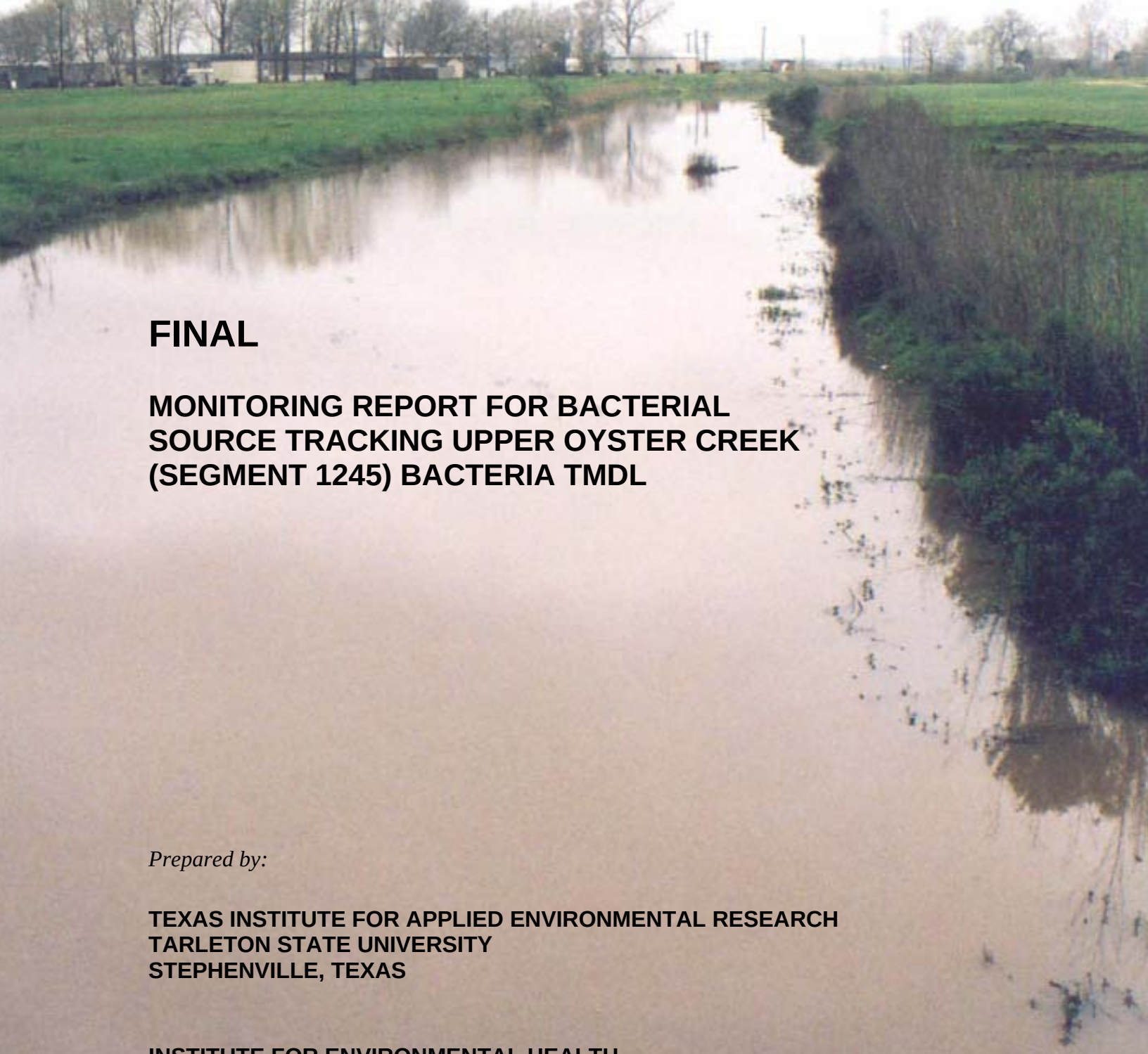

FINAL

MONITORING REPORT FOR BACTERIAL SOURCE TRACKING UPPER OYSTER CREEK (SEGMENT 1245) BACTERIA TMDL

Prepared by:

TEXAS INSTITUTE FOR APPLIED ENVIRONMENTAL RESEARCH
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ACRONYMS AND ABBREVIATIONS

ac/m	Acre-feet per month
BMP	Best management practice
BRA	Brazos River Authority
BST	Bacteria Source Tracking
CAFO	Confined animal feeding operation
CFR	Code of Federal Regulations
CFU	Colony-forming unit
CI	Confidence interval
CWA	Clean Water Act
FM	Farm to market road
GCWA	Gulf Coast Water Authority
ID	Identification or identifier
IEH	Institute for Environmental Health, Inc.
LA	Load allocation
MGD	Million gallons per day
MOS	Margin of safety
MS4	Municipal separate storm sewer system
MUD	Municipal utility district
NLCD	National Land Cover Data
NWDLS	North Water District Laboratory Services, Inc.
PFGE	Pulsed Field Gel Electrophoresis
QA	Quality assurance
QAO	Quality assurance officer
QAPP	Quality assurance project plan
QC	Quality control
RFLP	Restricted fragment length polymorphism
rRNA	Ribosomal ribonucleic acid
SH	State highway
SOP	Standard operating procedure
SWMP	Stormwater management program
TCEQ	Texas Commission on Environmental Quality
TDCJ	Texas Department of Criminal Justice
WWTP	Wastewater treatment plant

SECTION 1

INTRODUCTION

1.1 Purpose and Scope

Section 303(d) of the Clean Water Act (CWA) and U.S. Environmental Protection Agency (USEPA) Water Quality Planning and Management Regulations (40 Code of Federal Regulations [CFR] Part 130) require States to develop total maximum daily loads (TMDL) for water bodies not meeting designated uses where water quality-based controls are in place. TMDLs establish the allowable loadings of pollutants or other quantifiable parameters for a water body based on the relationship between pollution sources and in-stream water quality conditions, so States can implement water quality-based controls to reduce pollution from both point and nonpoint sources and restore and maintain the quality of its water resources (USEPA 1991).

Texas Commission on Environmental Quality (TCEQ) is leading an effort to assess the water quality of classified Segment 1245 of Oyster Creek, known as "Upper Oyster Creek." Segment 1245 was placed on the State of Texas 2002 303(d) list as impaired from the presence of fecal pathogen indicator bacteria and requires development of a TMDL for point and nonpoint sources of *Escherichia coli* (*E. coli)* bacteria. Segment 1245 is located within the Brazos River Basin, southwest of Houston, Texas in northern Fort Bend County (Figure 1-1 and 1-2). The segment begins at the Gulf Coast Water Authority (GCWA) Shannon Pump Station on the Brazos River and continues through Jones Creek to its confluence with Oyster Creek, through the City of Sugar Land to its confluence with Flat Bank Creek, through Flat Bank Creek to its confluence with the diversion canal, through the diversion canal to its confluence with Steep Bank Creek, and finally through Steep Bank Creek to its confluence with the Brazos River (Figure 1-2). Segment 1245 extends approximately 54 miles, and its watershed contains four incorporated areas: Fulshear, Sugar Land, Stafford, and Missouri City.

The TCEQ contracted with the Texas Institute for Applied Environmental Research (TIAER) to conduct the appropriate studies to estimate the relative magnitude of fecal coliform bacteria point and nonpoint sources contributing to the high *E. coli* levels on the main stem and tributary stations of the Upper Oyster Creek drainageway. TIAER's technical project team include Parsons Water & Infrastructure, Inc., who performed the majority of the field efforts associated with the work herein as well as taking the lead on writing this report; North Water District Laboratory Services, Inc., who performed the *E. coli* concentration enumeration; and the Institute for Environmental Health, Inc. (IEH), who performed the molecular fingerprinting analyses.

Recent studies show that isolates from human raw sewage and waste from various host species (*e.g.*, cattle, poultry, and swine) differ, both genetically (DNA) and phenotypically (physical traits). Use of genetic and biochemical tests, referred to as bacterial source tracking (BST), may allow the original host animal to be identified. Molecular tools appear to hold the greatest promise for BST and appear to provide the most conclusive pollution source

Insert Figure 1-1 Location of Segment 1245 (Upper Oyster Creek) from attached file Figures 1 & 2.wpd

Insert Figure 1-2 Relevant Geographical References in Upper Oyster Creek from attached file Figures 1 & 2.wpd

characterization and level of discrimination. Of the molecular tools available, ribosomal ribonucleic acid (rRNA) genetic fingerprinting (ribotyping) has emerged as a versatile and feasible BST technique, and was used by Parsons and IEH, along with TIAER to characterize the *E. coli* contamination in Upper Oyster Creek, which is the subject of this report.

1.2 Water Quality Standards

Water quality standards (WQS) consist of designated beneficial uses, water quality criteria to protect the uses, and antidegradation policies. These standards serve dual purposes of establishing water quality goals for the nation's water bodies and providing the regulatory basis for establishing certain treatment controls and strategies. The State of Texas WQSs applies to Upper Oyster Creek as described in the Texas Surface Water Quality Standards (TCEQ 2000). Designated uses of Segment 1245 are intermediate aquatic life use, contact recreation, and public water supply. This report addresses only the contact recreation use.

Water quality criteria list specific constituent levels to be maintained to ensure that designated uses are met. To protect contact recreation use, water quality criteria are based on concentrations of fecal coliform and *E. coli* bacteria in water. Fecal coliform bacteria are a group of moderately heat-tolerant coliform bacteria abundant in the intestines of warm-blooded animals, but are not believed to survive in the environment long-term. Because they are relatively easy to measure in water, they are used as an indicator of the possible presence of fecal pathogenic microorganisms in water, including other bacteria, viruses, and harmful protozoans. Most fecal coliform bacteria are not pathogenic. It has been established that *E. coli* is more closely associated with fecal pollution than other fecal coliform bacteria, some of which may normally reside and multiply in the environment. *E. coli* is often the most abundant species of the fecal coliform group of bacteria, and a few strains of *E. coli*, notably strain O157:H7, are pathogenic.

Applicable water quality criteria for *E. coli* state that the geometric mean concentration (in colony-forming units [cfu] of bacteria per 100 milliliters [ml]) should not exceed 126 per 100 ml, and the single sample concentration should not exceed 394 per 100 ml. Water quality criteria for fecal coliform state that the geometric mean concentration should not exceed 200 per 100 ml, and the single sample concentration should not exceed 400 per 100 ml (TCEQ 2000). The TCEQ prefers the use of *E. coli* as the fecal indicator organism rather than the fecal coliform, if sufficient data are available to allow assessment based on *E. coli*.

SECTION 2
HISTORICAL DATA REVIEW AND WATERSHED PROPERTIES

2.1 Watershed Hydrology and Climate

An important factor in assessing water quality of a water body such as Segment 1245 is the hydrology of the system. There are two distinct hydrologic reaches within the Upper Oyster Creek segment. The upper reach extends from the GCWA Shannon Pump Station on the Brazos River to Dam #3 within the City of Sugar Land. The lower reach begins at Dam #3 and continues downstream through Steep Bank Creek to its confluence with the Brazos River.

Hydrology of the upper reach is highly variable and has been modified by seasonal pumping of water into the segment from the Brazos River. The GCWA uses the reach above Dam #3 as a section of its Canal System A, which supplies water for irrigation, industrial, and public drinking supply to areas southeast of the watershed, in addition to uses in the vicinity of the City of Sugar Land. Canal System A is operated by the GCWA in tandem with Canal System B, located south of the Upper Oyster Creek watershed. To serve as a conveyance for the pumped water, Jones Creek and the portion of Oyster Creek above Dam #3 have been dredged to provide adequate capacity. The hydrologic modifications also include a diversion structure that allows the water pumped from the Brazos River into Jones Creek to be diverted into Oyster Creek, and the presence of three small dams or retention structures operated by the GCWA.

The discussion of these small dams and their operation is taken from Kolbe (1992) and personal observations by TIAER staff. Each retention structure is constructed of concrete with slots for horizontally placed wooden boards, which may be added or removed to control water level. The dams form impoundments to maintain nearly constant water levels for industrial and recreational uses and off-channel lakes that create "lakefront" property with commensurate aesthetic and monetary value. Dam #2 stores water for industrial use and forms Brooks and Cleveland Lakes. Dam #3 retains water for Alkire, Eldridge, and Horseshoe Lakes, and also serves to retain water for the GCWA Second Lift Station where water is pumped into the American Canal for transport to the Texas City area.

Hydrology of the reach below Dam #3 is highly impacted by the presence of Dam #3 and the Second Lift Station. Small amounts of seepage do occur through Dam #3, and there is uncontrolled excess rainfall runoff over the dam into the lower reach. The Second Lift Station, however, operates under most wet-weather conditions to capture portions of the rainfall-runoff, which reduces the amount released below Dam #3. This reach, therefore, contains no retention structures, and is characterized by reduced flow composed of seepage from Dam #3, contributions from municipal dischargers, natural contributions from the drainage area below Dam #3, and excess rainfall runoff from the upper reach above Dam #3. The reach below Dam #3, however, is also hydrologically modified, though not for conveyance of water supplies and impoundment of water, but rather for flood prevention. These modifications result in Oyster Creek being diverted into Flat Bank Creek and then into Steep Bank Creek via a diversion channel. These confluences and connections are not a result of natural stream

conveyance and hydrologic conveyance patterns, but as stated previously, serve the utility of flood flow conveyance.

Data from GCWA Shannon Pump Station and the Second Lift Station were evaluated for trends and general characteristics for the period 1986 through 2000. The records for the Shannon Pump Station were used to indicate general conditions of water demands supplied through Canal System A. Records from the Second Lift Station were used to characterize monthly hydrologic conditions in the upper reach of Upper Oyster Creek, because some, though not all, rainfall runoff is captured and pumped from that station. The hydrologic conditions and pattern reflected in the records of the Second Lift Station provide more accurate estimates of flow conditions for the reach above Dam #3 than do the records of the Shannon Pump Station; therefore, those records will be used in comparison with water quality data.

Data for the Second Lift Station indicate that the pumped flow increases through the spring (between 1,000 to 3,000 acre-feet per month [ac/m] on average) to a maximum in July. Pumped flow decreases through the fall and winter to its lowest average rate of 1,325 ac/m in February. Average annual pumped flow through the segment is over 50,000 acre-feet per year. A minimum of 28,889 acre-feet per year were pumped in 1997, and a maximum of 69,670 acre-feet per year were pumped in 1995. Historical flow data from the U.S. Geological Survey (USGS) station 08112500 suggest similar characteristics and patterns of pumped flow, for a period from 1931 to 1973. Seasonal high flow was observed in the USGS data for the months of April through September while lower flow is noted in March and October. A period of reduced flow is indicative of the months of November through February.

The hydrology of the reach above Dam #3 may be impacted if Sugar Land, Missouri City, Fort Bend Water Control and Improvement District (WCID) No. 2, and the western portions of the City of Houston continue with plans to reduce their total reliance on ground water for public water supply and supplement demand with surface water from the Brazos River. In a study for the GCWA and Texas Water Development Board (TWDB), a feasibility study by Montgomery Watson America, Inc. (2000) for a regional surface water treatment plant for Brazoria, Fort Bend, and west Harris counties indicated a two-fold need to supplement groundwater with surface water. First, groundwater pumpage was causing subsidence, which can greatly increase flooding, and second, large population growth in the area may exceed reliable groundwater supplies. Discussions by TIAER staff with both GCWA and the City of Sugar Land in 2001 indicated that a facility to supply surface water from the Brazos River is still being considered, though the exact timeframe, size, and location of the facility are unknown. However, any plans for a facility to supply surface water from the Brazos River appear to have hydrologic implications to the upper reach of Upper Oyster Creek. The exact location of the water treatment plant would determine how much of the reach above Dam #3 would be directly impacted. The size of surface water treatment plant being considered could be as large as 150 million gallons per day (MGD) (maximum of 13,800 ac/m), and conveyance would occur through Jones Creek and Oyster Creek, perhaps all the way to the Second Lift Station. Not only could the amount of additional flow in the upper reach of Segment 1245 be substantial, the historical seasonal component would be modified because of the water needs of municipalities are more constant than agricultural needs.

In summary, the hydrology of Segment 1245 is anthropogenically modified. There is a seasonal pattern of pumping water from the Brazos River into the reach of Upper Oyster Creek above Dam #3. Peak pumping occurs in the summer and minimum pumping occurs in the winter, which reflects the water demands for irrigation purposes and the use of the upper reach as conveyance for these water demands. Increasing municipal demands from rapid urbanization in the entire region west and south of the City of Houston compounded by needs to supplement the present exclusive use of groundwater with surface water could, over time, change this seasonal water pumping pattern to one with a less pronounced seasonal pattern.

The Upper Oyster Creek watershed lies within a climatic region classified as subtropical humid, which is defined as having hot summers and dry winters. An average annual rainfall of 49.3 inches was measured at Sugar Land airport between 1970 and 2000 (NOAA 2004). Over this same period, rainfall events of 0.1, 0.5, and 1 inch of rain were observed on average 64, 31, and 16 days per year, respectively. The Upper Oyster Creek watershed is within the upper portion of the Gulf Coast Prairies and Marshes ecoregion, an area characterized as containing nearly level, undissected plains with native vegetation types composed of tall grass prairie and post oak savanna. The elevation of the area is approximately 25 meters above mean sea level.

2.2 Review of Historical Fecal Coliform Monitoring Data

2.2.1 Data Acquisition

Investigations of historical water quality data, which involved evaluation of past and recent water quality data from Upper Oyster Creek, were performed. Data sources investigated included the TCEQ Texas Regulatory Activity and Compliance System (TRACS) database.

General assessment criteria methodologies established by TCEQ were used in data evaluations to determine the percentage of samples exceeding adopted criteria or screening levels for a water body.

2.2.2 Water Quality Assessment

Analyses of historical data were conducted by separating the data period 1988-2003 into two subsets, 1988-2001 and 2002-2003. For most monitoring stations, the majority of data was taken beginning in 1988 to the present, hence, the beginning date of 1988. More current data are discussed in the subset of 2002-2003.

2.2.3 Analysis of Bacteria

Nearly all fecal coliform bacteria data originate from three TCEQ stations– Station 12086, Oyster Creek at State Highway (SH) 6, Station 12083, Oyster Creek at Highway 90, and Station 12079, Oyster Creek at Highway 59. Refer to Figure 2-1 for specific station locations. Station 12083 is the only station for which data are available from 1970 to the present. There were occurrences of extremely high counts of bacteria at this station in the 1970s which have not occurred in the more recent data. The most downstream station, 12079, has less frequent excursions of fecal coliform above 400 cfu per 100 ml; however, the data are also more sparse at this station than at the other two stations.

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Insert Figure 2-1 Upper Oyster Creek showing sites for bacteria sampling of Oct. 2002 – Aug. 2003 from file attached Figure 2-1 and Figure 3-2 z-fold.doc

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Most of the fecal coliform data were collected in the impoundment area of Segment 1245. These bacterial data generally support the section 303(d) listing of the segment for nonsupport of the contact recreation use (Table 2-1), as fecal coliform concentrations above the criterion of 400 cfu per 100 ml occurred at a frequency of greater than 25 percent at one site.

Table 2-1 Historical Fecal Coliform Exceedances, 1988-2001

Oyster Creek (1988-2001)		
Station	**No. of Samples 1988-2001**	**% Exceedance Fecal coliform (cfu/100ml)**
12086	57	25%
12083	59	31%
12079	27	11%

The 2002 Texas Water Quality Inventory and 303(d) list indicated that the standard for the segment from Highway 90A to Dam #1, located 1.5 miles upstream of Harmon Street, was *not met* for contact recreational use. A *use concern* from Dam #1 to the confluence of Oyster Creek and Jones Creek was also listed. Table 2-2 lists the bacteria results for stations located in Segment 1245 as well as results from various tributaries and lakes for 12 survey events conducted during the time period of October 2002 to August 2003 (see Figure 2-1 for sampling station locations). These survey events were specifically designed to assist in assessment of the level of support of contact recreation use for Segment 1245. Appendix A contains in graphical format the results summarized below for fecal coliform and *E. coli* in both mainstem Upper Oyster Creek and some major tributaries and lakes.

For the mainstem stations on Upper Oyster Creek, the geometric mean for fecal coliform should not exceed 200 cfu per 100 ml. All stations exceeded the criteria during the time period from October 2002 to August 2003 except for Stations 17685, 12079, 17373, and 12077. The fecal coliform results for the stations that exceeded the geometric mean criteria were between slightly over the exceedance criteria to nearly 1700 cfu per 100 ml. The percent exceedance criteria is 42 percent (or the minimum number of samples in exceedance is 5) when the sample size is 12 as in this study (see assessment guidance TCEQ 2003a).[1] All stations on the

[1] The TCEQ applies the binomial method to establish the required number of exceedances to indicate nonsupport of contact recreation use. To determine nonsupport (i.e., greater than 25% of samples exceed the relevant criterion) and to keep the percent probability at less than 20% of inappropriately assessing a water body as not supporting when it is actually fully supporting, a minimum of 5 samples must be in exceedance for a sample size of 12.

mainstem ranged from 17 percent to 70 percent exceedance for fecal coliform. For the tributaries and lakes monitored, Stations 17686, 17688, and 17689 exceeded the criteria for fecal coliform. For Stations 11516, 11510, and 17687, the geometric means for fecal coliform were 66, 83 and 142 cfu per 100 ml, respectively. The percent exceedances for fecal coliform on the tributaries and lakes ranged from 17 percent to 82 percent.

Table 2-2 Historical Fecal Coliform and *E. Coli* Exceedances, 2002-2003*

Oyster Creek (2002-2003)				
Station	**Fecal coliform (cfu/100ml) geometric mean**	***E. coli* (cfu/100ml) Geometric mean**	**% Exceedance Fecal coliform (cfu/100ml)**	**% Exceedance *E. Coli***
17685	102	75	17	8
12091	470	363	45	42
12090	452	427	33	58
12089	414	364	42	50
12088	454	293	42	42
12087	427	301	50	50
12086	238	154	33	42
12083	560	333	33	33
12079	80	65	17	18
17373	79	58	18	8
12077	160	104	36	25
12075	710	948	44	58
12074	690	512	70	67
17690	423	417	50	50
Tributaries and Lakes (2002-2003)				
Station	**Fecal coliform (cfu/100ml) geometric mean**	***E. coli* (cfu/100ml) Geometric mean**	**% Exceedance Fecal coliform**	**% Exceedance *E. Coli***
17686	1182	943	67	67
11516	142	98	42	42
11510	83	59	25	17
17687	66	52	17	9
17688	1694	906	82	58
17689	819	522	73	58

- 12 samples were collected at each station

For the mainstem stations on Upper Oyster Creek, the geometric mean for *E. coli* should not exceed 126 cfu per 100 ml. The same stations that exceeded the criteria for fecal coliform also exceeded the criteria for *E. coli* during the same time period. All other stations exceeded the criteria between slightly over the exceedances to 948 cfu per 100 ml. The percent exceedances for all mainstem stations ranged from 8 percent to 67 percent. For the tributaries and lakes, Stations 17686, 17688, and 17689 exceeded the criteria by 943, 906, and 522,

respectively. The *E. coli* geometric mean results for Stations 11516, 11510, and 17687 were between 52, 59, and 98 cfu per 100 ml. The percent exceedances for the tributaries and lakes ranged from 9 to 67 percent for *E. coli.*

The fecal coliform and *E. coli* data from the 12 survey events in 2002-2003 confirmed the 303(d) listing of Segment 1245 and further indicated much of the segment did not support the contract recreation use.

2.3 Potential Sources of Fecal Indicator Bacteria

2.3.1 Permitted Wastewater Discharges

Under the Texas Pollution Discharge Elimination System (TPDES), nine active facilities within Segment 1245 hold permits to discharge wastewater (Table 2-3). One additional facility within the segment, the Texas Department of Criminal Justice (TDCJ), holds an irrigation/agricultural (no discharge) permit for a confined animal feeding operation (CAFO), and Hines Horticulture holds a permit to discharge storm/irrigation waters. All other entities holding active TPDES discharge permits are domestic wastewater (sewage) treatment facilities. From approximately 2000 to mid-2004, the reported average daily domestic wastewater discharge to Upper Oyster Creek was 11.9 MGD, which is well below the permitted daily flow of 21.1 MGD. Increasing discharge limits for some municipal permittees within the segment and adding new discharge permits in recent years indicate a steadily increasing wastewater input into the segment commensurate with the rapid urbanization of the watershed.

The City of Sugar Land and Fort Bend County WCID #2 permits allow the largest discharge of the wastewater facilities at over 5 MGD each. The other wastewater facilities with permitted wastewater discharges of greater than 1 MGD are Quail Valley Utility District and Missouri City. Most of the wastewater permits do not include specific limits and monitoring requirements for fecal coliform concentrations in their effluents, but most do require disinfection of wastewaters.

In 2001 TIAER staff reviewed the TPDES permit files to identify enforcement actions or other persistent problems with permitted discharge facilities within Segment 1245. This review was updated in 2005 by reviewing the discharge monitoring reports (DMR) from the Permit Compliance System (PCS) downloaded from the USEPA Envirofacts Data Warehouse. No enforcement actions were uncovered in the screening; however, some self-reporting, operation, and administration violations were noted in the files. The TDCJ facility has had some minor violations regarding uncertified personnel, operational requirements, and final effluent limitations; however, these violations surfaced during an annual inspection and were completely resolved within the required time frame. The TDCJ facility underwent a $4.5 million expansion during 2001-2002. Imperial Sugar Corporation resolved a recurring violation on the annual certification of accuracy for pumping capacity used to measure flow, which was observed on biannual inspections in 1996 and 1998, though this facility has ceased operation and discharge since late in 2003. Of potential relevance to this study was a violation of the fecal coliform bacteria daily maximum, 7-day average, and daily average criteria by Missouri City in August 2000. The problem occurred due to an off line aerator that had

Table 2-3 Permitted Wastewater Discharges to Upper Oyster Creek and its Tributaries

Permit ID	Facility	Dates Monitored	Monthly Average Flow (MGD)	Permitted Flow (MGD)	FC Daily Load	
					Geometric Mean	Maximum Monthly Average
TX0077178	Fort Bend County MUD #25	9/30/99-7/31/04	0.42	0.98	$8.97x10^7$/day	$2.78x10^9$/day
TX0116386	Fort Bend County MUD #118	8/31/00-5/31/04	0.064	0.30**	NR	NR
TX0089249	Fort Bend County MUD #41	11/30/99-5/31/04	0.25	0.43**	NR	NR
TX0021458	Fort Bend County WTCID #2	1/31/00-7/31/04	3.52	5.1	NR	NR
TX0035220	Quail Valley UD	1/31/00-5/31/04	1.77	4.0	NR	NR
TX0090484	Palmer Plantation MUD 001	11/30/99-5/31/04	0.29	0.60	NR	NR
TX0096881	City of Sugarland	1/31/00-7/31/04	4.61	7.5**	NR	NR
TX0089036	TDCJ Jester Unit III - WWTP	10/31/99-6/30/04	0.24	0.75	NR	NR
TX0114855	City of Missouri City	12/31/99-6/30/04	0.69	1.5**	$1.16x10^8$/day	$6.95x10^9$/day
Total			11.9	21.1		

Notes: ** Interim Permitted flow.
FC = Fecal Coliform.
NR = Not reported.
MGD = millions of gallons per day.
TDCJ Jester Unit 1 (TX0031674) is a retention pond facility and Hines Horticulture (TX0103608) discharges only storm water, therefore these facilities were not included within this table, but may occasionally discharge to segment 1245 during rainfall events.

accumulated a large amount of settled solids. Solids were redistributed throughout the plant when the unit was restarted, causing poor effluent quality. The problem was resolved immediately, and subsequent fecal readings indicated no long-term concerns. No other fecal coliform effluent quality violations were reported since that time.

Because efforts to improve water quality problems have a long history in Upper Oyster Creek, a number of significant changes and improvements have occurred resulting in improved water quality. Kolbe (1992) reports 1) the City of Sugar Land wastewater treatment plant

(WWTP) discharge was moved to its present location in 1975; 2) the Hines Horticulture direct discharge was removed in 1990 and reduced to stormwater overflow releases; and 3) wastewater treatment of the TDCJ units has improved and feedlot runoff is better managed. After June 1996, Imperial Sugar's major discharges were delivered to the Brazos River Authority (BRA) regional WWTP for treatment and subsequent discharge outside the watershed and, as previously mentioned, has totally ceased any discharge into Oyster Creek since 2003. Kolbe (1992) states that from 1987 through 1990, Imperial Sugar discharged an average of 17 to 21 MGD of wastewater at elevated temperature, which was allowed in their permits.

The National Pollutant Discharge Elimination System Phase II rule, promulgated in 1999, requires small municipalities in urban areas to obtain permits for their stormwater systems. These permits, known as Municipal Separate Storm Sewer System (MS4) permits, require cities to reduce discharges of pollutants in stormwater to the "maximum extent practicable" by developing and implementing a Stormwater Management Program (SWMP). The SWMPs require specification of BMPs for six minimum control measures:

- Public education and outreach;
- Public participation/involvement;
- Illicit discharge detection and elimination;
- Construction site runoff control;
- Post-construction runoff control; and
- Pollution prevention/good housekeeping.

Most of the eastern half of the Upper Oyster Creek watershed is covered under these permits, including the Cities of Missouri City, Stafford, and Sugar Land (Refer to Table 2-3 for permitted wastewater discharge details). The cities will likely obtain coverage during 2005 under a TCEQ General Permit for stormwater discharges. This program may positively impact water quality in Upper Oyster Creek.

2.3.2 Land Use

The Upper Oyster Creek watershed covers approximately 110 square miles, approximately 12.5 percent, of the area of Fort Bend County. Approximately 60 percent of the land in the watershed is used for agricultural purposes, but a significant portion (16-29%) is developed land. Two land use classifications for the Upper Oyster Creek Watershed were developed in the 1990s. The National Land Cover Data set (NLCD) (Vogelmann 2001) was developed from Landsat satellite photographs taken in the early 1990s. A separate study by Baylor University for the period 1996-97 identified increasingly urban land use, although differences in classification methodology are likely responsible for much of the difference (Baylor University 1997). Refer to Tables 2-4 and 2-5 and for details.

Table 2-4 Baylor Land Use Classification

Baylor Land Use Classification (1997)	Acres	% of Area
Open Water	93	0.1
Residential	8,737	12.7
Urban Mixed	11,427	16.7
Bare Rock	2,038	3.0
Exposed Soil	1,177	1.7
Deciduous Forest	33	0.05
Evergreen Forest	266	0.4
Rangeland	3,793	5.5
Pasture/Crop	41,053	59.8

Table 2-5 NLCD Land Use Classifications

NLCD Land Use Classification (early 1990s)	Acres	% of Area
Open Water	1,000	1.4
Low Intensity Residential	3,981	5.6
High Intensity Residential	3,152	4.5
Commercial/Industrial/Transportation	2,345	3.3
Bare Rock/Sand/Clay	363	0.5
Quarries/Strip Mines/Gravel Pits	77	0.1
Transitional	223	0.3
Deciduous Forest	9,302	13.1
Evergreen Forest	2,254	3.2
Mixed Forest	243	0.3
Shrubland	1,861	2.6
Grasslands/Herbaceous	1,232	1.7
Pasture/Hay	33,393	47.2
Row Crops	6,053	8.6
Small Grains	1,665	2.4
Urban/Recreational Grasses	1,162	1.6
Woody Wetlands	775	1.1
Emergent Herbaceous Wetlands	1,708	2.4

2.3.3 Population Density: Humans and Pets

The population of the Upper Oyster Creek watershed in 2000 was estimated to be 96,273 (31,573 households) with an overall average population density of 877 persons per square mile (U.S. Census Bureau 2000). The population of Fort Bend County is estimated by the U.S.

Census Bureau to have increased approximately 6 percent per year since the 2000 census, so the current (2005) population may exceed 125,000. Approximately 28,000 cats and 25,000 dogs are also estimated to reside with households within the watershed, based on the 2000 census data along with national averages of pets per household from the American Veterinary Medical Association (2002).

Fort Bend County is expected to increase in population by approximately 78 percent from 2000 to 2020, according to the TWDB (Montgomery Watson America, Inc. 2000). As a result, the county expects significant increases in water demand for municipal purposes (65% increase). Smaller increases are expected for manufacturing (17%), mining (8%), and steam electric (10%) uses. Table 2-6 sets out TWDB population growth estimates for selected cities within Fort Bend County from 2000 to 2005.

Table 2-6 Fort Bend County Population and Projected Increases by City, 2000 to 2020

City	2000 Census Population	2010 Population	2020 Population	Growth Rate (2000-2020)
Fulshear	716	883	1,056	47%
Missouri City	52,913	83,645	104,844	98%
Stafford	15,681	23,339	31,275	99%
Sugar Land	63,328	72,500	72,500	14%

Source: Montgomery Watson America, Inc. 2000.

The population estimates for Sugar Land are held constant after the year 2010 because the city is expected to be completely built-out by this date. Conversations with TWDB staff confirmed that previous TWDB estimates were made in error and did not account for the built-out issue. However, TWDB estimates may not account for future annexations that could occur. Annexations were used to drive population growth in the 1990s. The 2000 census figures indicate a 158 percent increase in the population of Sugar Land since 1990.

2.3.4 Sewered and Non-Sewered Areas

The method of sewage disposal for housing units in the Upper Oyster Creek watershed was estimated from the 1990 federal census at the block group level because these data were not collected in the 2000 census (U.S. Census Bureau 1990). Because of rapid urbanization in the watershed, estimates based on those data may no longer be accurate. At that time, approximately 6 percent of households in the watershed utilized septic tanks for sanitary waste disposal, while 93 percent were connected to a sanitary sewer system. Approximately 1,400 housing units in the watershed were reportedly not connected to a sanitary sewer system. The more rural western half of the watershed was primarily served by septic tanks; however, the highest density of septic tanks was in two areas: the Fifth Avenue area, bounded roughly by Cartwright Road on the south, American Canal on the north and east, and farm-to-market (FM) Road 1092 on the west, and the Four Corners area northwest of Sugar Land, bounded by SH 6 on the east, Old Richmond Road on the west, Voss Road on the south, and Boss-Gaston

Road on the north. The density of septic tanks in these two areas ranged from approximately 0.2 to 0.3 per acre.

2.3.5 Livestock Populations

The smallest unit for which livestock census data are available is the whole of Fort Bend County, which indicate beef cattle to be the dominant livestock species in the watershed (Table 2-7). Other livestock species present in the watershed include horses, goats, chickens, and hogs. Livestock populations were estimated from the 2002 agricultural census of the

Table 2-7 Estimated Potential Source Populations in Fort Bend County

Category	Livestock	Fort Bend County	Estimated Watershed Population
Human	Census		128,000[#]
Pets	Cats		28,000*
Pets	Dogs		25,000*
Livestock	Cattle & Calves-All	51,000 [†]	6,375
Livestock	Beef cows	35,000 [†]	4,375
Livestock	Milk cows	0 [‡]	0
Livestock	Horses	3,400 [‡]	425
Livestock	Mules, burros, & donkeys	116 [‡]	14
Livestock	Hogs & Pigs	1,367 [‡]	171§
Livestock	Goats-all	1,400 [†]	175
Livestock	Sheep & Lambs	622 [‡]	78
Livestock	Rabbits	311[‡]	39
Livestock	Bison	27 [‡]	3
Livestock	Domestic Deer	82 [‡]	10
Livestock	Chickens	2,226 [‡]	278
Livestock	Ducks-Domestic	172 [‡]	22
Livestock	Geese-Domestic	390 [‡]	49
Livestock	Turkeys-Domestic	49 [‡]	6
Livestock	Pheasants-Domestic	220 [‡]	28
Livestock	Quail-Domestic	1,382 [‡]	173
Livestock	Emus	47 [‡]	6
Livestock	Other poultry*	200 [‡]	25

[#] projected based on 2000 federal census and annual growth rate of 6%
* From *2002 U.S. Pet Ownership & Demographics Sourcebook*, American Veterinary Medical Association
[†] As of January 1, 2004 Texas Agricultural Statistics Service
[‡] 2002 Agricultural Census, USDA
[§] Probably an underestimate, based on observed population at prison farm

National Agricultural Statistics Service of the U.S. Department of Agriculture, or from more recent estimates of the Texas Agricultural Statistics Service, when available.

2.4 Summary of Sanitary Survey Observations

A sanitary survey to identify potential bacteria sources within the Upper Oyster Creek watershed was performed from May 3 to 5, 2004 by the Parsons sampling team. Fecal sampling was also performed as part of the survey when scat samples were observed.

The most evident feces observed adjacent to water bodies in urban areas were from waterfowl, specifically ducks and geese. A large number of Muscovy ducks were observed in central portions of the watershed, particularly in the many residential lake areas. This species is a non-native resident, often domesticated, and frequently white or white and black with a red bulbous bill. Duck fecal matter was very dense along the banks of impounded Oyster Creek at the Fluor-Daniel Road. Black-Bellied Whistling Ducks were also observed to defecate at this same location. Fecal samples of each were collected.

Pigeons and swallows were observed to be nesting on bridges over Oyster Creek at a number of locations, and perching on utility lines over the creek. Their dried fecal matter caked portions of the bridges. The swallows were only observed during the summer months. Other common birds in and near the creeks included several species of herons and egrets.

In rural areas, cattle and raccoon feces were observed the most. Cattle feces were more widespread and more abundant than others. Cattle were observed to be numerous in the western half of the watershed. Brangus and Limousine appeared to be the most abundant cattle breeds. Abundant raccoon feces were observed adjacent to smaller more sheltered waterways. It was observed during the March fecal sampling event that the raccoon diet appeared to consist mostly of blackberries, but crayfish parts littered the banks of these smaller water bodies as well. Road kill indicated the expected fauna of southeast Texas, including skunks, raccoons, armadillos, and opossum.

The Fifth Avenue and Four Corners areas of the watershed were thought to have mobile homes with poor-quality septic systems. After surveying the areas, it appeared homes in the area were now connected to sanitary sewers. Approximately two dozen chickens were observed throughout the neighborhoods within these areas.

Hog waste at the Jester Unit of the TDCJ was managed in three consecutive passive treatment lagoons. The pig housing was very clean at the time of the site visit, making sample collection difficult, but successful. No connection was noted between the lagoons and the adjacent Oyster Creek. The land on which the farm was located sloped away from the creek, but drained into a swale that appeared to curve around the hog area to the north and back of the creek.

2.5 Project Photo Log

During the course of this study the following were photos taken of various locations within the Oyster Creek watershed.

Photo 1: Station 12074, Flat Bank Creek at SH 6

Photo 2: Storm debris along the bank of Flat Bank Creek, Station 12074, at SH 6

Photo 3: Swallow nests under a bridge at Station 12074, Flat Bank Creek at SH 6

Photo 4: Station 17688, Stafford Run at El Dorado Boulevard

Photo 5: Station 17688, Stafford Run at El Dorado Boulevard

Photo 6: Station 12083, Oyster Creek at Highway 90A looking downstream

Photo 7: Station 12087, Oyster Creek at FM 1464 looking upstream

Photo 8: Station 12086, Oyster Creek at SH 6 looking downstream

Photo 9: Station 12086, Oyster Creek at SH 6 looking downstream

Photo 10: Station 11516, Red Gully at Richmond-Gaines Road

Photo 11: Wastewater discharge into Red Gully near sampling site

Photo 12: Coyote scat found on a road in the north-central part of the watershed

Photo 13: Egrets adjacent to the dog kennel waste lagoon at the Jester Unit of the TDCJ in Sugar Land

Photo 14: Drainage from the dog kennels waste lagoon at the TDCJ's Jester Unit

Photo 15: Hog farm at the TDCJ's Jester Unit

SECTION 3

OBJECTIVES AND METHODS

3.1 Fundamentals of Bacterial Source Tracking and Ribotyping

The BST method is based on two principles. The first is that the bacterial population genetic structure is clonal, a well-established element of microbial genetics. Bacteria reproduce by binary fission, or dividing in half, and the two daughter cells generated as a result of this cell division are virtually identical in all aspects. All descendents of a common ancestral cell are genetically related to each other. Over time, members of a given clone may accumulate genetic changes which will cause them to diverge from the main lineage and form one or several new clonal groups. BST makes use of the clonal population structure of bacteria to classify organisms into groups of clonal descent based on their genetic fingerprints.

The second principle of BST methodology is the assumption that within a given species of bacteria, various members have adapted to living/environmental conditions in specific hosts/environments. As a result, there is a high degree of host specificity among bacterial strains that are seen in the environment. A bacterial strain that has adapted to a particular environment or host (*e.g.*, animal intestinal tract) is capable of colonizing that environment and competing favorably with members of the hosts' indigenous flora. Such a bacterial strain is called a resident strain. Resident strains are usually shed from their host over a long period of time, thus providing a reliable, characteristic signature of their source. A transient strain is a bacterial strain that is introduced into a new environment or host but cannot colonize and persist in that environment. If a host is sampled over time for a given species of bacteria, a few resident strains are consistently being shed while a large number of transient strains are shed for brief lengths of time. A study conducted by Hartl and Dykhuizen (1984) illustrates this point. Over a period of 11 months, 22 fecal samples were taken from a single individual. A total of 550 *E. coli* isolates were characterized, of which two were considered to be resident strains, appearing 252 times. Data show that using this subtyping method (rRNA typing using two restriction enzyme reactions), more than 96 percent of *E. coli* strains are seen in only one host species (or group of related species) (Mazengia 1998). Thus, it appears that only about 4 percent of the *E. coli* strains are transient and not attributable to one specific source.

The key methodological problem in tracing sources of microbial contamination in the environment has been the lack of a universal single-reagent typing scheme for bacteria. This was overcome by the work of several investigators in the fields of population genetics, molecular systematics, and molecular epidemiology. In 1986, Grimont *et al*. showed that DNA probes corresponding to specific regions of the rRNA operon could be used to speciate bacteria. Stull *et al*. (1988) and Lipuma *et al*. (1988) used the rRNA operon to study the molecular epidemiology of several species of bacteria. To trace the indicator bacterium, *E. coli*, from water to its specific source, the bacterial strain must first be uniquely identified. Populations of *E. coli*, like other bacteria, are composed essentially of a mixture of strains of clonal descent. Due to the relatively low rates of recombination, these clones remain more or less independent (Selander *et al.* 1987). These clones, or strains of bacteria, are uniquely

adapted to their own specific environments. As a result, the *E. coli* strain that inhabits the intestines of one species is genetically different from the strain that might inhabit another.

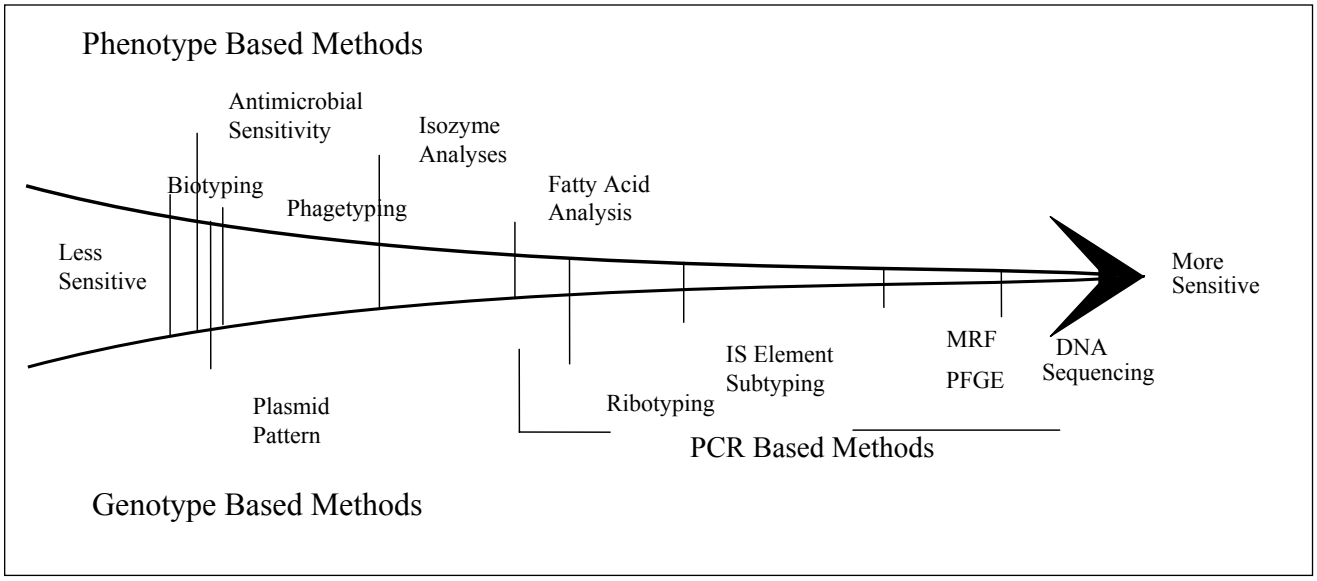
Ribosomal ribonucleic acids, which are integral to the machinery of all living cells and tend to be very highly conserved, make an ideal choice of target in interstrain differentiation. Since the *E. coli* chromosome contains seven copies of the rRNA operon, a ribosomal nucleic acid probe can be used as a definitive taxonomic tool (Grimont *et al.* 1986). That is, when digested with restriction enzymes, resolved by agarose gel electrophoresis, transferred to a membrane and hybridized with an rRNA probe, an *E. coli* chromosome will produce several bands to create a specific restriction fragment length polymorphism (RFLP) pattern that can be used to uniquely identify the bacterial strain.

The pattern of DNA fragments corresponding to the rRNA operon is referred to as the ribotype. Ribotyping has been useful in many studies to differentiate between bacterial strains that would have otherwise been difficult or impossible to distinguish. Fisher *et al.* (1993) followed the transmission of *Pseudomonas cepacia* from environmental sources to and between cystic fibrosis patients and discovered the majority of cases contracted cystic fibrosis from one of two treatment centers. Moyer *et al.* (1992) used rRNA typing to identify the Aeromonad strains responsible for several waterborne gastroenteritis episodes in a community and was able to trace the contamination to specific locations in water treatment and distribution systems. Barloga and Harlander (1991) compared several typing methods for distinguishing between strains of *Listeria monocytogenes* implicated in a food-borne illness and found that ribotyping was the preferred method due to its precision and reproducibility. Atlas *et al.* (1992) described the technology of ribotyping as applicable to the tracking of genetically engineered microorganisms (GEM) in the environment.

Dr. Samadpour's BST method, which was employed in this study, was developed on the basis of the principles of microbiology, epidemiology, molecular epidemiology, microbial population genetics, sanitary engineering, and hydrology. In any watershed, there are multiple contributing animal sources of microbial pollution, each of which has its own unique clones of bacteria that constitute their normal flora. Ribotyping is applied as part of an BST study in the following steps. First, collections of isolates from appropriate bacterial species are compiled from the polluted sites and the suspected animal sources of pollution, which are identified through a sanitary survey of the region surrounding the polluted site. Second, using an appropriate molecular subtyping method, all bacteria in the collection can be subtyped. Finally, the genetic fingerprints of the bacterial isolates from the polluted site are compared to those of the bacteria from the suspected animal sources. When a strain of bacteria with an identical genetic fingerprint is isolated from both a water sample and a suspected animal source, the animal is implicated as a contributor of that specific strain of the bacteria to the polluted site. The relative contributions of various sources are quantified based on the fraction of isolates from a representative set of ambient water samples that match ribotypes of resident strains from that source (human or nonhuman).

Figure 3-1 displays a conceptual sensitivity continuum of some of the widely used subtyping methods. Phenotypic based methods (methods based on the expression of phenotypes) are at the less sensitive domain of the continuum while genotypic based methods

Figure 3-1 Microbial Source Tracking Methods



Source: Samadpour 2001.

constitute the more sensitive end of the spectrum. The level of sensitivity depends on the choice of gene(s) and the size of fragment(s) sequenced.

Ribotyping was selected as the BST method for this project because it balances high source specificity with moderate requirements for library size. Pulse Field Gel Electrophoresis (PFGE), while excellent at resolving different source species, also requires a very large and expensive library due to the high variation in PFGE profiles. There is substantial uncertainty over the efficacy of antibiotic resistance analysis at distinguishing bacterial sources.

All bacterial source tracking methods, including the ribotyping used herein, are predicated on proper statistical sampling of the water body. Almost countless numbers of *E. coli* occur in the waters of Oyster Creek at any given time, and it is resource prohibitive to use ribotyping for source identification on but a few of those bacteria during any one sampling event. Through proper sampling design, however, statistically meaningful inferences can be made concerning identified sources within the watershed. Caution must be exercised so that the findings are not extended beyond their statistical validity, where, for instance, the findings for a sampling station during a single event may be misleading, but aggregation of data across multiple events or all events provides increasingly more reliable findings.

3.2 Study Design and Data Quality Objectives

The purpose of this project was to estimate the relative magnitude of fecal sources contributing to the observed high *E. coli* levels on the main stem and tributary stations of the Upper Oyster Creek drainageway. This project had two general objectives: (1) to assess the water quality in Upper Oyster Creek with regard to the relative contributions of fecal bacteria

from human, bovine, and other animal contributions, to the water bodies; and (2) to develop local genetic libraries that can be used in determining the animal or human nonpoint fecal source contamination of surface water.

This project involved several steps:

- A sanitary survey of the watershed to identify potential contributing sources of fecal microbes that needed to be considered.
- Development of libraries of ribotypes of *E. coli* isolated from fecal matter collected from known sources in the Upper Oyster Creek watershed.
- Collection and culturing of a representative set of *E. coli* isolates from Upper Oyster Creek.
- Determination of the ribotypes of these *E. coli* isolates from Upper Oyster Creek, followed by matching to those from the known source library to identify the sources of each *E. coli* isolate.
- Quantification of the accuracy and precision of the ribotyping source determinations.
- Estimation of the relative source contributions of *E. coli* in the Upper Oyster Creek watershed, and the confidence of these estimates, based on the above measurements.

3.2.1 Ambient Water Sampling

Ambient water sampling was performed to collect *E. coli* isolates from Upper Oyster Creek. These ambient water sampling events included 12 events, spaced approximately 3 weeks apart, beginning in March 2004 and ending in late November 2004. Originally, the monitoring plan included collecting samples for *E. coli* analysis at six stations dispersed throughout the main stem of the Upper Oyster Creek watershed and one tributary to the watershed. An amendment to the monitoring plan added three supplemental monitoring stations for the last four sampling events, beginning in September 2004, for a total of nine monitoring stations.

3.2.1.1 Monitoring Station Descriptions

Stations were selected with the objective of identifying the sources contributing to violations of water quality criteria for *E. coli* in Upper Oyster Creek. The sources contributing to *E. coli* violations were expected to vary from station to station. Given that Oyster Creek is somewhat hydrologically divided by dams and diversions into multiple reaches, most of these stations were assigned to adequately characterize various reaches and to isolate, whenever possible, major contributing areas. Stations within each reach were selected where high bacterial levels were indicated from the 2002-2003 sampling results. The lower portion of Upper Oyster Creek below Dam #3 provided challenges in station selection because of access issues in the extreme lower portion (Steep Bank and Diversion Canal portions) and the presence of Dam #3, which reduced bacteria concentrations along the portion of the sub-

segment immediately below the dam. Consequently, the station selection process necessitated inclusion of a tributary station (Station 17688 on Stafford Run) in addition to a main-stem station (12074). These two stations are listed in Table 3-1 with the four other selected stations, and set out in Figure 3-2.

Three additional stations were added to the project in September 2004. These three stations were chosen to provide important supplemental data to the six core stations and to assist in the determination of the spatial distribution of *E. coli* sources. Two of the new stations were located on major tributaries to Segment 1245, while the third is located on Jones Creek, which is designated as a main stem of Segment 1245. The two tributary stations were selected and designed to be used in characterizing potential bacterial sources to the Upper Oyster Creek. The station on Jones Creek, near the Shannon Lift Station, assisted in determining sources associated with the transfer of water via pumping that occurs from the Brazos River into the Upper Oyster Creek. The station descriptions for the three additional sites are listed below the original six stations in Table 3-1. Station descriptions follow.

Table 3-1 Ambient Water Sampling Stations

Water Body	Station ID
Jones Creek at FM 723	12090
Upper Oyster Creek at FM 1464	12087
Upper Oyster Creek at Highway 6	12086
Upper Oyster Creek at US 90A	12083
Stafford Run at El Dorado Boulevard	17688
Flat Bank Creek at Highway 6	12074
Red Gully at Richmond-Gaines Road	11516
Jones Creek at Bois D'Arc Lane	17685
Flewellen Creek at Briscoe Road	17686

* Shaded stations were added for events 9 – 12.

Station 12090 is located on Jones Creek at FM 723, 5.5 miles north of the City of Rosenberg. The station is in the upper portion of Segment 1245. The station was selected to represent bacterial sources from Flewellen Creek and water pumped from the Brazos River at the Shannon Lift Station.

Station 12087 is located on Upper Oyster Creek at FM 1464 west of Sugar Land. The station is in the middle portion of Segment 1245 and downstream from Station 12090. The station was selected to represent bacterial sources downstream of the TDCJ wastewater discharge, a CAFO, upstream of Fort Bend County municipal utility district (MUD) #21 and the confluence with Red Gully, a tributary with high bacteria concentrations during storm events.

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Insert Figure 3-2 Upper Oyster Creek showing BST sites from file attached

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Station 12086 is located on the Upper Oyster Creek at SH 6 near Hull Airport in Sugar Land. This station has been the focus of significant monitoring, including routine and special studies, occurring in Upper Oyster Creek since 1989. The station is in the middle portion of Segment 1245 and downstream from Station 12087. The station was selected to represent bacterial sources and characterize water quality upstream of Fort Bend County MUD #25 wastewater discharge and Dam #1, below the confluence with Red Gully.

Station 12083 is located on Upper Oyster Creek at Highway 90A in Sugar Land. This station has historically been the focus of water quality monitoring in Upper Oyster Creek. Beginning in 1970, monitoring has occurred at varying frequencies at this station in support of both special studies and routine monitoring efforts. The station is in the middle portion of Segment 1245 and downstream from Station 12086. The station was selected to characterize water quality below Dam #1 and above Dam #2, as well as potential impacts from the significant number of waterfowl that frequent this stretch of river from fall to spring.

Station 17688 is located on Stafford Run at El Dorado Boulevard in Missouri City. The station was selected to characterize water quality in Stafford Run, a major tributary on the lower portion of Segment 1245. The station is in the lower portion of Upper Oyster Creek and downstream from Station 12083.

Station 12074 is located on Flat Bank Creek at SH 6 near Dewalt. The station is in the lower portion of Upper Oyster Creek and downstream from Station 17688. The station was selected to characterize bacteria sources and water quality downstream of Palmer Plantation MUD #001 in the reach of Segment 1245 below Dam # 3.

Station 11516 is located on Red Gully at Richmond-Gaines Road, 2.4 miles northwest of Sugar Land. The station is in the middle portion of Segment 1245. This supplementary station was selected to characterize water quality in Red Gully, including the impact of septic systems and two small WWTPs. Ft. Bend MUD #25 and MUD #41 are upstream of this station. Observed *E. coli* concentrations have been very high following runoff.

Station 17685 is located on Jones Creek at Bois D'Arc Lane, 3.5 miles south of Fulshear. The station is in the upper portion of Segment 1245. This supplementary station was selected to represent bacteria sources to water pumped from the Brazos River into the segment by the GCWA Shannon Pump Station and to characterize water quality above the Jones Creek confluence with Flewellen Creek.

Station 17686 is located on Flewellen Creek at Briscoe Road, one quarter mile upstream of Jones Creek. The station is in the upper portion of Segment 1245. This supplementary station was selected to characterize bacteria sources from Flewellen Creek, which is a major tributary to Segment 1245. Flewellen Creek is the largest tributary to the Upper Oyster Creek watershed. It is largely rural and has many ranches. Cattle are often in or near the water at sampling stations in the vicinity.

3.2.1.2 Ambient Water Sample Collection and Analysis

Because *E. coli* populations have been found to vary on fine spatial and temporal scales, sampling representativeness was increased by collecting five independent water samples per station, 1-2 minutes and 3-10 feet apart, at each event. Typically, this was done by sampling five points evenly spaced around each station. Because six stations were sampled in the first eight events, and nine stations were sampled in the last four events, a total of 420 water samples were collected.

Typically, water samples were collected directly from the stream (approximately 1 foot below the surface) into sterile wide-mouthed polypropylene bottles supplied by the culturing laboratory. Care was exercised to avoid the surface microlayer of water, which may be enriched with bacteria and not representative of the water column. In cases where, for safety reasons, it was inadvisable to enter the stream bed, or access was not practical, staff used a long handled dipper to collect samples from the stream, and poured the water into the sample bottles. The dipper was thoroughly rinsed and sanitized with bleach between stations. At the time of water sample collection, field observations for current weather, flow severity, water conditions and days since last significant precipitation were made based on standard operating procedures (SOP) in *TCEQ's Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (TCEQ 2003b).

After collection, all water samples were placed on ice in a cooler and transported to North Water District Laboratory Services (NWDLS) for *E. coli* culturing and enumeration via the membrane filter modified mTEC method. A 6-hour holding time was adhered to for sample delivery to the laboratory.

Following the 24-hour incubation and enumeration, the *E. coli* cultures were shipped overnight at a temperature of 1-4^{o}C to the ribotyping lab for *E. coli* colony isolation and confirmation, archiving, and ribotyping analysis. The ribotyping lab selected at least two isolates from each culture for processing and analysis.

3.2.1.3 BST Ribotyping Procedure

The ribotyping was performed at the Seattle, Washington laboratories of Institute for Environmental Health, Inc. (IEH). The detailed ribotyping protocol is found in Appendix B.

3.2.2 Known Source Ribotype Library Development

3.2.2.1 Sanitary Survey

A key component of the monitoring plan was preparation of a sanitary survey for the Upper Oyster Creek watershed. Through the sanitary survey, potential sources and general categories of fecal contamination within the watershed were identified and listed. These included assessment of wildlife, livestock, concentrated waterfowl areas, bird rookeries or bat colonies, dogs, cats, and other domestic animals, and utilization of waterways by wildlife. Human influences were also identified, including malfunctioning septic systems, municipal WWTPs, and sewer overflows. Based on information derived from the sanitary survey, a field

collection strategy was defined for collecting known fecal source samples from throughout the watershed.

A sanitary survey of source regions, as well as information about land use, population density, wastewater and storm water infrastructure, agricultural practices, and wildlife provided information to assist in identifying the sources of fecal pollutants within the Upper Oyster Creek watershed study area. This was important for two reasons.

- First, identification of the possible sources throughout the watershed ensured that analysis of resident *E. coli* strains from each contributing source was accomplished.

- Second, this information provides TCEQ with information not only on the specific animal source of fecal contamination, but also assisted in pinpointing the sources geographically.

The Project Team reviewed available literature, data, and information germane to describing the contributions and defining sources of bacterial loading in the watersheds. Data analyses included discussion of temporal (inter-annual, seasonal) and spatial trends in water quality, an evaluation of potential sources, and an identification of data gaps. Special emphasis was placed on acquiring land use/land cover and human and agricultural census data. These data were integral in assisting in the planning and execution of the project. Several other types of existing data and information were useful in the sanitary survey, described in Section 2. These data included:

- Reported wastewater permit information, including permit limits, self-reported effluent quality data, violations, and inspection reports;

- Hydrologic and meteorological data;

- Land use, population density, and the extent to which on-site sewerage systems are used (septic tanks) in the watershed;

- Livestock density and agricultural practices in the watershed from the most recent county-level agricultural census, as well as the abundance and type of CAFOs;

- Estimated populations of domestic pets; and

- Special studies and published reports for the study area.

3.2.2.2 Known Source Library Sample Collection

Based on the sanitary survey, a list of targets for the known source library was compiled (Table 3-2). The original planned size of the library included 400 fecal and sewage samples from known sources. This local library supplemented the much larger IEH library of many thousand *E. coli* from known sources collected throughout the United States over several years. A complete list of library samples is included in Appendix C.

Sample collection for library development was targeted at 400 *E. coli* isolates, although 500 isolates were actually collected. The species collected included: hogs, horses, cattle, goats, dogs, cats, raccoon, feral hogs, coyotes, waterfowl, and other birds. Samples of sewage and septage were also collected throughout the Upper Oyster Creek watershed.

Fresh animal fecal samples were collected aseptically into sterile test tubes, capped, and sealed. To the extent possible, known source samples were collected directly from the source. An exception was human samples, which were collected from septic tanks, sewer lines, and WWTPs. In some cases, wildlife samples were collected indirectly, from "found" fecal samples. The sources of these "found" wildlife fecal samples were identified to the lowest practical taxonomic level by experienced field biologists. Following sample collection, samples were shipped on ice in coolers via overnight courier to IEH. All sample containers were labeled with the following information: sample type, host species, sample date and time, sample location, and sampler's initials. All the sample information was logged into a field log.

3.3 Quality Assurance/Quality Control

This project provides an estimate of the relative contributions from various fecal sources in the watershed to the observed *E. coli* levels in Upper Oyster Creek; however, it is important to understand the level of uncertainty that accompanied those estimates. Precision, accuracy, sensitivity, completeness, and representativeness are critical data quality issues affecting uncertainty. Representativeness must be controlled by developing an environmental monitoring program characteristic of actual environmental conditions. Accuracy, precision, sensitivity, and completeness can be similarly controlled through careful planning, but also should be quantified via quality control (QC) measures. These QC measures include analysis of replicate laboratory duplicate samples performed by the culture lab, and known standards for BST (samples of known origin).

3.3.1 Completeness

Completeness of the data is a measure of how much of the data is available for use compared to the total potential data. Ideally, 100 percent of the data should be available. However, the possibility of unavailable data due to accidents, insufficient sample volume, broken or lost samples, *etc*. is to be expected. Therefore, it was a general goal of the project(s) that 90 percent data completion be achieved.

An additional element of completeness is involved with BST. The sources of *E. coli* isolates which do not match those from a library of known sources cannot be identified. In all BST studies, a source cannot be identified with acceptable confidence for a portion of the *E. coli* isolates. This inability to identify some isolates is a function of 1) the size of the library relative to the true diversity of *E. coli* in the watershed; 2) ability of the method to distinguish sources with acceptable confidence; and 3) abundance of *E. coli* strains that colonize multiple sources, and thus cannot be used to uniquely identify a source. The project team developed a library of approximately 500 isolates collected from fecal sources within the Upper Oyster Creek watershed. This local library was supplemented by a much larger library previously

developed by IEH. It was a general goal of this project to identify the sources of 70 percent of the *E. coli* strains isolated from water.

Table 3-2 Summary of Fecal Source Sampling for Library Development

Upper Oyster Creek Segment 1245		**Target No. Samples to Collect**	**TotalSamples Collected**	**Sample % of Target**
Major Category	Minor Category			
Human/Sewage	human - raw sewage	35	55	
	Human septage	35	15	
Human/Sewage Total		70	70	100%
Pets	cat	20	14	
	dog	40	51	
	other	10	5	
Pets Total		70	70	100%
Livestock	cattle, dairy	0	1	
	cattle, beef	35	54	
	chicken	10	16	
	turkey	0	4	
	horses/ponies	16	41	
	goat	5	23	
	sheep	5	0	
	donkeys	10	7	
	hog (domestic)	15	37	
	Guinea fowl	0	4	
Livestock Total		96	187	195%
Wildlife-mammals	raccon	10	20	
	deer	10	0	
	Hog (feral)	3	10	
	mouse	5	0	
	Rat	5	0	
	rabbit	3	0	
	opossum	5	1	
	squirrel	5	1	
	armadillo	3	0	
	coyote	1	9	
	fox	1	0	
	beaver	1	0	
	nutria	1	0	
	skunk	3	0	
	other	5	1	
Wildlife-avian	ducks/geese	35	80	
	swallow	20	3	
	pigeon	20	0	
	heron	3	7	
	grackle	5	2	
	egret	3	16	
	martin	5	0	
	sparrow	2	0	

Upper Oyster Creek Segment 1245		Target No. Samples to Collect	TotalSamples Collected	Sample % of Target
	dove	5	3	
	other (birds)	5	1	
	mockingbird	0	1	
	starling	0	13	
	killdeer	0	4	
	crow	0	1	
Wildlife Total		164	173	104%
Other	compost	0	1	
	Grand Total	**400**	**501**	**125%**

3.3.2 Quantification of Accuracy and Precision in Ribotyping Source Determinations

BST does not lend itself easily to the same QC methods as chemical quantification. Blank samples may be irrelevant, and replicate water samples may often yield different *E. coli* strains. The method accuracy and precision was quantified through a special QC study with "double-blind" safeguards, as practiced in epidemiological QC.

The IEH prepared triplicate cultures of 30 *E. coli* isolates from known sources collected in the Upper Oyster Creek watersheds as part of this study. These isolates were selected from a variety of species. The 120 (40x3) cultures were placed in 120 identical culture tubes, each with a removable label indicating their source and the isolate number. These tubes were mailed to the Parsons Quality Assurance Officer (QAO). The Parsons QAO prepared and sent a list of the 40 isolate sources to the TIAER QAO, who selected from the list 20 isolates to be blind QC test samples. (By selecting a subset of only 50 percent of the prepared tubes, the laboratory had no basis for anticipating the identity of the unmarked blind samples when received.) The Parsons QAO identified the 60 culture tubes associated with those 20 isolates, replaced each label with a new label, numbered them from 1 to 60 in random fashion, and recorded those numbers on a key with the isolate number and source. The Parsons QAO sent those 60 culture tubes back to the IEH after verifying that there was no way for their source to be identified. Parsons sent the key to the TIAER QAO. The samples were processed through the ribotyping procedures in a blind fashion; that is, the laboratory did not know the sources. The IEH sent the results to the Parsons QAO, who made a copy of the key and results and provided it to the IEH and TIAER QAO. The Parsons QAO evaluated and prepared a brief report on the accuracy and precision of the methods, the results of which are found in Section 4.

In ribotyping, with the inherent high precision and accuracy of the rRNA methods, data completeness was most affected by the number of ribotypes found that match ribotypes in the known source library. Thus, a large library was important.

3.4 Sampling Event Summary

The intent of this section is to provide general information about the condition of the water bodies and observable weather at the time of water sampling. Rainfall occurring over the region at least 1 week prior to the sampling events was also noted. The maximum allowed holding times for water samples analyzed for *E. coli* was 6 hours. All samples were transferred to the lab and processed within the required holding time.

Fecal sampling occurred as sample material was identified during water sampling events, as well as during sampling events specific for fecal collection. In addition to water and fecal sampling events, a sanitary survey was conducted which is described in Section 2. The sanitary survey included potential bacteria source identification as well as fecal sampling.

Event 1

The first water sampling event took place on March 15, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). The weather was overcast and 1.42 inches of rain fell the day before sampling. The flow at all stations was strong and the water was brown and turbid. Swallows and pigeons were observed under bridges at the downstream segments. This event was considered a run-off event.

Event 2

The second sampling event took place on April 6, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). The weather was cloudy and humid. It had been 3 days since the prior significant rain event, but rain storms moved into the area during sampling. The flow at all stations was strong and the water was brown and turbid. Swallows and pigeons were observed under bridges at the downstream segments.

Heavy rain started at 11:20 am. Samples at Station 12090 were collected after rain had been falling for approximately 35 minutes; therefore; only Station 12090 was considered a runoff-influenced event.

Event 3

The third sampling event took place on May 4, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). This sampling event was observed by both TCEQ and TIAER personnel. No significant weather was noted, with clear skies, a light southwesterly breeze, and 80^{o}F temperature. Almost three inches of rain fell on May 1. The flow at all stations was slightly above normal and the water was brown and turbid with storm debris along the banks.

A fecal sampling event was scheduled to coincide with the water event occurring on May 5, 2004. Parsons and TIAER personnel met with personnel from GCWA who provided access

to privately owned land along Oyster and Flewellen Creeks. Wild and domestic animal scat was collected with help from the GCWA personnel. The TDCJ, Jester Unit was also visited by Parsons and TIAER personnel. Dog, hog and wild egret scat was collected during the visit.

Event 4

The fourth sampling event took place on May 25, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). No significant weather was noted. The previous significant rain fell on May 14, eleven days prior to the sampling event. The flow at all stations was normal or below normal and the water was brown and turbid.

Event 5

The fifth sampling event took place on June 22, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (12090, 12087, 12086, 12083, 12074 and 17688). The weather was hot, cloudy and humid with rain moving into the area. Rain fell over the sampling area five days prior to the sampling event. The flow at all stations was normal and the water was brown and turbid at Stations 12074 and 12083; clear and greenish at Station 17688; and tan with a little turbidity at Stations 12086 and 12087. Rain started to fall as sampling was completed. A definite odor of pigeon and swallow feces was noted under the bridge at Station 12074. Samples at the last station 12090 were collected after rain had been falling, therefore; only station 12090 was considered a run-off event.

Event 6

The sixth sampling event took place on July 13, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (12090, 12087, 12086, 12083, 12074 and 17688). No significant weather was noted. An inch and a half of rain fell over the sampling area two days prior to the sampling event. The flow at all stations was normal and the water was brown and slightly turbid at all stations except 12074, where it was tan and slightly turbid, and 17688 where the water was greenish with low turbidity. Recent storms show a debris line approximately 20 inches over the ordinary high water mark.

Dedicated fecal sampling occurred on July 14th and the 26th -29th. The fecal samples were placed on ice and transferred to the ribotyping lab. Wastewater treatment plant influent samples were collected at Sugarland WWTP and the Missouri City WWTP. The WWTP samples were placed on media plates and incubated at 35^{o}C for 2 hours after which the temperature was increased to 44.5^{o}C for 24 hours.

Event 7

The seventh sampling event took place on August 10, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). The weather was hot, clear and calm. The previous significant rainfall had fallen eight days before the sampling event. The flow at all stations was normal or lower than normal and the water was greenish-tan and had low turbidity.

Event 8

The eighth sampling event took place on August 24, 2004. The event included five bacteria water samples collected at each of the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074 and 17688). The weather was hot, clear and calm. The previous significant rainfall (0.56") had fallen 3 days before the sampling event; however 0.24 inches of rain had fallen just a day before. The flow at all stations was normal and the water was greenish-tan with low turbidity.

Event 9

The ninth sampling event took place on September 28, 2004. Water and fecal sampling initially occurred on September 14, but the lab was not prepared to handle the water samples. Re-sampling took place on September 28, 2004. The event included five bacteria water samples collected at each of the three new sampling sites, in addition to the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074, 17688, 11516, 12086, 17685 and 17688). The weather was warm, clear and calm. Significant rain (0.38 inches) fell over the sampling area three days prior to the sampling event. The flow at all stations was normal and the water was greenish-tan with low turbidity.

Event 10

The tenth sampling event took place on October 12, 2004. The event included five bacteria water samples collected at each of the three new sampling sites, in addition to the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074, 17688, 11516, 12086, 17685 and 17688). The weather was warm, clear, and calm. An inch of rain had fallen five days prior to the sampling event. The flow at all stations was normal and the water was greenish-tan and turbid.

Event 11

The eleventh sampling event took place during a large rain event on November 2, 2004. The event included five bacteria water samples collected at each of the three new sampling sites, in addition to the original six sampling locations (Stations 12090, 12087, 12086, 12083, 12074, 17688, 11516, 12086, 17685 and 17688). The weather was cool and cloudy with a slight breeze. A substantial amount of rain (2.83 inches) fell on November 1, with another 0.8 inch on the sampling day. The flow at all stations was very high and the water was brown and turbid. This event was considered a run-off event.

Event 12

The twelfth and last water sampling event took place on November 23, 2004. The event included five bacteria water samples collected at each of the three new sampling sites, in addition to the original six sampling locations (Station 12090, 12087, 12086, 12083, 12074, 17688, 11516, 12086, 17685 and 17688). The weather was cloudy, cool, humid, and calm with skies starting to clear. Heavy rain had fallen over the sampling area for several days until approximately six hours prior to the sampling event. The total rainfall for the four-day rain

event was 5.8 inches, with more than one-half inch each day. The flow at all stations was very high and the water was brown with high turbidity. This event was considered a run-off event.

Fecal samples were collected on November 22nd. Additional fecal sampling occurred on December 8 and 9, 2004 that included additional trips to private property on a ranch, the GCWA pump station property, and the TDCJ, Jester Unit. Wastewater treatment plant influent samples were collected at Sugarland WWTP and the Missouri City WWTP. A sample of composted planting soil was collected from Houston Nurseries. City of Rosenberg and Fort Bend County Animal Shelters were also visited for domestic animal fecal samples. The WWTP samples were placed on media plates and incubated at 35^{o}C for 2 hours after which the temperature was increased to 44.5^{o}C for 24 hours.

SECTION 4
RESULTS AND DISCUSSIONS

Ambient water sampling for this project lasted approximately 9 months and consisted of 12 sampling events beginning March 15, 2004 and ending November 23, 2004. The ambient water sampling sites included six core sites that were sampled 12 times, and three supplementary sites that were sampled during the last four events, beginning September 28, 2004.

Rainfall runoff washes fecal material from the land surface into water, and typically causes a pronounced increase in fecal bacteria levels. The objective of this sampling was to obtain representative sampling of Upper Oyster Creek under both runoff and non-runoff conditions, with the ratio of runoff to non-runoff samples typical of the natural frequencies of these conditions. A sampling event was considered to be influenced by runoff if more than one-quarter inch of rain was measured at the Hull (Sugar Land) Airport on the day of sampling (before the sample was collected) or on the previous day. From March through November 2004, one-quarter inch or more of rain fell on 45 days. Considering days of consecutive rainfall of one-quarter inch or more and the above definition of a runoff-influenced sampling event, 76 days out of 276 days (or 28 percent) would be considered runoff influenced. Thus, Upper Oyster Creek was expected to be influenced by runoff on one of every three or four days, on average.

Overall, 30 percent of the water samples were considered runoff-influenced, in general agreement with the natural frequency of this condition. However, this frequency was not uniform for all sites. For the three monitoring stations added in September 2004, samples were runoff-influenced on two of the four sampling dates. Thunderstorms began during two sampling events, and only the last samples collected on those dates were considered to be influenced by runoff. Thus, five of the 12 sampling events at Station 12090 were runoff-influenced. For the other five stations, three of 12 samples were considered to be runoff-influenced.

4.1 Quality Assurance/Quality Control Results

QA/QC measures utilized by NWDLS in the culturing and enumeration of *E. coli* from water samples, and by IEH in the ribotyping of *E. coli* are described separately below.

4.1.1 Culturing and Enumeration of *E. coli*

Method blanks were run by the laboratory with each group of samples delivered to the laboratory. Method blanks were sterile buffered dilution water free of *E. coli,* and were carried through the entire analytical process. All method blanks were negative for *E. coli*, reflecting a lack of contamination in the analytical procedure, including media, filters, dilution and rinse water, and glassware and equipment.

Positive and negative control cultures were also run with each group of ambient water samples. Positive controls were known *E. coli* cultures to ensure that the media supported

growth of *E. coli*. Negative controls were cultures of bacteria species other than *E. coli* to ensure that other types of bacteria did not grow on the media under the incubation conditions. All positive controls were positive, and all negative controls were negative.

Laboratory duplicate samples were analyzed at a rate of just under one in 10 samples. These samples were collected by analysis of two separate aliquots of an ambient water sample delivered to the laboratory. Laboratory duplicate samples were used to quantify variation in the analytical procedure. The relative percent deviation of the log-transformed *E. coli* concentrations of laboratory duplicates averaged less than 2 percent, did not exceed 7 percent, and remained within the control limits specified in the Quality Assurance Project Plan (QAPP). This indicates that very little variation was introduced during analysis.

4.1.2 Ribotyping and Source Identification

Ribotyping was extremely precise and repeatable. As described in Section 3, IEH analyzed 60 unknown *E. coli* cultures. These 60 cultures represented three copies each of 20 different *E. coli* isolates selected randomly by the TIAER QAO from a group of 120 (three copies each of 40 different *E. coli* isolates) cultures provided by IEH to the Parsons QAO from the IEH known source library of *E. coli* isolates. These included isolates from seagulls, humans, and cattle. Labels on the slant tubes containing the isolates were randomly changed by the Parsons QAO before being returned to IEH, so IEH could not identify the cultures except through ribotyping. For each of the 60 unknown *E. coli* cultures tested, IEH assigned the same ribotype identification (ID) to each of the three copies of a given isolate. In other words, with repeated analysis the method produced the same ribotype result each time; thus, precision of the method was judged to be 100 percent. Accuracy was judged by the ability of the lab to assign the correct ribotype ID to the unknown cultures. IEH assigned the correct ID to 57 of the 60 unknown cultures, for a correct rate of 95 percent. It should be noted that though the ribotype ID was incorrectly identified for one isolate (three cultures); the source species identified was actually correct. In other words, the correct source species was identified for 100 percent of the cultures. These precision and accuracy rates met the 90 percent accuracy and precision data quality objectives of the project.

4.2 *E. coli* Levels in Water

Measured *E. coli* levels were summarized by event and by site by the minimum, maximum, and geometric mean measured concentration, and are provided in Appendix D. The overall geometric mean *E. coli* level at each site, including both runoff-influenced and non-runoff event samples can be compared to the geometric mean water quality criterion of 126 cfu per 100 ml. However, it is more appropriate to make inter-comparisons between sites based on levels under either runoff or non-runoff conditions, but not the aggregate, as the frequency of runoff influence varied from site to site.

Table 4-1 summarizes the observed *E. coli* concentration under runoff and non-runoff conditions. The geometric mean *E. coli* levels exceed the 126 cfu per 100 ml water quality criterion at all sites except Station 12083, Upper Oyster Creek at Highway 90A in Sugar Land. Additionally, the measured *E. coli* levels exceeded the single sample maximum water quality

criterion (396 cfu per 100 ml) in more than 25 percent of the samples at all sites. These levels indicate general non-attainment of water quality criteria protecting contact recreation throughout the system.

Table 4-1 Measured *E. coli* Levels under All Conditions

Site Description	Station Number	Number of Events	*E. coli* Concentration (cfu/100 mL)			% Samples Exceeding 396 / 100 ml
			Min	Max	Geometric Mean	
Jones Creek at FM 273	12090	12	13	>20,000	563	52%
Upper Oyster Creek at FM 1464	12087	12	17	7,500	268	38%
Upper Oyster Creek at Hwy 6	12086	12	20	7,600	227	33%
Upper Oyster Creek at US 90A	12083	12	<1	5,400	114	33%
Stafford Run at El Dorado Blvd	17688	12	63	16,900	788	57%
Flat Bank Creek at Hwy 6	12074	12	<1	10,300	341	52%
Red Gully at Richmond-Gaines Rd	11516	4	<1	19,000	219	50%
Jones Creek at Bois D'Arc Lane	17685	4	11	18,000	358	50%
Flewellen Creek at Briscoe Road	17686	4	62	>20,000	972	50%

Table 4-2 summarizes the much lower *E. coli* levels observed under non-runoff conditions. Only three stations, Stafford Run, Flat Bank Creek, and Jones Creek at FM 273, exceeded water quality criteria. Repeated exceedances of the water quality criteria under non-runoff conditions may indicate disinfection problems with point source wastewater discharges, livestock in the stream, or localized wildlife impacts such as birds residing under the bridge at the monitoring station. The highest levels were observed at Stafford Run. Excluding the supplementary stations which were sampled only twice under non-runoff conditions, the lowest *E. coli* levels were observed in Upper Oyster Creek in and just upstream of Sugar Land (Stations 12083 and 12086).

Table 4-3 summarizes the high *E. coli* levels observed under runoff conditions. Some measurements exceeded 20,000 cfu/100 ml. On average, the highest levels were observed in Flewellen Creek, Red Gully, and Stafford Run, and the lowest levels were observed in Oyster Creek at Highway 90A in Sugar Land.

Table 4-2 Measured *E. coli* Levels under Non-runoff Conditions

Site Description	Station Number	Number of Events	*E. coli* concentration (cfu/100 ml)			% Samples Exceeding 396 / 100 ml
			Min	Max	Geometric Mean	
Jones Creek at FM 273	12090	7	13	880	135	17%
Upper Oyster Creek at FM 1464	12087	9	17	1080	121	18%
Upper Oyster Creek at Hwy 6	12086	9	20	630	75	11%
Upper Oyster Creek at US 90A	12083	9	<1	680	41	11%
Stafford Run at El Dorado Blvd	17688	9	63	7500	356	42%
Flat Bank Creek at Hwy 6	12074	9	<1	2900	157	36%
Red Gully at Richmond-Gaines Rd	11516	2	<1	18	4	0%
Jones Creek at Bois D'Arc Lane	17685	2	11	77	33	0%
Flewellen Creek at Briscoe Road	17686	2	62	95	76	0%

Table 4-3 Measured *E. coli* Levels under Runoff Conditions

Site Description	Station Number	Number of Events	*E. coli* Concentration (cfu/100 ml)			% Samples Exceeding 396/100 ml
			Min	Max	Geometric Mean	
Jones Creek at FM 273	12090	5	690	>20,000	4,165	100%
Upper Oyster Creek at FM 1464	12087	3	1,000	7,500	3,392	100%
Upper Oyster Creek at Hwy 6	12086	3	4,800	7,600	6,265	100%
Upper Oyster Creek at US 90A	12083	3	650	5,400	2,355	100%
Stafford Run at El Dorado Blvd	17688	3	3,400	16,900	8,565	100%
Flat Bank Creek at Hwy 6	12074	3	1,570	10,300	3,509	100%
Red Gully at Richmond-Gaines Rd	11516	2	5,900	19,000	10,871	100%
Jones Creek at Bois D'Arc Lane	17685	2	850	18,000	3,913	100%
Flewellen Creek at Briscoe Road	17686	2	7,100	>20,000	12,411	100%

Some general conclusions can be reached based on the observed *E. coli* levels. First, it does not appear that water entering the system by pumping from the Brazos River represents a major source of the observed *E. coli* levels. With its high bacteria levels, Stafford Run may exert a major influence on observed *E. coli* levels in the lower reaches of the segment. *E. coli*

levels in the middle reaches of Upper Oyster Creek may be reduced by the dams and impoundments, where the resulting lower water velocities permit bacteria to settle out of the water column. Finally, the profound influence of runoff on *E. coli* levels must be noted. The persistence of high *E. coli* levels following runoff is not well-quantified in this system, and may require further examination. Very high *E. coli* levels were observed during sampling event #1 (March 15), 1 day after a 1.4-inch rainfall event. Sampling event #6 (July 13) occurred 2 days after a 1.5-inch rain, and *E. coli* levels ranged between 130 cfu and 680 cfu per 100 ml, higher than but in the same range of magnitude as most other non-runoff-influenced events. While the influence of runoff does not persist too long, the frequency of rainfall in this area indicates that it may be difficult to meet water quality standards.

4.3 Identified Bacterial Sources Based on BST Technology

Overall, 1136 *E. coli* isolates from ambient water samples were ribotyped, substantially exceeding the stated project objective of ribotyping 840 isolates (120 per site for the six original stations plus 40 per site for the supplemental stations). Table 4-4 provides a summary of the isolates ribotyped by site. Data completeness met or exceeded 100 percent at all sites.

Table 4-4 Count of *E. coli* Ribotype Characterization by Site and Runoff Condition

Site Description	Station Number	Count of *E. coli* Isolates Ribotyped		
		Non-runoff	Runoff	Total
Jones Creek at FM 273	12090	99	70	169
Upper Oyster Creek at FM 1464	12087	110	47	157
Upper Oyster Creek at Hwy 6	12086	130	49	179
Upper Oyster Creek at US 90A	12083	92	47	139
Stafford Run at El Dorado Blvd	17688	135	48	183
Flat Bank Creek at Hwy 6	12074	119	49	168
Red Gully at Richmond-Gaines Rd	11516	20	20	40
Jones Creek at Bois D'Arc Lane	17685	25	23	48
Flewellen Creek at Briscoe Road	17686	29	24	53
All Sites Combined		759	377	1136

To interpret results of BST methodology, and summarize the fraction of fecal coliform in ambient water from specific sources, it is important to note that the relative weighting of individual water samples in the source summary is not equal. There are many reasons for this unequal weighting related to the sampling and analytical process. The primary reasons include:

- The number of water samples collected from each site was variable, considering that the three supplementary sites were sampled only four times;
- the number of discrete fecal coliform colonies that could be harvested by IEH from a plate was in some cases limited due to low *E. coli* counts or laboratory dilutions. In some cases, no *E. coli* were observed in a sample.

- the fraction of fecal coliform colonies harvested from a plate that, upon purification and testing, were verified to be *E. coli* and ribotyped, was variable; and
- discretion of the laboratory staff. (In some cases, fecal coliform filter were re-sampled to harvest additional colonies.)

To achieve an overall average of two isolates ribotyped from each water sample, IEH often selected as many as five or six isolates from an individual filter. The number of satisfactory ribotypes obtained from a single water grab sample ranged from zero to six. Thus, when reporting and interpreting the data, the reader must understand that when computing summary statistics regarding source identification, one site, sampling event, or individual water sample may have more influence on the summary results than another. Attempts to normalize the results to reduce this disparate influence could be made, but because many factors control the sample influence, there are as many different possible ways to normalize. For this reason, raw data in Appendix E will be provided to allow the user to interpret data according to their needs.

4.3.1 Source Categorization

The subjective grouping of ribotypes into source categories merits discussion. The categorization is based to some extent on the basis of biological similarity, but it is also influenced by co-occurrence of species. For example, cattle and guinea fowl are not biologically similar, but these categories can be grouped from a management viewpoint as livestock that tend to occur on farms and ranches.

E. coli strains that have been observed in more than one source type are considered transient strains. Because they cannot be used to identify a source, the source of *E. coli* is identified as "unknown." *E. coli* isolated from water samples that do not match any *E. coli* in the known source library are also identified as "unknown" sources.

When *E. coli* are observed in multiple species, but the species are closely related, they are not identified as transient strains, but the source category description is expanded. For example, strains that have been seen in dogs and coyotes will be assigned to the category "canine," and strains observed in bison and cattle will be assigned to the category "bovine." There is a biological basis for this grouping, because conditions in the gut of closely related species are expected to be similar, and gut conditions are believed to be the primary factor influencing which *E. coli* strains are abundant.

Strains of *E. coli* are often observed to occur in many different species of birds, but not in mammals. Thus, even when an *E. coli* isolate has been observed from only one type of bird, it is assumed that it may also occur in other species and is assigned to the generic "avian" source category, unless numerous observations confirm that its occurrence is specific to a particular type of bird. An exception is waterfowl, which appear to host some strains of *E. coli* that do not occur in other types of birds. These *E. coli* were assigned to the category "waterfowl," a subset of the avian category.

The category "human" is assigned to *E. coli* that have only been observed in raw sewage. Sewage, septage, and sewage sludge are assumed to consist primarily of human waste, but also

include fecal matter from other species. When source categories are grouped into "super-categories," sewage, septage, and sludge were grouped together with human sources.

Dogs, cats, and other non-native, non-livestock animals are grouped into the super-category "pets." All the native wild mammals, including rodents, coyotes, deer, *etc*., are grouped into the super-category "non-avian wildlife." When categories include both wild and domestic species, they are included in the respective domestic super-category totals because it is believed that abundance of the domestic species typically exceeds that of the wild species. For example, *E. coli* from the "canine" source category, which includes strains found in both dogs and coyotes, are included in the super-category "pets" rather than "non-avian wildlife." This may be a poor assumption in some cases, such as the porcine category, because wild hogs were observed to be abundant in the Upper Oyster Creek watershed, likely outnumbering their domestic cousins.

4.3.2 *E. coli* Source Contribution Estimates Based on Ribotyping

In this section, *E. coli* source contributions are estimated for the Upper Oyster Creek watershed as a whole and for individual sampling sites. Source contributions are calculated as the sum of isolates matching a particular source category or super-category, divided by the total number of *E. coli* for which sources are identified. Confidence intervals around the source contribution estimates were calculated from the following formula:

$$\overline{p} \pm z_{\alpha/2}\sqrt{\frac{p(1-p)}{n}}$$

where p is the estimated proportion of the *E. coli* from a given source, n is the total number of isolates, and $z_{\alpha/2}$ is the value of the standard normal distribution at confidence interval α.

4.3.3 All Sites Combined

In this section, results for all nine monitoring stations were pooled to estimate bacteria sources to Upper Oyster Creek as a whole. Table 4-5 and Figure 4-1 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. Wildlife represented the largest source of *E. coli*, accounting for 43 percent of the total observed in the stream. Among the wildlife, birds (23.2%) were a slightly more significant source than mammals (19.5%). Among birds, the *E. coli* specific to waterfowl accounted for approximately 7 percent of the total. Among mammals, rodents, including squirrels, were the major source, accounting for 11.4 percent of the total. Raccoons were also a significant (>4%) source of *E. coli*, in agreement with the observations of their abundance during the sanitary survey and subsequent sampling. Pets, primarily dogs, accounted for just fewer than 10 percent of the total *E. coli* observed. Cats were not a significant source. Livestock represented 19 percent of the total *E. coli* observed. Livestock contributions were primarily from bovine (7%, assumed to be cattle), swine (5.7%, hogs and pigs), and horses (5%). Goats and poultry were very minor sources. As stated earlier, the BST methodology does not distinguish wild from domestic hogs. Since wild hogs were observed to be abundant in the watershed, this source may more appropriately be assigned to the wildlife super-category. The source of approximately 15 percent of *E. coli* isolates could not be identified, either because there were

no matching ribotypes in the known source library or because the matching isolates were transients, *i.e.*, they are not host-specific having been observed in multiple types of host species.

Table 4-5 *E. coli* Source Characterization of Upper Oyster Creek under All Conditions

Super-category	Category	Source	Isolates	% Contribution	95% Confidence Interval
Human/sewage	human	human	18	1.6%	0.9-2.3%
Human/sewage	sewage	sewage	143	12.6%	10.7-14.5%
Human/sewage	subtotal		161	14.2%	12.1-16.2%
Livestock		bovine	79	7.0%	5.5-8.4%
Livestock		horse	57	5.0%	3.7-6.3%
Livestock		poultry	9	0.8%	0.3-1.3%
Livestock		Guinea fowl	1	0.1%	0.0-0.3%
Livestock		donkey	1	0.1%	0.0-0.3%
Livestock		goat	3	0.3%	0.0-0.6%
Livestock		porcine	65	5.7%	4.4-7.1%
Livestock	subtotal		215	18.9%	16.6-21.2%
Pets	canine	canine	85	7.5%	6.0-9.0%
Pets	canine	dog	17	1.5%	0.8-2.2%
Pets	feline	feline	5	0.4%	0.1-0.8%
Pets	subtotal		107	9.4%	7.7-11.1%
Wildlife	mammal	coyote	9	0.8%	0.3-1.3%
Wildlife	mammal	deer	20	1.8%	1.0-2.5%
Wildlife	mammal	rabbit	1	0.1%	0-0.3%
Wildlife	mammal	raccoon	47	4.1%	3.0-5.3%
Wildlife	mammal	rodent	128	11.3%	9.4-13.1%
Wildlife	mammal	opossum	14	1.2%	0.6-1.9%
Wildlife	mammal	skunk	1	0.1%	0.0-0.3%
Wildlife	mammal	squirrel	1	0.1%	0.0-0.3%
Wildlife	mammal	subtotal	221	19.5%	17.2-21.8%
Wildlife	avian	waterfowl	76	6.7%	5.2-8.1%
Wildlife	avian	avian	187	16.5%	14.3-18.6%
Wildlife	avian	subtotal	263	23.2%	20.7-25.6%
Wildlife	subtotal		484	42.6%	39.7-45.5%
Unknown		unknown	169	14.9%	12.8-16.9%
Grand Total			1136	100.0%	

Figure 4-1 *E. coli* Source Characterization of Upper Oyster Creek under All Conditions

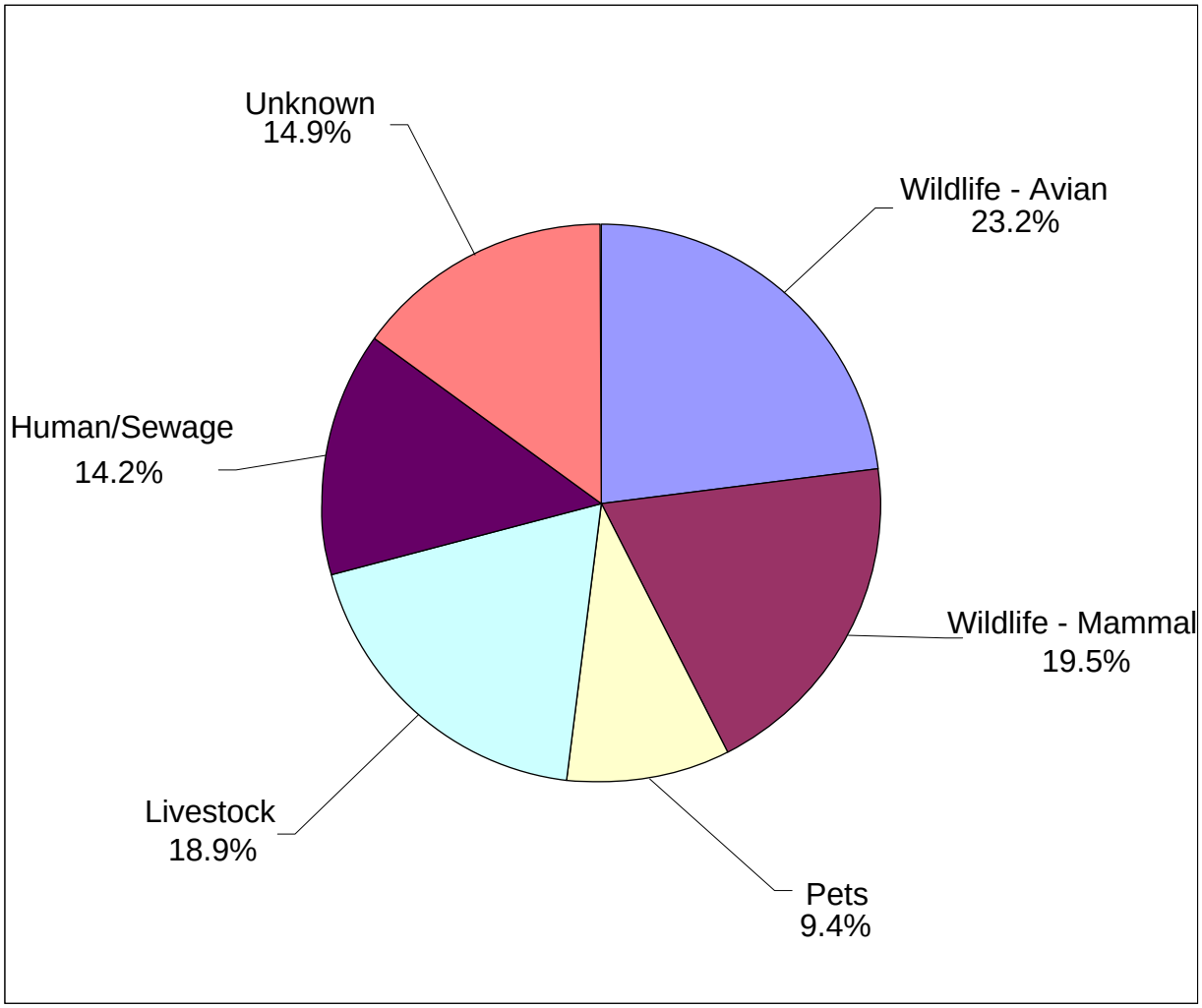


Table 4-6 summarizes and compares the sources of *E. coli* from all sites under runoff and non-runoff conditions. No statistically significant (α=0.05) differences in sources were observed between runoff and non-runoff conditions. While somewhat counter-intuitive, this result has been frequently observed in other BST projects.

Table 4-6 Comparison of *E. coli* Sources from all Sites under Runoff and Non-runoff Conditions

Super-Category	Category	Source	Non-runoff			Runoff		
			Isolates	% Contribution	95% CI	Isolates	% Contribution	95% CI
Human	Human	Human	11	1.4	0.6-2.3	7	1.9	0.5-3.2
Human	sewage	sewage	100	13.2	10.8-15.6	43	11.4	8.2-14.6
			111	14.6	12.1-17.1	50	13.3	9.8-16.7
Livestock		bovine	53	7.0	5.2-8.8	26	6.9	4.3-9.5
Livestock		horse	33	4.3	2.9-5.8	24	6.4	3.9-8.8
Livestock		poultry	7	0.9	0.2-1.6	2	0.5	0.0-1.3
Livestock		Guinea fowl	0	0.0		1	0.3	0.0-0.8
Livestock		donkey	1	0.1	0.0-0.4	0	0.0	
Livestock		goat	3	0.4	0.0-0.8	0	0.0	
Livestock		porcine	42	5.5	3.9-7.2	23	6.1	3.7-8.5
Livestock	subtotal		139	18.3	15.6-21.1	76	20.2	16.1-24.2%
Pets	canine	canine	57	7.5	5.6-9.4	28	7.4	4.8-10.1
Pets	canine	dog	14	1.8	0.9-2.8	3	0.8	0.0-1.7
Pets	feline	feline	5	0.7	0.1-1.2	0	0.0	
Pets	subtotal		76	10.0	7.9-12.1	31	8.2	5.4-11.0
Wildlife	mammal	coyote	6	0.8	0.2-1.4	3	0.8	0.0-1.7
Wildlife	mammal	deer	16	2.1	1.1-3.1	4	1.1	0.0-2.1
Wildlife	mammal	rabbit	1	0.1	0.0-0.4	0	0.0	
Wildlife	mammal	raccoon	38	5.0	3.5-6.6	9	2.4	0.8-3.9
Wildlife	mammal	rodent	81	10.7	8.5-12.9	47	12.5	9.1-15.8
Wildlife	mammal	opossum	9	1.2	0.4-2.0	5	1.3	0.2-2.5
Wildlife	mammal	skunk	0	0.0		1	0.3	0.0-0.8
Wildlife	mammal	squirrel	1	0.1	0.0-0.4	0	0.0	
Wildlife	mammal	subtotal	152	20.0	17.2-22.9	69	18.3	14.4-22.2
Wildlife	avian	waterfowl	50	6.6	4.8-8.4	26	6.9	4.3-9.5
Wildlife	avian	avian	118	15.5	13.0-18.1	69	18.3	14.4-22.2
Wildlife	avian	subtotal	168	22.1	19.2-25.1	95	25.2	20.8-29.6
Wildlife	subtotal		320	42.2	38.6-45.7	164	43.5	38.5-48.5
Unknown		unknown	113	14.9	12.4-17.4	56	14.9	11.3-18.4
Grand Total			759	100.0		377	100.0	

As noted earlier, it is possible to normalize results to reduce potential bias introduced in the source characterization by the unequal number of *E. coli* ribotyped for each site and sampling event. This normalization was performed by calculating the source contribution at each site for each sampling event, then averaging those results by site, and finally calculating an overall average source contribution percentage for all sites. Table 4-7 provides results for this normalization for all sites and events combined. Table 4-7 can be compared to the non-normalized results in Table 4-5. All differences are very minor, and none are statistically significant at the 95 percent confidence level.

Table 4-7 Normalized *E. coli* Source Characterization of Upper Oyster Creek under All Conditions

Super-category	Category	Source	% Contribution	95% Confidence Interval
Human/sewage	human	Human	2.5%	1.6-3.4%
Human/sewage	sewage	Sewage	11.4%	9.6-13.3%
Human/sewage	subtotal		13.9%	11.9-15.9%
Livestock		Bovine	6.4%	5.0-7.9%
Livestock		Horse	6.1%	4.7-7.5%
Livestock		Poultry	0.6%	0.1-1.0%
Livestock		Guinea fowl	0.1%	0.0-0.2%
Livestock		Donkey	0.1%	0.0-0.3%
Livestock		Goat	0.1%	0.0-0.3%
Livestock		Porcine	4.3%	3.1-5.5%
Livestock	subtotal		17.7%	15.5-20.0%
Pets	canine	canine	8.6%	7.0-10.2%
Pets	canine	dog	1.6%	0.9-2.4%
Pets	feline	feline	0.8%	0.3-1.3%
Pets	subtotal		11.0%	9.2-12.8%
Wildlife	mammal	coyote	0.7%	0.2-1.2%
Wildlife	mammal	deer	1.2%	0.5-1.8%
Wildlife	mammal	rabbit	0.1%	0.0-0.2%
Wildlife	mammal	raccoon	3.4%	2.3-4.4%
Wildlife	mammal	rodent	11.7%	9.8-13.55
Wildlife	mammal	opossum	0.9%	0.3-1.4%
Wildlife	mammal	skunk	0.1%	0.0-0.3%
Wildlife	mammal	squirrel	0.1%	0.0-0.2%
Wildlife	mammal	subtotal	18.0%	15.8-20.2%
Wildlife	avian	waterfowl	5.6%	4.3-7.0%
Wildlife	avian	avian	17.6%	15.4-19.8%
Wildlife	avian	subtotal	23.2%	20.8-25.7%
Wildlife	subtotal		41.2%	38.3-44.1%
Unknown		unknown	16.1%	14.0-18.3%

Neither the raw nor normalized source characterizations presented to this point in this report reflect the observed variations in *E. coli* concentrations in Upper Oyster Creek. Typically, two to four *E. coli* were ribotyped from each water sample, regardless of whether there were 20 or 20,000 *E. coli* in the water sample. Thus, sources that contribute large numbers of *E. coli* under runoff conditions when *E. coli* concentrations in water are high may be minimized by the unweighted methodology. Table 4-8 summarizes the source

Table 4-8 Concentration-weighted *E. coli* Source Characterization of Upper Oyster Creek under All Conditions

Super-category	Category	Source	% Contribution	95% Confidence Interval
Human/sewage	human	human	1.6%	0.8-2.3%
Human/sewage	sewage	sewage	12.8%	10.8-14.7%
Human/sewage	subtotal		14.3%	12.3-16.4%
Livestock		bovine	6.9%	5.4-8.3%
Livestock		horse	5.2%	3.9-6.5%
Livestock		poultry	0.8%	0.3-1.3%
Livestock		Guinea fowl	0.1%	0.0-0.3%
Livestock		donkey	0.1%	0.0-0.3%
Livestock		goat	0.3%	0.0-0.5%
Livestock		porcine	5.8%	4.4-7.2%
Livestock	subtotal		19.1%	16.8-21.4%
Pets	canine	canine	7.2%	5.7-8.7%
Pets	canine	dog	1.5%	0.8-2.2%
Pets	feline	feline	0.3%	0.0-0.7%
Pets	subtotal		9.0%	7.4-10.7%
Wildlife	mammal	coyote	0.8%	0.3-1.3%
Wildlife	mammal	deer	1.6%	0.8-2.3%
Wildlife	mammal	rabbit	0.1%	0.0-0.2%
Wildlife	mammal	raccoon	4.1%	2.9-5.2%
Wildlife	mammal	rodent	11.2%	9.4-13.0%
Wildlife	mammal	opossum	1.2%	0.6-1.9%
Wildlife	mammal	skunk	0.1%	0.0-0.3%
Wildlife	mammal	squirrel	0.1%	0.0-0.2%
Wildlife	mammal	subtotal	19.2%	16.9-21.5%
Wildlife	avian	waterfowl	6.6%	5.2-8.1%
Wildlife	avian	avian	16.6%	14.4-18.8%
Wildlife	avian	subtotal	23.2%	20.8-25.7%
Wildlife	subtotal		42.4%	39.5-45.3%
Unknown		unknown	15.1%	13.1-17.2%
Grand Total			100.0%	

characterization that results from weighting sources by the *E. coli* concentration in each water sample from which it was harvested. Table 4-8 can be compared to the non-normalized results in Table 4-5. All differences are very minor, and none are statistically significant at the 95 percent confidence level.

4.3.4 Station 12090 Jones Creek at FM 273

In this section, results for Station 12090 are described individually. This source characterization is based on 99 isolates collected in seven non-runoff events and 70 isolates collected in five runoff events. Table 4-9 and Figure 4-2 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. The human and sewage source contribution was 13 percent overall, similar to that of the watershed as a whole, and did not vary significantly under runoff versus non-runoff conditions. The livestock source contributions were higher at this site than for the watershed as a whole, which was expected considering the rural nature of the western watershed. *E. coli* from both horses and cattle were significantly more abundant at this site than at most other sites. A stable for miniature horses is located near this station. Deer and opossum also represented a larger source of *E. coli* at this site than most others. The source of approximately 14 percent of the *E. coli* isolates could not be identified.

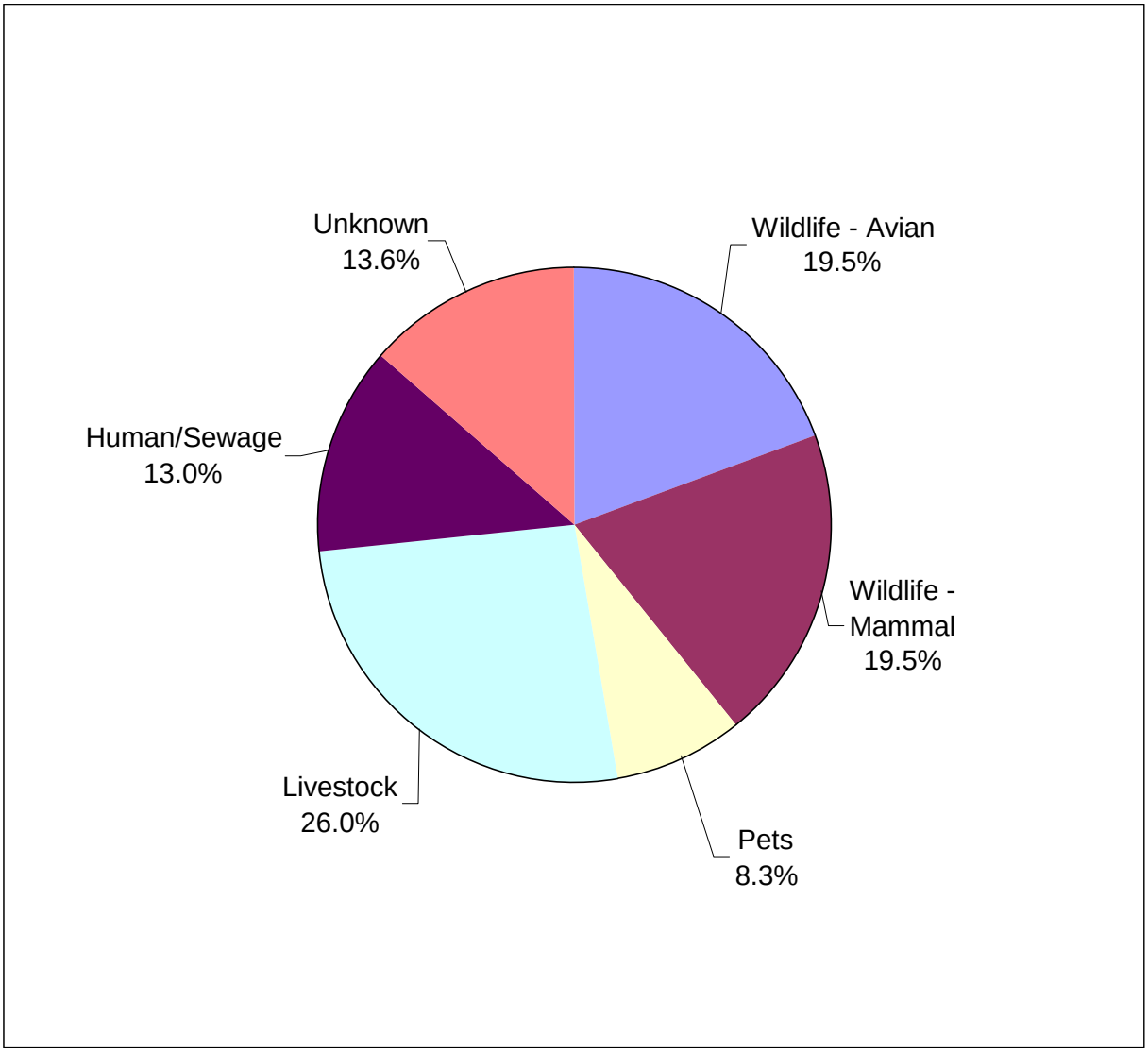
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, unweighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-9 *E. coli* Source Characterization of Station 12090

Super - category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human	Human	Human	1.0%	0.0-3.0%	1.4%	0.0-4.2%	1.2%	0.0-2.8%
Human	sewage	sewage	13.1%	6.5-19.8%	10.0%	3.0-17.0%	11.8%	7.0-16.7%
Human	subtotal		14.1%	7.3-21.0%	11.4%	4.0-18.9%	13.0%	7.9-18.1%
Livestock		bovine	12.1%	5.7-18.6%	14.3%	6.1-22.5%	13.0%	7.9-18.1%
Livestock		horse	8.1%	2.7-13.4%	11.4%	4.0-18.9%	9.5%	5.1-13.9%
Livestock		poultry	0.0%		0.0%		0.0%	
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	2.0%	0.0-4.8%	5.7%	0.3-11.2%	3.6%	0.8-6.3%
Livestock	subtotal		22.2%	14.0-30.4%	31.4%	20.6-42.3%	26.0%	19.4-32.7%
Pets	canine	canine	10.1%	4.2-16.0%	4.3%	0.0-9.0%	7.7%	3.7-11.7%
Pets	canine	dog	1.0%	0.0-3.0%	0.0%		0.6%	0.0-1.7%
Pets	feline	feline	0.0%		0.0%		0.0%	
Pets	subtotal		11.1%	4.9-17.3%	4.3%	0.0-9.0%	8.3%	4.1-12.4%
Wildlife	mammal	coyote	3.0%	0.0-6.4%	0.0%	0.0-0.0%	1.8%	0.0-3.8%
Wildlife	mammal	deer	4.0%	0.2-7.9%	2.9%	0.0-6.8%	3.6%	0.8-6.3%
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	4.0%	0.2-7.9%	1.4%	0.0-4.2%	3.0%	0.4-5.5%
Wildlife	mammal	rodent	7.1%	2.0-12.1%	11.4%	4.0-18.9%	8.9%	4.6-13.2%
Wildlife	mammal	opossum	1.0%	0.0-3.0%	4.3%	0.0-9.0%	2.4%	0.1-4.7%
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	

Super - category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	
Wildlife	mammal	subtotal	19.2%	11.4-26.9%	20.0%	10.6-29.4%	19.5%	13.6-25.5%
Wildlife	avian	waterfowl	6.1%	1.4-10.8%	4.3%	0.0-9.0%	5.3%	1.9-8.7%
Wildlife	avian	avian	17.2%	9.7-24.6%	10.0%	3.0-17.0%	14.2%	8.9-19.5%
Wildlife	avian	subtotal	23.2%	14.9-31.6%	14.3%	6.1-22.5%	19.5%	13.6-25.5%
Wildlife	subtotal		42.4%	32.7-52.2%	34.3%	23.2-45.4%	39.1%	31.7-46.4%
Unknown		unknown	10.1%	4.2-16.0%	18.6%	9.5-27.7%	13.6%	8.4-18.8%

Figure 4-2 *E. coli* Source Characterization of Station 12090 under All Conditions



4.3.5 Station 12087 Upper Oyster Creek at FM 1464

In this section, results for Station 12087 are described individually. This source characterization is based on 110 isolates collected in nine non-runoff events and 47 isolates collected in three runoff events. Table 4-10 and Figure 4-3 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. The human and sewage source contribution was only 3.2 percent overall, significantly less than that of the watershed as a whole, and was not observed at this site under runoff conditions. Livestock source contributions were high at this site due to the porcine contributions of almost 15 percent. The porcine contribution was particularly high under runoff conditions. This high porcine contribution may reflect the influence of the hog farm on TDCJ property at the Jester Unit, a short distance upstream of this station. The source of approximately 14 percent of the *E. coli* isolates could not be identified.

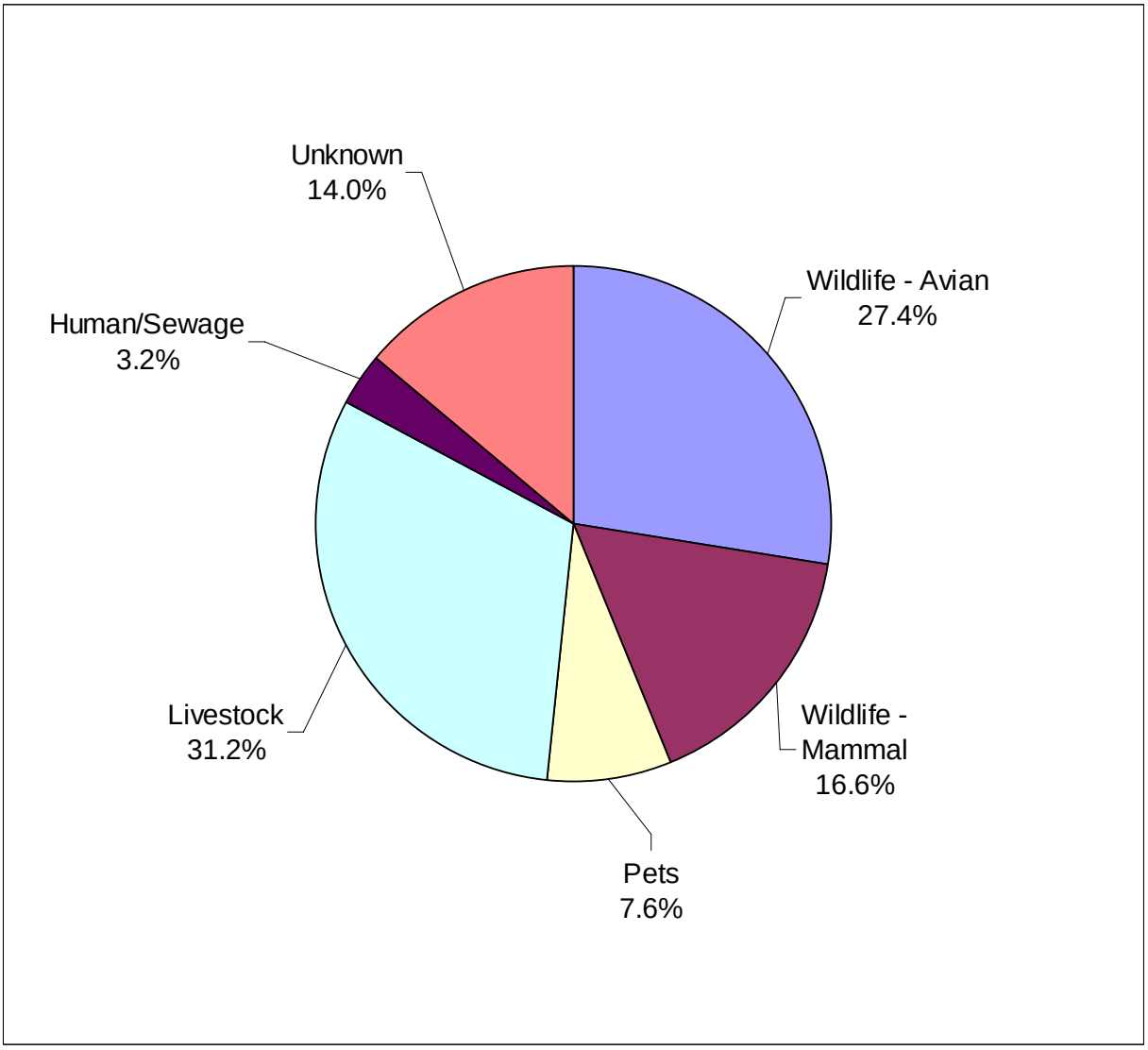
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, unweighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-10 *E. coli* Source Characterization of Station 12087

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/ sewage	human	human	0.9%	0.0-2.7%	0.0%	0.0-0.0%	0.6%	0.0-1.9%
Human/ sewage	sewage	sewage	3.6%	0.1-7.1%	0.0%	0.0-0.0%	2.5%	0.1-5.0%
Human/ sewage	subtotal		4.5%	0.7-8.4%	0.0%	0.0-0.0%	3.2%	0.4-5.9%
Livestock		bovine	11.8%	5.8-17.9%	4.3%	0.0-10.0%	9.6%	5.0-14.2%
Livestock		horse	2.7%	0.0-5.8%	12.8%	3.2-22.3%	5.7%	2.1-9.4%
Livestock		poultry	0.9%	0.0-2.7%	0.0%	0.0-0.0%	0.6%	0.0-1.9%
Livestock		Guinea fowl	0.0%	0.0-0.0%	0.0%	0.0-0.0%	0.0%	0.0-0.0%
Livestock		donkey	0.0%	0.0-0.0%	0.0%	0.0-0.0%	0.0%	0.0-0.0%
Livestock		goat	0.9%	0.0-2.7%	0.0%	0.0-0.0%	0.6%	0.0-1.9%
Livestock		porcine	12.7%	6.5-19.0%	19.1%	7.9-30.4%	14.6%	9.1-20.2%
Livestock	subtotal		29.1%	20.6-37.6%	36.2%	22.4-49.9%	31.2%	24.0-38.5%
Pets	canine	canine	5.5%	1.2-9.7%	6.4%	0.0-13.4%	5.7%	2.1-9.4%
Pets	canine	dog	2.7%	0.0-5.8%	0.0%	0.0-0.0%	1.9%	0.0-4.1%
Pets	feline	feline	0.0%	0.0-0.0%	0.0%	0.0-0.0%	0.0%	0.0-0.0%
Pets	subtotal		8.2%	3.1-13.3%	6.4%	0.0-13.4%	7.6%	3.5-11.8%
Wildlife	mammal	coyote	0.0%	0.0-0.0%	2.1%	0.0-6.3%	0.6%	0.0-1.9%
Wildlife	mammal	deer	0.9%	0.0-2.7%	4.3%	0.0-10.0%	1.9%	0.0-4.1%
Wildlife	mammal	rabbit	0.0%	0.0-0.0%	0.0%	0.0-0.0%	0.0%	0.0-0.0%
Wildlife	mammal	raccoon	2.7%	0.3-5.8%	2.1%	0.0-6.3%	2.5%	0.1-5.0%
Wildlife	mammal	rodent	7.3%	2.4-12.1%	12.8%	3.2-22.3%	8.9%	4.5-13.4%
Wildlife	mammal	opossum	1.8%	00.0-4.3%	2.1%	0.0-6.3%	1.9%	0.2-4.1%
Wildlife	mammal	skunk	0.0%	0.0-0.0%	2.1%	0.0-6.3%	0.6%	0.0-1.9%
Wildlife	mammal	squirrel	0.0%	0.0-0.0%	0.0%	0.0-0.0%	0.0%	0.0-0.0%
Wildlife	mammal	subtotal	12.7%	6.5-19.0%	25.5%	13.1-38.0%	16.6%	10.7-22.4%

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	avian	waterfowl	11.8%	5.8-17.9%	4.3%	0.0%-10.0%	9.6%	5.0-14.2%
Wildlife	avian	avian	16.4%	9.5-23.3%	21.3%	9.6-33.0%	17.8%	11.8-23.8%
Wildlife	avian	subtotal	28.2%	19.8-36.6%	25.5%	13.1-38.0%	27.4%	20.4-34.4%
Wildlife	subtotal		40.9%	31.7-50.1%	51.1%	36.8-65.4%	43.9%	36.2-51.7%
Unknown		unknown	17.3%	10.2-24.3%	6.4%	0.0-13.4%	14.0%	8.6-19.4%

Figure 4-3 *E. coli* Source Characterization of Station 12087 under All Conditions



4.3.6 Station 12086 Upper Oyster Creek at SH 6

In this section, results for Station 12086 are described individually. This source characterization is based on 130 isolates collected in nine non-runoff events, and 49 isolates collected in three runoff events. Table 4-11 and Figure 4-4 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. The human and sewage source contribution was 15.6 percent overall, slightly higher than that of the watershed as a whole. Horses represented only 2.2 percent of the *E. coli* sources. Rodents were a major source at this site, representing over 17 percent of the *E. coli* typed. In general *E. coli* sources at this station closed resembled those of the watershed as a whole.

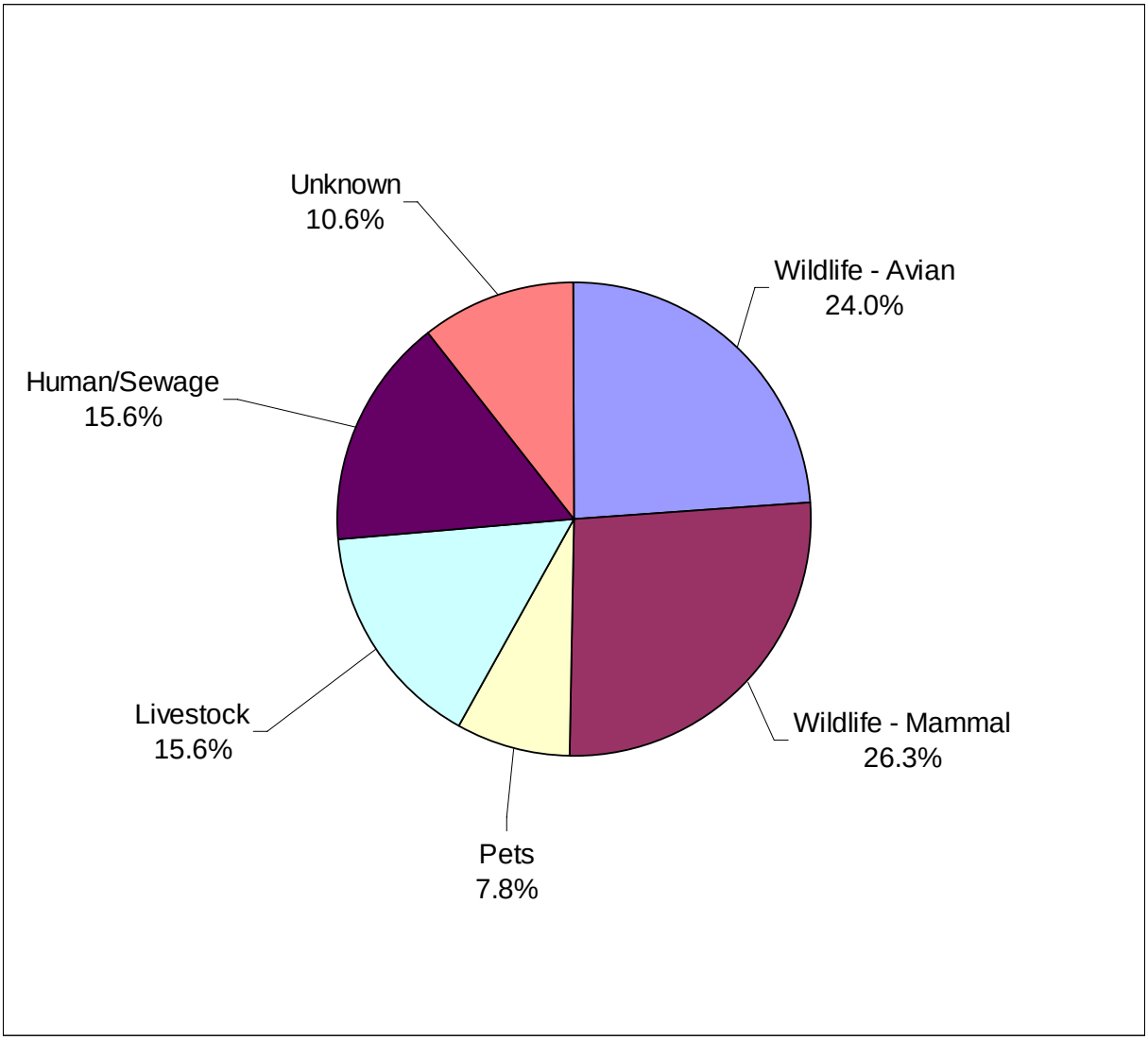
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-11 *E. coli* Source Characterization of Station 12086

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	3.1%	0.1-6.0%	2.0%	0.0-6.0%	2.8%	0.4-5.2%
Human/sewage	sewage	sewage	13.8%	7.9-19.8%	10.2%	1.7-18.7%	12.8%	7.9-17.8%
Human/sewage	subtotal		16.9%	10.5-23.4%	12.2%	3.1-21.4%	15.6%	10.3-21.0%
Livestock		bovine	7.7%	3.1-12.3%	8.2%	0.5-15.8%	7.8%	3.9-11.8%
Livestock		horse	2.3%	0.0-4.9%	2.0%	0.0-6.0%	2.2%	0.1-4.4%
Livestock		poultry	0.8%	0.0-2.3%	2.0%	0.0-6.0%	1.1%	0.0-2.7%
Livestock		Guinea fowl	0.0%		2.0%	0.0-6.0%	0.6%	0.0-1.7%
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	4.6%	1.0-8.2%	2.0%	0.0-6.0%	3.9%	1.1-6.8%
Livestock	subtotal		15.4%	9.2-21.6%	16.3%	6.0-26.7%	15.6%	10.3-21.0%
Pets	canine	canine	8.5%	3.7-13.2%	4.1%	0.0-9.6%	7.3%	3.5-11.1%
Pets	canine	dog	0.0%		0.0%		0.0%	
Pets	feline	feline	0.8%	0.0-2.3%	0.0%		0.6%	0.0-1.7%
Pets	subtotal		9.2%	4.3-14.2%	4.1%	0.0-9.6%	7.8%	3.9-11.8%
Wildlife	mammal	coyote	0.0%		4.1%	0.0-9.6%	1.1%	0.0-2.7%
Wildlife	mammal	deer	2.3%	0.0-4.9%	0.0%		1.7%	0.0-3.6%
Wildlife	mammal	rabbit	0.8%	0.0-2.3%	0.0%		0.6%	0.0-1.7%
Wildlife	mammal	raccoon	5.4%	1.5-9.3%	2.0%	0.0-6.0%	4.5%	1.4-7.5%
Wildlife	mammal	rodent	16.2%	9.8-22.5%	20.4%	9.1-31.7%	17.3%	11.8-22.9%
Wildlife	mammal	opossum	1.5%	0.0-3.7%	0.0%		1.1%	0.0-2.7%
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	subtotal	26.2%	18.6-33.7%	26.5%	14.2-38.9%	26.3%	19.8-32.7%
Wildlife	avian	waterfowl	7.7%	3.1-12.3%	8.2%	0.5-15.8%	7.8%	3.9-11.8%
Wildlife	avian	avian	14.6%	8.5-20.7%	20.4%	9.1-31.7%	16.2%	10.8-21.6%
Wildlife	avian	subtotal	22.3%	15.2-29.5%	28.6%	15.9-41.2%	24.0%	17.8-30.3%
Wildlife	subtotal		48.5%	39.9-57.1%	55.1%	41.2-69.0%	50.3%	43.0-57.6%
Unknown		unknown	10.0%	4.8-15.2%	12.2%	3.1-21.4%	10.6%	6.1-15.1%

Figure 4-4 *E. coli* Source Characterization of Station 12086 under All Conditions



4.3.7 Station 12083 Upper Oyster Creek at Highway 90A in Sugar Land

In this section, results for Station 12083 are described individually. This source characterization is based on 92 isolates collected in nine non-runoff events and 47 isolates collected in three runoff events. Table 4-12 and Figure 4-5 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. The human and sewage source contribution was 20.9 percent overall, substantially higher than that of the watershed as a whole. Livestock contributions were minor at this site, and particularly low for cattle and horses. These observations agree with the urbanized nature at this station. The source of approximately 14 percent of the *E. coli* isolates could not be identified.

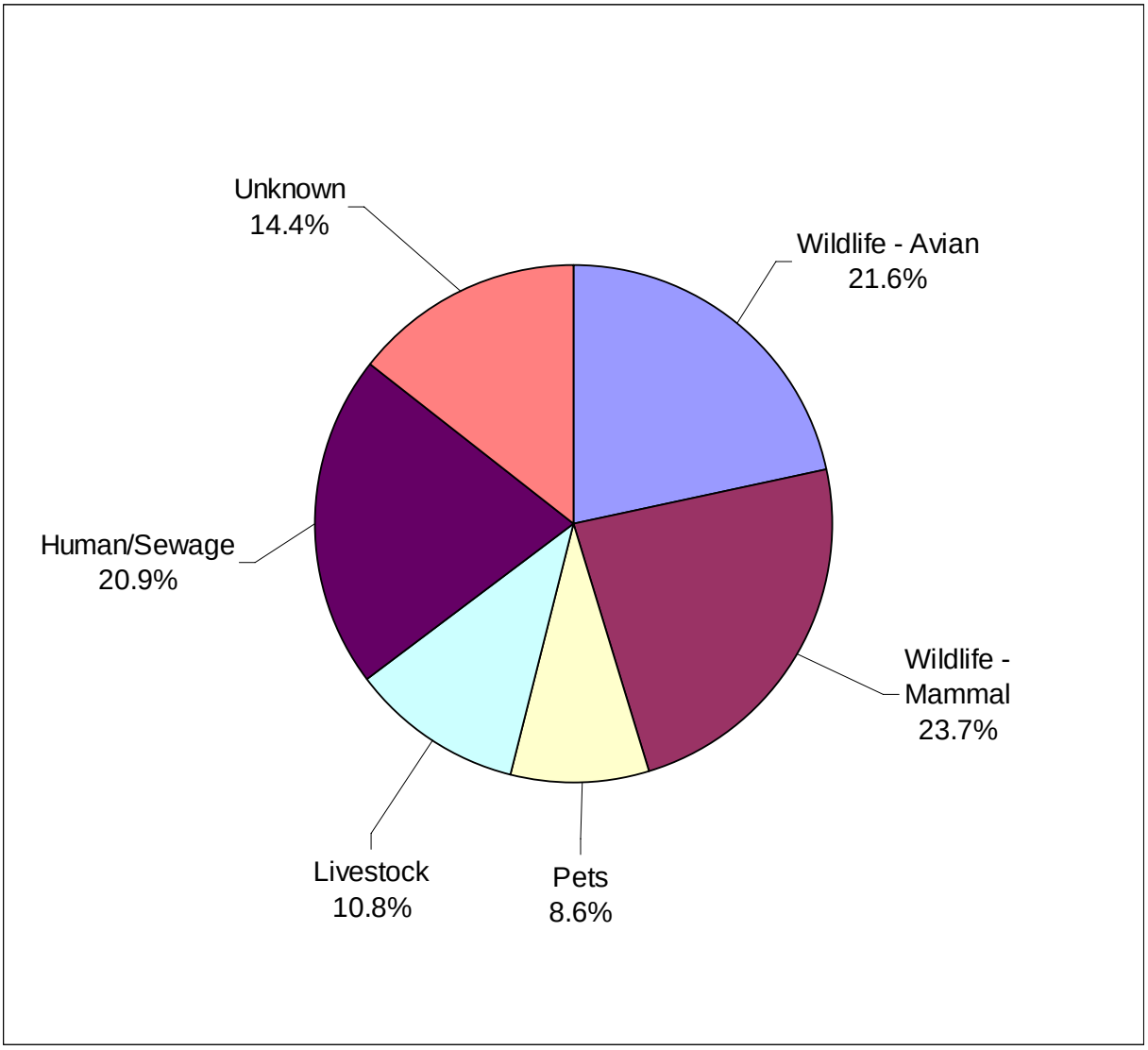
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-12 *E. coli* Source Characterization of Station 12083

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	1.1%	0.0-3.2%	2.1%	0.0-6.3%	1.4%	0.0-3.4%
Human/sewage	sewage	sewage	16.3%	8.8-23.9%	25.5%	13.1-38.0%	19.4%	12.8-26.0%
Human/sewage	subtotal		17.4%	9.6-25.1%	27.7%	14.9-40.4%	20.9%	14.1-27.6%
Livestock		bovine	3.3%	0.0-6.9%	2.1%	0.0-6.3%	2.9%	0.1-5.7%
Livestock		horse	3.3%	0.0-6.9%	0.0%		2.2%	0.0%-4.6%
Livestock		poultry	0.0%		0.0%		0.0%	
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	4.3%	0.2-8.5%	8.5%	0.5-16.5%	5.8%	1.9-9.6%
Livestock	subtotal		10.9%	4.5-17.2%	10.6%	1.8-19.5%	10.8%	5.6-15.9%
Pets	canine	canine	7.6%	2.2-13.0%	4.3%	0.0-10.0%	6.5%	2.4-10.6%
Pets	canine	dog	1.1%	0.0-3.2%	2.1%	0.0-6.3%	1.4%	0.0-3.4%
Pets	feline	feline	1.1%	0.0-3.2%	0.0%		0.7%	0.0-2.1%
Pets	subtotal		9.8%	3.7-15.9%	6.4%	0.0-13.4%	8.6%	4.0-13.3%
Wildlife	mammal	coyote	2.2%	0.0-5.2%	0.0%		1.4%	0.0-3.4%
Wildlife	mammal	deer	1.1%	0.0-3.2%	0.0%		0.7%	0.0-2.1%
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	9.8%	3.7-15.9%	4.3%	0.0-10.0%	7.9%	3.4-12.4%
Wildlife	mammal	rodent	15.2%	7.9-22.6%	10.6%	1.8-19.5%	13.7%	8.0-19.4%
Wildlife	mammal	opossum	0.0%		0.0%		0.0%	
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	
Wildlife	mammal	subtotal	28.3%	19.1-37.5%	14.9%	4.7-25.1%	23.7%	16.7-30.8%
Wildlife	avian	waterfowl	3.3%	0.0-6.9%	12.8%	3.2-22.3%	6.5%	2.4-10.6%
Wildlife	avian	avian	14.1%	7.0-21.2%	17.0%	6.3-27.8%	15.1%	9.2-21.1%
Wildlife	avian	subtotal	17.4%	9.6-25.1%	29.8%	16.7-42.9%	21.6%	14.7-28.4%
Wildlife	subtotal		45.7%	35.5-55.8%	44.7%	30.5-58.9%	45.3%	37.0-53.6%
Unknown		unknown	16.3%	8.8-23.9%	10.6%	1.8-19.5%	14.4%	8.6-20.2%

Figure 4-5 *E. coli* Source Characterization of Station 12083 under All Conditions



4.3.8 Station 17688 Stafford Run at El Dorado Boulevard

In this section, the results for Station 17688 are described individually. This source characterization is based on 135 isolates collected in nine non-runoff events and 48 isolates collected in three runoff events. Table 4-13 and Figure 4-6 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. In most respects, the source contributions at this site mirrored that of the watershed as a whole. Perhaps the only significant difference was that the source of approximately 22 percent of the *E. coli* isolates could not be identified, and one third of those were collected under runoff conditions.

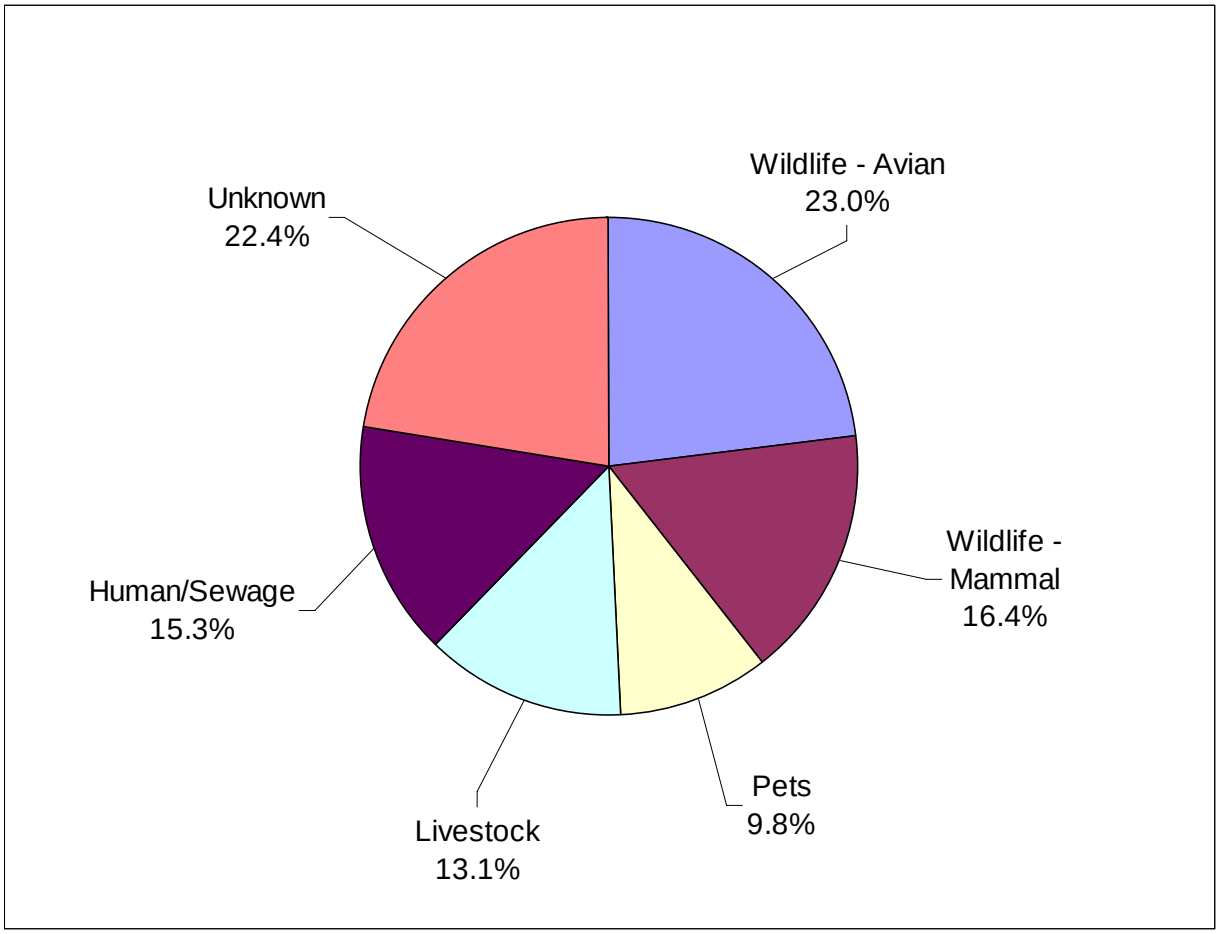
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-13 *E. coli* Source Characterization of Station 17688

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	0.7%	0.0-2.2%	4.2%	0.0-9.8%	1.6%	0.0-3.5%
Human/sewage	sewage	sewage	15.6%	9.4-21.7%	8.3%	0.5-16.2%	13.7%	8.7-18.6%
Human/sewage	subtotal		16.3%	10.1-22.5%	12.5%	3.1-21.9%	15.3%	10.1-20.5%
Livestock		bovine	3.0%	0.1-5.8%	6.3%	0.0-13.1%	3.8%	1.0-6.6%
Livestock		horse	4.4%	1.0-7.9%	0.0%		3.3%	0.7-5.9%
Livestock		poultry	2.2%	0.0-4.7%	0.0%		1.6%	0.0-3.5%
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	1.5%	0.0-3.5%	0.0%		1.1%	0.0-2.6%
Livestock		porcine	3.7%	0.5-6.9%	2.1%	0.0-6.1%	3.3%	0.7-5.9%
Livestock	subtotal		14.8%	8.8-20.8%	8.3%	0.5-16.2%	13.1%	8.2-18.0%
Pets	canine	canine	8.9%	4.1-13.7%	4.2%	0.0-9.8%	7.7%	3.8-11.5%
Pets	canine	dog	2.2%	0.0-4.7%	0.0%		1.6%	0.0-3.5%
Pets	feline	feline	0.7%	0.0-2.2%	0.0%		0.5%	0.0-1.6%
Pets	subtotal		11.9%	6.4-17.3%	4.2%	0.0-9.8%	9.8%	5.5-14.2%
Wildlife	mammal	coyote	0.7%	0.0-2.2%	0.0%		0.5%	0.0-1.6%
Wildlife	mammal	deer	2.2%	0.0-4.7%	0.0%		1.6%	0.0-3.5%
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	7.4%	3.0-11.8%	0.0%		5.5%	2.2-8.8%
Wildlife	mammal	rodent	7.4%	3.0-11.8%	8.3%	0.5-16.2%	7.7%	3.8-11.5%
Wildlife	mammal	opossum	1.5%	0.0-3.5%	0.0%		1.1%	0.0-2.6%
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	subtotal	19.3%	12.6-25.9%	8.3%	0.5-16.2%	16.4%	11.0-21.8%
Wildlife	avian	waterfowl	3.7%	0.5-6.9%	10.4%	1.8-19.1%	5.5%	2.2-8.8%
Wildlife	avian	avian	14.8%	8.8-20.8%	25.0%	12.8-37.3%	17.5%	12.0-23.0%
Wildlife	avian	subtotal	18.5%	12.0-25.1%	35.4%	21.9-48.9%	23.0%	16.9-29.0%
Wildlife	subtotal		37.8%	29.6-46.0%	43.8%	29.7-57.8%	39.3%	32.3-46.4%
Unknown		unknown	19.3%	12.6-25.9%	31.3%	18.1-44.4%	22.4%	16.4-28.4%

Figure 4-6 *E. coli* Source Characterization of Station 17688 under All Conditions



4.3.9 Station 12074 Flat Bank Creek at SH 6

In this section, results for Station 12074 are described individually. This source characterization is based on 119 isolates collected in nine non-runoff events and 49 isolates collected in three runoff events. Table 4-14 and Figure 4-7 summarize the identified sources of *E. coli* from all 12 events, including both runoff and non-runoff conditions. The sewage contribution at this site was 21.4 percent, higher than that of the watershed as a whole. The porcine contribution was also relatively high under both runoff and non-runoff conditions.

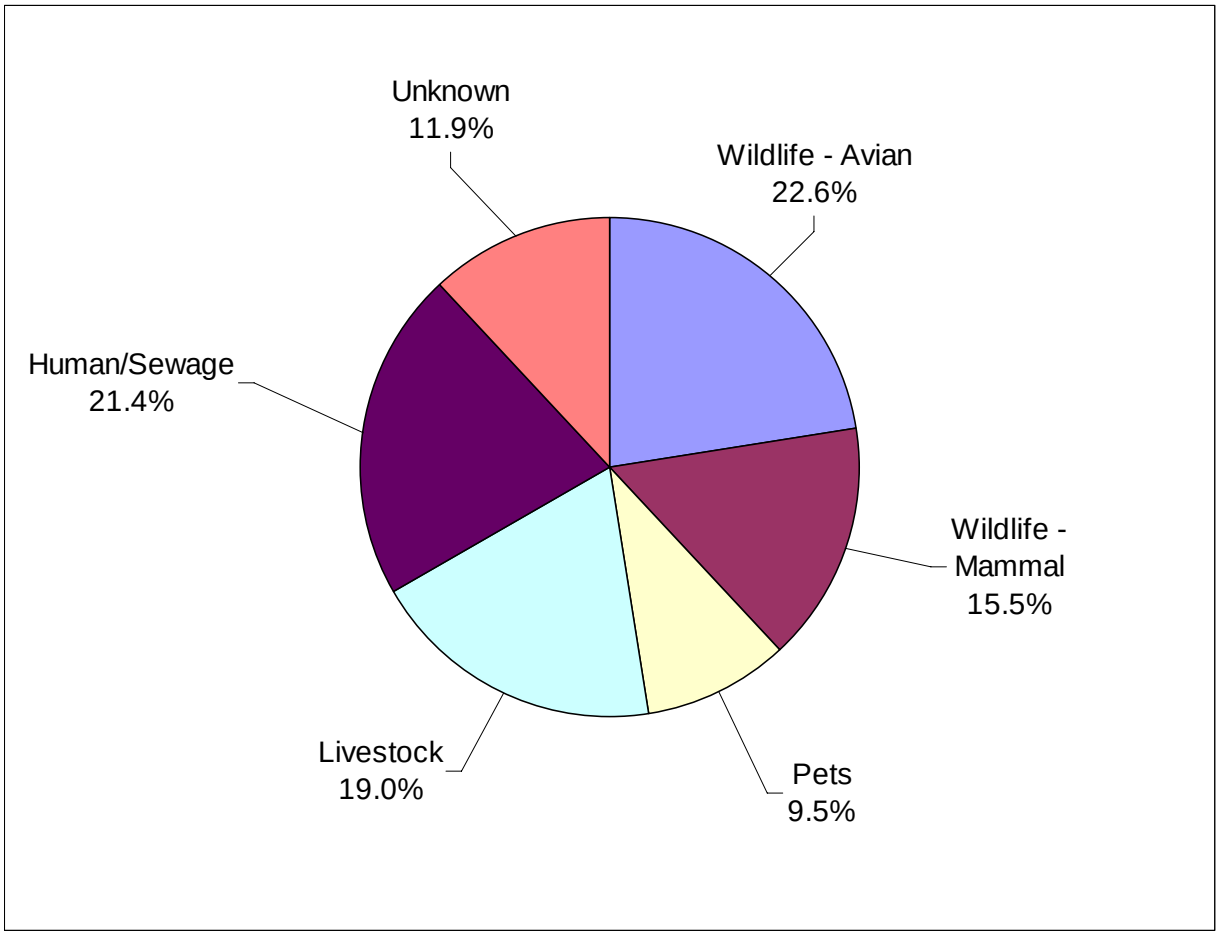
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-14 *E. coli* Source Characterization of Station 12074

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	0.8%	0.0-2.5%	2.0%	0.0-6.0%	1.2%	0.0-2.8%
Human/sewage	sewage	sewage	21.0%	13.7-28.3%	18.4%	7.5-29.2%	20.2%	14.2-26.3%
Human/sewage	subtotal		21.8%	14.4-29.3%	20.4%	9.1-31.7%	21.4%	15.2-27.6%
Livestock		bovine	5.9%	1.7-10.1%	2.0%	0.0-6.0%	4.8%	1.5-8.0%
Livestock		horse	3.4%	0.1-6.6%	4.1%	0.0-9.6%	3.6%	0.8-6.4%
Livestock		poultry	1.7%	0.0-4.0%	2.0%	0.0-6.0%	1.8%	0.0-3.8%
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.8%	0.0-2.5%	0.0%		0.6%	0.0-1.8%
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	8.4%	3.4-13.4%	8.2%	0.5-15.8%	8.3%	4.2-12.5%
Livestock	subtotal		20.2%	13.0-27.4%	16.3%	6.0-26.7%	19.0%	13.1-25.0%
Pets	canine	canine	2.5%	0.0-5.3%	10.2%	1.7-18.7%	4.8%	1.5-8.0%
Pets	canine	dog	4.2%	0.6-7.8%	4.1%	0.0-9.6%	4.2%	1.1-7.2%
Pets	feline	feline	0.8%	0.0-2.5%	0.0%		0.6%	0.0-1.8%
Pets	subtotal		7.6%	2.8-12.3%	14.3%	4.5-24.1%	9.5%	5.1-14.0%
Wildlife	mammal	coyote	0.0%		0.0%		0.0%	
Wildlife	mammal	deer	3.4%	0.1-6.6%	0.0%		2.4%	0.1-4.7%
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	2.5%	0.0-5.3%	4.1%	0.0-9.6%	3.0%	0.4-5.5%
Wildlife	mammal	rodent	5.0%	1.1-9.0%	16.3%	6.0-26.7%	8.3%	4.2-12.5%
Wildlife	mammal	opossum	0.8%	0.0-2.5%	2.0%	0.0-6.0%	1.2%	0.0-2.8%
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	
Wildlife	mammal	squirrel	0.8%	0.0-2.5%	0.0%		0.6%	0.0-1.8%

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	subtotal	12.6%	6.6-18.6%	22.4%	10.8-34.1%	15.5%	10.0-20.9%
Wildlife	avian	waterfowl	8.4%	3.4-13.4%	6.1%	0.0-12.8%	7.7%	3.7-11.8%
Wildlife	avian	avian	16.0%	9.4-22.5%	12.2%	3.1-21.4%	14.9%	9.5-20.3%
Wildlife	avian	subtotal	24.4%	16.7-32.1%	18.4%	7.5-29.2%	22.6%	16.3-28.9%
Wildlife	subtotal		37.0%	28.3-45.6%	40.8%	27.1-54.6%	38.1%	30.8-45.4%
Unknown		unknown	13.4%	7.3-19.6%	8.2%	0.5-15.8%	11.9%	7.0-16.8%

Figure 4-7 *E. coli* Source Characterization of Station 12074 under All Conditions



4.3.10 Station 11516 Red Gully at Richmond-Gaines Road

In this section, results for supplemental Station 11516 are described individually. This source characterization is based on 20 isolates collected in two non-runoff events and 20 isolates collected in two runoff events. Due to the relatively small number of isolates, the confidence intervals around the source estimates are broad. With the qualification that the source estimates are based on only 40 isolates, canines and birds were well-represented among those, while the human/sewage category was not. Table 4-15 and Figure 4-8 summarize the identified sources of *E. coli* from all four events, including both runoff and non-runoff conditions.

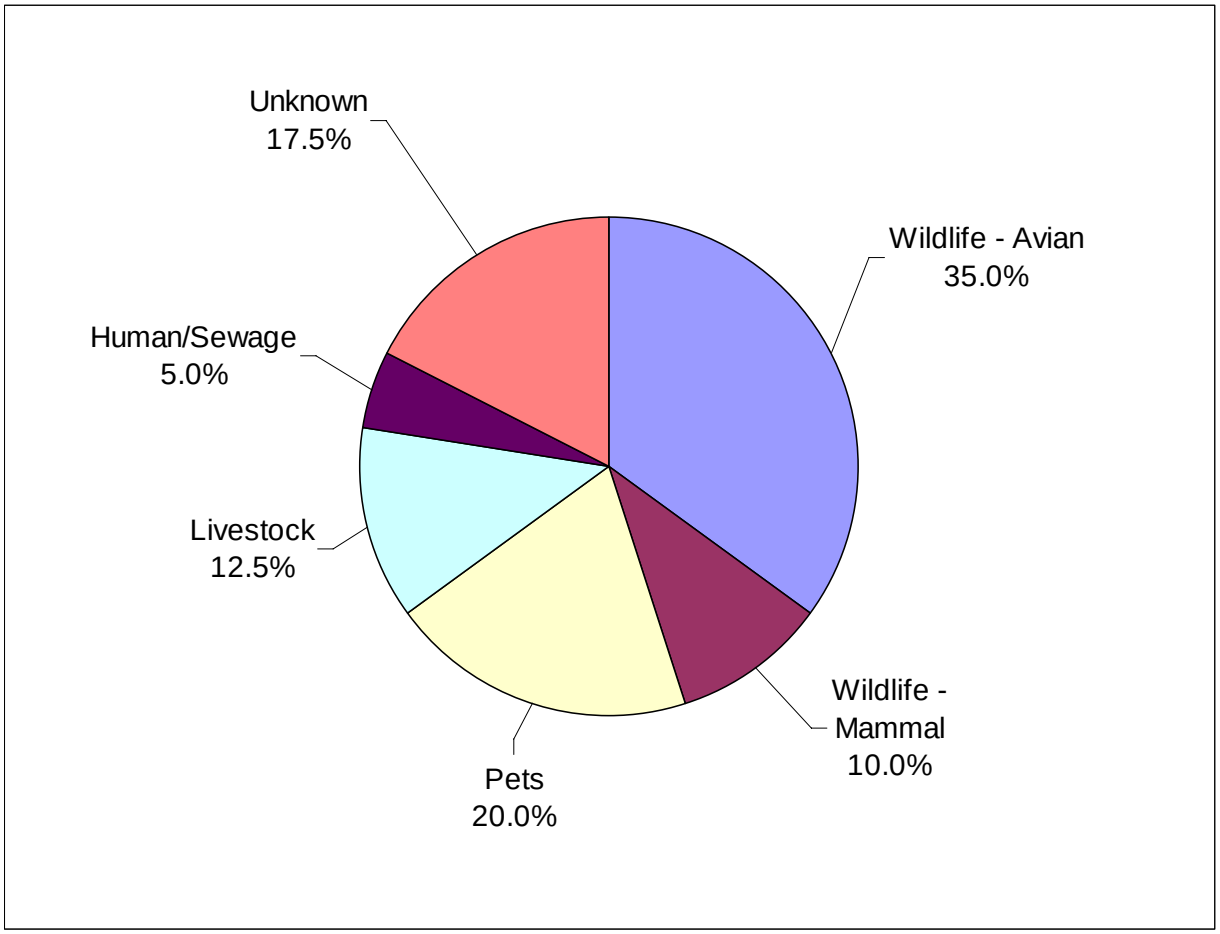
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-15 *E. coli* Source Characterization of Station 11516

			Non-runoff		Runoff		All Conditions	
Super-category	Category	Source	% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	5.0%	0.0-14.6%	0.0%		2.5%	0.0-7.3%
Human/sewage	sewage	sewage	5.0%	0.0-14.6%	0.0%		2.5%	0.0-7.3%
Human/sewage	subtotal		10.0%	0.0-23.1%	0.0%		5.0%	0.0-11.8%
Livestock		bovine	0.0%		10.0%	0.0-23.1%	5.0%	0.0-11.8%
Livestock		horse	0.0%		15.0%	0.0-30.6%	7.5%	0.0-15.7%
Livestock		poultry	0.0%		0.0%		0.0%	
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	0.0%		0.0%		0.0%	
Livestock	subtotal		0.0%		25.0%	6.0-44.0%	12.5%	2.3-22.7%
Pets	canine	canine	20.0%	2.5-37.5%	15.0%	0.0-30.6%	17.5%	5.7-29.3%
Pets	canine	dog	0.0%		0.0%		0.0%	
Pets	feline	feline	5.0%	0.0-14.6%	0.0%		2.5%	0.0-7.3%
Pets	subtotal		25.0%	6.0-44.0%	15.0%	0.0-30.6%	20.0%	7.6-32.4%
Wildlife	mammal	coyote	0.0%		0.0%		0.0%	
Wildlife	mammal	deer	0.0%		0.0%		0.0%	
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	0.0%		5.0%	0.0-14.6%	2.5%	0.0-7.3%
Wildlife	mammal	rodent	5.0%	0.0-14.6%	10.0%	0.0-23.1%	7.5%	0.0-15.7%
Wildlife	mammal	opossum	0.0%		0.0%		0.0%	
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	subtotal	5.0%	0.0-14.6%	15.0%	0.0-30.6%	10.0%	0.7-19.3%
Wildlife	avian	waterfowl	5.0%	0.0-14.6%	0.0%		2.5%	0.0-7.3%
Wildlife	avian	avian	35.0%	14.1-55.9%	30.0%	9.9-50.1%	32.5%	18.0-47.0%
Wildlife	avian	subtotal	40.0%	18.5-61.5%	30.0%	9.9-50.1%	35.0%	20.2-49.8%
Wildlife	subtotal		45.0%	23.2-66.8%	45.0%	23.2-66.8%	45.0%	29.6-60.4%
Unknown		unknown	20.0%	2.5-37.5%	15.0%	0.0-30.6%	17.5%	5.7-29.3%

Figure 4-8 *E. coli* Source Characterization of Station 11516 under All Conditions



4.3.11 Station 17685 Jones Creek at Bois D'Arc Lane

In this section, results for supplemental Station 17685 are described individually. This source characterization is based on 25 isolates collected in two non-runoff events and 23 isolates collected in two runoff events. Due to the relatively small number of isolates, the confidence intervals around the source estimates are broad. Table 4-16 and Figure 4-9 summarize the identified sources of *E. coli* from all four events, including both runoff and non-runoff conditions. Few isolates at this site were from human and sewage sources, which is expected given the rural nature of this western area of the watershed; otherwise the sources were similar to the watershed as a whole. Because of downstream proximity of this station to the Shannon Pump Station, the sources characterized here also reflect conditions in the Brazos River at the pump station during the four sampled events.

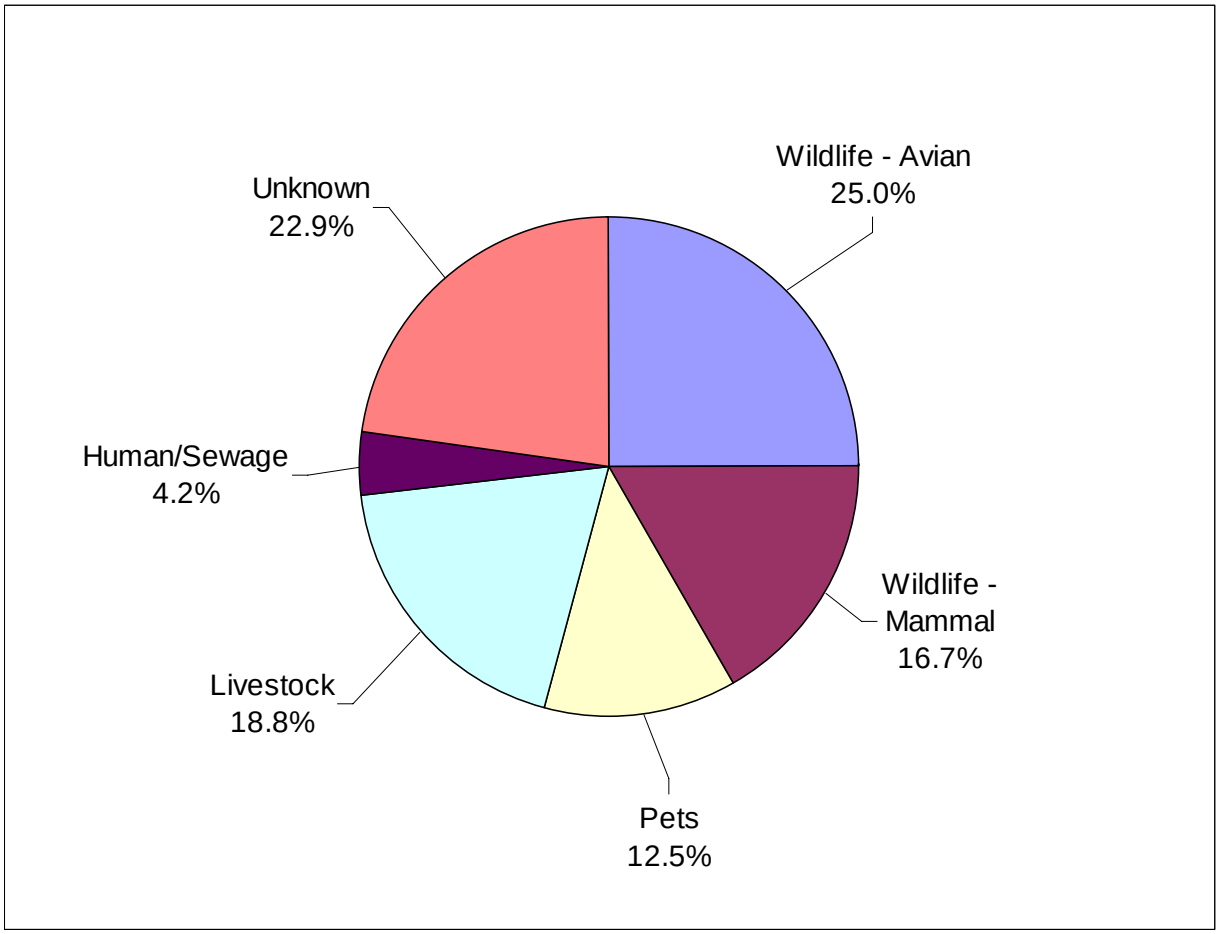
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-16 *E. coli* Source Characterization of Station 17685

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	0.0%		0.0%		0.0%	
Human/sewage	sewage	sewage	4.0%	0.0-11.7%	4.3%	0.0-12.7%	4.2%	0.0-9.8%
Human/sewage	subtotal		4.0%	0.0-11.7%	4.3%	0.0-12.7%	4.2%	0.0-9.8%
Livestock		bovine	12.0%	0.0-24.7%	8.7%	0.0-20.2%	10.4%	1.8-19.1%
Livestock		horse	8.0%	0.0-18.6%	8.7%	0.0-20.2%	8.3%	0.5-16.2%
Livestock		poultry	0.0%		0.0%		0.0%	
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	0.0%		0.0%		0.0%	
Livestock	subtotal		20.0%	4.3-35.7%	17.4%	1.9-32.9%	18.8%	7.7-29.8%
Pets	canine	canine	0.0%		21.7%	4.9-38.6%	10.4%	1.8-19.1%
Pets	canine	dog	4.0%	0.0-11.7%	0.0%		2.1%	0.0-6.1%
Pets	feline	feline	0.0%		0.0%		0.0%	
Pets	subtotal		4.0%	0.0-11.7%	21.7%	4.9-38.6%	12.5%	3.1-21.9%
Wildlife	mammal	coyote	0.0%		0.0%		0.0%	
Wildlife	mammal	deer	0.0%		0.0%		0.0%	
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	0.0%		0.0%		0.0%	
Wildlife	mammal	rodent	32.0%	13.7-50.3%	0.0%		16.7%	6.1-27.2%
Wildlife	mammal	opossum	0.0%		0.0%		0.0%	
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	

Super-category	Category	Source	Non-runoff		Runoff		All Conditions	
			% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Wildlife	mammal	subtotal	32.0%	13.7-50.3%	0.0%		16.7%	6.1-27.2%
Wildlife	avian	waterfowl	0.0%		13.0%	0.0-26.8%	6.3%	0.0-13.1%
Wildlife	avian	avian	12.0%	0.0-24.7%	26.1%	8.1-44.0%	18.8%	7.7-29.8%
Wildlife	avian	subtotal	12.0%	0.0-24.7%	39.1%	19.2-59.1%	25.0%	12.8-37.3%
Wildlife	subtotal		44.0%	24.5-63.5%	39.1%	19.2-59.1%	41.7%	27.7-55.6%
Unknown		unknown	28.0%	10.4-45.6%	17.4%	1.9-32.9%	22.9%	11.0-34.8%

Figure 4-9 *E. coli* Source Characterization of Station 17685 under All Conditions



4.3.12 Station 17686 Flewellen Creek at Briscoe Road

In this section, results for supplemental Station 17686 are described individually. This source characterization is based on 29 isolates collected in two non-runoff events and 24 isolates collected in two runoff events. Due to the relatively small number of isolates, the confidence intervals around the source estimates are broad. Table 4-17 and Figure 4-10 summarize the identified sources of *E. coli* from all four events, including both runoff and non-runoff conditions. The large number of sewage isolates at this site under runoff conditions was somewhat surprising, given the rural nature of this western area of the watershed.

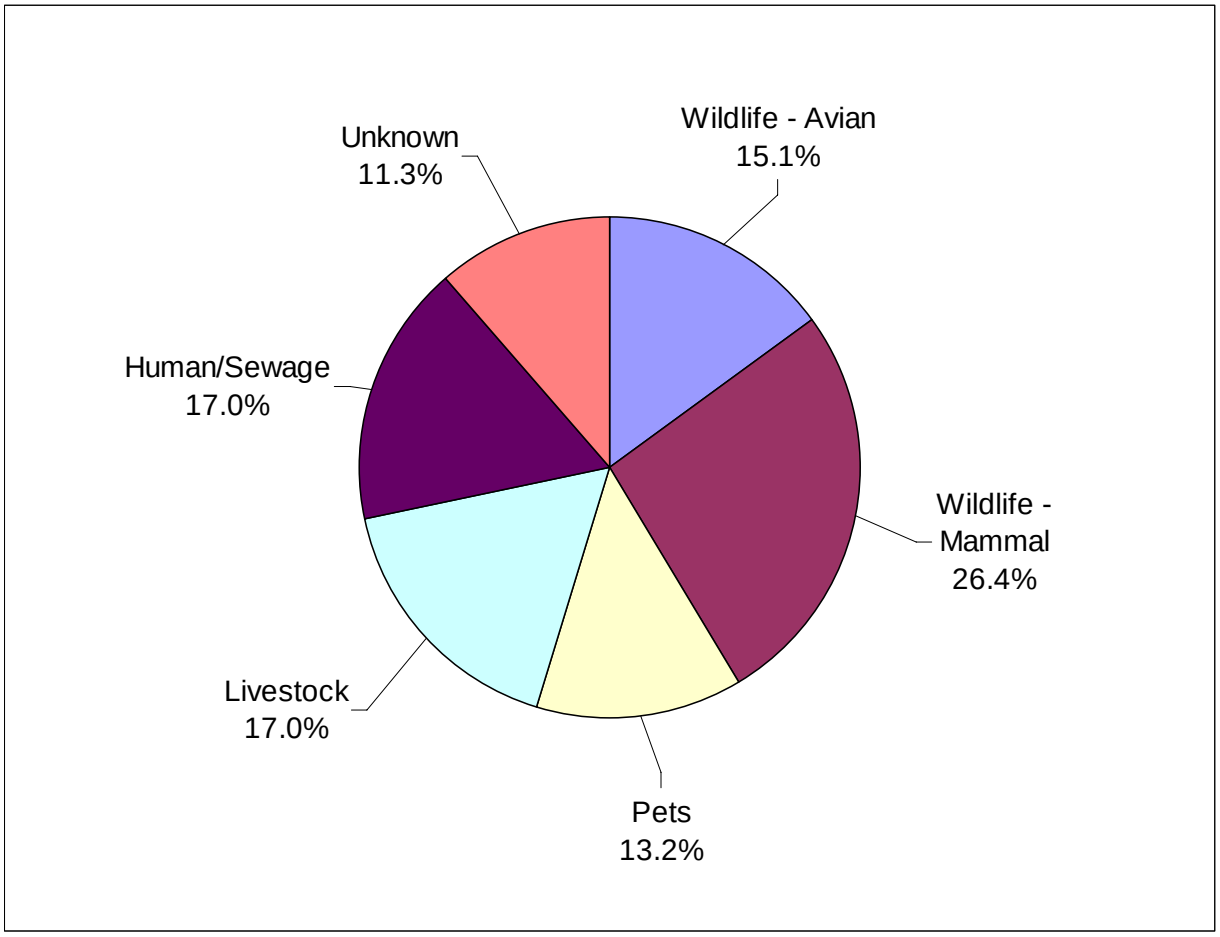
The event normalizations and concentration-weighted source characterizations are not presented for this site because there were no significant differences from the non-normalized, un-weighted results. Also, there were no statistically significant differences in source contributions under runoff versus non-runoff conditions.

Table 4-17 *E. coli* Source Characterization of Station 17686

			Non-runoff		Runoff		All Conditions	
Super-category	Category	Source	% Contribution	95% CI	% Contribution	95% CI	% Contribution	95% CI
Human/sewage	human	human	3.4%	0.0-10.1%	4.2%	0.0-12.2%	3.8%	0.0-8.9%
Human/sewage	sewage	sewage	6.9%	0.0-16.1%	20.8%	4.6-37.1%	13.2%	4.1-22.3%
Human/sewage	subtotal		10.3%	0.0-21.4%	25.0%	7.7-42.3%	17.0%	6.9-27.1%
Livestock		bovine	3.4%	0.0-10.1%	4.2%	0.0-12.2%	3.8%	0.0-8.9%
Livestock		horse	13.8%	1.2-26.3%	8.3%	0.0-19.4%	11.3%	2.8-19.9%
Livestock		poultry	0.0%		0.0%		0.0%	
Livestock		Guinea fowl	0.0%		0.0%		0.0%	
Livestock		donkey	0.0%		0.0%		0.0%	
Livestock		goat	0.0%		0.0%		0.0%	
Livestock		porcine	3.4%	0.0-10.1%	0.0%		1.9%	0.0-5.5%
Livestock	subtotal		20.7%	5.9-35.4%	12.5%	0.0-25.7%	17.0%	6.9-27.1%
Pets	canine	canine	13.8%	1.2-26.3%	12.5%	0.0-25.7%	13.2%	4.1-22.3%
Pets	canine	dog	0.0%		0.0%		0.0%	
Pets	feline	feline	0.0%		0.0%		0.0%	
Pets	subtotal		13.8%	1.2-26.3%	12.5%	0.0-25.7%	13.2%	4.1-22.3%
Wildlife	mammal	coyote	0.0%		0.0%		0.0%	
Wildlife	mammal	deer	0.0%		0.0%		0.0%	
Wildlife	mammal	rabbit	0.0%		0.0%		0.0%	
Wildlife	mammal	raccoon	6.9%	0.0-16.1%	4.2%	0.0-12.2%	5.7%	0.0-11.9%
Wildlife	mammal	rodent	20.7%	5.9-35.4%	16.7%	1.8-31.6%	18.9%	8.3-29.4%
Wildlife	mammal	opossum	3.4%	0.0-10.1%	0.0%		1.9%	0.0-5.5%
Wildlife	mammal	skunk	0.0%		0.0%		0.0%	

			Non-runoff		Runoff		All Conditions	
Super-category	**Category**	**Source**	**% Contribution**	**95% CI**	**% Contribution**	**95% CI**	**% Contribution**	**95% CI**
Wildlife	mammal	squirrel	0.0%		0.0%		0.0%	
Wildlife	mammal	subtotal	31.0%	14.2-47.9%	20.8%	4.6-37.1%	26.4%	14.5-38.3%
Wildlife	avian	waterfowl	6.9%	0.0-16.1%	0.0%		3.8%	0.0-8.9%
Wildlife	avian	avian	6.9%	0.0-16.1%	16.7%	1.8-31.6%	11.3%	2.8-19.9%
Wildlife	avian	subtotal	13.8%	1.2-26.3%	16.7%	1.8-31.6%	15.1%	5.5-24.7%
Wildlife	subtotal		44.8%	26.7-62.9%	37.5%	18.1-56.9%	41.5%	28.2-54.8%
Unknown		unknown	10.3%	0.0-21.4%	12.5%	0.0-25.7%	11.3%	2.8-19.9%

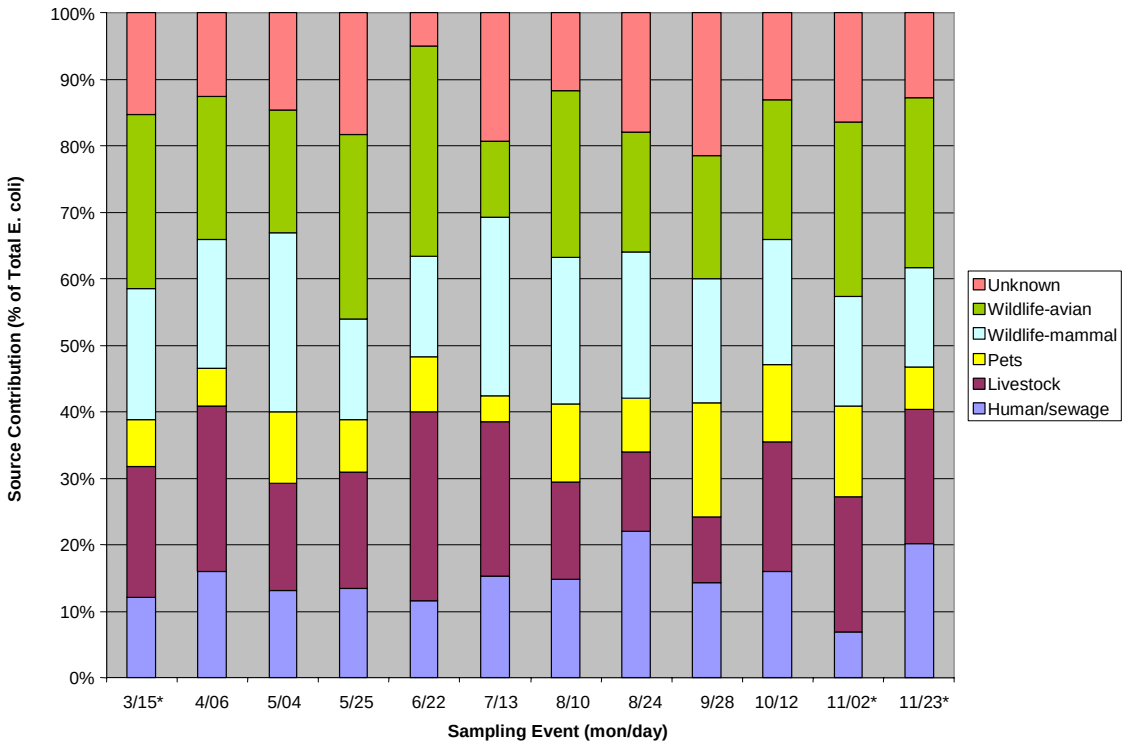
Figure 4-10 *E. coli* Source Characterization of Station 17686 under All Conditions



4.3.13 Seasonality of *E. coli* Sources

An analysis of the source contributions for each sampling event was performed to identify changes in source composition from season to season. Figure 4-11 does not indicate a pronounced or systematic variation in the relative magnitude of major source categories from event to event. This figure includes data from all sites. Note that events one, eleven and twelve were runoff events.

Figure 4-11 Seasonal *E. coli* Source Characterization.



*Runoff influenced event

SECTION 5
SUMMARY AND CONCLUSIONS

To support the present bacteria TMDL, fecal coliform and *E. coli* data were collected at 20 stations during 12 surveys over the period October 2002 through August 2003. The findings of this data collection support the State of Texas 2002 303(d) listing of Upper Oyster Creek (Segment 1245) as impaired from the presence of fecal pathogen indicator bacteria and nonsupport of contact recreation use. Some stations would meet the pertinent bacteria criteria under dry weather conditions, though the very high *E. coli* levels observed under rainfall-runoff events combined with the frequency of rainfall in the watershed results in consistently high indicator bacteria levels. Typically, the highest *E. coli* levels were observed in several tributaries of Oyster Creek, including Stafford Run and Flewellen Creek. Red Gully exhibited very low *E. coli* levels under dry weather conditions, probably due to the chlorinated municipal WWTP effluent discharged into it, but very high *E. coli* levels under runoff conditions. Generally low levels of *E. coli* were indicated in the waters pumped from the Brazos River as shown at the most upstream sampling station in Segment 1245. *E. coli* levels tended to be lower in the broad-channeled impounded reaches of Oyster Creek, where the resulting low water velocities may permit settling of *E. coli* from the water column to sediments. In fact in the impounded reaches fecal coliform and *E. coli* data indicated support of the contact recreation use.

To determine sources of the *E. coli* in Oyster Creek ribotyping was selected as the bacterial source tracking (BST) method. Six core water sampling stations were established where high bacteria levels were identified from the 2002-2003 sampling. These core stations were sampled during 12 events from March through November 2004. Three supplemental stations were added in September 2004 to provide additional information on the spatial distribution of sources. Overall 1,136 *E. coli* isolates from ambient water samples were ribotyped. At all six core stations, the project objective of 120 ribotyped isolates was exceeded, sometimes substantially.

A mixture of sources contributing to observed *E. coli* levels was identified by BST. No single source category comprised the dominant source of *E. coli* at any station. Wildlife were the source of approximately 43% of the *E. coli* when data from all stations were combined, and the wildlife sources were roughly evenly split between avian and mammals. Major wildlife contributors included rodents (11%), waterfowl (7%), and raccoons (4%). Livestock (primarily cattle, horses, and hogs) accounted for approximately 19% of the observed *E. coli*. Dogs accounted for approximately 9% of the *E. coli*. Human and sewage accounted for approximately 14% of the *E. coli*. The source of approximately 15% of the *E. coli* could not be identified, either because there were no matching ribotypes in the known source library or because the matching isolates were transients, i.e., they are not host-specific having been observed in multiple types of host species.

There were no statistically significant differences in sources under rainfall-runoff versus non-runoff conditions when the data were evaluated either collectively or station-by-station. Similarly, event normalizations and concentration-weighted source characterizations did not result in any significant differences from the non-normalized, unweighted results for any station. No significant patterns of seasonal variation in source contribution were observed when the data were evaluated collectively. Seasonality on a station-by-station basis was not performed, because insufficient *E. coli* isolates were ribotyped for any one event by station to allow statistically valid evaluations at this spatial and temporal level.

In general, source identification results for individual sites did not depart significantly from the results of the combined data set (Table 5-1). The station-to-station differences in source composition that were observed could in some instances be related to watershed characteristics. For example, the human and sewage influence was less apparent and the livestock influence more apparent in the more rural western portions of the watershed. Also, the porcine influence, though not shown in Table 5-1, was highest at station 12087, which is just downstream of the TDCJ Jester Unit hog farm. Even at this station, porcine only constituted approximately 15% of the *E. coli*, and at the next downstream station, 12086, the porcine contribution was back to the average across all stations.

As shown in Table 5-1 a diversity of sources contributed to the observed *E. coli* at each station, and no source dominated contribution at any station. As examples, human and sewage never comprised over a quarter of the contribution at any station, and livestock never comprised over a third of the contribution at any station. The combination of mammal and avian wildlife categories comprised the largest percent contribution and the percent is generally in the 40% range. The wide spectrum of *E. coli* sources and the absence of dominance by any particular source indicate that approaches to reduce *E. coli* in Upper Oyster Creek will need to be broad and consider a wide variety of control measures.

Table 5-1 *E. coli* Source Characterization Summarization for Upper Oyster Creek

Station	Human & Sewage	Livestock	Pets	Wildlife (mammal)	Wildlife (avian)	Unknown
All	14.2%	18.9%	9.4%	19.5%	23.2%	14.9%
12090	13.0%	26.0%	8.3%	19.5%	19.5%	13.6%
12087	3.2%	31.2%	7.6%	16.6%	27.4%	14.0%
12086	15.6%	15.6%	7.8%	26.3%	24.0%	10.6%
12083	20.9%	10.8%	8.6%	23.7%	21.6%	14.4%
17688	15.3%	13.1%	9.8%	16.4%	23.0%	22.4%
12074	21.4%	19.0%	9.5%	15.5%	22.6%	11.9%
11516*	5.0%	12.5%	20.0%	10.0%	35.0%	17.5%
17685*	4.2%	18.8%	12.5%	16.7%	25.0%	22.9%
17686*	17.0%	17.0%	13.2%	26.4%	15.1%	11.3%

* Supplemental station

SECTION 6

REFERENCES

American Veterinary Medical Association 2002. http://www.avma.org (April 21, 2005)

Atlas, R.M., G. Sayler, R.S. Burlage, and A.K. Bej. 1992. Molecular approaches for environmental monitoring of microorganisms. *BioTechniques* 12(5):706-717.

Barloga, A.O. and S.K. Harlander. 1991. Comparison of methods for discrimination between strains of *Listeria monocytogenes* from epidemiological surveys. *Appl Environ Microbiol.* 57(8):2324-2331.

Baylor University, The Center for Applied Geographic and Spatial Assessment. 1997. GIS land use and land cover classification provided for an area of the Brazos River watershed below Waco, TX. Provided under contract to Texas Institute for Applied Environmental Research.

Fisher, M.C., J.J. Lipuma, S.E. Dasen, G.C. Caputo, J.E. Mortenson, K.L. McGowen, and T.L. Stull. 1993. Source of *Pseudomonas cepacia*: Ribotyping of isolates from patients and the environment. *J. Pediatr.* 123: 745-747.

Grimont, F. and Grimont, P.A.D. 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Annules de L'Institut Pasteur/Microbiology.*137 B(2):165-175.

Hartl, D.L. and D.E. Dykhuizen. 1984. The population genetics of *Escherichia coli*. *Ann. Rev. Genetics* 18:31-68.

Kolbe 1992. Texas Water Commission. An Assessment of Water Quality and Fish Kills in Upper Oyster Creek, Segment 1245. SR 92-05. Texas Water Commission, Austin, Texas.

Lipuma, J.J., J.E. Mortenson, S.E. Dasen, T.D. Edlind, D.V. Schidlow, J.L. Burns, and T.L. Stull. 1988. Ribotype Analysis of Pseudomonas cepacia from cystic fibrosis treatment centers. *J. Pediatr.* 113: 859-862.

Mazengia, E. 1998. Microbial source tracking: utility of a clonal database. M.S. Thesis.University of Washington, Seattle, WA.

Montgomery Watson America, Inc. 2000. Regional Surface Water Plant Feasibility Study for Brazoria, Fort Bend, and West Harris Counties. Prepared for the Gulf Coast Water Authority and the Texas Water Development Board, Dickinson and Austin, Texas.

Moyer, N.P., G.M. Luccini, L.A. Holcomb, N.H. Hall, and M. Altwegg. 1992. Application of ribotyping for differentiating aeromonads isolated from clinical and environmental sources. *Appl. Environ. Microbiol*. 58: 1940-1944.

NOAA 2004. National Oceanic and Atmospheric Administration. http://www.noaa.gov (April 21, 2005)

PBS&J 2000. Bacterial Indicator Study, Final Report. (Doc. No. 000195). Prepared for Guadalupe-Blanco River Authority and Texas Natural Resource Conservation Commission. PBS&J, Austin, Texas.

Farag, A.M., J.N. Goldstein, D. F. Woodward, and M. Samadpour. 2001. Water quality in three creeks in the backcountry of Grand Teton National Park, USA. Journal Fresh Water Ecology, 16:135-143.

Selander, R.K., D.A. Caugant, and T.S. Whittam. 1987. Genetic structure and variation in natural populations of *Escherichia coli*. In: *Cellular and Molecular Biology*, F.C. Neidhardt et al. (eds.). American Society for Microbiology, Washington D.C.

Stull, T.L., et al. 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *J. Infectious Diseases*. 157(2): 280-286.

TCEQ. 2000. Texas Natural Resources Conservation Commission, Texas Surface Water Quality Standards. §307.1-307.10. Adopted by the Commission: July 26, 2000; Effective August 17, 2000 as the state rule. Austin, Texas.

TCEQ. 2003a. Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data, 2004.

TCEQ. 2003b. TCEQ's Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue

TCEQ. 2005. Industrial and Municipal (Domestic) Wastewater Permit Applications Query Results. http://www.tnrcc.state.tx.us (April 18, 2005)

Texas Agricultural Statistics Services. 2004. http://www.nass.usda.gov/tx/index.htm (April 21, 2005)

U.S. Census Bureau, 1990. http://www.census.gov (April 21, 2005)

U.S. Census Bureau, 2000. http://www.census.gov (April 21, 2005)

USEPA, 1991. Guidance for Water Quality-.21 Based Decisions: The TMDL Process. Office of Water, USEPA 440/4-91-001.

USEPA, 2005. Envirofacts Warehouse – PCS. http://www.epa.gov/enviro/ (April 15, 2005)

Vogelmann, J.E., S.M. Howard, L. Yang, C.R. Larson, B.K. Wylie, and N. Van Driel. 2001. Completion of the 1990s National Land Cover Data Set for the Conterminous United States from Landsat Thematic Mapper Data and Ancillary Data Sources, Photogrammetric Engineering and Remote Sensing. 67:650-652.