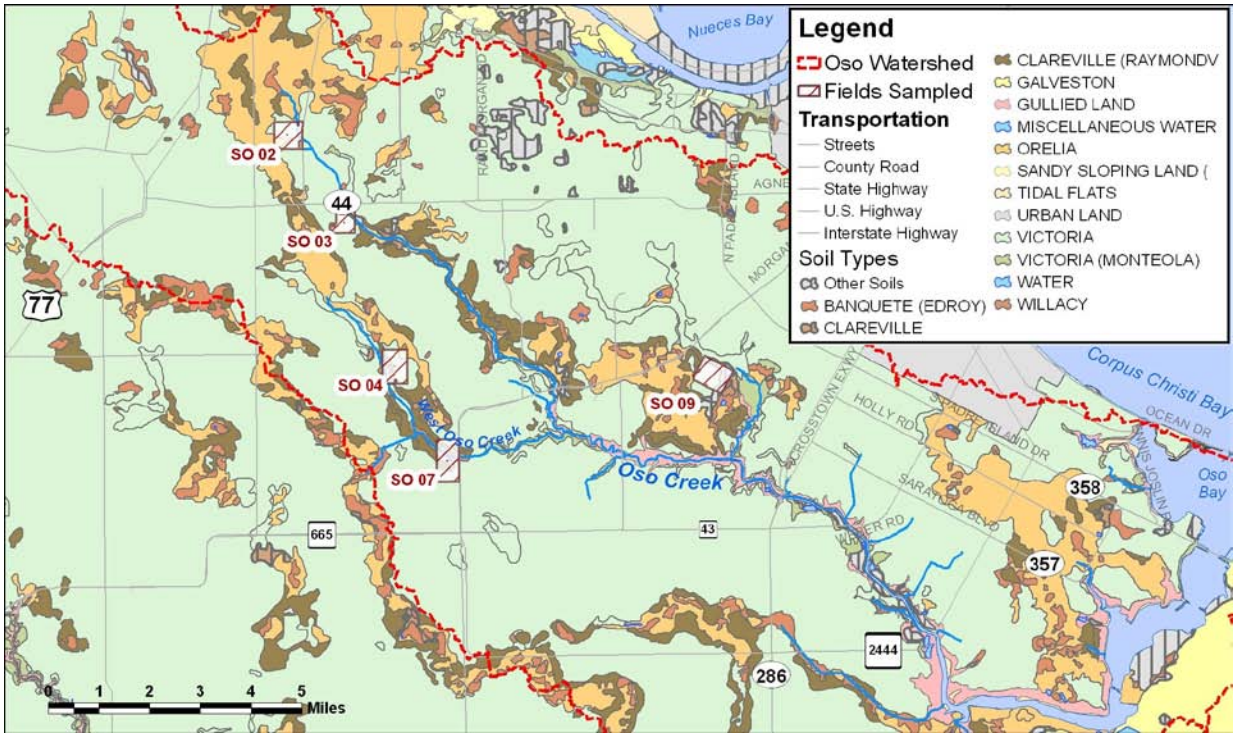

Figure 5. Map showing locations of the fields in the Upper Oso Watershed.

Table 4. Soil sampling sites (note, fields were sampled both during the crop growing season and during periods where no crop was being grown).

Site	Location (Soil types)	UTM14-E	UTM14-N	Crop Cover Year 1	Crop Cover Year 2
SO 02	W. side CR 24 S. of FM 1889 (Victoria/Orelia)	637072	3076280	Sorghum	Cotton
SO 03	S. side SH 44 E. of CR 61 W. of Oso Creek (Victoria)	638880	3073450	No crop cover	Cotton
SO 04	CR 30 N. side E. of CR 61 - (Victoria/Clareville)	640500	3068810	Sesame	Cotton
SO 07	FM 665 W. side N. of FM 43- (Victoria/Hidalgo/Clareville)	642182	3065770	Pasture	Pasture
SO 09	N. side of SH357 between CR35 and CR37 (Victoria)	650405	3068271	Cotton	Cotton

Fecal Samples

Fecal samples were collected as per the QAPP for the project and approved in a special study plan "Application of antibiotic resistance patterns to differentiate sources of *E. coli* in coastal waters of Texas" (2000), prepared by Dr. Mott for TCEQ. Samples were collected in pre-packaged sterile, polypropylene, screw cap specimen containers or using pre-packaged sterile BD BBL EZ Culture swabs. Procedures differed depending on the source of the fecal material. Human/sewage samples were collected directly from untreated (influent) and treated (effluent) sewage at the Robstown WWTF using sterile specimen containers. Livestock and companion animal (pet) samples were collected using sterile swabs, with the appropriate permission from owners or from veterinary clinics and with the stipulations that the animals must reside within the watershed and antibiotics had not been administered to the animals within six months. Fecal samples from avian wildlife (birds) were collected only from visually observed depositions. Wildlife samples (avian and non-avian) were obtained in collaboration with TPWD personnel using TPWD approved methods, from fecal samples from areas where animals were visually observed defecating or from gut samples from animals recently killed by cars (within 24 hours) or legally harvested by hunters who agreed to work with TAMU-CC. Samples taken directly from the gut were collected by using sterile swabs inserted anally or by swabbing the intestine after dissection of abdomen using a sterile scalpel. All swabs and specimen containers were held in sterile biohazard bags and placed on ice until delivery to the lab. Specific locations were documented on field data sheets.

Water and sediment sampling for enterococci isolations

Water samples were collected during four seasonal sampling events (three ambient dry and one following rainfall) from the three historic upstream stations (18499, 18500, 18501) for isolation of approximately 600 unknown source creek water enterococci. Additionally approximately 200 isolates were obtained from sediment collected at five historic stations (18499, 18500, 18501, 20559, 20198).

Laboratory Analytical Methods

Isolation of Enterococcus from Water, Sediment, and Soil Samples

Laboratory analysis of samples followed procedures from *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008)), except for analysis for which no standard methods exist (i.e., BST).

Enterococci in water samples were enumerated following EPA Method 1600: Membrane Filter Test Method for *Enterococcus* in Water (1997, 2000, 2002). Sediment and soil samples (50 g) were placed in 500 mL Phosphate Buffer Saline (PBS) and shaken for

one hour using a Burrell Wrist Action Shaker (Zuberer, 1994). Supernatant was then extracted and filtered following Method 1600. All plates were counted and the numbers of colony forming units (CFUs) were recorded. Laboratory duplicates and method blanks were run with each analytical batch for quality control. For each membrane filter test sterility of the media, petri dishes, membrane filters, dilution water and apparatus were checked using about 20 ml sterile water. The analysis of equipment blanks should yield values less than the minimum analytical limit (1 colony per volume). A blank was run at the start and end of each sample analyzed. Normally data from samples with growth on blanks were omitted; however, in cases where extremely high levels of bacteria were present in the sample, the blank run at the end of the group should have less than 1% of the colonies on the sample filter of the highest volume filtered. Corrective action was implemented if these values were exceeded.

Isolation of Enterococcus from Fecal Samples

Enterococci were isolated from fecal samples following methods included in the QAPP; mEnterococcus plates (Difco) were streaked for isolation with fecal swabs, using standard microbiological techniques, and incubated for 48 hours at 41°C. Wastewater influent and effluent samples were analyzed with two different methods. Initially sterile culture swabs were dipped into well shaken sample containers and streaked onto mE agar followed by incubation for 48 hours at 41°C. This technique did not prove as effective for analysis of these samples so subsequent isolations of enterococci were performed as follows. Influent and effluent samples were analyzed with membrane filtration following EPA Method 1600: Membrane Filter Test Method for *Enterococcus* in Water (1997, 2000). For effluent varying volumes from 1, 3, 10, 30, and 100mL were filtered and for influent volumes varied from 0.01 mL to 10 mL. Isolated colonies were transferred to mE agar plates to obtain pure cultures. At least two pure isolates with a maroon hue were transferred from each plate to Difco Tryptic Soy Agar (Becton Dickinson and Co.) slants for storage until further analysis.

Bacterial Source Tracking Analysis-Carbon Source Utilization

Enterococci isolates considered to be pure after isolation from fecal samples were confirmed as *Enterococcus*, speciated, and profiled for carbon source utilization using the Microlog™ Microbial Identification System (Biolog, Inc., Hayward, CA) following the MicroLog™ System Release 4.0 User Guide (Biolog, 1999). Isolates were transferred to Biolog Universal Growth (BUG) plates containing 5% sheep's blood and incubated at 35°C for at least 24 hours. Suspensions of each isolate were made up to a turbidity of 20%T ± 2% in Inoculating Fluid, according to Biolog procedures and these were used to inoculate GP2 MicroPlates™. After incubation, plates were read using a Biolog MicroStation™ Reader to obtain well color intensity data. If the isolate was confirmed at the genus level but the species was not identified, the sample was also read manually and this was noted on the sample ID sheet.

Dependent on the proportion of isolates identified to genus but not to species by the Biolog Microstation™ Reader, those isolates not speciated were swabbed onto mEI

Agar plates and incubated at 41^{o}C for 24h. Isolates whose colonies exhibit a blue halo on mEI Agar (i.e. that would be identified as enterococci by EPA Method 1600) may be included in the database.

Enterococci isolates from environmental water and sediment samples (~25 per sample), collected through one of the sampling events described earlier, were transferred from mEI filtration plates to mEnterococcus plates and incubated at 41°C for 48 hours to obtain pure cultures before following the procedures described above for fecal isolates using the Microlog™ Microbial Identification System. Every effort was made to isolate the required number of isolates. However, it should be noted that in some instances bacteria were not present in sufficient concentrations to achieve this objective. In such cases, the volume filtered and the number of isolates obtained was recorded and analyses proceeded using those isolates that could be obtained.

All cultures analyzed with the Microlog™ Microbial Identification System were maintained on tryptic soy agar (TSA) slants throughout stages of analysis. Isolates were stored permanently in a -80°C freezer.

Quality control for CSU analysis followed protocols from the MicroLog™ System Release 4.0 User Guide (Biolog, 1999). Each GP2 MicroPlate™ has been tested and must have met internal quality control standards before being released for sale. A set of three gram-positive control strains: *Corynebacterium minutissimum* (ATCC 23348), *Rhodococcus equi* (ATCC 6939), and *Staphylococcus aureus* (ATCC 12600) were streaked onto BUG plates, inoculated onto GP2 MicroPlates™ and analyzed with the Biolog MicroStation™ Reader for quality control purposes. An additional positive control of *Enterococcus faecalis*, ATCC strains 19433 or 29212 or both, were run with each analytical batch. The MicroLog™ Microbial Identification System also provides a % similarity of each isolate with known bacteria in the Biolog database.

The CSU profiles of isolates from known source fecal samples collected in this study were supplemented with enterococci CSU profiles from previously collected fecal samples in the Coastal Bend, stored in a database at TAMU-CC which were reviewed and accepted in accordance with a previously approved work plan for a 2002-2003 Texas General Land Office project (Mott et al. 2003). Only animal sources considered relevant to the Oso Creek area were included.

Bacterial Source Tracking Analysis-Antibiotic Resistance Profiling

For isolates confirmed as enterococci by the Microlog™ Microbial Identification System, detailed above, antibiotic resistance profiling was conducted. Additional isolates characterized as enterococci in a previous study from animals collected in the watershed were re-grown from storage at -80°C and also analyzed for antibiotic resistance also. Note that not all isolates could be re-grown and only 382 of the original 421 CSU profiled enterococci were analyzed through ARP. The Kirby Bauer Disk Diffusion method with a panel of antibiotics was used to develop antibiotic resistance

profiles for isolates, following the standard method of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2006, 2008a, 2008b). Zones of inhibition were measured using an automated image analyzer, the Biomic™ Microbiology System (Giles Scientific USA, Santa Barbara, CA, USA), to ensure uniformity for future comparisons with *Enterococcus* isolates from unknown sources as detailed in the TAMU-CC Standard Operating Procedure (SOP) (available on request) following National Committee for Clinical Laboratory Standards (2006) as approved in a previous QAPP (2003) "Development of an E. coli bacterial source tracking library and assessment of bacterial sources impacting Lake Waco and Lake Belton" (TSSWCB project 02-10). Antibiotics used for antibiotic resistance profiling are shown in Table 5. A standard bacterial strain from ATCC™ (American Type Culture Collection, Manassas, VA) for which acceptable zone diameters to certain antibiotics have been determined by CLSI, were included with each analytical batch to ensure consistency and to validate results, following published clinical standards, specifically *Staphylococcus aureus* spp. *aureus* (ATCC 25923) (CLSI 2006, 2008a). Laboratory duplicates were also run with each batch at a rate of 10%. When zone diameters for duplicates differed more than +/- 3mm from the original sample for more than a single antibiotic, the data was examined by the TAMU-CC QAO and professional judgment was used to determine validity of the sample and whether any other course of action was warranted (i.e. re-training of analyst).

Table 5. Antibiotic Concentrations Used for Antibiotic Resistance Profiling.

Antibiotic	Abbreviation	Concentration
Ampicillin	AM	10 µg
Augmentin	AmC	30 µg
Cefazolin	CZ	30 µg
Cefotaxime	CTX	30 µg
Ceftazideme	CAZ	30 µg
Cefttriaxone	CRO	30 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Doxycycline	D	30 mg
Enrofloxacin	ENO	5 µg
Gentamicin	GM	10 µg
Imipenem	IPM	10 µg
Kanamycin	K	30 µg
Nalidixic acid	NA	30 µg
Neomycin	N	30 µg
Spectinomycin	SPT	100 µg
Streptomycin	S	10 µg
Sulfamethoxazole Trimethoprim	SXT	23.75µg/1.25 µg
Sulfisoxazole	G	0.25 mg
Tetracycline	Te	30 µg
Vancomycin	V	30 µg

Bacterial Source Tracking Analysis-esp Gene Analysis

A subset of water samples was analyzed for the presence of the *esp* gene following the TAMU-CC/USF Harwood protocol described in the project QAPP, based on McQuaig et al. (2006).

Environmental water samples were first filtered following USEPA Method 1600, but with incubation time extended to increase growth. Colonies of *Enterococcus* were then washed from filters, and DNA was extracted from the sample with Qiagen DNeasy Blood and Tissue Kit and ASL buffer (Qiagen, Inc.). Extraction was performed utilizing InhibitEX (Qiagen, Inc.) to minimize inhibition of PCR analysis. PCR was then performed on a reaction mix using GoTaq Green mix (Promega) according to manufacturer's instruction and primers specific for the *esp* gene—*esp* forward primer (5'-TAT GAA AGC AAC AGC ACA AGT T-3') (Scott et al. 2005) and *esp* reverse primer (5'-ACG TCG AAA GTT CGA TTT CC-3') (Hammerum and Jensen 2002). The product was visualized by agarose gel electrophoresis using a Promega ladder with the expected product at the 680bp mark. A no-DNA PCR negative control, an extraction blank and a positive control of *Enterococcus faecium* C68 genomic DNA were included in each analytical batch. EB, a DNA extraction blank, was run using materials from the DNA extraction kit without the addition of bacterial culture. Additional controls, SP1, SP2 and MB were carried through the entire analysis from sample processing through electrophoresis. SP1 was composed of 300mL of composite sample (all environmental samples combined) spiked with 100µL of overnight culture of *Enterococcus faecium* C68, filtered through a 0.45µm nitrocellulose filter. SP2 was composed of 300mL of buffered dilution water spiked with 100µL of overnight culture of *Enterococcus faecium* C68, filtered through a 0.45µm nitrocellulose filter. MB was composed of 300mL buffered dilution water filtered through a 0.45µm nitrocellulose filter. SP1 and SP2 were used to check for inhibitors present in the environmental samples. A positive result for both SP1 and SP2 indicated no inhibition, whereas a positive for SP2 and a negative for SP1 denoted possible inhibition.

Enterococcus Survival Studies in Soil

Based on initial sampling results, additional small scale studies to examine survival and growth of enterococci in agricultural field soil were conducted. Several factors that potentially influence survival: air temperature, soil moisture and bacterial strain, were investigated. Preliminary studies revealed *Enterococcus mundtii* to be a widespread species of *Enterococcus* in local soils (based on analysis of soil isolates using the Microlog™ Microbial Identification System) and a strain of this species isolated from soil at SO02G on 02/11/11 was therefore selected as the test strain for the experiments. The studies examined survival of both a laboratory (ATCC 43186) and the environmental strain of *E. mundti* at various temperatures under moist and dry conditions in soil collected from soil sampling site SO02.

Topsoil was collected in June 2011, following a period of dry weather, mixed and placed in columns. The initial *Enterococcus* concentration in the soil and all enterococci enumerations for the experiments were determined following procedures outlined above under *Isolation of Enterococcus from Water, Sediment, and Soil Samples.* Initial concentratons were found to be less than 1 cfu/gram dry weight of soil. Columns were then subdivided by moisture content. Samples considered "dry" soil had an initial moisture content of 9.16% and had no additional water added. Samples considered "wet" soil had ~1" of water added (to simulate rainfall effects), bringing the moisture content up to 36.42%. All columns were inoculated with either environmental or ATCC strain of *E. mundtii.,* with the exception of control columns which were not inoculated and were used to demonstrate that *Enterococcus* concentrations remained less than 1 cfu/gdw throughout each experiment. Both dry and wet soil samples were incubated at 10, 25, and 40°C and assayed over time for levels of *Enterococcus mundtii*. For all assays triplicate samples were removed from the experimental conditions and enterococci levels determined. Each experiment was conducted twice.

Additional Quality Control Measures

All QA/QC measures detailed in the QAPP for the project were followed. QC samples were run (positive and negative controls for selective media and positive controls and sterility checks for all batches of media) as specified in Section 9020 B. *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 2005). Media which supported growth of a negative control, did not support growth of the positive control or failed the sterility check was discarded. Other QC standards specific for individual methods were included as mentioned under the method and detailed in the QAPP.

Data Analysis

Enterococci levels in samples from the various environmental matrices (water, sediment and soil) were tabulated and compared with historical data and in relation to rainfall. Levels in groundwater, subsurface seepage, sediment and soil were evaluated to identify which of these sources contained levels of enterococci that would identify them as potential sources contributing enterococci to the creek.

Bacterial source tracking data (color intensity data for carbon source utilization and zone diameter data for antibiotic resistance of fecal source and environmental source enterococci) were transferred to SPSS® spreadsheets and analyzed with the statistical technique Discriminant Analysis (DA) using IBM® SPSS® Statistics Version 19. The enterococci in fecal samples and the environment were also identified to genus or species.

A library of CSU and ARA profiles of known source fecal enterococci isolates was constructed using isolates collected in this study and supplemented with some existing

data (~ 421 CSU profiles for *Enterococcus*) in a database stored at TAMU-CC which was previously collected, reviewed and accepted in accordance with a previously approved work plan (Texas General Land Office 2002 – see above). Some of the animal sources from the previous study were judged not to be representative of Oso watershed sources and were omitted. Data from the existing isolates was compared with the new isolates for consistency and decisions made on inclusion of all/some/none of the previously collected isolates would be included in the library. Data obtained from CSU and ARA profiles of unknown source enterococci collected in the study were then compared with those in the constructed library using DA to categorize the unknown source isolates to animal source.

Results

Field measurement data and observations are included in Appendices.

Water sampling of historic TCEQ stations

Water at the three historic stations (18499, 18500 and 18501) was sampled quarterly for a total of 10 sampling events from April 2009 to August 2011. Station 18501 was dry or only had small pools of water for certain sampling events and thus could not always be sampled. A total of 65 instream surface water samples were able to be collected and were analyzed for enterococci levels in duplicate (samples A & B). A third, Sample C, was collected at each station on October 2010 to provide additional subsamples for *esp* gene analysis. Three events were considered wet events (following rainfall): 1/20/10, 7/19/10, and 1/19/11. Results are included in Table 6 and showed consistently high levels of enterococci at all three stations for each sampling event. The average *Enterococcus* level for station 18499 was 1,410 cfu/100mL, with levels ranging from 113-5,500 cfu/100mL throughout the sampling period. At 18500 and 18501 the averages were 1,605 cfu/100mL and 1,468 cfu/100mL, respectively with a range of 165-6,433 cfu/100mL at 18500 and 2-6,690 cfu/100mL at 18501. Only one station (18501), for one sampling event (04/20/11) had levels below the U.S. EPA single sample standard of 104 cfu/100mL. While numbers tended to be higher following rainfall, levels were also elevated in dry weather. The average levels at each site for dry events versus wet events were: 18499 (dry-935cfu/100mL; wet-2,564cfu/100mL), 18500 (dry-834cfu/100mL; wet-3,475cfu/100mL), and 18501 (dry-261cfu/100mL; wet-3,192cfu/100mL). The enterococci levels found during the period of the study were compared with those collected in previous studies (Hay and Mott, 2005, 2006) for each station (Figure 6, Figure 7, Figure 8) and demonstrated the trend of consistently elevated levels at all three stations has continued through 2011.

Table 6. *Enterococcus* levels in surface water sample from historic TCEQ stations (results expressed in colonies per 100mL of water).

Date	Site	Sample A	Sample B	Sample C	Rainfall within last 7 days (inch)
04/24/09	18499	150	113	NA	0.00
04/24/09	18500	165	167	NA	0.00
04/24/09	18501	DRY	DRY	NA	0.00
07/20/09	18499	300	240	NA	0.00
07/20/09	18500	570	320	NA	0.00
07/20/09	18501	DRY	DRY	NA	0.00
10/19/09	18499	5500	3100	NA	0.44
10/19/09	18500	2600	2100	NA	0.44
10/19/09	18501	430	320	NA	0.44
01/20/10	18499	1210	1470	NA	2.68
01/20/10	18500	710	750	NA	2.68
01/20/10	18501	4320	6690	NA	2.68
04/28/10	18499	400	430	NA	0.01
04/28/10	18500	760	520	NA	0.01
04/28/10	18501	270	270	NA	0.01
07/19/10	18499	3033	2667	NA	1.24
07/19/10	18500	6433	8633	NA	1.24
07/19/10	18501	1033	733	NA	1.24
10/18/10	18499	500	310	730	0.44
10/18/10	18500	1010	910	1030	0.44
10/18/10	18501	590	260	420	0.44
01/19/11	18499	3200	2433	3933	2.38
01/19/11	18500	2433	2600	2767	2.38
01/19/11	18501	3267	3200	3100	2.38
04/20/11	18499	167	167	123	0.01
04/20/11	18500	608	600	500	0.01
04/20/11	18501	2	26	30	0.01
08/08/11	18499	1600	1200	867	0.00
08/08/11	18500	700	766	867	0.00
08/08/11	18501	DRY	DRY	DRY	0.00

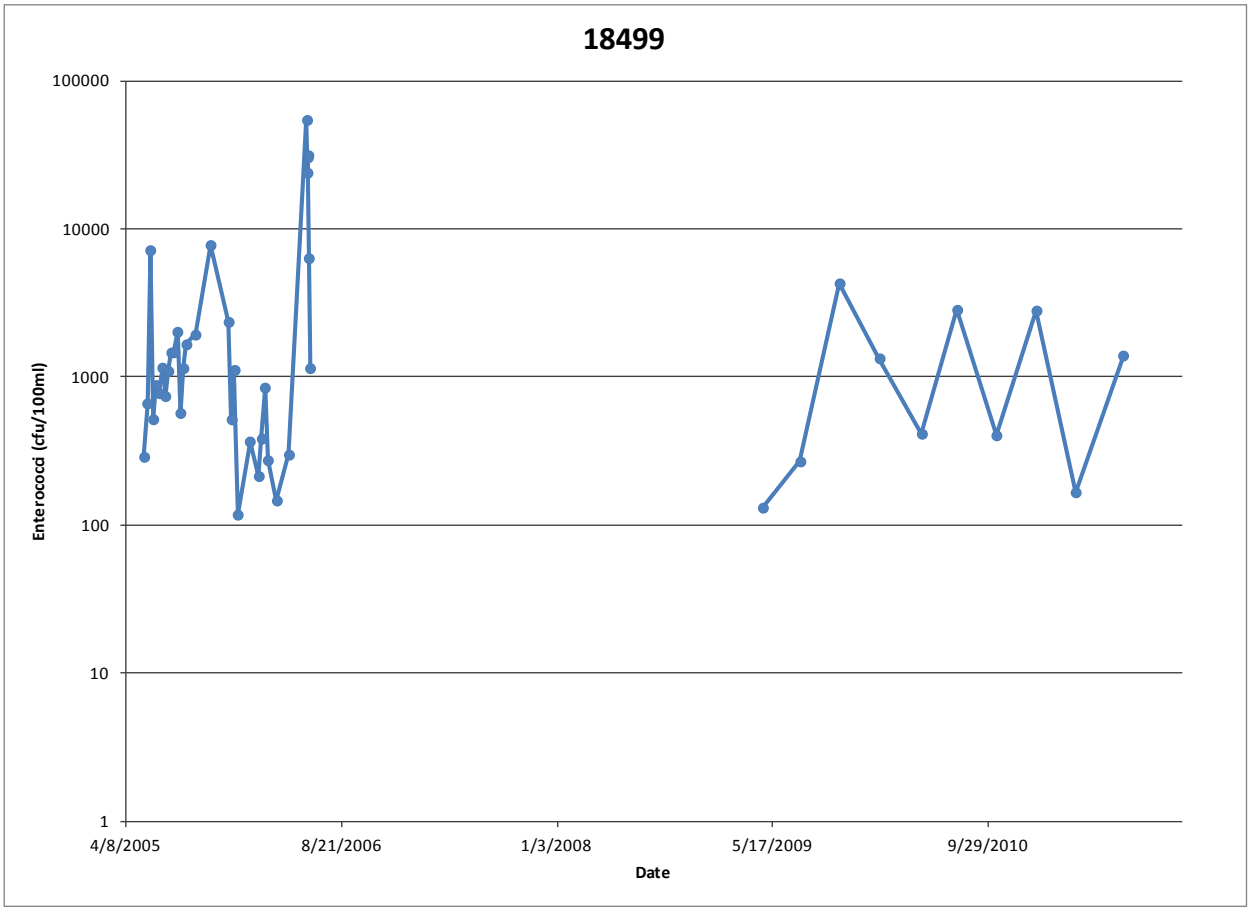

Figure 6. Enterococci levels at historic station 18499 from current and previous studies conducted under approved QAPPs (Hay and Mott 2005, 2006).

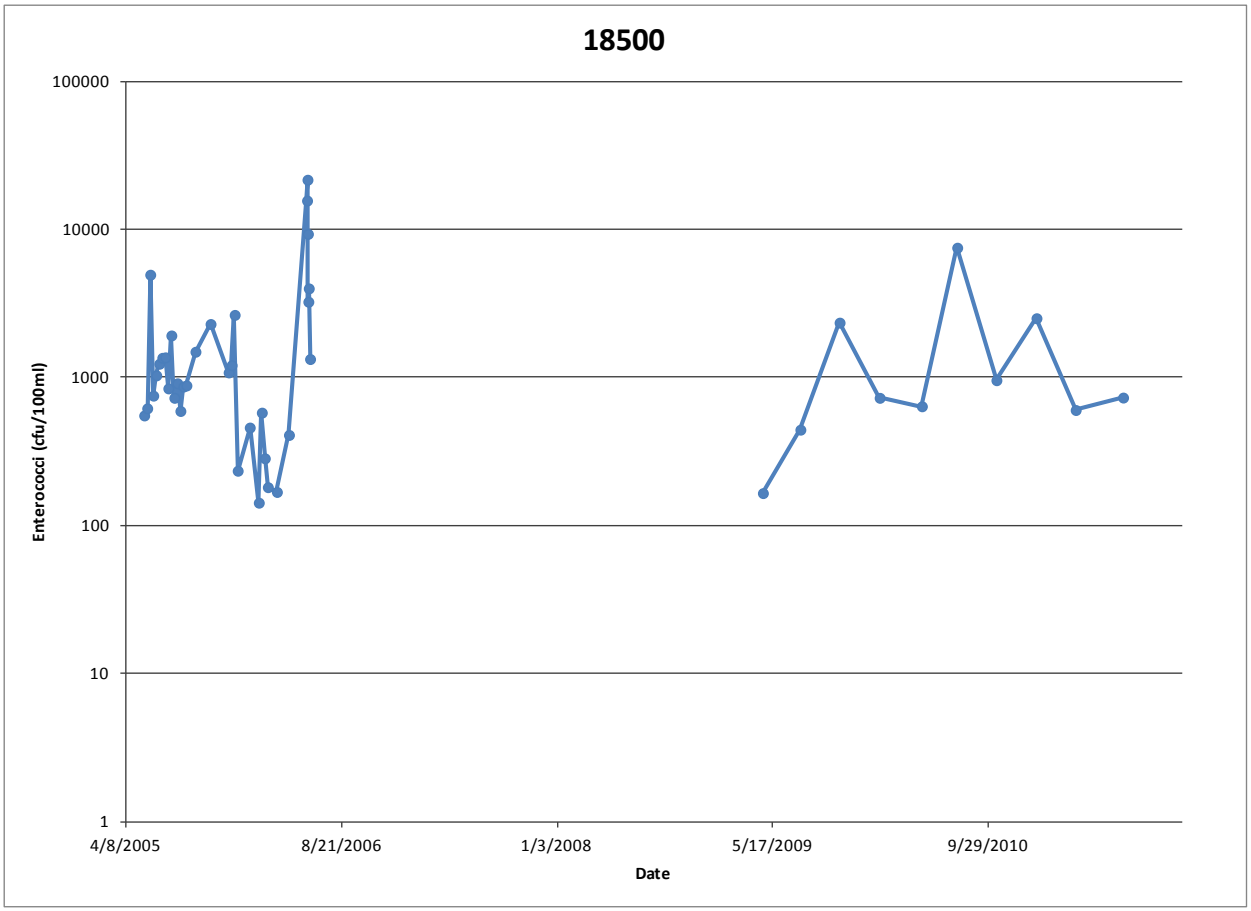

Figure 7. Enterococci levels at historic station 18500 from current and previous studies conducted under approved QAPPs (Hay and Mott 2005, 2006).

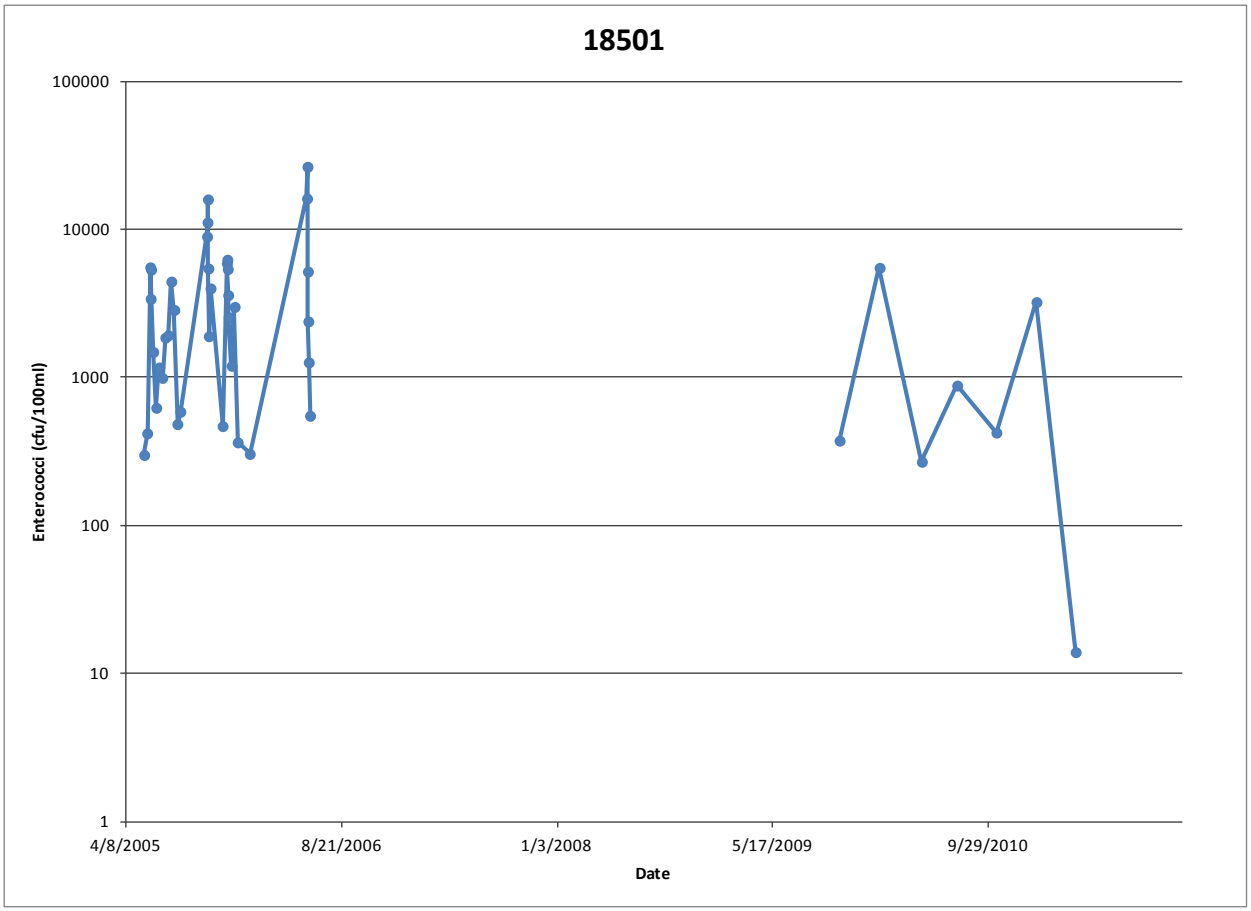

Figure 8. Enterococci levels at historic station 18501 from current and previous studies conducted under approved QAPPs (Hay and Mott 2005, 2006).

A previous study (Mott and Hay, 2009. Oso Creek Bacteria Contamination Investigation. CBBEP Project Number 0916. Publication CBBEP – 61 CBBEP Contract Number: 0816. 2008-2009), included enterococci data collected under an approved Quality Assurance Project Plan. In the study, the entire length of Oso Creek was observed and locations of any discharges were recorded and photographed, along with analysis of water samples for enterococci levels from actively discharging sites for the entire length of Oso Creek (Figure 9). As can be seen from the map (Figure 9), despite numerous potential discharge points in the upper creek, only one was actively flowing during the period of the study (shown by green, vs. red points), and was sampled (OCB003). Table 7 shows enterococci level results from this site. This inflow generally contained low levels of *Enterococcus* (72% samples were within the EPA single sample criterion of 104 cfu/100mL) with the exception of two sampling events where numbers exceeded 2000 cfu/100 mL. No potential or active inflows were observed along west Oso Creek.

Four additional flowing discharges were observed slightly downstream – (OCB063-066). Of these, only one site (OCB066) was found to discharge water with elevated levels of enterococci into the creek (Table 8). The site is located approximately 500 m downstream from FM-2292. It is a large ditch that empties into Oso Creek between Old

Brownsville Rd and FM-2292. For access reasons, the samples were taken from a road that crosses the ditch just upstream from the intersection of the ditch and the creek. The *Enterococcus* levels at this site ranged from 23 to 3,650 cfu/100mL. The average level was 1,057 cfu/100mL, and the median was 1,020 cfu/100mL.

The other three sites had very low or undetectable levels of enterococci in water samples collected during the study (Table 9, Table 10, Table 11). OCB063 located just downstream from Old Brownsville Rd. is a small plastic pipe coming from the People's Baptist Church WWTP. The water from this pipe flows down a small embankment into Oso Creek. *Enterococcus* levels at this site were extremely low, ranging from <1 to 1 cfu/100mL throughout the sampling events. Mean and median were 1 cfu/100mL, and mode was <1 cfu/100mL. OCB064, located just upstream from FM-763, is a site where water appears out of a hole in the ground. The water flows down the bank and directly into Oso Creek. Not a single *Enterococcus* colony was isolated from the nine sampling events at this location. All levels were reported as <1 cfu/100mL, the minimum detectable limit. OCB065 is located 1.7 km upstream from FM-763. It is a pipe from the Cuddihy WWTP on the same land as OCB063, which is owned by the People's Baptist Church. A pool of water forms over the pipe and gradually flows down a small path towards Oso Creek. *Enterococcus* levels at this site ranged from <1 to 240 cfu/100mL. The average level was 40 cfu/100mL, with a median of 4 cfu/100mL and a mode of <1 cfu/100mL.

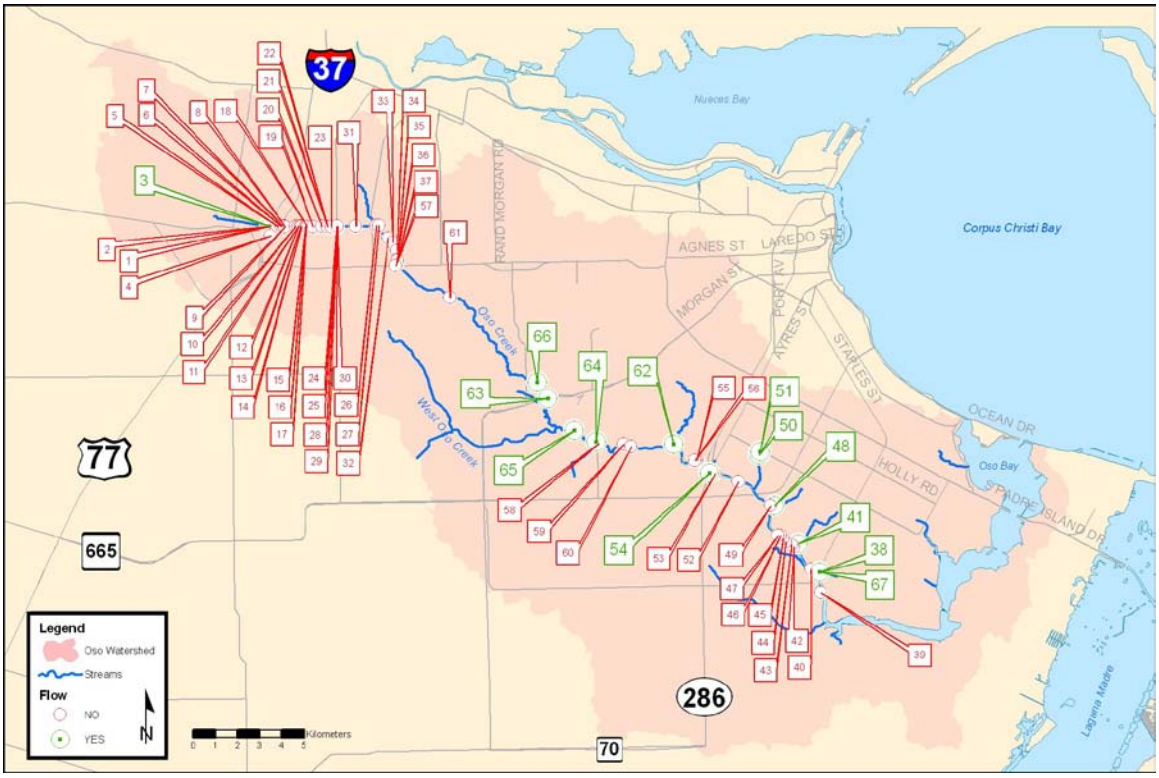

Figure 9. Map showing observed discharge points into Oso Creek.

Table 7. Summary of bacteriological data for OCB003 (Robstown WWTP).

Site #	Date	*Enterococcus* CFU/100 ML				Flow (cfs)
		Sample #1	Sample #2	Duplicate	Average	
OCB003	12/03/08	10	6		8	1.71
OCB003	12/17/08	22	14		18	1.55
OCB003	01/22/09	147	230		189	1.86
OCB003	01/28/09	123	180		152	1.86
OCB003	02/18/09	>2000	>2000	>2000	>2000	1.55
OCB003	02/25/09	12	9		11	1.55
OCB003	03/04/09	23	29		26	1.86
OCB003	03/11/09	127	73		100	2.02
OCB003	03/25/09	130	16		73	1.86
OCB003	04/01/09	14	6		10	1.86
OCB003	04/08/09	2590	2570		2580	1.86
OCB003	04/15/09	4	11		8	1.86
OCB003	04/20/09	< 1	1		1	1.86
OCB003	04/29/09	< 1	< 1		1	1.71
OCB003	05/06/09	16	16	14	15	2.02
OCB003	05/11/09	< 1	< 1	9	4	2.02
OCB003	05/20/09	26	30		28	1.71
OCB003	05/25/09	61	63		62	1.71

Table 8. Summary of bacteriological data for OCB066 (Clarkwood Ditch).

Site #	Date	Enterococcus CFU/100 ML				Flow (cfs)
		Sample #1	Sample #2	Duplicate	Average	
OCB066	04/20/09	47	80		64	Stagnant
OCB066	04/29/09	208	260		234	Stagnant
OCB066	05/06/09	40	23		32	Stagnant
OCB066	05/11/09	3650	3520		3585	Stagnant
OCB066	05/20/09	1260	1210	1100	1235	Stagnant
OCB066	05/25/09	1320	1020		1170	Stagnant

Table 9. Summary of bacteriological data for OCB065 (Cuddihy WWTP).

Site #	Date	*Enterococcus* CFU/100 ML				Flow (cfs)
		Sample #1	Sample #2	Duplicate	Average	
OCB065	04/01/09	< 1	< 1		< 1	< 0.01
OCB065	04/08/09	< 1	4		3	< 0.01
OCB065	04/15/09	1	< 1		1	0.09
OCB065	04/20/09	1	22		12	0.08
OCB065	04/29/09	< 1	< 1		< 1	0.08
OCB065	05/06/09	< 1	< 1		< 1	0.03
OCB065	05/11/09	1	< 1		1	0.03
OCB065	05/20/09	< 1	240		121	0.02
OCB065	05/25/09	< 1	8		5	0.02

Table 10. Summary of bacteriological data for OCB064 (FM763).

Site #	Date	*Enterococcus* CFU/100 ML				Flow (cfs)
		Sample #1	Sample #2	Duplicate	Average	
OCB064	04/01/09	< 1	<1		<1	0.01
OCB064	04/08/09	< 1	< 1		<1	0.01
OCB064	04/15/09	< 1	< 1		<1	<0.01
OCB064	04/20/09	< 1	< 1		<1	<0.01
OCB064	04/29/09	< 1	< 1	< 1	<1	<0.01
OCB064	05/06/09	< 1	< 1		<1	<0.01
OCB064	05/11/09	< 1	< 1		<1	<0.01
OCB064	05/20/09	< 1	< 1		<1	0.01
OCB064	05/25/09	< 1	< 1		<1	0.01

Table 11. Summary of bacteriological data for OCB063 (People's Baptist WWTP).

Site #	Date	*Enterococcus* CFU/100 ML				Flow (cfs)
		Sample #1	Sample #2	Duplicate	Average	
OCB063	04/01/09	<1	<1	<1	<1	0.01
OCB063	04/08/09	<1	<1		<1	0.01
OCB063	04/15/09	< 1	< 1		<1	<0.01
OCB063	04/20/09	< 1	< 1		<1	0.01
OCB063	04/29/09	< 1	< 1		<1	0.01
OCB063	05/06/09	1	1		1	<0.01
OCB063	05/11/09	< 1	< 1		<1	<0.01
OCB063	05/20/09	< 1	< 1		<1	0.01
OCB063	05/25/09	< 1	< 1		<1	0.01

Groundwater (Well) samples

Groundwater samples were collected during six sampling events from June 2009 through February 2011 from ten paired - shallow (sample A) and deep (sample B) paired - wells. Sampling events in September 2009 and July 2010 were considered wet events (following rainfall). *Enterococcus* levels ranged from <1 to 2,900 cfu/100mL overall (Table 12). However, the average level for each site (with Samples A and B averaged together over time) ranged from 1 to 656 cfu/100mL, with GW15 having the lowest average (1 cfu/100mL) and GW14 and having the highest average of 656 cfu/100mL (Table 13).

Table 12. Enterococcus results from groundwater sampling (Sample A=shallow well average of two samples, Sample B=deep well average of two samples).

Date	Site	Sample A (cfu/100mL)	Sample B (cfu/100mL)	Rainfall within last 7 days (inch)
06/09/09	GW08	NA	80	0.98
06/09/09	GW09	9	100	0.98
06/10/09	GW10	6	15	0.98
06/09/09	GW14	2800	25	0.98
06/10/09	GW15	NA	<1	0.98
09/14/09	GW08	136	2533	4.51
09/14/09	GW09	850	<1	4.51
09/14/09	GW10	<1	10	4.51
09/14/09	GW14	173	<1	4.51
09/14/09	GW15	<1	NA	4.51
10/26/09	GW08	NA	61	1.23
10/26/09	GW09	74	250	1.23
10/26/09	GW10	< 1	73	1.23
10/26/09	GW14	4	< 1	1.23
10/26/09	GW15	NA	< 1	1.23
03/29/10	GW08	NA	<1	0.06
03/29/10	GW09	53	<1	0.06
03/29/10	GW10	<1	<1	0.06
03/29/10	GW14	<1	<1	0.06
03/29/10	GW15	<1	<1	0.06
07/06/10	GW08	<1	<1	6.23
07/06/10	GW09	2	<1	6.23
07/06/10	GW10	<1	<1	6.23
07/06/10	GW14	1967	2900	6.23
07/06/10	GW15	<1	<1	6.23
02/07/11	GW08	NA	23	0.05

Date	Site	Sample A (cfu/100mL)	Sample B (cfu/100mL)	Rainfall within last 7 days (inch)
02/07/11	GW09	35	<1	0.05
02/07/11	GW10	<1	<1	0.05
02/07/11	GW14	<1	<1	0.05
02/07/11	GW15	<1	<1	0.05

Table 13. Average enterococci level for groundwater samples by sampling site; Shallow and deep well combined by sampling site.

Site	Average *Enterococcus* Count (cfu/100mL)
GW08	355
GW09	115
GW10	9
GW14	656
GW15	1

Additional information on nutrient and groundwater levels in the watershed obtained from another project conducted by the Center for Water Supply Studies (2011) (CBBEP Oso Watershed Characterization – Ground Water Monitoring Quality Assurance Project Plan. CBBEP Project No. 0541. 2008-2009), is included here. Temperature data was collected to be used as a proxy for effective flow to establish surface and groundwater connections, but was not helpful in determining groundwater flux to the creek. Equation 1 was used as an alternative method. Table 14 shows the data from the CBBEP groundwater/surface water interaction project. Groundwater samples collected from all deep monitoring wells, were submitted to Xenco Laboratory, and analyzed for the presence of nitrogen and phosphorous.

Table 14. Nutrient and bacteria results from monitoring wells.

Well_ID	DEPTH (feet)	N-NH3 mg/l	N-Nitrite mg/l	N-Nitrate mg/l	N-Kjeldahl mg/l	P-Total mg/l	P-Dissolved mg/l	P-Ortho mg/l	Entero (Max) cfu/100ml	Entero (Min) cfu/100ml
GW08A	-	-	-	-	-	-	-	-	136	1
GW08B	9.55	0.214	0.152	0.113	0.326	0.120	0.014	8.540	2533	1
GW09A	-	-	-	-	-	-	-	-	850	2
GW09B	8.67	0.228	0.152	0.113	0.411	0.370	0.024	4.720	250	1
GW10A	-	-	-	-	-	-	-	-	6	1
GW10B	7.29	0.130	0.152	16.400	0.245	1.680	0.091	4.430	73	1
GW14A	-	-	-	-	-	-	-	-	2800	1
GW14B	6.88	0.089	0.152	3.060	0.399	1.530	0.081	4.520	2900	1
GW15A	-	-	-	-	-	-	-	-	1	1

Well_ID	DEPTH	N-NH3	N-Nitrite	N-Nitrate	N-Kjeldahl	P-Total	P-Dissolved	P-Ortho	Entero (Max)	Entero (Min)
GW15B	9.84	0.186	0.050	0.669	0.742	1.570	0.071	0.020	1	1

- Not Sampled

Nitrogen occurs in an oxidized state such as nitrate (NO_3^-) and nitrite (NO_2^-) or a reduced state like ammonium (NH_4^+) and cyanide (CN^-). Nitrogen can also be bound to organic matter as amino acids. Microbes generally facilitate oxidation and reduction of nitrogen in groundwater and soil. Ammonia for example can be oxidized to a highly reactive nitrite, which is in turn oxidized rapidly to nitrate. Nitrites in groundwater are usually found in low concentrations because of this reactivity (Fetter, 1999). Nitrate concentrations in shallow unconsolidated sand beneath forest or permanent pasture were found to be less than 1 mg/l, however beneath heavily fertilized potato fields, concentrations were found in excess of 10 mg/l (Hill, 1982). In Nueces County groundwater, average concentrations of nitrate, nitrite and ammonia (expressed as nitrogen) are 0.914 mg/l, 0.056 mg/l and 0.082 mg/l respectively (Shafer, 1968; TWDB, 2005)

Phosphorous in natural waters occurs primarily in the +5 valance state. Dissolved phosphorous occurs primarily as orthophosphate ions and is readily sorbed to soil, making it's mobility limited in the subsurface (Fetter, 1999). Average concentrations of dissolved phosphorous and total phosphorous in Nueces County groundwater are 0.01mg/l and 0.053 mg/l respectively (Shafer, 1968; TWDB, 2005). Phosphorous values measured in this study averaged 1.121 mg/l (total phosphorous) and were generally high throughout the basin. Dissolved phosphorous values averaged 0.107 mg/l, but are notably higher in the vicinity of Oso Bay and the lower tidal section of Oso Creek. Dissolved Orthophosphate averaged 2.67 mg/l and was notably higher in the non-tidal and fresher sections of the watershed.

Nutrients were measured in surface water at 20198 on West Oso Creek in an agricultural runoff study of Oso Creek Watershed (Ockerman, 2008). Nitrate, nitrite, and ammonia (as nitrogen) measured in groundwater was 16.4 mg/l, <0.152 mg/l, 0.13 mg/l, and respectively. Total nitrogen and ammonia (as nitrogen) measured in surface water was much lower than groundwater at this site. Surface water was collected during runoff events, when total nitrogen and ammonia (as nitrogen) averaged 3.15 mg/l and 0.087 mg/l respectively. Total phosphorous in surface water averaged 0.31 mg/l (Ockerman and Fernandez, 2010) and 1.68 mg/l in groundwater.

The flux of a constituent from the groundwater to the creek can be modeled based on measured values of hydraulic conductivity, average concentrations of the constituent in groundwater, average creek width, average creek depth, segment of interest length, and the maximum measured water table elevation range in each segment. A slug test was performed on many of the wells to provide a value of hydraulic conductivity for modeling groundwater movement. The creek was divided up into segments defined by the location of each monitoring site along the main reach of Oso Creek, and a flux for

each constituent was calculated based on the measured values in this study and Darcy's Law (Equation 1).

Table 15. Nitrogen flux to Oso Creek segments.

Reach	Total Nitrogen Flux to Reach (mg/day)	Increased Total Nitrogen Concentration In Segment (mg/l)
Above 18499	6.55E+0	992.91E-9
18499-18500	2.58E+0	254.33E-9
18500-13029	193.71E-3	45.79E-9

Flux values for total nitrogen (Table 16) ranged from 193.71 $x10^{-3}$ mg/day added to the segment above between 18500 and 13029 to 6.55 mg/day added to the segment above 18499. None of these daily fluxes calculated where great enough to increase the surface water concentrations of a segment by more than 9.93 $x10^{-7}$ mg/l.

Table 16. Phosphorous flux to Oso Creek segments.

Reach	Total Phosphorous Flux to Reach (mg/day)	Increased Total Phosphorous Concentration In Segment (mg/l)
Above 18499	13.87E+0	2.10E-6
18499-18500	2.32E+0	228.96E-9
18500-13029	71.30E-3	16.86E-9

Flux values for total phosphorous from the groundwater to the creek ranged from 71.3 $x10^{-3}$ mg/day added to the segment between 18500-13029 to 13.87 mg/day added to the segment above 18499. None of the calculated daily fluxes were great enough to increase the surface water concentrations by more than $2.10x10^{-6}$ mg/l.

Equation 1. Darcy's Law.

$$Q = -KA\left(\frac{dh}{dl}\right)$$

Where:
Q = discharge
K = hydraulic conductivity
A = Cross-sectional Area
dh/dl = hydraulic gradient

Enterococci concentrations were measured during wet and dry conditions over the course of the project to evaluate groundwater as a potential source of fecal bacteria to the Oso Creek/Oso Bay hydrologic system. Previous studies (Hay and Mott, 2005,

2006), suggested that groundwater influx could be a source of bacteria to Oso Creek and Oso Bay, particularly during dry weather conditions.

Enterococcus concentrations were generally low in groundwater with some exceptions. High values of *Enterococcus* were measured at the groundwater sampling event occurring on 9/14/2009, following the first significant rainfall since well installation. The wells were installed during extremely dry weather and as a result the bentonite grout seal in the annulus of the well between the casing and the soil failed due to lack of initial moisture. This seal relies on moisture to swell the low permeability bentonite clay in the grout so that surface water flowing overland around the well head cannot migrate into the well through this annulus. *Enterococcus* concentrations decreased substantially in subsequent sampling events. Other excursions occurred at the first sampling event (6/9/2009) of GW14A and later in the 7/6/2010 sampling event. High enterococci concentrations during the first sampling event are suspected to have occurred due to the shallowness of the well and the mostly saturated soil. These factors as well as flooding contributed to the high values measured on the 7/6/2010 event. Therefore, high enterococci concentrations measured in the wells can be primarily attributed to contamination from surface water and runoff and have been removed from the remaining discussion since they are not considered to be representative of the ambient groundwater conditions.

Average groundwater concentration of enterococcus was 230 cfu/100ml, but if the events attributed to surface water/runoff contamination are removed it is reduced to 18 cfu/100ml. Only one well exceeded the Texas State Water Quality Standard single sample criteria for Enterococcus of 104 cfu/100ml, GW09B at 250 cfu/100ml. The single sample criterion was exceeded in 5.8% of the samples and the geometric mean criterion of 35 cfu/100 ml was exceeded by 29.4% of the samples.

Approximately 58% of the groundwater samples had *Enterococcus* concentrations of less than 2 cfu/100 ml. By contrast, historic *Enterococcus* concentrations in the surface waters of Oso Creek and Oso Bay averaged 2,918 cfu/100 ml over all events and stations (Hay and Mott, 2006).

The non-tidal Oso Creek had three monitoring sites, 13029, 18500, and 18499 that were co-located with groundwater monitoring wells and had historic *Enterococcus* measurements (Hay and Mott, 2006). Average *Enterococcus* concentration in surface water at station 13029 measured in a previous study was 3,709 cfu/100 ml. Groundwater adjacent to this location (GW08B) in this study averaged 29 cfu/100 ml of *Enterococcus*. Average *Enterococcus* concentration in surface water at station 18500 measured in a previous study was 2,939 cfu/100 ml. Groundwater concentrations of *Enterococcus* adjacent to this location (GW09A&B) in this study averaged 95 cfu/100 ml. Average *Enterococcus* concentration in surface water at station 18499 measured in a previous study 6,183 cfu/100 ml. Groundwater adjacent to this location (GW15A&B) in this study averaged 1.5 cfu/100 ml of *Enterococcus.*

Additionally, three tributaries have water quality stations from two previous studies (Hay and Mott, 2006; Ockerman, 2008) with data that includes *Enterococcus* measurements, 18501 (West Oso Creek at FM665), 08211517 (West Oso Creek at Merritt Road), and 08211525 (Unnamed tributary and FM 2444). Site 08211525 (Ockerman and Fernandez, 2010) is co-located with groundwater monitoring wells at Site 12 (GW07A and GW07B). Historic *Enterococcus* concentrations in surface water at this site averaged 32,560 cfu/100 ml while groundwater data from this study averaged 8 cfu/100 ml. Site 08211525 (Ockerman, 2008) is co-located with Site 16 (GW10A and GW10B). Historic enterococci concentrations in surface water at this site averaged 75,340 cfu/100 ml while groundwater data from this study averaged 19 cfu/100 ml. Surface water data collection at these two sites was focused on high flow events, which typically contain high concentrations of bacteria and nutrients. Surface water data collected at site 18501 (Hay and Mott, 2006) measured average *Enterococcus* concentrations of 4,323 cfu/100 ml and is co-located with Site 25 (GW14A and GW14B) where groundwater concentrations of enterococci averaged 3 cfu/100 ml.

Overall, groundwater concentrations of *Enterococcus* were several orders of magnitude less than surface water. Groundwater concentrations of *Enterococcus* in contaminated fractured limestone aquifers have been reported at concentrations generally below 10 cfu/100 ml, but as high as about 115 cfu/100 ml with about 58% of the samples having greater that 1 cfu/100 ml (Celico et al., 2004) suggesting that even in contaminated aquifers groundwater concentrations of enterococci are very low compared to the adjacent surface water measurement.

Table 17. Estimated flux of Enterococcus from groundwater to surface water in Oso Creek.

Segment Identified by Site ID	Enterococcus Flux (cfu/day)	Increased Enterococcus Concentration In Segment (cfu/m^3)
Above 18499	132.48E+0	20.07E-3
18499-18500	5.97E+3	587.86E-3
18500-13029	172.32E+0	40.74E-3

A flux of enterococci from the groundwater to the creek can be modeled similar to the flux of nutrients with the additional assumption that the bacteria are transported freely through the advection of groundwater. The creek was divided up into segments defined by the location of each monitoring site located along the main reach of Oso Creek and a flux was calculated base on the previously mentioned parameters and Darcy's Law (Equation 1). Flux values ranged from 132 cfu/day added to the non-tidal portion of the segment above 18499 to 5,970 cfu/day added to the segment between 18499 and 18500. None of these daily fluxes calculated were great enough to increase the surface water concentrations by more than 0.59 cfu/m^3.

Sub-surface Seepage samples

Samples were collected from seepage meters constructed for this study, following rainfall for four sampling events from November 2009 through July 2010. Due to the design of the equipment, initially a single sample was collected and analyzed for enterococci, but an additional sample was added for the last two sampling events. Samples contained low numbers of enterococci (<1 to 62 cfu/100mL) and average levels for 18499, 18500, and 18501 were 2, 11 and 27 cfu/100mL, respectively (Table 18). Based on these results, sub-surface seepage was deemed not to be a significant contributing source of enterococci into the upper creek and sampling was discontinued.

Table 18. Enterococci levels in sub-surface seepage samples.

Date	Site	Sample A (cfu/100mL)	Sample B (cfu/100mL)	Rainfall within last 7 days (before placement)	Rainfall within last 7 days (meter removed)
11/11/2009	18499	<1	NA	0	0.43
11/11/2009	18500	2	NA	0	0.43
11/11/2009	18501	49	NA	0	0.43
11/18/2009	18499	1	NA	0	0.07
11/18/2009	18500	57	NA	0	0.07
11/18/2009	18501	1	NA	0	0.07
2/3/2010	18499	6	1	0	1.43
2/3/2010	18500	3	1	0	1.43
2/3/2010	18501	49	62	0	1.43
7/19/2010	18499	<1	<1	0.04	1.24
7/19/2010	18500	<1	<1	0.04	1.24
7/19/2010	18501	<1	<1	0.04	1.24

Agricultural soil samples

Soil samples were collected for enterococci analysis from five locations with two subsites each (one well drained and one poorly drained) from July 2009 through June 2011 for a total of nine sampling events (Table 19). Two sampling events followed at least one inch of rainfall, September 2009 and December 2009. The averages for each site, including the breakdown by well drained and poorly drained subsite averages are summarized in Table 20 below. There did not appear to be a trend in numbers in poorly drained vs well drained soil or under different crop covers. Many samples were collected outside the crop growing season. Numbers during dry summer months were low, but increased following rainfall in the fall of 2009 and enterococci were found during the winter and spring months, (Note- results are reported per gram dry weight and thus levels appear low in comparison with water samples reported per 100mL). However, when the amount of soil displaced during runoff events is considered, these enterococci concentrations can be extremely high as reported in the Oso Creek watershed runoff report by Ockerman and Fernandez 2010 and summarized above. It was therefore

decided to conduct additional laboratory experiments to examine the potential ability of enterococci to survive in Oso Creek watershed soils.

Table 19. Enterococci levels in soil samples from two sites at each of five fields.

Date	Site	Well drained site (cfu/gdw soil)	Poorly drained site (cfu/gdw soil)	Rainfall within last 7 days (inch)
7/29/2009	SO02	159	< 1	NA
8/18/2009	SO03	1	3	0.06
7/29/2009	SO04	< 1	< 1	NA
8/5/2009	SO07	< 1	< 1	NA
8/5/2009	SO09	< 1	< 1	NA
9/19/2009	SO02	9	50	3.95
9/19/2009	SO03	5	98	
9/19/2009	SO04	75	207	
9/19/2009	SO07	13	26	
9/19/2009	SO09	13	2	
12/2/2009	SO02	74	5	1.45
12/2/2009	SO03	4	31	
12/2/2009	SO04	3	31	
12/2/2009	SO07	12	14	
12/2/2009	SO09	< 5	56	
3/3/2010	SO02	1	4	0.13
3/3/2010	SO03	3	10	
3/3/2010	SO04	11	16	
3/3/2010	SO07	1	1	
3/3/2010	SO09	1	97	
5/12/2010	SO02	27	8	0.00
5/12/2010	SO03	4	< 1	
5/12/2010	SO04	< 4	6	
5/12/2010	SO07	2	1	
5/12/2010	SO09	< 5	8	
7/26/2010	SO02	< 4	24	0.35
7/26/2010	SO03	< 4	4	
7/26/2010	SO04	5	141	
7/26/2010	SO07	26	< 1	
7/26/2010	SO09	< 5	< 4	
10/25/2010	SO02	< 1	< 4	0.10
10/25/2010	SO03	< 1	< 4	
10/25/2010	SO04	< 1	< 4	
10/25/2010	SO07	< 1	< 1	
10/25/2010	SO09	< 1	2	
2/9/2011	SO02	< 4	< 5	0.11
2/9/2011	SO03	< 5	5	
2/9/2011	SO04	< 4	< 5	
2/9/2011	SO07	2	< 1	
2/9/2011	SO09	< 5	< 4	

Date	Site	Well drained site (cfu/gdw soil)	Poorly drained site (cfu/gdw soil)	Rainfall within last 7 days (inch)
6/1/2011	SO02	<3	<3	0.00
6/1/2011	SO03	<3	1	
6/1/2011	SO04	<3	1	
6/1/2011	SO07	<3	<3	
6/1/2011	SO09	2	11	

Table 20. Average level of enterococci at soil sampling sites and subsites.

Site	Average (cfu/gdw soil)	Well drained (cfu/gdw soil)	Poorly drained (cfu/gdw soil)
SO02	21	30	11
SO03	10	2	17
SO04	28	11	45
SO07	6	7	5
SO09	11	2	20

Paired surface water and sediment samples

Paired samples of water and sediment were collected from five different sites (18499, 18500, 18501, 20559, 20198) during nine sampling events from May 2009 through March 2011. Three sampling events followed rainfall (11/23/09, 02/17/10, and 09/13/10). Enterococci levels for surface water samples ranged from 24-12,500 cfu/100mL, and levels for sediment samples ranged from <9-684 cfu/100mL (Table 21). The average levels at each site for surface water and sediment samples are summarized in Table 22. It should be noted that the surface water average for 20198 was generated from a single sampling event, as this site was dry for all other surface water sampling dates. Based on the finding that enterococci were present in sediments following rainfall during year 1, sampling was continued through year 2 of the project. However, in year 2 enterococci were frequently not detected in the sediment. Overall, enterococci appeared to increase in the sediments following rainfall, but were very low during dry weather.

Table 21. Levels of enterococci in paired surface water and sediment samples.

Date	Site	Water Sample A (cfu/100mL)	Water Sample B (cfu/100mL)	Water Sample C (cfu/100mL)	Sediment Sample A (cfu/gdw)	Sediment Sample B (cfu/gdw)	Rainfall (within last 7 days) inch
05/08/09	18499	235	270	NA	1	2	0.0
05/08/09	18500	107	110	NA	3	2	0.0
05/08/09	18501	DRY	DRY	DRY	DRY	DRY	0.0
05/08/09	20559	290	280	NA	1	1	0.0
08/11/09	18499	390	933	NA	108	313	0.0
08/11/09	18500	520	410	NA	46	8	0.0
08/11/09	18501	DRY	DRY	DRY	DRY	DRY	0.0
08/11/09	20559	650	480	NA	117	185	0.0
11/11/09	18499	1540	1800	NA	34	NA	0.43
11/11/09	18500	520	640	NA	54	NA	0.43
11/11/09	18501	68	30	NA	< 6	NA	0.43
11/11/09	20559	157	140	NA	15	NA	0.43
11/23/09	18499	137	328	NA	23	109	2.74
11/23/09	18500	64	40	NA	30	40	2.74
11/23/09	18501	64	65	NA	64	60	2.74
11/23/09	20559	249	83	NA	31	27	2.74
02/17/10	18499	1070	1020	NA	814	684	1.49
02/17/10	18500	270	230	NA	3	8	1.49
02/17/10	18501	36	24	NA	7	15	1.49
02/17/10	20559	61	107	NA	9	2	1.49
05/31/10	18499	1350	1520	NA	9	< 7	0.06
05/31/10	18500	620	300	NA	< 4	15	0.06
05/31/10	18501	270	250	NA	1	< 9	0.06
05/31/10	20198	DRY	DRY	NA	10	5	0.06
09/13/10	18499	350	300	567	36	289	2.93
09/13/10	18500	733	1233	1300	4	1	2.93
09/13/10	18501	350	430	500	7	1	2.93
09/13/10	20559	260	360	333	<4	<2	2.93
09/13/10	20198	12500	7500	4700	131	168	2.93
12/06/10	18499	1067	667	1200	17	16	0.02
12/06/10	18500	300	390	400	4	< 1	0.02
12/06/10	18501	833	280	37	1	< 1	0.02
12/06/10	20559	103	83	83	<1	< 5	0.02
12/06/10	20198	DRY	DRY	DRY	<5	< 5	0.02
03/09/11	18499	533	500	867	<1	<1	0.03
03/09/11	18500	450	600	460	<1	5	0.03
03/09/11	18501	83	108	100	28	<5	0.03
03/09/11	20559	190	223	160	<5	<6	0.03
03/09/11	20198	DRY	DRY	DRY	5	5	0.03

Table 22. Average enterococci levels for surface water and sediment samples.

Site	Water (col/gdw)	Sediment (col/gdw)
18499	757	145
18500	441	12
18501	196	14
20599	215	26
20198	8233	39

Bacteria source tracking

A total of 1,120 known source enterococci isolates were included in the construction of the library of CSU and ARP profiles used to compare with unknown source profiles through bacteria source tracking (Table 23 and Table 24). The total number of isolates analyzed with both ARA and CSU are represented by the total number of isolates column (grand total of 1120). Seven different major sources of fecal samples were used to construct the known source library. Included in the bird category were samples from sparrow, grackles, and other birds. Human isolates were collected under this project from wastewater treatment plant influent and effluent. Human isolates collected under a prior project were not included in this known source library as they were primarily from individuals and were judged less representative for the current project. Wildlife isolates were taken from animal feces including skunk, raccoon and other miscellaneous road kill and wildlife sources.

Table 23. Summary of fecal sample enterococci isolates used in the library for bacterial source tracking.

Animal Type	TOTAL		TAMU-CC Existing Library		Current Project Library	
	Number of Samples	Number of Isolates	Number of Samples	Number of Isolates	Number of Samples	Number of Isolates
Bird	25	258	5	14	16	244
Cow	63	221	40	70	23	151
Dog	57	168	49	108	8	60
Horse	17	92	0	0	17	92
Human	17	111	0	0	17	111
Gull	38	87	38	87	0	0
Wildlife	27	183	0	0	27	183
TOTAL	**244**	**1120**	**132**	**279**	**108**	**841**

Table 24. Summary of animal groups used in the known source enterococci profile library.

Type of Animal	Number of Isolates
Bird-brown pelican	3
Bird-duck	16
Bird-roseate spoonbill	2
Bird-owl	3
Bird-parakeet	3
Bird-white crane	1
Bird-sparrow/blackbird	27
Bird-cardinal	17
Bird-hering	13
Bird-cowbird	7
Bird-unknown	166
Cow	221
Dog	168
Horse	92
Human	111
Gull	87
Wildlife-skunk	45
Wildlife-raccoon	42
Wildlife-unknown/road kill	96
TOTAL	**1,120**

Results of species identifications of enterococci isolated from animals and from creek samples are shown in Table 25. The known source library developed in this study consisted of a total of 11 different *Enterococcus* species with the majority (46.8%) of the isolates identified as *E. faecalis*. Two additional species isolated from animal fecal samples that when combined accounted for another 25% of the library were *E. casseliflavus* and *E. faecium* (13.6% and 12.5% respectively). *Enterococcus casseliflavus* and *E. faecalis* were the most common species isolated from human sources (21.84% and 20.69%), while *E. faecalis* were the largest proportion of species in non-human sources (49.79%). *E. pseudoavium* and *E. malodoratus* were only found in human sources, while *E. solitarius* was exclusive to only non-human sources.

Enterococcus mundtii was the most common species isolated from Oso Creek water and sediments (45.4%), with *E. faecalis,* accounting for nearly a third of all speciated creek water and sediment isolates (29.2%) (Table 26). Thus almost 75% of the creek isolates were identified as *E. mundtii* and *E. faecalis. E. dispar, E. raffinosus,* and *E. sulfureus* were exclusively isolated from Oso Creek water and *E. sulfureus* also being isolated from Oso Creek sediments.

Additional details on species in different animals vs. Oso Creek water and sediments can be found in Rodriguez, 2012.

Table 25. Enterococcus species identified from animal sources.

Source	Species	# Isolates	Percentages (%)
Bird	*E. faecalis*	168	63.2
	E. mundtii	30	11.3
	E. faecium	28	10.5
	E. casseliflavus	16	6.0
	E. gallinarum	10	3.8
	E. flavescens	6	2.3
	E. hirae	5	1.9
	E. durans	3	1.1
Dog	*E. faecium*	37	63.8
	E. faecalis	13	22.4
	E. gallinarum	4	6.9
	E. mundtii	3	5.2
	E. hirae	1	1.7
Cow	*E. casseliflavus*	57	48.3
	E. flavescens	25	21.2
	E. faecalis	15	12.7
	E. gallinarum	8	6.8
	E. mundtii	7	5.9
	E. faecium	5	4.2
	E. hirae	1	0.9
Horse	*E. flavescens*	18	19.0
	E. faecalis	17	17.9
	E. faecium	17	17.9
	E. mundtii	16	16.8
	E. casseliflavus	13	13.7
	E. gallinarum	9	9.5
	E. hirae	4	4.2
	E. solitarius	1	1.1
Human	*E. casseliflavus*	19	21.8
	E. faecalis	18	20.7
	E. gallinarum	15	17.2
	E. flavescens	12	13.8
	E. faecium	9	10.3
	E. mundtii	8	9.2
	E. pseudoavium	4	4.6
	E. durans	1	1.2
	E. malodoratus	1	1.2
Non-Avian Wildlife	*E. faecalis*	146	78.5
	E. gallinarum	11	5.9
	E. mundtii	9	4.8
	E. flavescens	8	4.3
	E. casseliflavus	5	2.7
	E. faecium	5	2.7
	E. hirae	2	1.1

Table 26. Species, number of isolates, and percentages of each *Enterococcus* species isolated from Oso Creek.

Creek Isolates	# Isolates	Percentage (%)
E. mundtii	336	45.4
E. faecalis	216	29.2
E. faecium	62	8.4
E. hirae	44	6.0
E. casseliflavus	26	3.5
E. gallinarum	27	3.7
E. flavescens	22	3.0
E. sulfureus	4	0.5
E. solitarius	1	0.1
E. raffinosus	1	0.1
E. dispar	1	0.1
Total	740	
Genus ID'ed	824	

The known source library was analyzed for the most appropriate and most accurate models for classification, and the results are summarized in Table 27 below. The final model used combined both Carbon Source Utilization (CSU) analysis and Antibiotic Resistance Analysis (ARA). The average rates of correct classification (ARCC) were relatively high, exceeding 91% for a two-way (human vs. non-human) classification. The cross-validated column displays the values used in cross-validation analysis (CV), which provides an evaluation of the library's performance. CV classifies each case by the functions derived from all cases other than that case. For the two-way classification the CV value of 88% suggests the library can classify isolates accurately into these two sources. ARCCs decreased with increasing numbers of categories, as expected, but remained relatively high even with a 7-way classification 75% or 63% (CV) compared with a random classification which would generate a rate of ~14.3% correct classification.

Table 27. Average rates of correct classification for known source library, CSU and ARA profiles combined.

Classification of Sources	Sources Included	ARCC (%)	CV (%)
2 way classification	Human	91.7	88.1
	Nonhuman(dog, cow, horse, wildlife, bird, gull)		
4 way classification	Human	79.8	73.2
	Dog		
	Domesticated (cow & horse)		
	Wildlife (non-avian wildlife, bird & gull)		
5 way classification	Human	78.8	71.1
	Dog		
	Domesticated (cow & horse)		
	Wildlife (non-avian wildlife & bird)		
	Gull		
7 way classification	Human	75.6	62.9
	Dog		
	Cow		
	Horse		
	Wildlife (non-avian)		
	Bird		
	Gull		

Table 28 summarizes the rates of correct classification (RCC) for each known source in the library in the four final statistical models combining CSU and ARA profiles. All models produced relatively high RCCs ranging from 69.8-92.3%.

Table 28. Rates of correct classification (RCC) for known source library.

Classification of Sources	Sources Included	RCC (%)
2 way classification	Human	86.5
	Nonhuman(dog, cow, horse, wildlife, bird, gull)	92.3
4 way classification	Human	82.0
	Dog	86.3
	Domesticated (cow & horse)	73.5
	Wildlife (non-avian wildlife, bird & gull)	81.1
5 way classification	Human	79.3
	Dog	79.2
	Domesticated (cow & horse)	73.8
	Wildlife (non-avian wildlife & bird)	79.8
	Gull	90.8
7 way classification	Human	78.4
	Dog	76.8
	Cow	74.2
	Horse	72.8
	Wildlife (non-avian)	76.5
	Bird	69.8
	Gull	92.0

Table 29 (below) summarizes the quantity and type of environmental source isolates analyzed for bacteria source tracking with both carbon source utilization and antibiotic resistance analysis. “Wet” indicates a sampling event taken after sufficient rainfall to be considered a runoff event.

Table 29. Unknown source surface water and sediment enterococci isolates analyzed for bacteria source tracking purposes.

Total Samples		Surface Water Samples		Sediment Samples	
Site	Number of Isolates	Site	Number of Isolates	Site	Number of Isolates
18499 dry	201	18499 dry	172	18499 dry	29
18499 wet	90	18499 wet	56	18499 wet	34
18500 dry	146	18500 dry	140	18500 dry	6
18500 wet	79	18500 wet	52	18500 wet	27
18501 dry	72	18501 dry	71	18501 dry	1
18501 wet	120	18501 wet	106	18501 wet	14
20198 dry	0	20198 dry	0	20198 dry	0
20198 wet	73	20198 wet	0	20198 wet	73

Total Samples		Surface Water Samples		Sediment Samples	
Site	**Number of Isolates**	**Site**	**Number of Isolates**	**Site**	**Number of Isolates**
20559 dry	3	20559 dry	0	20559 dry	3
20559 wet	3	20559 wet	0	20559 wet	3
TOTAL	**787**	**TOTAL**	**597**	**TOTAL**	**190**

Table 30 is a summary of the classification of unknown source water and sediment isolates according to the statistical models created with the known sources outlined in Table 24. All models classified less than 9% of the 787 total unknown source isolates into the human category. The majority of isolates (between 87 and 91% depending on the statistical model) were classified as domesticated animals (cow and horse), wildlife (non-avian), and bird. For each model ~60% of the isolates classified as wildlife, including bird, with about 30% classifying as cow or horse. The other ~10% were classified as human or dog. Less than 1% classified as gull, which were not observed in the upper creek area, lending confidence to the results.

Table 30. Classification of unknown source water and sediment enterococci isolates.

Classification of Sources	Sources Included	Number of Unknown Isolates Classified	Percentage of Unknown Isolates Classified
2 way classification	Human	60	7.6
	Nonhuman(dog, cow, horse, wildlife, bird, gull)	727	92.4
4 way classification	Human	49	6.2
	Dog	40	5.1
	Domesticated (cow & horse)	213	27.1
	Wildlife (non-avian wildlife, bird & gull)	485	61.6
5 way classification	Human	51	6.5
	Dog	30	3.8
	Domesticated (cow & horse)	223	28.3
	Wildlife (non-avian wildlife & bird)	477	60.6
	Gull	6	0.8
7 way classification	Human	30	3.8
	Dog	26	3.3
	Cow	117	14.9
	Horse	120	15.2
	Wildlife (non-avian)	226	28.7
	Bird	264	33.5
	Gull	4	0.5

Table 31 summarizes the classification of unknown source water and sediment isolates from each sampling site. Unknown source isolates were least commonly classified as gull and were most commonly classified as wildlife (non-avian) or bird at all five sites. For 18499, 18500 and 18501, <8% were classified as human source. A larger proportion of isolates at 20198 and 20559 were classified as human. However, results for these two sites must be treated with caution due to the limited number of isolates collected from these sites (only 73 isolates were analyzed from site 20198 all collected under wet conditions, and only six isolates were analyzed from site 20559 (3 collected under dry and 3 collected under wet conditions)). 18499 is upstream from 18500, while 18501 is located on the west branch of the creek. Proportions of isolates from sources were similar at the stations although there appears to be about a 10% difference in allocation between domesticated and wildlife categories at 18501 vs. 18499 and 18500 (more than 75% from wildlife at 18501 with more domesticated at 18499 and 18500).

Table 31. Bacterial Source Tracking Classification Results for Unknown Source Water and Sediment Isolates by Sampling Site

		Percentage of Unknown Isolates Classified				
Classification of Sources	**Sources Included**	**18499**	**18500**	**18501**	**20198**	**20559**
2 way classification	Human	7.6	4.9	4.2	23.3	33.3
	Nonhuman(dog, cow, horse, wildlife, bird, gull)	92.4	95.1	95.8	76.7	66.7
4 way classification	Human	6.2	3.6	2.1	23.3	33.3
	Dog	9.6	4.0	1.6	0.0	0.0
	Domesticated (cow & horse)	28.5	24.0	17.7	54.8	33.3
	Wildlife (non-avian wildlife, bird & gull)	55.7	68.4	78.6	21.9	33.3
5 way classification	Human	6.5	4.0	2.1	23.3	33.3
	Dog	7.2	3.1	1.0	0.0	0.0
	Domesticated (cow & horse)	30.6	25.3	17.7	56.2	33.3
	Wildlife (non-avian wildlife & bird)	55.3	66.7	77.6	20.5	33.3
	Gull	0.3	0.9	1.6	0.0	0.0
7 way classification	Human	3.8	1.8	2.1	13.7	16.7
	Dog	5.8	3.6	0.5	0.0	0.0
	Cow	16.2	18.2	9.4	13.7	16.7
	Horse	17.5	8.9	8.9	42.5	16.7
	Wildlife (non-avian)	17.9	30.7	43.2	24.7	33.0
	Bird	38.5	36.0	35.4	2.7	16.7
	Gull	0.3	0.9	0.5	0.0	0.0

When isolates were separated by wet vs. dry sampling events for the three historic stations from which most isolates were collected (18499, 18500, 18501) the percentages attributed to human source remained low at all sites (Table 32).

Classifications of other unknowns at site 18499 remained fairly consistent between dry and wet events with about 32-35% cow/horse and 51-59% wildlife/bird. For sites 18500 and 18501 a noticeable percentage of unknown isolates classified differently between wet and dry events. For both sites the percentage of unknown source isolates which classified as wildlife (non-avian) increased from ~20-22% of the isolates in the dry event to over 50% of the isolates in the wet event. Isolates classifying as bird decreased from ~46% in dry events to between 18 and 28% for wet events. At 18501 the proportion of isolates classifying as cow and horse also decreased in wet weather, while at 18500 the percentage classifying as cow remained similar under both wet and dry conditions.

Table 32. Seven-way classification of unknown source water and sediment isolates by wet versus dry events (% of Total).

	18499		18500		18501	
Known Source	Dry	Wet	Dry	Wet	Dry	Wet
Human	3.0	5.6	2.1	1.3	4.2	0.8
Dog	5.0	7.8	3.4	3.8	0.0	0.8
Cow	15.9	16.7	18.5	17.7	13.9	6.7
Horse	17.4	17.8	10.3	6.3	12.5	6.7
Wildlife(non-avian)	20.9	11.1	19.2	51.9	22.2	55.8
Bird	37.8	40.0	45.9	17.7	47.2	28.3
Gull	0.0	1.1	0.7	1.3	0.0	0.8

Esp marker analysis

Samples for analysis to detect the *esp* gene human-specific marker were collected with either the quarterly historic station samples (three stations) or with the water and sediment paired sampling (five stations). Thus water from stations 20559 and 20198 were not always analyzed. Samples marked with “NA” in Table 33 indicate that a sample was not run for that sampling event. Additionally, station 20198 rarely had water and 18501 was also dry for one sampling event.

The *esp* marker was detected at three of the five sites from which samples were collected, over the course of this study (Table 33). InhibitEX tablets (Qiagen) were used according to the protocol for inhibitory samples for each sampling event. Beginning with the samples from 9/13/10, additional PCR controls were added to test individual sites for inhibition; one sample from each site was analyzed with and without the addition of *E. faecium* C68 DNA to the PCR chamber. Each of these spikes tested positive for *esp*, indicating that inhibition was not occurring, except for the sample from 18501 on 9/13/10.

All environmental samples were negative for *esp* on 7/7/10, 9/13/10, and 1/19/11 with the waters having *Enterococcus* concentrations ranging from 700 to 1900 cfu/100 ml , 300 to 12,500 cfu/100 ml and 2600 to 3189 cfu/100 mL respectively. On 10/18/10, one sample from 18499 tested positive for *esp*. The *Enterococcus* concentration for this sample was 310 cfu/100mL. One sample from 20559, located downstream of the Robstown wastewater treatment facility, was positive for *esp* on 12/6/10 despite a low

enterococci concentration of 83 cfu/100 mL. Another sample from this site tested positive for the marker on 3/9/11 with an *Enterococcus* concentration of 160 cfu/100 mL[1].

The only site where the *esp* gene was detected in all three samples was at 18500 on 4/20/11 with *Enterococcus* concentrations ranging from 500 to 608 cfu/100 ml, and the gene was also detected in two samples from this site on 8/8/11. Both these sampling events were not preceded by any rainfall.

Table 33. Results of *esp* analysis.

Site	07/07/10	09/13/10	10/18/10	12/06/10	01/19/11	03/09/11	04/20/11	08/08/11
18499A	-	-	-	-	-	-	-	-
18499B	-	-	+	-	-	-	-	-
18499C	-	-	-	-	-	-	-	-
18500A	-	-	-	-	-	-	+	-
18500B	-	-	-	-	-	-	+	+
18500C	-	-	-	-	-	-	+	+
18501A	-	-	-	-	-	-	-	NA
18501B	-	-	-	-	-	-	-	NA
18501C	-	-	-	-	-	-	-	NA
20198A	NA	-	NA	NA	NA	NA	NA	NA
20198B	NA	-	NA	NA	NA	NA	NA	NA
20198C	NA	-	NA	NA	NA	NA	NA	NA
20559A	NA	-	NA	-	NA	-	NA	NA
20559B	NA	-	NA	+	NA	-	NA	NA
20559C	NA	-	NA	-	NA	+	NA	NA
SP1	+	+	+	+	+	+	-	+
SP2	+	+	+	+	+	+	+	+
MB	-	-	-	-	-	-	-	-
EB	-	-	-	-	-	-	-	-
PCR +	+	+	+	+	+	+	+	+
PCR -	-	-	-	-	-	-	-	-
18499A + C68 Genomic DNA	NA	+	+	+	+	+	+	+
18500A + C68 Genomic DNA	NA	+	+	+	+	+	+	+
18501A + C68 Genomic DNA	NA	-	+	+	+	+	+	NA
20198A + C68 Genomic DNA	NA	+	NA	NA	NA	NA	NA	NA
20559A + C68 Genomic DNA	NA	+	NA	+	NA	+	NA	NA

Enterococcus Survival Studies in Soil

The environmental strain of *Enterococcus mundtii* grown in soil unamended with water was able to survive periods of more than 80 days with only approximately a log decrease in number at 10 and 25°C. However incubation at 40°C resulted in a rapid die off within 14 days (Figure 10). Results using an ATCC strain showed similar results (Figure 11). Note: all results shown are averages of two experiments with three replicates sampled at each time interval per experiment.

At 10°C (Figure 12), there was an initial growth (at Day 3) of the environmental *E. mundtii* strain but not the ATCC strain in the dry soil samples. Both populations continued to decrease slowly from Day 3 until the end of the experiment at 91 days. It should be noted that as per Day 0 bacteria levels, the inoculum for the ATCC strain was less concentrated than that of the environmental strain. For moist soil samples grown at 10°C, both environmental and lab strains showed initial growth from Day 0 to Day 3. From Day 3 until the end of the study (Day 91), both populations showed a marked decrease in bacterial levels.

All strains in soils incubated at 25°C demonstrated a decline in growth from time zero to the first time point. Enterococci levels in dry soil remained high, declining only slightly over the course of the study (35 days), in contrast, levels for both ATCC and environmental strains in wet soil declined steadily over time (Figure 13). Figure 14 displays the results from the survival studies in wet and dry soil incubated at 40°C. In dry soil, the environmental *E. mundtii* strain showed initial growth after 8 hours of inoculation, in contrast to the ATCC strain which showed an initial decrease in bacterial levels. Bacterial levels for both the laboratory and environmental strains of *E. mundtii* grown in moist soil increased in the first 8 hours. After this initial incubation both strains, in wet and dry soil decreased over 14 days to very low levels.

Figure 15 and Figure 16 summarize the data for both the environmental and ATCC *E. mundtii* strains for the survival studies. Environmental and ATCC strains followed similar patterns of growth under all types of lab conditions with the exception of initial increases in bacterial levels under dry conditions at 10°C; while both strains grew in wet 10°C soil the ATCC strain did not show the increase in numbers after inoculation under dry conditions, exhibited by the environmental strain. Both strains died off or possibly entered a viable but not culturable state (so did not grow when the soil was assayed) in both wet and dry soil incubated at 40°C within about 14 days. Similarly both strains survived in wet and dry soil incubated at 10°C for 90 days with only an approximately 2 log decrease in numbers over the time period. At 25°C numbers in wet soil died off after approximately 35 days but in dry soil at the same incubation temperature the bacteria persisted, following a survival curve similar to those incubated at 10°C.

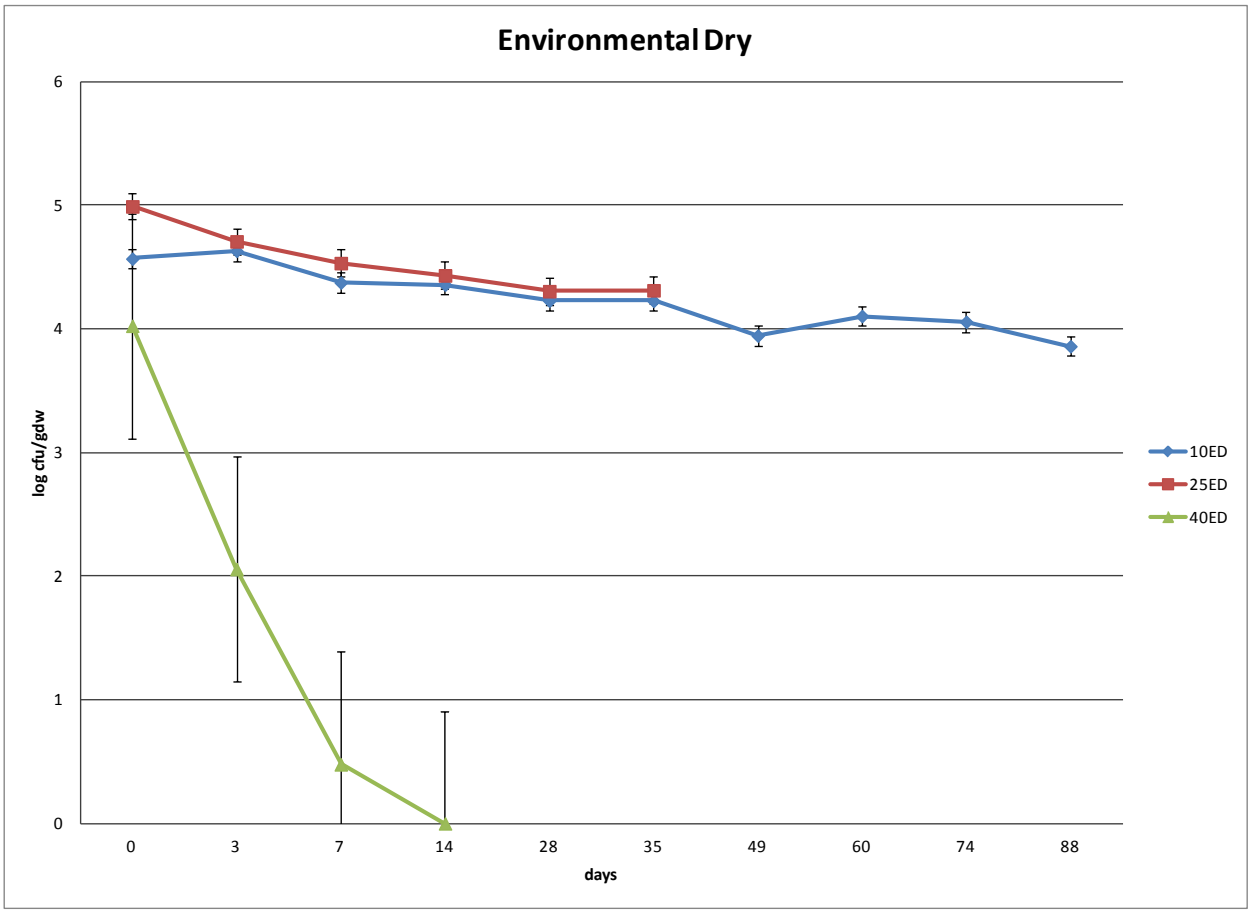

Figure 10. Numbers of an environmental *E. mundtii* strain over time incubated at different temperatures in dry soil

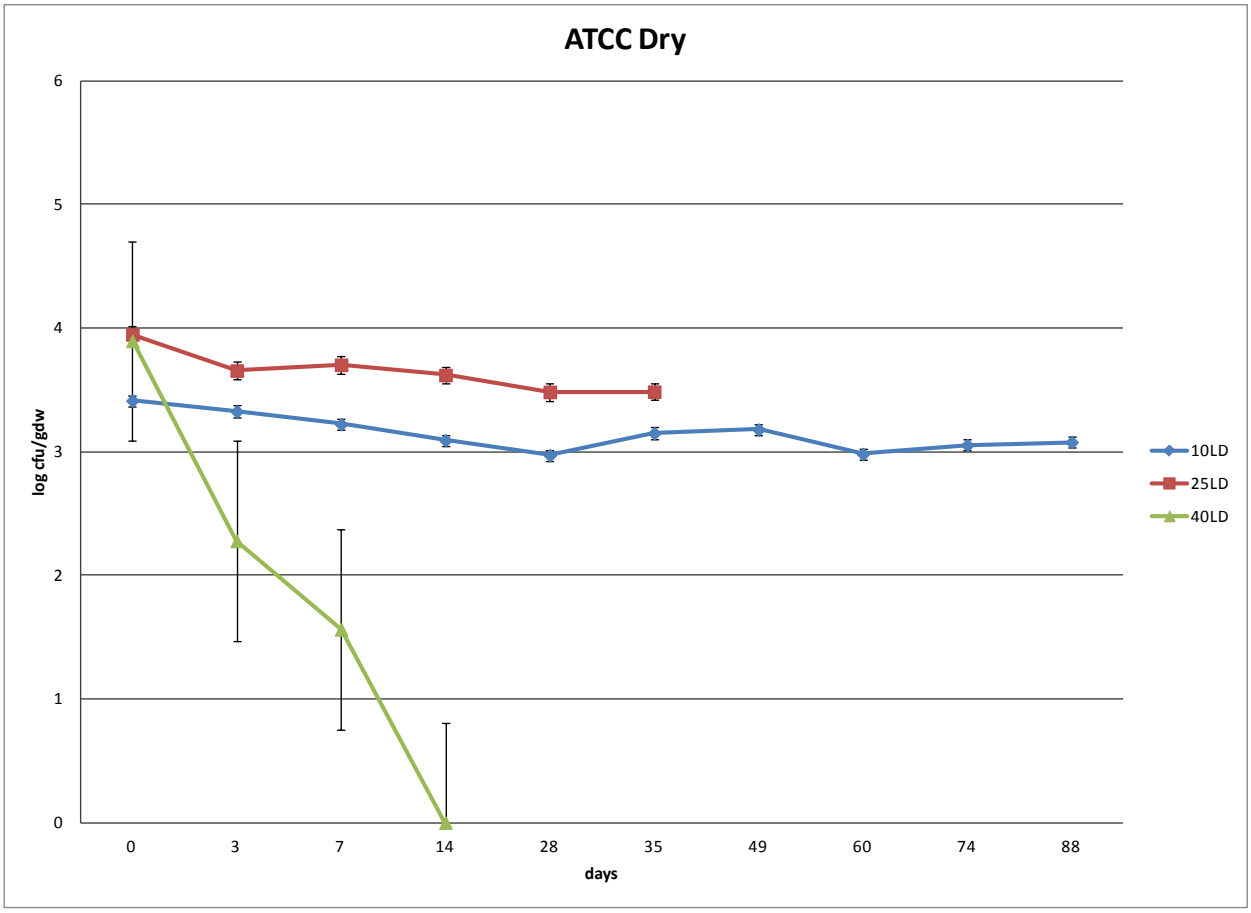

Figure 11. Numbers of the ATCC 43186 *E. mundtii* strain over time incubated at different temperatures in dry soil

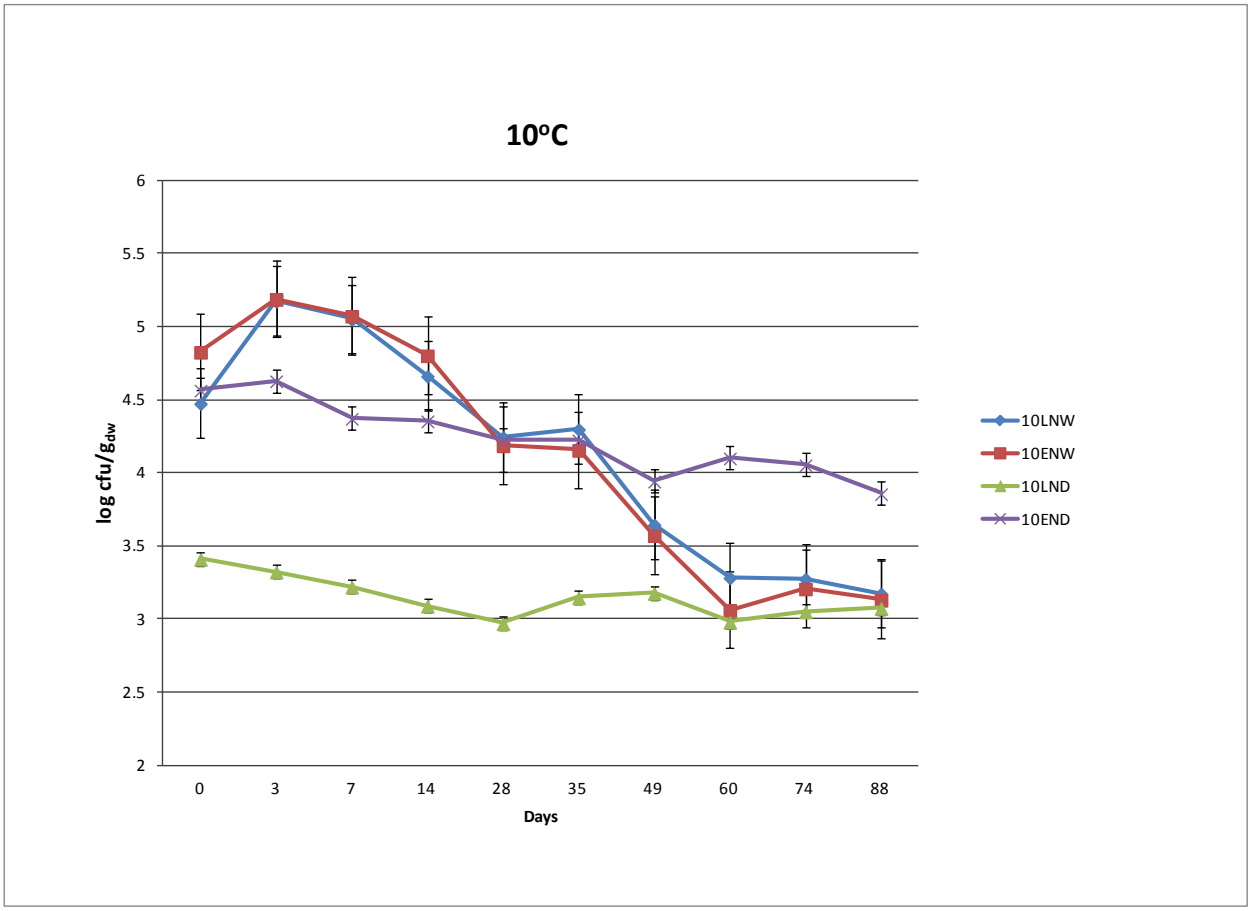

Figure 12. Numbers of *E.mundtii* over time incubated in wet and dry soils at 10°C (L=ATCC strain; E=environmental strain; W = wet soil; D = dry soil)

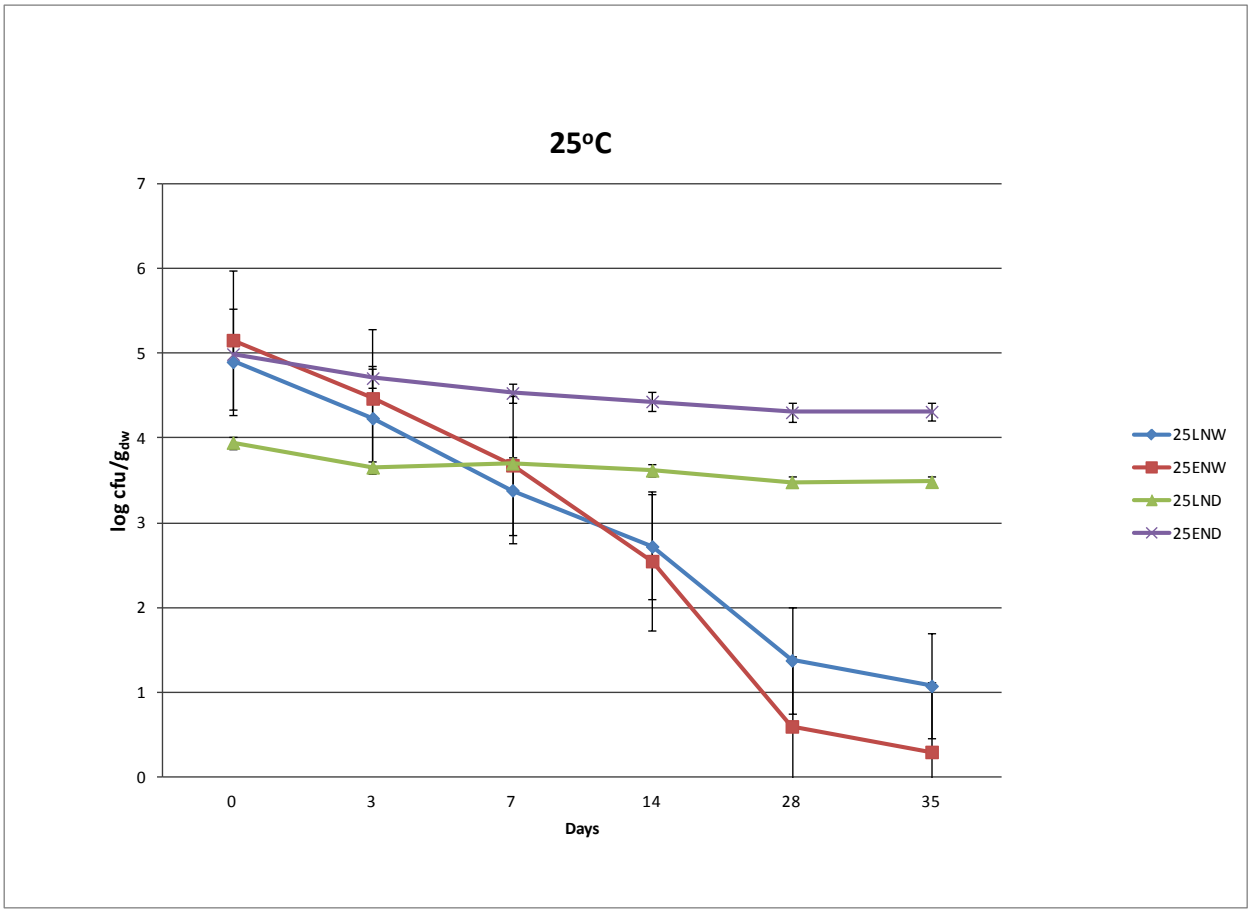

Figure 13. Numbers of E. mundtii over time incubated in wet and dry soils at 25°C (L=ATCC strain; E=environmental strain; W=wet; D=dry)

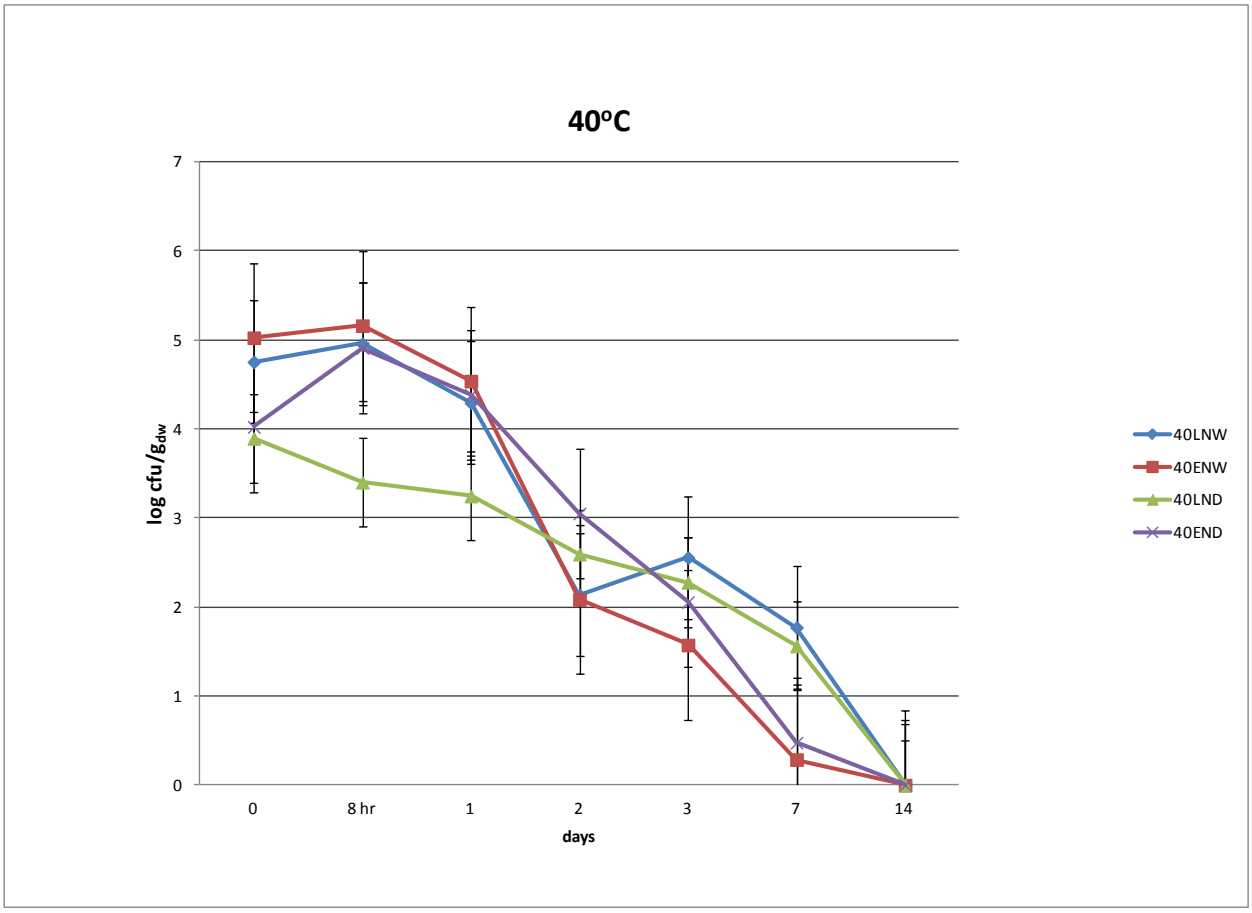

Figure 14. Numbers of *E. mundtii over time* incubated in wet and dry soils at 40°C (L=ATCC strain; E=environmental strain; W=wet; D=dry)

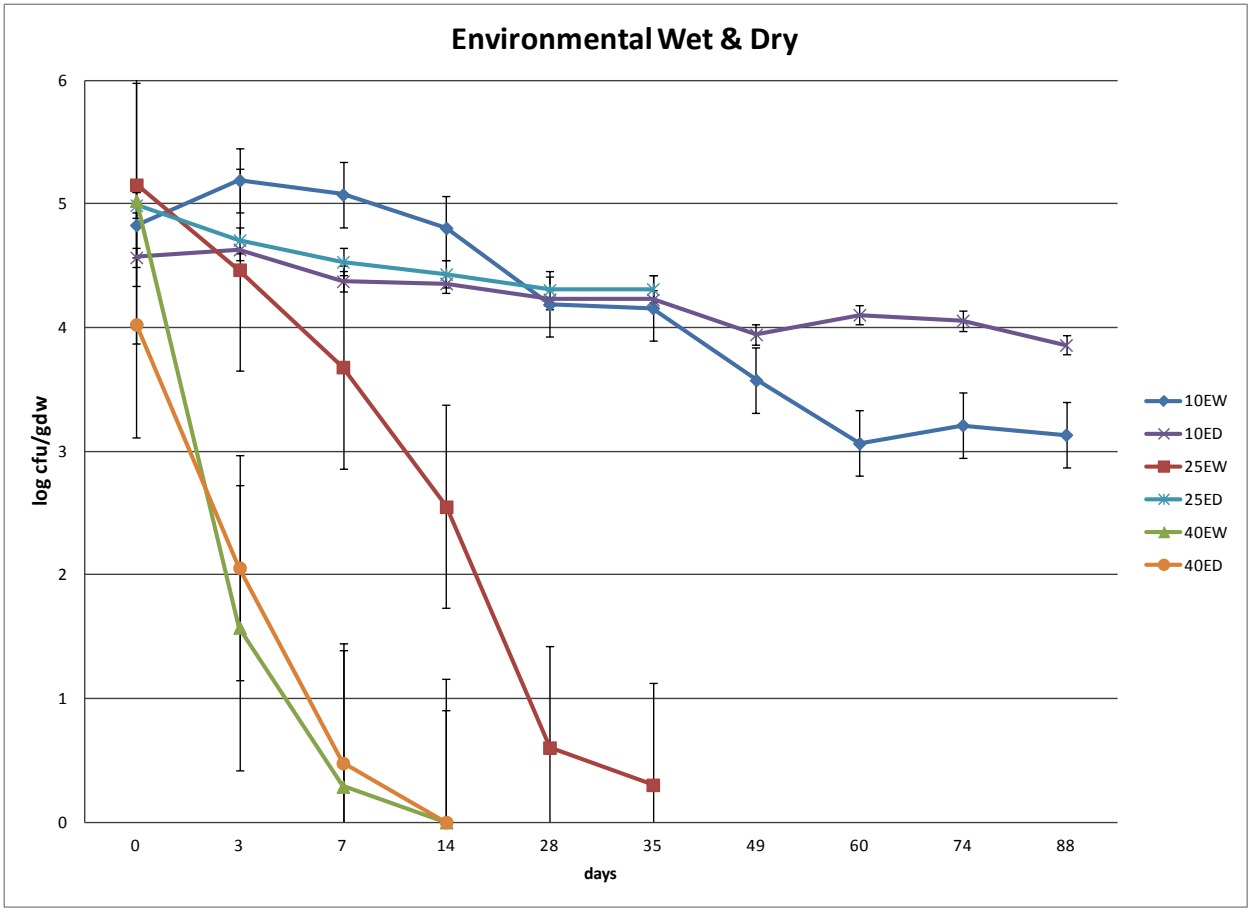

Figure 15. Numbers of an environmental E. mundtii strain over time incubated in wet and dry soils at different temperatures.

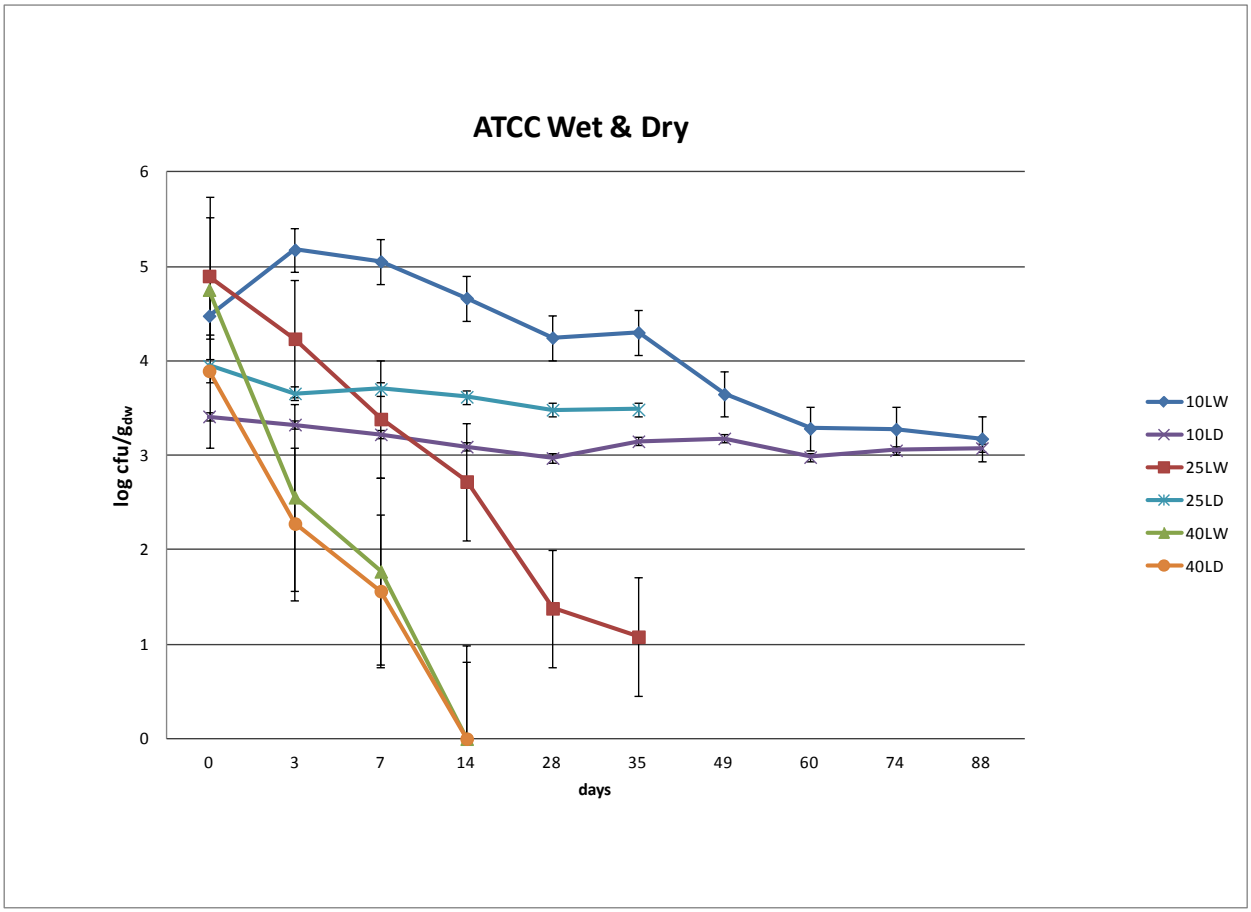

Figure 16. Numbers of the ATCC 43186 E. mundtii strain over time incubated in wet and dry soils at different temperatures

Discussion and Conclusions

The series of Oso Creek studies following the 2002 listing of the creek (Segment 2485A) on the Texas Water Quality Inventory and 303(d) List aligns with the approach and sequence of steps recommended by the Texas Interagency Task Force on Bacteria TMDLs from 2007 (Jones et al., 2009). This study constitutes the step of targeted sampling and BST to identify specific sources of contamination in the upper section of the creek, following monitoring and modeling of the entire creek to assess contamination and sources along the segment. The identification of the upper section as having elevated enterococci levels without obvious sources of contamination led to the study while also allowing a more focused BST analysis.

Nonpoint sources of enterococci in the upper section of Oso Creek

The sample design for this study was based on the project goals to determine the nonpoint sources of enterococci in the upper sections of the creek and the animal sources contributing to the loading.

Historic station water sampling

In order to continue a record of enterococci levels at the three TCEQ historic stations in the upper creek, which had been sampled during previous TMDL studies, these stations were monitored throughout the study period on a quarterly basis, generating data from ten sampling events. In order to obtain representative results, ambient water sampling occurred on a routine schedule, capturing dry and runoff-influenced events at their natural frequency. There was no prejudice against rainfall or high flow events. Seven events were classified as dry and three as wet weather events. While levels of enterococci ranged from 2 to 8,633 cfu/100mL and wet weather averages were considerably higher than dry weather averages for each site, all samples (with the exception of 18501 on 4/20/11) exceeded the U.S. EPA single sample surface water quality guidelines, confirming that dry weather loading continues to constitute a significant problem in the creek, as revealed in prior studies. Comparisons with earlier results do not indicate that there has been a reduction in enterococci levels in the upper creek. While a separate study (Mott and Hay 2009), examining potential inflows into the creek, found multiple potential discharges, upstream of the historic stations, only one was actively flowing – from the Robstown WWTP. Only two sampling events of the 18 total, showed high levels of enterococci (>2000 cfu/100mL) at this site, suggesting this while this inflow may occasionally contribute enterococci to the creek it is not a consistent source of contamination.

Additional data on enterococci levels further downstream in the creek and bay are available through the Nueces River Authority (NRA) which conducts routine Clean Rivers Program (CRP) monitoring at stations 13028 (Oso Creek at at SH 286 South of Corpus Christi) and 13440 (Oso Bay at Padre Island Drive (SH 358) quarterly (FY2009) (http://cms.lcra.org/).

Evaluation of potential NPS contributions to the upper creek

The first year of sampling provided a synoptic dataset of the study area to generate information on potential sources of enterococci and allowed optimization of sampling resources for follow-up sampling and laboratory experiments by focusing on those areas identified as having high enterococci concentrations. Samples collected from a variety of potential sources - creek sediments, soils, sub-surface seepage and groundwater were analyzed to evaluate contributions from these sources. Based on these results additional sampling was conducted in years 2 and 3. Additionally, small-scale laboratory experiments were conducted once soil was identified as a potential contributing source of enterococci to the creek.

Groundwater

Paired groundwater wells, one shallow and one deep, were sampled during six sampling events. Two sampling events were considered wet weather events and the remaining four were dry weather events. Groundwater well 14 had the highest average bacterial levels (656 cfu/100mL). There was no discernable pattern regarding bacterial levels in samples from shallow or deeper wells. High bacteria levels occurred in samples both in dry and wet weather events; most of these occurrences were related to surface water entering the well due to flooding or failure of the annular seal and not representative of ambient groundwater conditions. Overall, most groundwater samples had relatively low enterococci counts and were shown to be low compared to historic values of bacteria in surface water. One exception, the set of paired wells, GW09A&B, at Oso Creek and FM 665 had elevated levels for both wet weather and dry weather sampling events. It was concluded that groundwater is not a significant contributor to the enterococci levels in the creek. Based on the year 1 results only limited year 2 sampling was conducted.

Bacteria fluxes were modeled in the separate study discussed under results, using the aquifer properties determined by aquifer testing, and were found to be very low due to the poor hydraulic conductivity and low hydraulic gradient. The highest flux of bacteria calculated in the upper portion of the watershed was 5,970 cfu/day, an equivalent to increasing the enterococcus concentration in the creek by 0.59 cfu/m^3.

Nutrients (measured under separate study as discussed in the results section, were comparable to surface water in some cases, however several sites raise concern for high concentrations of nitrogen and many monitoring wells in the overall study had total phosphorous values in the high range for groundwater. Modeling groundwater movement to the creek and bay provided some flux values for the transport of nutrient to surface waters. The modeling efforts indicated that only a very small flux of nutrients to the surface water is possible due to the low hydraulic conductivity of the formations and the low hydraulic head to provide a gradient for advection.

Although there were individual measurements of nutrients and bacteria that were high, modeled fluxes confirmed that the potential of groundwater to be a significant pathway for either of these constituents to surface water is very low.

Sub-surface Seepage

Seepage samples collected during four sampling events from sites 18499, 18500, and 18501 were found to contain low numbers of enterococci (<1 to 62 cfu/100mL) with average levels at each of the three sites less than 30 cfu/100mL, indicating that sub-surface water seepage into the creek is not a significant contributor to contamination of the creek. Sampling was therefore discontinued after these sampling events.

Soil

A possible contribution to the creek of enterococci from soils was suggested by results from another project (TSSWCB project 02-13; Dr. Fernandez, Texas AgriLife Research at Corpus Christi; USGS 2008), with fecal indicator analyses conducted by TAMU-CC (Mott). Extremely high concentrations of enterococci were found in runoff from agricultural fields in the watershed (Ockerman and Fernandez, 2010).

Locations for sampling in this study were chosen to provide a range of agriculture fields, in close proximity to the creek, with no vegetation, or planted to a range of crops including cotton, sorghum, corn and sesame. One field was a pasture throughout the study period. Soil sampling focused primarily on surface soils (top 6 inches) and a poorly drained and well drained site at each field was sampled. Viable enterococci were found in soils during year 1, particularly during the fall, following rainfall and during the winter months. It was therefore decided to continue sampling of soils. However, there was little rainfall during year 2 and numbers of enterococci were very low or not detected in dry summer months. Based on the fact that enterococci were present in soils under certain conditions and have been recorded in extremely high numbers in Oso Creek watershed agricultural field runoff it was decided to conduct follow-up studies in the laboratory to examine persistence in soils. When the amount of soil potentially displaced into the creek through runoff is considered, and the fact that enterococci can be detected at per gram dry weight levels, soils appear to constitute a potential source of enterococci at least during heavy runoff periods.

It should be noted that with the complexity of factors involved in site selection for soil sampling, it is recognized that our results provide only a very limited dataset and comparisons between crop covers and poorly drained vs. well drained soils could not be well addressed. Additional studies are needed to more fully investigate enterococci levels in Oso Creek watershed soils.

Surface Water and Sediment Sampling

Paired surface water and sediment samples were collected for two years at each of the following five stations, 18499, 18500, 18501, 20559, 20198 to examine the relationship between levels of enterococci in the water column and in the sediments For all five sites, high levels were found consistently in surface water samples, regardless of rainfall, similar to levels seen in the quarterly monitoring of the three historic sites. In other studies sediments, particularly at marine sites, have been reported as a source of enterococci for surface waters. In our study, although enterococci were found in the sediment, the numbers and times found, suggest that while they may be deposited following rainfall, there is limited survival in sediment during dry periods. Sediments do not seem to act as a reservoir and contribute significantly to the enterococci loading in the water of Oso Creek

Overall, of the matrices sampled to identify NPS contributions to the enterococci levels in the upper Oso Creek, groundwater and subsurface seepage do not appear to act as major sources. Our results indicate some enterococci survival in sediment but numbers were relatively low especially during dry conditions, suggesting die-off over time. Soil appears to have the potential to contribute enterococci to the creek, especially as evidenced by previous studies of levels in agricultural field runoff. However, conditions for survival and persistence need to be further investigated and laboratory experiments were conducted to examine effects of temperature and moisture on survival (See below).

Animal sources of creek contamination as determined by Bacterial Source Tracking

Library based BST – ARA-CSU profiles

Enterococci isolates from fecal samples and Oso Creek were speciated as part of the development of carbon source utilization profiles for the isolates. The finding that different animal types in the watershed contained very different proportions of species based on CSU data, supports the premise that CSU profiles can be used to distinguish between animal sources for source tracking purposes.

A total of 1,120 known source isolates (841 collected during the current project and the others from a previous project) were used in the construction of the known source library of isolate profiles to classify unknown source isolates through comparison of their profiles with those in the library. Not all isolates from the previous project could be re-grown for ARA analysis. All known source isolates were analyzed with both antibiotic resistance analysis and carbon source utilization profiling. Multiple models were generated using DA with this known source library to achieve the most accurate and appropriate model for classification of sources. This included evaluating whether the ARA profiles or CSU profiles alone or together would produce the best model. The final model selected utilized a combination of the two bacterial source tracking methods, ARA and CSU. All discriminant analysis models used in this study were generated with the statistical package IBM SPSS Statistics 19. The data was also analyzed using

Random Forests (Rodriguez, 2012) but for this dataset, DA was shown to be the better statistical tool.

Initially, 32 chicken profiles collected during this project were included in the known source library (for a total of 1,152 known source isolates). However, during the process of evaluating the statistical models it was determined that the chicken isolates did not increase the accuracy or value of the model. Current estimates of the number of chickens in Nueces County indicate these animals are negligible sources of contamination in the watershed, supporting the decision to exclude this small group from the known source library.
(http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_2_County_Level/Texas/st48_2_013_013.pdf)

Selection of the final model also included evaluation of the appropriateness of inclusion of bacterial profiles from the existing BST library. Human isolates from the existing BST library were from individuals, rather than sewage influent/effluent, and this was considered a less relevant source for the watershed, specifically considering that the creek is driven by sewage effluent. The profiles significantly differed from those generated from isolates collected from sewage under this project. They were therefore removed from the known source library.

To finalize the known source library, various models were evaluated for performance and accuracy. Accuracy of the library is measured by the Average Rate of Correct Classification (ARCC), a value that represents the overall rate that known sources are correctly classified. Performance of the library is measured by the Cross Validation analysis (CV). CV, also known as leave-one-out analysis, removes an isolate from the library and classifies it based on all of the remaining ones. If the ARCC and CV differ by less than ten percent, the library is considered to be appropriate, representative and does not "overfit" (bends the results to fit the data). A model using a large number of variables tends to have a larger difference between ARCC and CV values than models with 20 variables or less. Our model used 21 antibiotic variables and 95 carbon source utilization variables for a total of 116 variables, which would generally cause a larger difference between ARCC and CV than just the antibiotic variables alone. In all models, excluding the 7 way classification, the difference between ARCC and CV was less than 10%. However, in the 7 way classification the difference was 12.7%. The high level of differentiation between sources combined with the very large number of variables in the model leads to smaller CV values (larger differences between ARCC and CV), but it does not indicate this model is not appropriate or representative. It simply means this will be the greatest accuracy the model can achieve for a data set with this level of complexity.

In terms of accuracy all four models (2 way, 4 way, 5 way and 7 way classification) performed well, equal or above percentages for ARCCs reported in the literature with other data sets. In particular, the 7 way classification model, which had an ARCC of 75.6% performed well above what random classification would produce (~14.0%). As the model becomes more complex in terms of differentiation between sources, it is

expected that there will be some loss in accuracy due to very similar sources (e.g. horse and cow) now classifying separately.

In terms of rates of correct classification (RCC) for each specific source, all four models performed well, with RCCs ranging from 69.8-92.3%. Within a specific model, RCCs stayed relatively consistent with each other, avoiding extreme lows or extreme highs. These consistently high RCCs lend confidence to the ARCC and the strength of the models.

After the final models were selected, the ~800 unknown source surface water and sediment isolates were analyzed with all four models. The key finding was the small percentage of all the isolates that classified as human sources (<8.0%). The majority of the unknown source isolates (~60%) were classified as wildlife and bird, with the next large group of unknowns (~30%) classifying as domesticated (cow and horse). Relatively few isolates classified as either dog or gull source. The lack of isolates classifying as gull was supported by observations throughout the study that gulls were not among the birds seen in the upper creek watershed area.

The unknown source isolates were also split out by sampling site and analyzed with the four statistical models. As mentioned in the results section, the data for sites 20198 and 20559 which had higher percentages of isolates classified as human sources than did the remaining three sites, should be viewed with caution due to the small number and characteristics of these isolates. Site 20198 isolates only originated from sediment samples taken during a wet weather event, and only six isolates were used for Site 20559. Sites 18499, 18500 and 18501 were more representative of both wet and dry weather. At all sites, unknowns were least often (< 2.0%) classified as gull. For sites 18499, 18500 and 18501 the majority of isolates (> 50%) classified as wildlife (non-avian) and bird. Site 20198 had ~43% isolates which classified as horse. The small sample size (n=6), precludes drawing conclusions about specific sources at Site 20559, except to note that isolates were classified more often as nonhuman than human.

The unknown source isolates were also analyzed by sampling site and preceding weather (dry vs. wet). The proportions of unknown source isolates from Site 18499 classifying into each group were similar for both dry and wet weather events. However, for sites 18500 and 18501 ~20% isolates from dry events classified as wildlife (non-avian), while >50% classified in this category for wet weather events. In contrast isolates classifying as bird decreased from ~46% in dry events to between 18 and 28% for wet events At 18501, the proportion of isolates classifying as cow and horse also decreased in wet weather, while at 18500 the percentage classifying as cow remained similar under both wet and dry conditions. These results suggest that perhaps following rainfall, runoff carries wildlife feces into the creek, whereas during dry weather there is more direct deposition of bird feces into the water.

Library independent BST – human specific marker (Esp Analysis)

The detection of the *esp* marker in Oso Creek waters indicates that human fecal contamination contributes to bacterial loading. However, the esp marker was detected at only three of the five sites tested and for only a small number of surface water samples from the sites. The gene was only detected in more than one sample for a sampling event for 18500. The lowest enterococci concentration of a positive sample was 83 cfu/100mL which is much lower than the enterococci concentrations of positive samples in similar studies (Scott *et al.* 2005; McDonald *et al*. 2006; Ahmed *et al*. 2008; Korajkic *et al.* 2009). Ahmed *et al.* (2008) determined that 48 $\pm$ 7 sewage origin enterococci colonies in a water sample were necessary for *esp* detection, and Scott *et al.* (2005) found that a concentration of 58 $\pm$ 24 enterococci CFU 100 ml^{-1} was necessary for *esp* detection.

The absence of the human associated marker in samples with high levels of *Enterococcus* suggests that other sources are contributing to enterococci levels in the creek. These *esp* results provide independent support for the results from the antibiotic resistance analysis and carbon source utilization data in suggesting that human sources contribute only a small percentage of the fecal contamination in the upper Oso Creek.

Overall, the BST results demonstrate that the main sources of enterococci in the creek are non-human in origin, and that the Robstown WWTP or other sewage leaks are not significant sources of contamination, The results suggest wildlife (avian and non-avian) and livestock both contribute to the contamination. Additional studies are needed to further elucidate where and when these sources reach the creek and potential pathogens that may be carried into the creek with the contamination.

While the toolbox approach using multiple datasets and both library dependent and independent methods adds confidence in the data and follows recommendations of the Texas task force on bacteria TMDLs (Jones et al., 2009), a number of limitations are always involved with BST. The decision to use enterococci as the fecal indicator affected the choice of BST methods and eliminated the option to use the statewide *E. coli* library for this study. A previous pilot study using CSU profiles of enterococci (Mott et al 2003) and other studies (e.g. Hagedorn et al., 2003) suggested this approach could be successful, and combined with ARA data, provide a useful library (similar to an earlier study using ARA and CSU *E. coli* profiles together to generate a composite library for Whiteoak and Buffalo Bayous in Houston). Results of TSSWCB Project 02-10: Development of an *E. coli* bacterial source tracking library and assessment of bacterial sources impacting Lake Waco and Lake Belton, suggested ARA data in combination with another method could be used successfully for BST.

The use of the *esp* marker was successful for this study as a human-specific marker, but because no single BST method developed to date can identify all pollution sources with 100% accuracy, it is not recommended to use this method as the only test for human fecal contamination. Instead, a toolbox approach using a set of MST methods is preferred in order to accurately assess health risk to the public (Ahmed et al. 2009).

The *esp* marker is not present in the feces of all individuals and may vary in concentration (Casarez et al. 2007; Field and Samadpour 2007), and the assay has a minimum limit of detection; thus, the lack of a positive result does not completely rule out the presence of human fecal contamination. Further studies in the frequency of the *esp* gene in the human-specific *E. faecium* population of this region, as well as quality control studies using raw sewage from local sewage treatment plants to determine the limit of detection, would be useful in determining the reliability of this method.

Enterococcus Survival Studies in Soil

Experiments on survival of enterococci in soil under different conditions were performed utilizing an ATCC and a local environmental strain of *Enterococcus mundtii*, a species shown in preliminary studies to be widespread both in local soils and in the creek. Multiple parameters were examined in the experiments, including ambient (incubation) temperature and soil moisture content. The three temperatures (10°C, 25°C, and 40°C) were selected to represent average local winter, average room, and average local summer temperatures, respectively. The environmental strain was able to proliferate initially in both wet and dry soils at both 10°C and 40°C, representing the full range of local seasonal temperatures. The ATCC strain was only able to initially grow in wet soil at both 10°C and 40°C. However, both strains, in wet and dry soils at both temperatures showed a decrease in bacterial numbers after the second time point, in all studies. Results demonstrated that for *E. mundtii*, both an ATCC and a local environmental strain can persist for a relatively long time in soil from the Oso Creek watershed at lower, winter temperatures. At 25 °C persistence was much greater in dry than wet soil. These results suggest that enterococci persist in soils during the cooler parts of the year and this is supported by the field data on enterococci levels in soils from this study.

Evaluation of Project Results and Environmental Implications

This multi-year, multi-level project provides important information about sources of the bacterial contamination (as measured by enterococci levels) in the upper portions of Oso Creek that can be used in the development of an implementation plan for the bacteria TMDL.

High levels of enterococci under dry weather conditions, identified in previous projects as problematic, are still persistent in Oso Creek. Although wet weather averages of bacterial levels for surface water samples are higher than those under dry conditions, dry event averages still largely exceed the single sample contact recreation standard for Enterococci (104 cfu/100mL).

Of the multiple types of environmental samples analyzed in this project, levels of enterococci in groundwater and sub-surface seepage suggest that these sources are not major contributors to bacterial contamination of the upper creek. While enterococci were found in creek sediments, they did not persist during dry weather implying that

they were deposited in the sediment, but do not survive over time. Thus potential sources of enterococci appear to be within the water of the creek (i.e. direct fecal deposition) or through runoff or erosion carrying soil containing enterococci into the creek. Based on this study additional work is needed to investigate the origins of the enterococci associated with soil – animal deposition and survival/re-growth, plant-associated, or indigenous soil bacteria, and to elucidate the movement of the bacteria with soil into the creek.

Bacteria source tracking was conducted to determine animal sources of contamination using two approaches: phenotypic library-dependent (antibiotic resistance analysis, carbon source utilization), and genotypic library–independent (*esp* gene analysis) BST methods, demonstrated that human sources make only a small contribution (<10%) to enterococci contamination of the upper region of Oso Creek. The percentage of creek isolates classified as human sources was consistent for dry and wet weather sampling, suggesting that any human contamination was not originating from run-off sources. Data from the Robstown WWTP outflow, obtained under a separate study, indicate occasional elevated levels of enterococci, a controllable source.

For all five sampling sites, ~30% of unknown surface water and sediment isolates classified as livestock (cow and horse) with the (~60%) of the remainder of the isolates classified as wildlife (non-avian) and bird. Proportions of isolates from different sources differed at sampling sites with respect to wet (preceding rainfall) and dry weather events. Percentages of isolates classified as specific known sources remained fairly constant throughout dry and wet weather for Site 18499, suggesting runoff sources do not play a significant role at this sampling site. Site 18499 did have higher bacterial levels in sediment than did the other sampling sites, which could indicate sediments might be contributing to enterococci in the water at this particular stretch of the creek. Further studies are needed to confirm this.

Although sites 18499 and 18500 had a relatively similar percentage of isolates which classified as livestock in both wet and dry events, site 18501 had an almost 50% reduction in isolates classifying in this group between dry and wet events. Domesticated animal input does appear to play a role in both dry and wet loading, but according to the data from this study does not play a large role in runoff driven loading of Oso Creek, suggesting this source reaches the creek by direct deposition in dry weather. Observations of cow fecal material in the creek were made by sampling personnel and animals were free to enter the creek.

Approximately 70% of all known sources at both sites 18500 and 18501 could be attributed to a combination of wildlife (non-avian) and bird sources. However, the percentages of each source differed between wet and dry weather events. More isolates classified as wildlife (non-avian) at both sites following rainfall, while fewer classified as bird under these conditions. This suggests that at these two sampling sites birds play a larger role in dry day loading, but under rainfall conditions wildlife contributions are greater, presumably through runoff containing wildlife feces. Crop

cover could affect wildlife usage of different sections of the creek and should be further investigated.

In summary, the results of the study suggest that the bulk of bacterial loading into the creek is directly attributed to surface water contributions, with sediment possibly playing a minor role as a reservoir for enterococci at some portions of the creek, and soil (either indigenous bacteria/animal feces) as a potential source during wet weather. The sources of the enterococci appear to be largely wildlife (non-avian) and bird, with wildlife (non-avian) sources increasing in terms of contribution during runoff driven events (wet weather).

In terms of mitigation, the finding that non-human sources are the major contributors to contamination, and that wildlife (non-avian) and bird sources may be significant suggest control and reduction of enterococci levels will be difficult to achieve in Oso Creek. Contributions from agricultural activity can be reduced with cooperation from farmers using BPM's for livestock and erosion/sediment control.

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APPENDIX A. Field Data

Table A.1. Field Data Instream Surface Water Samples 2009-2011

Site	Date	Time Received	Time Collected	Depth (cm)	Flow (cfs)	Air Temp (°C)	Wind Intensity	Wind Direction	Weather	Water Temp (°C)	Conductivity (mhos/com)	Salinity (ppt)
OST18499	4/24/09	1443	1350	20		30.5	3	6	2	24.87	1.956	0.99
OST18500	4/24/09	1443	1310	12	1.085	28.3	3	6	2	24.39	3.4	1.78
OST 18501	4/24/09	DRY	DRY	DRY	No flow	DRY	DRY	DRY	DRY	DRY	DRY	DRY
OST18499	7/20/09	1122	0928		6.203	32	3	6	1	28.57	1.617	0.81
OST18500	7/20/09	1122	0820	9.9	1.684	28.1	1	6	1	27.84	3.185	1.38
OST18501	7/20/09	DRY	DRY	DRY	No flow	DRY	DRY	DRY	DRY	DRY	DRY	DRY
OST18499	10/19/09	1345	1127	15	2.616	25.4	2	7	2	20.99	1.435	0.81
OST18501	10/19/09	1345	1255	15	No flow	30.4	2	7	2	20.02	0.4011	0.22
OST18500	10/19/09	1643	1525	15	1.479	31.9	2	7	2	NA	NA	NA
OST18499	1/20/10	1725	1435	15	2.552	20.8	2	2	3	19.31	1.122	0.55
OST18500	1/20/10	1725	1622	15	12.293	26.6	1	2	2	18.67	1.551	0.78
OST18501	1/20/10	1725	1545	15	No flow	25.8	1	2	1	19.92	0.238	0.11
OST18499	4/28/10	1345	1159	15	No flow	24.4	3	6	1	22.13	2.705	1.4
OST18500	4/28/10	1345	1241	15	1.348	26.1	3	6	1	21.31	6.24	3.41
OST18501	4/28/10	1345	1225	15	No flow	26.1	3	6	1	20.9	0.521	0.25
OST18499	7/19/10	1230	1055	15	No flow	32.2	2	2	2	29.59	1.43	0.71
OST18500	7/19/10	1230	1151	15	1.449	31.8	2	2	2	28.5	4.361	2.31
OST18501	7/19/10	1230	1130	15	No flow	32.8	2	2	2	30.93	0.436	0.21
OST18499	10/18/10	1310	1136	15	No flow	26.8	2	6	1	22.1	2.744	1.42
OST18500	10/18/10	1310	1228	15	1.532	29.3	1	6	1	21.95	6.509	3.56
OST18501	10/18/10	1310	1205	15	No flow	28.5	2	6	1	22.23	0.412	0.2
OST18499	1/19/11	1330	1155	15	No flow	19	3	5	3	15.85	15.07	0.76
OST18500	1/19/11	1330	1235	15	8.654	21.7	1	5	3	14.96	1.773	0.9
OST18501	1/19/11	1330	1222	15	No flow	20.7	3	5	3	15.31	0.376	0.18
OST18499	4/20/11	1040	0926	15	No flow	25.9	3	6	3	25.75	2.445	1.25
OST18500	4/20/2011	1040	0946	15	1.348	26.8	2	6	3	24.76	5.217	2.8
OST18501	4/20/2011	1040	1005	15	No flow	26.9	2	6	3	24.43	1.294	0.64

Site	Date	DO (mg/L)	pH (s.u.)	Secchi Disk (m)	Water color	Odor	Surface	Days Since Last Rainfall	Rainfall (1 day) (inches)	Rainfall (7 days) (inches)	Comments
OST18499	4/24/09	7.02	8.07	0.09	6-grey	6	2	6	trace	trace	light trash
OST18500	4/24/09	8.88	7.9	0.17	1	6	2	6	trace	trace	
OST18501	4/24/09	DRY	DRY	DRY	DRY	DRY	DRY	6	trace	trace	
OST18499	7/20/09	NA	7.76	0.08	1	6	2	8	NA	NA	next to major highway, moderate trash
OST18500	7/20/09	NA	7.32	0.071	6-Grey	6	2	8	NA	NA	
OST18501	7/20/09	DRY	DRY	DRY	DRY	DRY	DRY	8	NA	NA	Dry
OST18499	10/19/09	NA	6.96	0.05	3	3	1	6	0.00	0.44	
OST18501	10/19/09	NA	7.49	0.2	1	3	2	6	0.00	0.44	Feces all in creek, next to cow pasture
OST18500	10/19/09	NA	NA	0.079	6-clear green	6	2	6	0.00	0.44	YSI malfunctioned could not retrieve data, Heavy trash
OST18499	1/20/10	NA	7.71	0.128	3	6	1	1	0.00	2.68	Evidence of flooding
OST18500	1/20/10	NA	7.68	0.09	1	6	2	1	0.00	2.68	Evidence of flooding, Water still high
OST18501	1/20/10	NA	8.1	0.04	1	6	1	1	0.00	2.68	Evidence of flooding, 66 cows upstream, cow feces on fence in water (point source)
OST18499	4/28/10	6.68	7.74	0.062	6-green/ yellow	6	1	4	0.00	0.01	
OST18500	4/28/10	NA	7.52	0.11	3	6	2	4	0.00	0.01	
OST18501	4/28/10	4.41	7.77	0.05	1	6	1	4	0.00	0.01	
OST18499	7/19/10	4.62	7.29	0.062	1	6	1	1	1.24	1.24	
OST18500	7/19/10	5.94	7.54	0.16	6	6	2	1	1.24	1.24	
OST18501	7/19/10	2.59	7.61	0.1	1	6	1	1	1.24	1.24	
OST18499	10/18/10	3.73	7.51	0.17	6-Brown/ green	3	1	22	0.00	0	Creek blocked by what looked like corn husks from adjacent fields, heavy trash
OST18500	10/18/10	NA	7.58	0.2	5	6	2	22	0.00	0	Air smelled like rotten eggs, heavy trash
OST18501	10/18/10	NA	7.88	0.15	1	6	1	22	0.00	0	Heavy trash, no cows in adjacent fields
OST18499	1/19/11	5.38	7.61	0.13	1	6	1	3	0.00	2.38	Blockage still under bridge, heavy trash
OST18500	1/19/11	8.32	7.67	0.1	5	6	2	3	0.00	2.38	Trash, flow higher than normal
OST18501	1/19/11	7.73	7.91	0.14	1	6	1	3	0.00	2.38	Cows in pasture upstream, heavy trash.
OST18499	4/20/11	3.54	7.56	0.11	1	2	1	9	0.00	0.01	Green algae and sticks covered water, blockage still present
OST18500	4/20/11	5.87	7.61	0.11	5	6	1	9	0.00	0.01	Bridge that runes over creek was being re-asphalted the same day.

OST18501	4/20/11	6.91	7.89	0.13	1	3	1	9	0.00	0.01	Water level very low, green algae throughout site, no cows in any fields, heavy trash. Road had been recently re-asphalted.

Table A.2. Field Data Groundwater Samples 2009-2011

Site	Date	Time Received	Time Collected	Air Temp (°C)	Wind Intensity	Wind Direction	Weather	Water color	Water Odor	Days Since Last Rainfall	Rainfall (1 day) (inches)	Rainfall (7 days) (inches)	Comments
OSTGW08A	6/9/09	0858	DRY	28.7	1	none	1	DRY	DRY	6	0.00	0.98	DRY
OSTGW08B	6/9/09	0858	0744	28.7	1	none	1	6	6	6	0.00	0.98	
OSTGW09A	6/9/09	0858	0805	28.9	1	none	1	5	6	6	0.00	0.98	
OSTGW09B	6/9/09	0858	0806	28.9	1	none	1	5	6	6	0.00	0.98	
OSTGW10A	6/10/09	1052	0923	29.2	3	6	1	6-cream	6	7	0.00	0.98	birds in air
OSTGW10B	6/10/09	1052	0956	29.2	3	6	1	5	6	7	0.00	0.98	birds in air
OSTGW14A	6/9/09	0858	0820	29.3	1	none	1	6-grey	6	6	0.00	0.98	Cow pasture on both sides of creek
OSTGW14B	6/9/09	0858	0821	29.3	1	none	1	5	6	6	0.00	0.98	Cow pasture on both sides of creek
OSTGW15A	6/10/09	1052	DRY	31.5	3	6	1	DRY	DRY	7	0.00	0.98	DRY
OSTGW15B	6/10/09	1052	1017	31.5	3	6	1	5	6	7	0.00	0.98	Heavy trash, 30 birds in air
OSTGW08A	9/14/09	1335	1132	28.3	2	7	1	1	6	2	0	4.51	
OSTGW08B	9/14/09	1335	1140	28.3	2	7	1	1	6	2	0	4.51	
OSTGW09A	9/14/09	1335	1155	28.4	2	7	1	2	6	2	0	4.51	
OSTGW09B	9/14/09	1335	1156	28.4	2	7	1	5	6	2	0	4.51	
OSTGW10A	9/14/09	1335	1229	29.4	2	4	1	1	6	2	0	4.51	
OSTGW10B	9/14/09	1335	1232	29.4	2	4	1	1	6	2	0	4.51	
OSRGW14A	9/14/09	1335	1219	29.1	2	4	1	5	6	2	0	4.51	
OSTGW14B	9/14/09	1335	1220	29.1	2	4	1	5	6	2	0	4.51	
OSTGW15A	9/14/09	1335	DRY	30.1	3	7	1	DRY	DRY	2	0	4.51	DRY
OSTGW15B	9/14/09	1335	1315	30.1	3	7	1	1	6	2	0	4.51	Moderate trash
OSTGW01A	10/28/09	1455	1248	28.5	3	4	2	6-yellow	1	2	0	1.75	
OSTGW03A	10/28/09	1455	1222	29.5	3	4	2	DRY	DRY	2	0	1.75	water in casing, DRY
OSTGW03B	10/28/09	1455	1222	29.5	3	4	2	5	6	2	0	1.75	

OSTGW04A	10/28/09	1455	1216	29.3	4	4	2	5	6	2	0	1.75	
OSTGW04B	10/28/09	1455	1216	29.3	4	4	2	5	6	2	0	1.75	
OSTGW05A	10/28/09	1455	1203	29.2	4	4	2	6-yellow	3	2	0	1.75	
OSTGW05B	10/28/09	1455	1203	29.2	4	4	2	6-yellow tan	3	2	0	1.75	
OSTGW06A	10/28/09	1455	1042	27	3	4	2	6-yellow	6	2	0	1.75	
OSTGW06B	10/28/09	1455	1042	27	3	4	2	5	6	2	0	1.75	
OSTGW07A	10/28/09	1455	1103	28.2	4	4	2	5	6	2	0	1.75	water inside casing
OSTGW07B	10/28/09	1455	1106	28.2	4	4	2	5	6	2	0	1.75	water inside casing
OSTGW08A	10/26/09	1415	1215	22.5	4	1	3	DRY	DRY	1	0.94	1.23	Cold front moving in, wind picked up and is raining
OSTGW08B	10/26/09	1415	1215	22.5	4	1	3	6-tan	6	1	0.94	1.23	
OSTGW09A	10/26/09	1415	1206	31.6	4	3	3	5	6	1	0.94	1.23	Cold front just came through
OSTGW09B	10/26/09	1415	1206	31.6	4	3	3	1	6	1	0.94	1.23	Cold front just came through
OSTGW10A	10/26/09	1415	1109	28.8	3	3	2	1	6	1	0.94	1.23	
OSTGW10B	10/26/09	1415	1109	28.8	3	3	2	5	6	1	0.94	1.23	
OSTGW11A	10/28/09	1455	1337	30.5	3	4	2	5	6	2	0	1.75	
OSTGW11B	10/28/09	1455	1337	30.5	3	4	2	5	6	2	0	1.75	
OSTGW12A	10/28/09	1455	1235	29.2	3	4	2	5	3	2	0	1.75	
OSTGW12B	10/28/09	1455	1235	29.2	3	4	2	5	3	2	0	1.75	
OSTGW13A	10/28/09	1455	1049	28.1	3	4	2	6-yellow	6	2	0	1.75	
OSTGW13B	10/28/09	1455	1049	28.1	3	4	2	5	6	2	0	1.75	3 fishermen
OSTGW14A	10/26/09	1415	1140	29.1	3	3	2	1	6	1	0.94	1.23	
OSTGW14B	10/26/09	1415	1140	29.1	3	3	2	5	6	1	0.94	1.23	
OSTGW15A	10/26/09	1415	1049	28	4	3	2	DRY	DRY	1	0.94	1.23	DRY
OSTGW15B	10/26/09	1415	1049	28	4	3	2	5	6	1	0.94	1.23	
OSTGW16A	10/28/09	1455	1118	29.1	4	4	2	5	3	2	0	1.75	
OSTGW16B	10/28/09	1455	1118	29.1	4	4	2	5	3	2	0	1.75	
OSTGW17A	10/28/09	1455	1128	29.6	3	4	2	6-tan	6	2	0	1.75	
OSTGW17B	10/28/09	1455	1128	29.6	3	4	2	6-clear yellow	6	2	0	1.75	
OSTGW19A	10/28/09	1455	1144	30.2	3	4	2	5	6	2	0	1.75	

OSTGW19B	10/28/09	1455	1144	30.2	3	4	2	5	6	2	0	1.75	Feces on concrete well outside casing
OSTGW20A	10/28/09	1455	1027	26.6	3	4	2	5	6	2	0	1.75	
OSTGW20B	10/28/09	1455	1027	26.6	3	4	2	5	6	2	0	1.75	
OSTGW01A	3/30/10	1225	1154	26	2	6	1	5	6	6	0	0.06	
OSTGW03A	3/30/10	1225	1125	25.6	2	6	1	6-light brown	6	6	0	0.06	
OSTGW03B	3/30/10	1225	1126	25.6	2	6	1	5	6	6	0	0.06	
OSTGW04A	3/30/10	1225	1118	24.9	2	6	1	5	6	6	0	0.06	
OSTGW04B	3/30/10	1225	1119	24.9	2	6	1	5	6	6	0	0.06	
OSTGW05A	3/30/10	1225	1105	24.2	2	6	1	6	3	6	0	0.06	
OSTGW05B	3/30/10	1225	1106	24.2	2	6	1	6-yellow	6	6	0	0.06	
OSTGW06A	3/29/10	1350	1142	22.6	1	6	1	5	6	5	0	0.06	
OSTGW06B	3/29/10	1350	1143	22.6	1	6	1	5	6	5	0	0.06	
OSTGW07A	3/29/10	1350	1110	21.43	1	6	1	5	6	5	0	0.06	
OSTGW07B	3/29/10	1350	1111	21.43	1	6	1	5	6	5	0	0.06	
OSTGW08A	3/29/10	1350	DRY	24.5	1	6	1	DRY	DRY	5	0	0.06	
OSTGW08B	3/29/10	1350	1206	24.5	1	6	1	2	6	5	0	0.06	
OSTGW09A	3/29/10	1350	1317	24.5	1	6	1	5	6	5	0	0.06	
OSTGW09B	3/29/10	1350	1318	24.5	1	6	1	5	6	5	0	0.06	
OSTGW10A	3/29/10	1350	1252	25.9	1	6	1	5	6	5	0	0.06	
OSTGW10B	3/29/10	1350	1253	25.9	1	6	1	5	6	5	0	0.06	
OSTGW11A	3/30/10	1225	1206	26	2	6	1	6-light brown	6	6	0	0.06	
OSTGW11B	3/30/10	1225	1207	26	2	6	1	6-brown-red	6	6	0	0.06	
OSTGW12A	3/30/10	1225	1140	26.4	2	6	1	5	3	6	0	0.06	
OSTGW12B	3/30/10	1225	1139	26.4	2	6	1	5	6	6	0	0.06	
OSTGW13A	3/29/10	1350	1129	22.5	1	6	1	2	6	5	0	0.06	Heavy trash, construction on the other side of bridge
OSTGW13B	3/29/10	1350	1130	22.5	1	6	1	5	6	5	0	0.06	Heavy trash, construction on the other side of bridge
OSTGW14A	3/29/10	1350	1307	24.5	1	6	1	5	6	5	0	0.06	
OSTGW14B	3/29/10	1350	1308	24.5	1	6	1	5	6	5	0	0.06	

OSTGW15A	3/29/10	1350	1230	25.1	1	6	1	6-light brown	6	5	0	0.06	Moderate Trash
OSTGW15B	3/29/10	1350	1231	25.1	1	6	1	1	6	5	0	0.06	Moderate Trash
OSTGW16A	3/30/10	1225	1023	22.5	2	6	1	5	6	6	0	0.06	
OSTGW16B	3/30/10	1225	1024	22.5	2	6	1	5	3	6	0	0.06	
OSTGW17A	3/30/10	1225	1034	23.5	2	6	1	5	6	6	0	0.06	
OSTGW17B	3/30/10	1225	1035	23.5	2	6	1	5	6	6	0	0.06	
OSTGW19A	3/30/10	1225	1048	24.4	2	6	1	5	6	6	0	0.06	
OSTGW19B	3/30/10	1225	1049	24.4	2	6	1	5	6	6	0	0.06	
OSTGW20A	3/29/10	1350	1152	23.8	1	6	1	5	6	5	0	0.06	
OSTGW20B	3/29/10	1350	1153	23.8	1	6	1	5	6	5	0	0.06	
GW08A	7/6/10	1235	1030	28.8	1	NA	2	5	6	4	0	6.23	
GW08B	7/6/10	1235	1030	28.8	1	NA	2	5	6	4	0	6.23	
GW09A	7/6/10	1235	1042	31.4	2	3	2	5	6	4	0	6.23	
GW09B	7/6/10	1235	1042	31.4	2	3	2	6 Brown-Yellow	6	4	0	6.23	
GW10A	7/6/10	1235	1051	30.4	2	3	2	5	6	4	0	6.23	
GW10B	7/6/10	1235	1053	30.4	2	3	2	5	6	4	0	6.23	
GW14A	7/6/10	1235	1120	34	1	NA	2	5	6	4	0	6.23	Well cap was broken by farmer plowing over it.
GW14B	7/6/10	1235	1121	34	1	NA	2	1	6	4	0	6.23	Well cap was broken by farmer plowing over it.
GW15A	7/6/10	1235	1141	34.1	1	NA	2	1	6	4	0	6.23	
GW15B	7/6/10	1235	1148	34.1	1	NA	2	5	6	4	0	6.23	
GW08A	2/7/11	1330	DRY	16.7	4	4	1	NA	NA	3	0	0.05	
GW08B	2/7/11	1330	1255	16.7	4	4	1	5	6	3	0	0.05	
GW09A	2/7/11	1330	1210	15.7	4	4	1	5	6	3	0	0.05	
GW09B	2/7/11	1330	1210	15.7	4	4	1	5	6	3	0	0.05	
GW10A	2/7/11	1330	1112	14.2	4	4	1	6-Light brown	6	3	0	0.05	
GW10B	2/7/11	1330	1112	14.2	4	4	1	1	6	3	0	0.05	
GW14A	2/7/11	1330	1130	16.7	4	4	1	1	6	3	0	0.05	
GW14B	2/7/11	1330	1130	16.7	4	4	1	1	6	3	0	0.05	

GW15A	2/7/11	1330	1050	12.6	4	4	1	5	6	3	0	0.05
GW15B	2/7/11	1330	1050	12.6	4	4	1	1	6	3	0	0.05

Table A.3. Field Data Water and Sediment Sampling 2009-2011

Site	Date	Time Received	Time Collected	Depth (cm)	Air Temp (°C)	Wind Intensity	Wind Direction	Weather	Water Temp (°C)	Conductivity (mhos/cm)
OST18499	5/8/09	1245	1015	15.0	34.7	3	2	2	26.99	1.760
OST18500	5/8/09	1245	1155	4.0	34.2	2	6	2	27.77	3.275
OST20559	5/8/09	1245	1105	12.0	32.7	3	6	2	27.82	1.739
OST18499(SW)	8/11/09	1113	810	29.5	28.2	1	6	1	28.79	1.644
OST18499(S)	8/11/09	1113	826	15.0	28.2	1	6	1	NA	NA
OST18500(SW)	8/11/09	1113	952	11.0	30.3	2	2	2	28.45	2.580
OST18500(S)	8/11/09	1113	958	15.0	30.3	2	2	2	NA	NA
OST20559(SW)	8/11/09	1113	923	12.0	29.7	2	6	2	30.22	1.553
OST20559(S)	8/11/09	1113	925	15.0	29.7	2	6	2	NA	NA
OST20198(SW)	8/11/09	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
OST18499(SW)	11/11/09	1320	1018	15.0	23.5	1	1	1	20.12	1.829
OST18499(S)	11/11/09	1320	1005	15.0	23.5	1	1	1	20.12	1.829
OST18500(SW)	11/11/09	1320	1149	15.0	28.2	2	1	1	20.15	2.779
OST18500(S)	11/11/09	1320	1149	15.0	28.2	2	1	1	20.15	2.779
OST18501(SW)	11/11/09	1320	1135	15.0	27.7	2	1	1	19.35	0.268
OST18501(S)	11/11/09	1320	1135	15.0	27.7	2	1	1	19.35	0.268
OST20559(SW)	11/11/09	1320	1040	15.0	27.5	1	1	1	25.91	1.115
OST20559(S)	11/11/09	1320	1040	15.0	27.5	1	1	1	25.91	1.115
OST18499(SW)	11/23/09	1300	1025	15.0	25.0	1	6	1	17.23	0.894
OST18499(S)	11/23/09	1300	1044	15.0	25.0	1	6	1	17.23	NA
OST18500(SW)	11/23/09	1300	1145	15.0	30.1	2	6	1	16.82	0.788
OST18500(S)	11/23/09	1300	1145	15.0	30.1	2	6	1	16.82	NA
OST18501(SW)	11/23/09	1300	1131	15.0	28.3	2	6	1	16.70	0.224

OST18501(S)	11/23/09	1300	1131	15.0	28.3	2	6	1	16.70	NA
OST20559(SW)	11/23/09	1300	1105	15.0	26.1	2	6	1	22.41	1.679
OST20559(S)	11/23/09	1300	1105	15.0	26.1	2	6	1	22.41	NA
OST18499(SW)	2/17/10	1325	1047	15.0	18.7	1	3	1	12.07	1.771
OST18499(S)	2/17/10	1325	1048	15.0	18.7	1	3	1	12.07	NA
OST18500(SW)	2/17/10	1325	1230	15.0	18.4	1	5	1	12.53	3.580
OST18500(S)	2/17/10	1325	1231	15.0	18.4	1	5	1	12.53	NA
OST18501(SW)	2/17/10	1325	1210	15.0	16.7	2	5	1	12.00	0.351
OST18501(S)	2/17/10	1325	1213	15.0	16.7	2	5	1	12.00	NA
OST20559(SW)	2/17/10	1325	1120	15.0	16.9	2	3	1	18.59	1.176
OST20559(S)	2/17/10	1325	1123	15.0	16.9	2	3	1	18.59	NA
OST18499(SW)	5/31/10	1143	0957	15.0	28.4	2	8	1	26.28	2.098
OST18499(S)	5/31/10	1143	1002	15.0	28.4	2	8	1	NA	NA
OST18500(SW)	5/31/10	1143	1058	15.0	28.7	1	8	1	25.69	4.706
OST18500(S)	5/31/10	1143	1101	15.0	28.7	1	8	1	NA	NA
OST18501(SW)	5/31/10	1143	1032	15.0	28.1	1	8	2	25.63	0.657
OST18501(S)	5/31/10	1143	1037	15.0	28.1	1	8	2	NA	NA
OST20559(SW)	5/31/10	1143	NA	NA	28.0	2	8	1	NA	NA
OST20559(S)	5/31/10	1143	NA	NA	28.0	2	8	1	NA	NA
OST20198(SW)	5/31/10	1143	DRY	DRY	30.4	2	8	1	DRY	DRY
OST20198(S)	5/31/10	1143	1020	15.0	30.4	2	8	1	NA	NA
OST18499(SW)	9/13/10	1510	1209	15.0	33.9	2	3	2	31.30	1.756
OST18499(S)	9/13/10	1510	1210	15.0	33.9	2	3	2	NA	NA
OST18500(SW)	9/13/10	1510	1410	15.0	34.0	2	3	2	29.41	2.979
OST18500(S)	9/13/10	1510	1413	15.0	34.0	2	3	2	NA	NA
OST18501(SW)	9/13/10	1510	1349	15.0	33.9	2	3	2	29.55	0.537
OST18501(S)	9/13/10	1510	1351	15.0	33.9	2	3	2	NA	NA
OST20559(SW)	9/13/10	1510	1240	15.0	31.4	2	3	2	30.72	1.502
OST20559(S)	9/13/10	1510	1242	15.0	31.4	2	3	2	NA	NA
OST20198(SW)	9/13/10	1510	1326	15.0	33.9	2	3	2	32.19	0.589
OST20198(S)	9/13/10	1510	1331	15.0	33.9	2	3	2	NA	NA

OST18499(SW)	12/6/10	1420	1143	15.0	18.8	3	5	2	14.73	3.025
OST18499(S)	12/6/10	1420	1147	15.0	18.8	3	5	2	NA	NA
OST18500(SW)	12/6/10	1420	1323	15.0	20.4	3	5	2	13.96	6.071
OST18500(S)	12/6/10	1420	1323	15.0	20.4	3	5	2	NA	NA
OST18501(SW)	12/6/10	1420	1305	15.0	18.2	3	5	2	12.2	0.517
OST18501(S)	12/6/10	1420	1305	15.0	18.2	3	5	2	NA	NA
OST20559(SW)	12/6/10	1420	1215	15.0	18.7	3	5	2	21.33	2.506
OST20559(S)	12/6/10	1420	1215	15.0	18.7	3	5	2	NA	NA
OST20198(SW)	12/6/10	1420	DRY	DRY	15.3	3	5	2	NA	NA
OST20198(S)	12/6/10	1420	1242	15.0	15.3	3	5	2	NA	NA
OST18499(SW)	3/9/11	1220	0940	15.0	20.0	2	1	2	21.41	3.348
OST18499(S)	3/9/11	1220	0940	15.0	20.0	2	1	2	NA	NA
OST18500(SW)	3/9/11	1220	1116	15.0	21.8	4	1	3	21.19	6.398
OST18500(S)	3/9/11	1220	1145	15.0	21.8	4	1	3	NA	NA
OST18501(SW)	3/9/11	1220	1055	15.0	21.0	4	1	3	20.63	1.157
OST18501(S)	3/9/11	1220	1055	15.0	21.0	4	1	3	NA	NA
OST20559(SW)	3/9/11	1220	1010	15.0	20.6	3	1	2	22.41	2.891
OST20559(S)	3/9/11	1220	1010	15.0	20.6	3	1	2	NA	NA
OST20198(SW)	3/9/11	1220	DRY	DRY	20.7	4	1	3	NA	NA
OST20198(S)	3/9/11	1220	1035	15.0	20.7	4	1	3	NA	NA

Site	Date	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Secchi Disk (m)	Water color	Water Odor	Water Surface	Days Since Last Rainfall	Rainfall (1 day) (inches)	Rainfall (7 days) (inches)	Comments
OST18499	5/8/09	0.89	4.92	7.64	0.10	6	6	1	9	0.00	0.00	2 large turtles
OST18500	5/8/09	1.70	6.97	7.92	0.10	1	6	2	9	0.00	0.00	Light trash
OST20559	5/8/09	0.87	5.45	7.34	0.55	3	6	1	9	0.00	0.00	Light trash
OST18499(SW)	8/11/09	0.82	NA	7.11	5.00	3	2	1	30	NA	NA	Thick layer of algae on surface of water, stagnant
OST18499(S)	8/11/09	NA	NA	NA	NA	NA	NA	NA	30	NA	NA	
OST18500(SW)	8/11/09	1.32	NA	7.65	7.00	1	6	2	30	NA	NA	Heavy trash, algae
OST18500(S)	8/11/09	NA	NA	NA	NA	NA	NA	NA	30	NA	NA	
OST20559(SW)	8/11/09	0.77	NA	7.35	36.00	5	6	1	30	NA	NA	
OST20559(S)	8/11/09	NA	NA	NA	NA	NA	NA	NA	30	NA	NA	
OST20198(SW)	8/11/09	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
OST18499(SW)	11/11/09	1.05	NA	7.61	0.70	1	6	1	3	0.00	0.43	
OST18499(S)	11/11/09	1.05	NA	7.61	0.70	1	6	1	3	0.00	0.43	
OST18500(SW)	11/11/09	1.62	NA	7.66	0.08	1	6	2	3	0.00	0.43	
OST18500(S)	11/11/09	1.62	NA	7.66	0.08	1	6	2	3	0.00	0.43	
OST18501(SW)	11/11/09	0.15	NA	7.68	0.03	1	6	1	3	0.00	0.43	
OST18501(S)	11/11/09	0.15	NA	7.68	0.03	1	6	1	3	0.00	0.43	
OST20559(SW)	11/11/09	1.07	NA	7.37	0.39	5	6	1	3	0.00	0.43	Debris dam preventing water flow downstream. Soft sediment layer is 33 cm deep.
OST20559(S)	11/11/09	1.07	NA	7.37	0.39	5	6	1	3	0.00	0.43	Debris dam preventing water flow downstream. Soft sediment layer is 33 cm deep.
OST18499(SW)	11/23/09	0.44	6.99	7.50	0.07	1	6	1	3	0.00	2.74	Tree down, debris creating dam, light trash
OST18499(S)	11/23/09	NA	NA	NA	NA	NA	NA	NA	3	0.00	2.74	Tree down, debris creating

												dam, light trash
OST18500(SW)	11/23/09	0.39	9.52	7.68	0.06	1	6	2	3	0.00	2.74	Flooding, fast flowing water
OST18500(S)	11/23/09	NA	NA	NA	NA	NA	NA	NA	3	0.00	2.74	Flooding, fast flowing water
OST18501(SW)	11/23/09	0.11	8.82	7.77	0.05	1	6	1	3	0.00	2.74	Cows in adjacent field
OST18501(S)	11/23/09	NA	NA	NA	NA	NA	NA	NA	3	0.00	2.74	Cows in adjacent field
OST20559(SW)	11/23/09	0.85	7.13	7.50	0.23	1	6	1	3	0.00	2.74	Strong rotten egg smell
OST20559(S)	11/23/09	NA	NA	NA	NA	NA	NA	NA	3	0.00	2.74	Strong rotten egg smell
OST18499(SW)	2/17/10	0.90	10.19	7.95	0.11	6	3	1	3	0.00	1.49	No flow
OST18499(S)	2/17/10	NA	NA	NA	NA	NA	NA	NA	3	0.00	1.49	
OST18500(SW)	2/17/10	1.90	13.89	7.91	0.12	5	6	2	3	0.00	1.49	
OST18500(S)	2/17/10	NA	NA	NA	NA	NA	NA	NA	3	0.00	1.49	
OST18501(SW)	2/17/10	0.17	1.04	8.27	0.08	1	3	1	3	0.00	1.49	2 cows in adjacent field, roughly 30 cows in 3 fields over, No flow
OST18501(S)	2/17/10	NA	NA	NA	NA	NA	NA	NA	3	0.00	1.49	2 cows in adjacent field, roughly 30 cows in 3 fields over, No flow
OST20559(SW)	2/17/10	0.87	9.70	7.76	0.20	5	1	1	3	0.00	1.49	Debris at least 6ft high blocking over 75% of drain. Water stagnant, pungent smell, rotten egg, heavy trash and creek is very low.
OST20559(S)	2/17/10	NA	NA	NA	NA	NA	NA	NA	3	0.00	1.49	Debris at least 6ft high blocking over 75% of drain. Water stagnant, pungent smell, rotten egg, heavy trash and creek is very low.
OST18499(SW)	5/31/10	1.07	3.58	7.48	0.05	6-light brown	6	1	6	0	0.06	Heavy trash, rotten egg smell from sediment, 40 birds under bridge, overgrown weeds, and algae and tree debris on the water surface.
OST18499(S)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	Heavy trash, rotten egg smell from sediment, 40 birds under bridge, overgrown weeds, and algae and tree debris on the water surface.

OST18500(SW)	5/31/10	2.51	6.26	7.75	0.1	6-light brown	6	2	6	0	0.06	
OST18500(S)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	
OST18501(SW)	5/31/10	0.32	2.41	7.73	0.05	1	6	1	6	0	0.06	
OST18501(S)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	
OST20559(SW)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	Unable to sample, too hazardous. No flow
OST20559(S)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	Unable to sample, too hazardous. No flow
OST20198(SW)	5/31/10	DRY	DRY	DRY	DRY	DRY	DRY	DRY	6	0	0.06	
OST20198(S)	5/31/10	NA	NA	NA	NA	NA	NA	NA	6	0	0.06	
OST18499(SW)	9/13/10	0.88	NA	7.88	0.10	1	6	1	1	0.58	2.93	Heavy trash
OST18499(S)	9/13/10	NA	NA	NA	NA	NA	NA	NA	1	0.58	2.93	Heavy trash
OST18500(SW)	9/13/10	1.54	5.90	7.61	0.12	5	6	2	1	0.58	2.93	
OST18500(S)	9/13/10	NA	NA	NA	NA	NA	NA	NA	1	0.58	2.93	
OST18501(SW)	9/13/10	0.26	3.63	7.77	0.05	6	6	1	1	0.58	2.93	+10 cows in pasture, moderate trash
OST18501(S)	9/13/10	NA	NA	NA	NA	NA	NA	NA	1	0.58	2.93	+10 cows in pasture, moderate trash
OST20559(SW)	9/13/10	0.75	3.96	7.2	0.91	5	6	1	1	0.58	2.93	Drain dammed with debris, moderate trash, heavy over growth
OST20559(S)	9/13/10	NA	NA	NA	NA	NA	NA	NA	1	0.58	2.93	Drain dammed with debris, moderate trash, heavy over growth
OST20198(SW)	9/13/10	0.28	NA	7.59	0.95	6-Dark Brown	7-fecal matter	1	1	0.58	2.93	Red algae on top of water
OST20198(S)	9/13/10	NA	NA	NA	NA	NA	NA	NA	1	0.58	2.93	Red algae on top of water
OST18499(SW)	12/6/10	1.59	4.51	7.47	0.11	5	6	1	1	0.02	0.02	Crop blockage still present, heavy trash
OST18499(S)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	Crop blockage still present, heavy trash
OST18500(SW)	12/6/10	3.32	9.45	7.72	0.23	5	6	2	1	0.02	0.02	Heavy trash
OST18500(S)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	Heavy trash
OST18501(SW)	12/6/10	0.25	9.28	8.18	0.09	1	7	1	1	0.02	0.02	3 cows upstream in the creek. Heavy trash, dead animal carcass.

OST18501(S)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	3 cows upstream in the creek. Heavy trash, dead animal carcass.
OST20559(SW)	12/6/10	1.29	8.99	7.56	0.73	5	6	1	1	0.02	0.02	Sides of ravine dozed to remove plants, heavy trash, creek had widened, but became shallower. (0.2 ft deep now)
OST20559(S)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	Sides of ravine dozed to remove plants, heavy trash, creek had widened, but became shallower. (0.2 ft deep now)
OST20198(SW)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	More than 100 blackbirds around, both in air and on ground, site. Creek was dry.
OST20198(S)	12/6/10	NA	NA	NA	NA	NA	NA	NA	1	0.02	0.02	More than 100 blackbirds around, both in air and on ground, site. Creek was dry.
OST18499(SW)	3/9/11	1.76	3.62	7.49	0.10	1	6	1	4	0.00	0.03	Green algae on top of water, blockage still present
OST18499(S)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	
OST18500(SW)	3/9/11	3.50	7.28	7.68	0.13	1	6	2	4	0.00	0.03	
OST18500(S)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	
OST18501(SW)	3/9/11	0.57	8.06	7.91	0.12	1	6	1	4	0.00	0.03	
OST18501(S)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	
OST20559(SW)	3/9/11	1.50	8.63	7.57	0.70	5	6	1	4	0.00	0.03	Sides to steep to take PYGMY meter down.
OST20559(S)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	
OST20198(SW)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	
OST20198(S)	3/9/11	NA	NA	NA	NA	NA	NA	NA	4	0.00	0.03	

Table A.4. Field Data Seepage Sampling

Site	Sampling Date	Placement Date	Removal Date	Time Placed	Time Removed	Time Received	Air Temp (°C)	Wind Intensity	Wind Direction	Weather
18499-P	11/6/09	11/6/09	NA	1430	NA	NA	30.5	3	3	2
18499-R	11/11/09	NA	11/11/09	NA	1320	1320	23.5	1	1	1
18500-P	11/6/09	11/6/09	NA	1525	NA	NA	29.1	2	3	2
18500-R	11/11/09	NA	11/11/09	NA	1149	1320	28.2	2	1	1
18501-P	11/6/09	11/6/09	NA	1510	NA	NA	29.5	2	3	2
18501-R	11/11/09	NA	11/11/09	NA	1135	1320	27.7	2	1	1
18499-P	11/16/09	11/16/09	NA	1120	NA	NA	19.8	4	1	3
18499-R	11/18/09	NA	11/18/09	NA	1127	1355	22.6	1	8	1
18500-P	11/16/09	11/16/09	NA	1207	NA	NA	20.6	4	1	2
18500-R	11/18/09	NA	11/18/09	NA	1249	1355	23.4	1	8	1
18501-P	11/16/09	11/16/09	NA	1146	NA	NA	19.2	4	1	3
18501-R	11/18/09	NA	11/18/09	NA	1234	1355	26.4	1	8	1
18499-P	1/29/10	1/29/10	NA	1320	NA	NA	16.7	3	1	2
18499-R	2/3/10	NA	2/3/10	NA	1127	1335	18.0	4	5	4
18500-P	1/29/10	1/29/10	NA	1401	NA	NA	16.5	2	1	2
18500-R	2/3/10	NA	2/3/10	NA	1220	1335	19.0	4	5	4
18501-P	1/29/10	1/29/10	NA	1348	NA	NA	16.1	3	1	2
18501-R	2/3/10	NA	2/3/10	NA	1245	1335	20.8	4	5	4
18499-P	7/16/10	7/16/10	NA	1117	NA	NA	30.0	1	NA	2
18499-R	7/19/10	NA	7/19/10	NA	1055	1230	32.2	2	2	2
18500-P	7/16/10	7/16/10	NA	1153	NA	NA	30.3	1	NA	2
18500-R	7/19/10	NA	7/19/10	NA	1151	1230	31.8	2	2	2
18501-P	7/16/10	7/16/10	NA	1137	NA	NA	30.4	1	NA	2
18501-R	7/19/10	NA	7/19/10	NA	1130	1230	30.4	2	2	2

Site	Sampling Date	Water Temp (°C)	Conductivity (mhos/com)	Salinity (ppt)	DO (mg/L)	pH (s.u.)	Water color	Water odor	Water surface	Days Since Last Rainfall	Rainfall (1 day) (inches)	Rainfall (7 days) (inches)
18499-P	11/6/09	23.02	NA	0.01	NA	7.43	1	6	1	8	0.00	0.00
18499-R	11/11/09	20.12	1.829	1.05	NA	7.61	1	6	1	3	0.00	0.43
18500-P	11/6/09	20.12	2.498	1.46	NA	7.49	1	6	1	8	0.00	0.00
18500-R	11/11/09	20.15	2.779	1.62	NA	7.66	1	6	2	3	0.00	0.43
18501-P	11/6/09	17.73	0.246	0.15	NA	7.51	1	6	1	8	0.00	0.00
18501-R	11/11/09	19.35	0.268	0.15	NA	7.68	1	6	1	3	0.00	0.43
18499-P	11/16/09	21.31	1.984	1.11	NA	7.58	1	6	1	8	0.00	0.00
18499-R	11/18/09	14.38	2.238	1.15	NA	7.65	1	6	1	2	0.00	0.07
18500-P	11/16/09	20.63	3.315	1.84	NA	7.71	5	6	2	8	0.00	0.00
18500-R	11/18/09	14.52	3.329	1.76	NA	7.9	5	6	2	2	0.00	0.07
18501-P	11/16/09	19.27	0.281	0.15	NA	7.68	1	6	1	8	0.00	0.00
18501-R	11/18/09	14.75	0.313	0.15	NA	7.58	1	6	1	2	0.00	0.07
18499-P	1/29/10	18.39	2.268	1.17	6.66	7.69	1	6	1	14	0.00	0.00
18499-R	2/3/10	14.72	2.012	1.03	6.16	7.67	1	6	1	0	1.07	1.43
18500-P	1/29/10	18.26	5.290	3.304	6.47	7.55	5	6	2	14	0.00	0.00
18500-R	2/3/10	15.14	2.307	1.19	6.66	7.64	1	6	2	0	1.07	1.43
18501-P	1/29/10	17.67	0.368	0.18	4.5	7.69	1	6	2	14	0.00	0.00
18501-R	2/3/10	17.89	0.002	0	6.57	8.03	1	6	1	0	1.07	1.43
18499-P	7/16/10	30.23	1.367	0.68	5.79	7.72	1	3	1	7	0.00	0.04
18499-R	7/19/10	29.59	1.430	0.71	4.62	7.29	1	6	1	1	1.24	1.24
18500-P	7/16/10	28.77	4.204	2.22	5.96	7.47	5	6	2	7	0.00	0.04
18500-R	7/19/10	28.5	4.361	2.31	5.94	7.54	6-yellow brown	6	2	1	1.24	1.24
18501-P	7/16/10	29.92	0.440	0.2	2.02	7.5	1	6	1	7	0.00	0.04
18501-R	7/19/10	30.93	0.436	0.21	2.59	7.61	1	6	1	1	1.24	1.24

A.5. Field Data Soil Sampling

Site	Date	Time Received	Time Collected	Depth (cm)	Air Temp (°C)	Wind Intensity	Wind Direction	Weather	Days Since Last Rainfall	Rainfall (1 day) (inches)	Rainfall (7 days) (inches)	Comments
SO02	7/29/09	1103	0902	15	28.9	3	6	2	17	NA	NA	House next to field near upland elevation, 8 birds in air
SO04	7/29/09	1103	0944	15	31.3	4	6	2	17	NA	NA	
SO03	8/18/09	1105	0958	15	30.5	2	6	2	6	NA	0.06	
SO07	8/5/09	1045	0926	15	29.5	3	6	1	14	NA	NA	
SO09	8/5/09	1045	0852	15	28.4	3	6	1	24	NA	NA	
SO02G	9/18/09	1400	1230	15	31.5	2	1	1	6	0.00	1.15	Very soft soil, no crops in field, no animal tracks, no trash
SO02P	9/18/09	1400	1237	15	31.5	2	1	1	6	0.00	1.15	Very wet soil, standing water on top of soil, Rodent tracks, smelled like animal feces, no birds no trash
SO03G	9/18/09	1400	1259	15	32.5	2	1	1	6	0.00	1.15	Top soil showing evidence of drying, small cracks were formed, lower part still moist, no animals, no trash, field was barren, Rainfall- on 8th day 2.8"
SO03P	9/18/09	1400	1313	15	32.5	2	1	1	6	0.00	1.15	No trash, no birds, no animals, moist soft soil, field was barren
SO04G	9/17/09	1705	1600	15	32.9	2	1	1	5	0.00	3.95	Small cracks in soil, no animals, no trash
SO04P	9/17/09	1705	1605	15	32.9	2	1	1	5	0.00	3.95	Softer soil then SO04G, small cracks, no trash no animals
SO07G	9/17/09	1705	1523	15	32.8	2	1	1	5	0.00	3.95	Ground was hard, heavy green grass growth, no trash, 26 cows in field, no birds, cow feces in area
SO07P	9/17/09	1705	1534	15	32.8	2	1	1	5	0.00	3.95	Moist soil, tall green grass-1 to 2 ft high, cow dropping

SO09G	9/18/09	1700	1626	15	32.2	2	1	2	6	0.00	1.15	near by, creek beside field was full of water, 26 cows in field
SO09P	9/18/09	1700	1617	15	32.2	2	1	2	6	0.00	1.15	Soft soil, no cracks,plowed recently, cotton laying on top of field and soil, dead twigs, no growing plants, no animals, light trash-plastic bags, plastic caps
SO02G	12/2/09	1330	1035	15	16.0	2	4	1	1	1.19	1.45	
SO02P	12/2/09	1330	1040	15	16.0	2	4	1	1	1.19	1.45	
SO03G	12/2/09	1330	1055	15	18.2	2	4	1	1	1.19	1.45	
SO03P	12/2/09	1330	1100	15	18.2	2	4	1	1	1.19	1.45	
SO04G	12/3/09	1030	0831	15	10.0	3	1	1	2	1.19	1.45	
SO04P	12/3/09	1030	0839	15	10.0	3	1	1	2	1.19	1.45	Standing water in field
SO07G	12/3/09	1030	0859	15	13.7	3	1	1	2	1.19	1.45	Cows in field to the left.
SO07P	12/3/09	1030	0907	15	13.7	3	1	1	2	1.19	1.45	
SO09G	12/3/09	1030	0921	15	17.1	3	1	1	2	1.19	1.45	
SO09P	12/3/09	1030	0932	15	17.1	3	1	1	2	1.19	1.45	
SO02G	3/3/10	1200	1018	15	17.9	2	8	1	2	0.00	0.13	
SO02P	3/3/10	1200	1021	15	17.9	2	8	1	2	0.00	0.13	Standing water
SO03G	3/3/10	1200	1030	15	21.3	2	8	1	2	0.00	0.13	Standing water in ditch
SO03P	3/3/10	1200	1032	15	21.3	2	8	1	2	0.00	0.13	
SO04G	3/3/10	1200	1050	15	26.3	2	8	1	2	0.00	0.13	
SO04P	3/3/10	1200	1053	15	26.3	2	8	1	2	0.00	0.13	
SO07G	3/3/10	1200	1107	15	25.5	2	8	1	2	0.00	0.13	Estimated 60 cows in adjacent field, gates were open, cows were allowed in and out of both fields.

SO07P	3/3/10	1200	1110	15	25.5	2	8	1	2	0.00	0.13	Estimated 60 cows in adjacent field, gates were open, cows were allowed in and out of both fields.
SO09G	3/3/10	1200	1126	15	18.5	2	8	1	2	0.00	0.13	
SO09P	3/3/10	1200	1131	15	18.5	2	8	1	2	0.00	0.13	
SO02G	5/12/10	1135	0950	15	27.6	4	2	2	18	0.00	0.00	Grain planted, 1 bird in air
SO02P	5/12/10	1135	0952	15	27.6	4	2	2	18	0.00	0.00	Grain planted, 1 bird in air
SO03G	5/12/10	1135	1009	15	27.2	4	2	2	18	0.00	0.00	Soil cracking
SO03P	5/12/10	1135	1012	15	27.2	4	2	2	18	0.00	0.00	
SO04G	5/12/10	1135	1022	15	28.5	4	2	2	18	0.00	0.00	
SO04P	5/12/10	1135	1025	15	28.5	4	2	2	18	0.00	0.00	
SO07G	5/12/10	1135	1035	15	29.1	4	2	2	18	0.00	0.00	10 cows in field
SO07P	5/12/10	1135	1039	15	29.1	4	2	2	18	0.00	0.00	10 cows in field
SO09G	5/12/10	1135	1057	15	28.4	4	2	2	18	0.00	0.00	Feces found in field, grain
SO09P	5/12/10	1135	1101	15	28.4	4	2	2	18	0.00	0.00	Feces found in field, grain
SO02G	7/26/10	1205	1007	15	27.5	2	5	3	3	0.00	0.35	>100 black birds, crop harvested
SO02P	7/26/10	1205	1012	15	27.5	2	5	3	3	0.00	0.35	>100 black birds, crop harvested
SO03G	7/26/10	1205	1024	15	27.5	2	5	3	3	0.00	0.35	Field not harvested
SO03P	7/26/10	1205	1026	15	27.5	2	5	3	3	0.00	0.35	Field not harvested
SO04G	7/26/10	1205	1044	15	28.0	2	5	3	3	0.00	0.35	Crop harvested, lots of baby toads in field
SO04P	7/26/10	1205	1046	15	28.0	2	5	3	3	0.00	0.35	Crop harvested, lots of baby toads in field
SO07G	7/26/10	1205	1101	15	28.8	2	5	3	3	0.00	0.35	Cows are in surrounding pastures
SO07P	7/26/10	1205	1109	15	28.8	2	5	3	3	0.00	0.35	Cows are in surrounding pastures
SO09G	7/26/10	1205	1127	15	28.8	2	5	3	3	0.00	0.35	>100 birds in field
SO09P	7/26/10	1205	1131	15	28.8	2	5	3	3	0.00	0.35	>100 birds in field
SO02G	10/25/10	1330	1130	15	29.1	3	2	1	2	0.00	0.10	4 dove in air. Old creek plowed through. Barren field
SO02P	10/25/10	1330	1126	15	29.1	3	2	1	2	0.00	0.10	4 dove in air. Old creek plowed through. Barren field

SO03G	10/25/10	1330	1148	15	29.2	3	2	1	2	0.00	0.10	Barren field
SO03P	10/25/10	1330	1142	15	29.2	3	2	1	2	0.00	0.10	Barren field
SO04G	10/25/10	1330	1216	15	29.2	3	2	1	2	0.00	0.10	Barren field, 24 white cranes flying above and around field
SO04P	10/25/10	1330	1212	15	29.2	3	2	1	2	0.00	0.10	Barren field, 24 white cranes flying above and around field
SO07G	10/25/10	1330	1232	15	29.3	3	2	1	2	0.00	0.10	No cows in field, or adjacent fields
SO07P	10/25/10	1330	1242	15	29.3	3	2	1	2	0.00	0.10	No cows in field, or adjacent fields
SO09G	10/25/10	1330	1301	15	31.0	3	2	1	2	0.00	0.10	Barren field
SO09P	10/25/10	1330	1304	15	31.0	3	2	1	2	0.00	0.10	Barren field
SO02G	2/9/11	1130	0928	15	18.1	4	1	4	1	0.06	0.11	Heavy trash, weeds growin gin field, no crops
SO02P	2/9/11	1130	0932	15	18.1	4	1	4	1	0.06	0.11	Heavy trash, weeds growin gin field, no crops
SO03G	2/9/11	1130	0944	15	17.4	4	1	4	1	0.06	0.11	Barren
SO03P	2/9/11	1130	0946	15	17.4	4	1	4	1	0.06	0.11	Barren
SO04G	2/9/11	1130	1008	15	18.5	4	1	4	1	0.06	0.11	Weeds growing in field, no crops
SO04P	2/9/11	1130	1010	15	18.5	4	1	4	1	0.06	0.11	Weeds growing in field, no crops
SO07G	2/9/11	1130	1034	15	16.3	4	1	4	1	0.06	0.11	No cows in pasture
SO07P	2/9/11	1130	1042	15	16.3	4	1	4	1	0.06	0.11	No cows in pasture
SO09G	2/9/11	1130	1057	15	12.9	4	1	4	1	0.06	0.11	
SO09P	2/9/11	1130	1102	15	12.9	4	1	4	1	0.06	0.11	
SO02G	6/1/11	1105	1004	15	32.7	2	6	2	20	0	0	Cotton growing on field
SO02P	6/1/11	1105	1009	15	32.7	2	6	2	20	0	0	Red color on surface of soil. Cotton growing on field
SO03G	6/1/11	1105	1024	15	32.3	2	6	2	20	0	0	Cotton growing on field
SO03P	6/1/11	1105	1028	15	32.3	2	6	2	20	0	0	Cotton growing on field
SO04G	6/1/11	1105	938	15	32.7	2	6	2	20	0	0	Cotton growing on field
SO04P	6/1/11	1105	941	15	32.7	2	6	2	20	0	0	Cotton growing on field
SO07G	6/1/11	1105	916	15	31.3	2	6	2	20	0	0	1 cow in adjacent field, adjacent creek was dry, cow feces throughout field.

SO07P	6/1/11	1105	926	15	31.3	2	6	2	20	0	0	1 cow in adjacent field, adjacent creek was dry, cow feces throughout field.
SO09G	6/1/11	1105	903	15	29.9	2	6	2	20	0	0	Cotton growing on field
SO09P	6/1/11	1105	900	15	29.9	2	6	2	20	0	0	Cotton growing on field

Table A. Field Data Sampling for Animal Fecal Collections

Location	Latitude	Longitude	Animal Type
Agrilife Research Facility	27.7794027	-97.5732110	Bird
Rodeo Run Arena	27.7278083	-97.5007250	Horse
Pee Wee's Animal Shelter	27.7225361	-97.4622610	Dog, Horse
TCEQ Station 18500 | Memorial Gardens Cemetary	27.7300500	-97.5164600	Wildlife, Bird
Hwy 44 & Violet Rd Intersection	27.7840500	-97.5855830	Wildlife
Private Residence (3727 Country Rd 61)	27.7700270	-97.5969720	Cow, Horse
Private Residence (4104 FM Rd 1694)	27.7980500	-97.6183610	Horse
Robstown Waste Water Treatment Facility	27.8002700	-97.6503800	Human
Corpus Christi International Airport	27.7767700	-97.4950830	Wildlife, Bird
Hwy 22 & Hwy 2444 Intersection	27.6795500	-97.4546100	Wildlife
TCEQ Station 18499 | Train Tracks Bridge	27.7840700	-97.5927380	Wildlife

Location	Latitude	Longitude	Animal Type
TCEQ Station S2 | Yorktown Rd & Sun Valley Dr Bridge	27.6842050	-97.4226694	Bird
Roadside 7673 Weber Rd	27.6885900	-97.4325200	Dog
Nueces County Animal Control Kennels	27.7969200	-97.6536830	Dog
TCEQ Station 18501 | Fields	27.7095410	-97.5541980	Cow
Roadside 2642 FM Rd 763	27.6958300	-97.5016660	Bird
Nueces Veterinary Hospital (11027 Leopard St)	27.8416880	-97.5836720	Dog
Private Residence (2065 Co Rd 20A)	27.6536880	-97.5361840	Dog, Horse, Sheep, Bird
Roadside 8600-8698 S Staples St	27.6530410	-97.4099970	Wildlife
Gulf Coast Animal Shelter (3118 Cabaniss Rd)	27.7039780	-97.4300630	Dog
Private Residence (S Violet Rd)	27.7682800	-97.5639500	Cow, Bird, Dog, Wildlife