EVALUATION OF METHODS TO ASSESS AND REDUCE BACTERIAL CONTAMINATION OF SURFACE WATER FROM GRAZING LANDS

A Dissertation

by

KEVIN LEE WAGNER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2011

Major Subject: Agronomy

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Approved by:

Co-Chairs of Committee,	Terry Gentry
	Larry Redmon
Committee Members,	R. Daren Harmel
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August 2011

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ABSTRACT

Evaluation of Methods to Assess and Reduce Bacterial Contamination of Surface Water from Grazing Lands. (August 2011)

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Co-Chairs of Advisory Committee: Dr. Terry Gentry
Dr. Larry Redmon

Excessive bacterial levels are a major water quality concern. Better methods are needed to quantify the proportion of bacterial loading contributed by various sources, and best management practices are needed to restore water quality. This study assessed the ability of alternative water supplies and grazing management to reduce *E. coli* loading from cattle and evaluated the ability of quantitative polymerase chain reaction analysis of total and bovine-associated *Bacteroides* markers (AllBac and BoBac, respectively) to determine the percentage of bovine-associated fecal contamination. Runoff from seven small watersheds, representing ungrazed, properly stocked, and overstocked conditions, was analyzed for *E. coli*, AllBac, and BoBac to assess grazing management impacts on *E. coli* runoff and the effectiveness of *Bacteroides* markers. To determine the effectiveness of alternative water, instream *E. coli* levels and cattle movement were evaluated before and after alternative water was provided.

The study found that when alternative off-stream water was provided, the amount of time cattle spent in the creek was reduced 43% and the direct deposition of *E. coli* into Clear Fork of Plum Creek was estimated to be reduced from 1.11E+07 to 6.34E+06

colony forming units per animal unit per day. Observed pre- and post-treatment *E. coli* loads suggested similar reductions; however, this study could not conclusively attribute observed *E. coli* loading reductions to providing alternative water because of the lack of statistical significance of these observations, possibly due to decreased streamflow during Year 2 (due to drought) and a corresponding increase in *E. coli* levels.

The study found that rotational stocking, if timed appropriately, was very effective at reducing *E. coli* runoff. The impact of grazing timing in relation to runoff events was more significant than the impact of grazing management (i.e. ungrazed properly stocked or overstocked) or stocking rate. When runoff occurred more than two weeks following grazing, *E. coli* levels in runoff were decreased more than 88%.

Finally, data suggest that AllBac and BoBac markers are good indicators of recent fecal contamination from cattle. However, although elevated BoBac/AllBac ratios generally aligned well with cattle presence, this ratio appeared to underestimate the percentage of bovine-associated fecal contamination.

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CHAPTER I

GENERAL INTRODUCTION

1.1 Problem Statement

Excessive levels of fecal indicator bacteria (e.g. *E. coli*) are a major cause of water quality impairment in Texas (TCEQ 2008a) and other regions (Weidhaas et al. 2011). Total Maximum Daily Loads (TMDLs), TMDL Implementation Plans (I-Plans), and watershed protection plans (WPPs) are being developed to address these impairments. However, watersheds can be affected by microbial pollution from a wide variety of sources (TCEQ 2008d; Weidhaas et al. 2011). Nevertheless, livestock are increasingly under scrutiny (Weidhaas et al. 2011). Grazing cattle are often the most abundant livestock species in impaired watersheds in Texas and are frequently identified as a source needing reductions (TCEQ 2008b, 2008c). Because of the potential regulatory implications of TMDLs, it is critical to accurately differentiate the potential bacterial contributions of livestock from those of wildlife or humans (TCEQ 2007c).

Further, concerns about fecal contamination of water bodies by cattle arise from documented human waterborne disease outbreaks associated with animal-impacted surface waters (Ferguson et al. 2003). *Campylobacter*, *Salmonella,* enterohemorrhagic *E. coli*, *Cryptosporidium*, *Listeria* and *Giardia* have been found in cattle manure.

This dissertation follows the style of Journal of Soil and Water Conservation.

Ingestion of these pathogens can be dangerous to human health and may cause gastrointestinal illness, and occasionally, renal failure and death (University of Wisconsin 2007a, 2007b). To protect human health, best management practices (BMPs) are needed to reduce fecal contamination by cattle.

1.2 Motivation

To address bacterial contributions by cattle, (1) analytical methods are needed to better assess bacterial loading from cattle and (2) if bacterial loading from cattle is found to be excessive, then BMPs are needed to reduce these bacterial loadings.

Analytical methods are needed to quantify the proportion of bacterial loading contributed by livestock versus humans, pets, and wildlife so that appropriate restoration goals can be established and restoration efforts targeted. The TMDLs, I-Plans, and WPPs being developed to address these impairments require determination of pollutant sources and allocation of load reductions to identified sources. Library-dependent bacteria source tracking (BST) can assist in these determinations; however, due to the need for library development, these methods are burdensome, time-consuming, and costly. Library-independent methods avoid the need for library development and are less expensive and less labor intensive than library dependent methods and can potentially provide results within hours of sample collection. As such, investigation of promising library-independent BST methods is needed (Jones et al. 2009). One such potential BST method uses *Bacteroides* quantitative polymerase chain reaction (qPCR) assays to assess sources of bacteria. A method, developed by Layton et al. (2006), simultaneously identifies and quantifies *Bacteroides* markers in water samples and estimates total (AllBac) and

bovine-associated (BoBac) fecal pollution in water. However, the ability of this method to reliably and correctly determine fecal contributions from cattle in environmental water samples has not been thoroughly tested to date.

Once appropriate goals are established for reductions of bacterial loading from cattle, then BMPs are needed to address the livestock contribution. Development and implementation of appropriate BMPs to reduce bacterial loadings and concentrations from grazing lands is critical to the success of water resource improvement and protection efforts in impaired water bodies. Success of TMDLs, I-Plans, and WPPs, and willingness of individuals to adopt various control practices and BMPs may be increased by scientific studies showing efficacy of various practices under a variety of conditions; therefore, such studies are important endeavors (Jones et al. 2009).

Bacterial contamination of streams can arise through direct deposition of feces into streams, surface runoff, and subsurface flows (Collins et al. 2005). Further, the extent to which cattle contribute bacteria from grazing lands is generally a function of the number and size of cattle, the location of fecal deposits in relation to water bodies, characteristics of the deposition site affecting adsorption and runoff, and bacterial survival between time of fecal deposition and runoff events (Larsen et al. 1994). A number of potential BMPs have been identified to reduce bacterial contributions from grazing lands. The primary focus of these BMPs is to protect riparian areas and maintain adequate ground cover in order to improve the filtering capacity of the vegetation and enhance infiltration of rainfall and runoff (NRCS 2007). Two such BMPs are alternative water supplies and prescribed grazing. These BMPs provide benefits to both the

producer and the environment; however, published data on *E. coli* reductions resulting from implementation of these practices are sparse and from studies conducted in regions much different than Texas. Although this existing information is valuable, it may not be universally transferable to Texas. Thus, the research objectives were:

- To assess the effect of providing an off-stream watering facility (i.e. water trough) on the percent time cattle spend in streams and riparian zones and the level of bacterial contamination of streams.
- To assess *E. coli* concentrations and loads in runoff at the small watershed scale from grazed and ungrazed pastures and assess the effect of grazing management (e.g. ungrazed, properly stocked, overstocked), timing of grazing, and stocking rate on *E. coli* levels in runoff.
- To determine the ability of the BoBac marker to assess the quantity of *E. coli* loading originating from cattle at the small watershed scale and to further evaluate the relationship between total *Bacteroides* (AllBac) and *E. coli* and its relevance as a fecal indicator.

In Chapter II, field experiment results for the alternative off-stream water evaluation are presented. Global positioning system (GPS) collar results showing the percent time cattle spent instream and within the riparian zone are discussed and compared to the instream *E. coli* measurements observed before and after implementation of alternative off-stream water.

In Chapter III, results for the grazing management assessment are evaluated. *E. coli* levels measured in runoff collected from seven small watersheds are evaluated

against timing of grazing and a variety of grazing management scenarios and stocking rates, as well as the water quality standards, to assess the efficacy of this best management practice (BMP).

In Chapter IV, the results of an assessment of the utility of qPCR assays for two *Bacteroides* markers are presented. Levels of the total *Bacteroides* (AllBac) marker and bovine-associated *Bacteroides* (BoBac) marker are compared to differing stocking rates, grazing management regimes, and *E. coli* levels. The use of the BoBac/AllBac ratio is evaluated for its ability to determine percent bovine-associated fecal contamination.

Finally, in Chapter V, the results of the evaluation of alternative water supplies and proper grazing management as best management practices for reducing bacterial contamination are summarized. Further, a synopsis of the assessment of the utility of qPCR assays for total and bovine-associated *Bacteroides* markers as bacterial pollution indicators is provided.

CHAPTER II

EFFECTS OF AN OFF-STREAM WATERING FACILITY ON CATTLE BEHAVIOR AND INSTREAM *E. COLI* LEVELS

2.1 Overview

Excessive levels of fecal indicator bacteria are the leading cause of water quality impairment in Texas. Livestock with direct access to water bodies are identified as a significant source of these bacteria. To help address this source, the effect of providing alternative off-stream watering facilities to reduce manure, and thus bacterial deposition in or near surface waters was evaluated from July 2007 to July 2009 in Clear Fork of Plum Creek in central Texas. An upstream-downstream, pre- and post-treatment monitoring design was used with off-stream water provided only during the second year of the study. Stream samples were analyzed semi-monthly for *E. coli* and turbidity, and flow was determined for each sample event. Cattle movement was tracked quarterly using global positioning system collars to assess the effect of providing alternative water on cattle behavior. The study found that when alternative off-stream water was provided, the amount of time cattle spent in the creek was reduced 43% from 3.0 to 1.7 min/AU/day. As a result of this, direct deposition of *E. coli* into Clear Fork of Plum Creek was estimated to be reduced from 1.11E+07 to 6.34E+06 cfu/AU/day. Observed pre- and post-treatment *E. coli* loads suggested similar reductions; however, this study could not conclusively attribute observed *E. coli* loading reductions to providing alternative water because of the lack of statistical significance of these observations,

possibly due to decreased streamflow during Year 2 (due to drought) and a corresponding increase in *E. coli* levels. Drought during Year 2, which reduced flows by 79% and influenced ranch management decisions to increase stocking rate 34%, may explain some of the increase in *E. coli* levels observed. Other probable factors impacting the observed *E. coli* levels include natural variability, changes in fate and transport due to drought, and potential contributions from wildlife.

Finally, unlike previous studies, this study did not find turbidity to be a good predictor of *E. coli*. Thus, it was concluded that use of turbidity as an indicator must be determined on a case-by-case basis and used with caution.

2.2 Introduction

Excessive levels of fecal indicator bacteria (i.e. *E. coli*, *Enterococcus*, and fecal coliforms) are the number one cause of water quality impairment in Texas. According to the Texas Commission on Environmental Quality (TCEQ) 2008 Water Quality Inventory and 303(d) List, over half of the water quality impairments in Texas (295 of the 516 impairments) result from excessive levels of bacteria (TCEQ 2008a). Fecal indicator bacteria are common inhabitants of the intestines of all warm-blooded animals, including livestock. Livestock having direct access to water bodies are identified as significant sources of bacteria in numerous bacterial total maximum daily loads (TMDLs) (TCEQ 2007a, 2007b).

Cattle are drawn to streams and adjacent riparian areas by water, shade, and the quality and variety of forage present (Kauffman and Krueger 1984). The length of time cattle spend in a stream, however, plays a significant role in fecal contamination (Mosley

et al. 1999). When cattle have stream access, a portion of their fecal matter is deposited directly into the stream (Larsen et al. 1988) and can be a significant source of contamination. Gary et al. (1983) observed cattle spent 5% of the day in or adjacent to the stream and 6.7 to 10.5% of defecations were deposited directly in the stream. Feces deposited in streams have a greater impact on water quality than that deposited away from streams. Larsen et al. (1994) found manure deposited 0.6 m (2 ft.) and 2.1 m (7 ft.) from a stream contributed 83% and 95% less bacteria, respectively, than that deposited directly in a stream.

Tiedemann et al. (1987) and Mosley et al. (1999) suggested that animal access to streams had a greater impact on stream bacterial levels than stocking density. Thus, riparian protection is needed to reduce manure deposition in or near surface waters (Ball et al. 2002). Exclusion of livestock from riparian areas by fencing of streams is frequently recommended to reduce manure inputs to surface water (Godwin and Miner 1996; McIver 2004). Numerous studies have shown fencing of streams, alone or in combination with other best management practices (BMPs), can reduce *E. coli* levels by 37-46% (Meals 2001, 2004), *Enterococcus* by 57% (Line 2003), and fecal coliforms by 30-94% (Brenner et al. 1994; Brenner 1996; Cook 1998; Hagedorn et al. 1999; Lombardo et al. 2000; Meals 2001; Line 2002; Line 2003; Meals 2004). However, this BMP is costly to install and maintain (Godwin and Miner 1996; Sheffield et al. 1997; Byers et al. 2005), results in loss of grazing area and ranching income, restricts access to reliable water sources, and may be inconvenient and impractical for many ranches. Thus, it is opposed by many ranchers (McIver 2004). Other concerns have recently been raised

regarding the impact of increasing wildlife populations in fenced riparian zones, potentially negating *E. coli* loading reductions provided by restricting livestock access.

Another practice available to protect riparian areas and reduce manure deposition in or near surface waters is development of alternative watering facilities (FCA 1999; Tate et al. 2003; Byers et al. 2005). A permanent or portable off-stream water supply (e.g. trough) provides livestock another drinking water source, which can be used alone or in conjunction with other practices to reduce the amount of time livestock spend near surface waters and in riparian areas. To achieve optimum uniformity of grazing and greatest use of alternative water sources, cattle should not have to travel more than 200 to 300 m (656 to 984 ft) to water (McIver 2004). Alternative water sources benefit livestock producers by improving grazing distribution, reducing herd health risks due to drinking or standing in contaminated water, decreasing herd injuries from cattle traversing steep or unstable streambanks, increasing water supply reliability during droughts, and increasing weight gains in beef cattle by 0.1 to 0.2 kg/day (0.2 to 0.4 lb/day) (Willms et al. 1994; Buchanan 1996; Porath 2002; Willms et al. 2002; Veira 2003; Dickard 1998).

Alternative off-stream water supplies can also provide environmental benefits including reduced manure deposition and bacterial contamination of surface waters and reduced streambank destabilization and erosion due to trampling and overgrazing of banks. Previous research has demonstrated that cattle spend 85 to 94% less time in streams (Miner et al. 1992; Clawson 1993; Sheffield et al. 1997) and 51 to 75% less time within 4.6 m (15 ft.) of streams when an off-stream watering facility was available

(Godwin and Miner 1996; Sheffield et al. 1997). As a result, Godwin and Miner (1996) deduced that under baseflow conditions, off-stream watering was nearly as effective as fencing in reducing manure inputs to surface water, thus reducing water quality impacts of grazing cattle at a reduced cost. Sheffield et al. (1997) confirmed this, finding that as a result of the reduction in time cattle spent in and near streams, instream fecal coliforms concentrations were reduced by an average of 51%. However, results varied among sites with statistically significant reductions in fecal coliform levels of 99%, 87%, and 57% being observed at three sites and a 53% increase, which was not statistically significant, being observed at one site. Further, Byers et al. (2005) found that providing water troughs decreased the amount of time cattle spent within 12 m (39 ft.) of a stream but that the result was dependent on time of year with a reduction of 40% observed in March 2002, 96% in December 2002, and approximately 60% in July 2003. Byers et al. (2005) also found that although alternative water did not impact stormwater *E. coli* concentrations, median base flow *E. coli* loads decreased 95% in one pasture and 85% in another when water troughs were available. However, streamflow was 51% smaller when the troughs were available, thus impacting the load differences.

With the exception of the study conducted by Byers et al. (2005) which used Global Positioning System (GPS) collars, previous studies used light beam counters (Godwin and Miner 1996), visual observations (Miner et al. 1992; Sheffield et al. 1997), and time-lapse cameras (Clawson 1993) to evaluate cattle behavior during daylight hours. However, night time observations can be critical because cattle exhibit bimodal grazing patterns (early morning and evening) with certain breeds spending a greater

portion of the night grazing as compared to day time (Pandey et al. 2009). The use of GPS and Geographic Information System (GIS) technology allows livestock behavior to be evaluated with greater spatial and temporal resolution. Animals can be tracked 24 hours a day using GPS receivers incorporated into collars worn by the animals (Pandey et al. 2009). Agouridis et al. (2005) evaluated GPS collars to determine accuracy for applications pertaining to animal tracking in grazed watersheds and found the collars were accurate within 4 to 5 m (13 to 16 ft) and thus acceptable for most cattle operational areas (Pandey et al. 2009).

Observation periods of these earlier studies were also generally of short duration, focusing on specific seasons. These studies also targeted the Pacific Northwest (Miner et al. 1992; Clawson 1993; Godwin and Miner 1996), Eastern (Sheffield et al. 1997), and Southeastern U.S. (Byers et al. 2005), regions where conditions are different than much of Texas and the mid-section of the country where a majority of U.S. cattle production occurs. Finally, these studies, with the exception of Byers et al., did not evaluate the impacts of off-stream water on *E. coli* levels (2005).

The objectives of this study were to assess the effect of providing an off-stream watering facility (i.e. water trough) on reducing the percent time cattle spend in streams and riparian zones and the level of bacterial contamination of streams. This information is needed by stakeholders, natural resource agencies, and others working to improve water quality in Texas, the mid-section of the U.S., and other regions around the world with similar climates and grazing systems, not only to better understand the effectiveness

of alternative water as a water quality BMP, but to improve the predictive capabilities of water quality models used for TMDLs and watershed protection plans.

2.3 Materials and Methods

2.3.1 Site Description

This study was conducted on a commercial cow-calf operation located in Caldwell County, TX bisected by Clear Fork of Plum Creek. Although the drainage area above the ranch is only 26 km^2 (10 mi^2), Clear Fork of Plum Creek is typically a perennial stream as a result of a number of springs located along it. The creek is 0.3 to 10.3 m (1.0 to 33.5 ft.) wide and less than 1 m (3.3 ft.) deep. Thus, the creek was generally not of sufficient depth for cattle to cool off in. The average slope of the stream is 0.3% while the average slope perpendicular to the stream is 5.4%. Clear Fork of Plum Creek is a tributary of Plum Creek, which is listed as impaired by excessive levels of *E. coli* on the 303(d) List and is the focus of watershed restoration efforts through a watershed protection plan.

The ranch is in the Texas Blackland Prairies Ecoregion (Omernik 1987) where annual precipitation averages 89 cm (35 in). However, as the result of a severe drought which began in the spring of 2008, only 56 cm (22 in) of rainfall was received in Year 1 and 40 cm (15.7 in) received in Year 2. Average annual temperatures were normal [20^{o}C (68^{o}F)] in Year 1 and higher than average [20.6^{o}C (69^{o}F)] during Year 2.

The flood plain soils along the creek are dominated by the Tinn series, a very deep, moderately well drained, very slowly permeable soil formed in calcareous clayey

alluvium. Upgradient of the Tinn soil is the Branyon clay, which like the Tinn soil, is a very deep, moderately well drained, very slowly permeable soil. Finally, soils in the upland areas of the ranch are comprised of Lewisville soils, very deep, well drained, moderately permeable soils on slopes of 0 to 10%.

The predominant forage in the creek pasture is common bermudagrass (*Cynodon dactylon* L.). Vegetation in the three other pastures is WW-B Dahl Bluestem (*Bothriochloa bladhii* L.), Old World Bluestem [(*Bothriochloa ischaemum* L.); (*Dicanthium* sp. L.)], and native grasses. Vegetation along the creek consisted primarily of common bermudagrass with few trees and other typical riparian vegetation present. Less than 5% of the stream and its riparian area were shaded; thus, shade was not a major attractant of cattle to the creek and riparian zone. With the exception of the creek pasture, most of the operation had been in row crops until 2003 when it was converted to pastureland in 2004.

2.3.2 Pasture Management

Four pastures, ranging in size from 12 to 15 ha (30 to 37 ac) were utilized during the study. Cattle had complete and continuous access to the creek and creek pasture throughout the study. Cattle were allowed access to the other pastures as needed. During the first year of the study (July 2007 to July 2008), pastures were stocked with 54 crossbred cows with calves and 2 bulls (57 AUs). During the second year of the study (July 2008 to July 2009), the pastures were stocked with 72 cows with calves and 3 bulls (76 AUs). The stocking rate was increased in the second year as the cooperating cow-calf operation consolidated herds from two ranches in response to the severe drought,

making it easier to feed, water, and care for the livestock until conditions improved. Water troughs supplying well water were present in all pastures but were turned off during the first year of the study (with the exception of two weeks in January 2008) forcing the cattle to water in the creek only. In January 2008, several calves became ill with bovine respiratory disease and water troughs were activated for a period of two weeks then turned off again and remained off until July 6, 2008. The troughs were turned on for the second year of the study and provided cattle an alternative water source. Distance between the water trough and stream in the creek pasture was approximately 137 m (150 yd.).

2.3.3 Global Positioning System (GPS) Tracking of Cattle

Each quarter throughout the two year study (Appendix A), six to eight randomly selected cows were collared with Lotek® GPS 3300LR collars (Lotek Wireless Inc., Newmarket, Ontario, Canada). The collar manufacturer reports that, with differential correction applied, horizontal accuracies of position readings have errors less than 5 m (16 ft). Positional readings were collected at a 5 min fixed interval, providing up to 6,624 locations by each collar each quarter. Cattle movement was tracked for 21 to 23 days and then the collars were removed.

Collar data were downloaded using Lotek host software and differentially corrected using data from the National Geodetic Survey (NGS), Continuously Operating Reference Stations (CORS) base-station nearest to the location of the trial for the day before the start of the trial through the day after the end of the trial. Differentially-

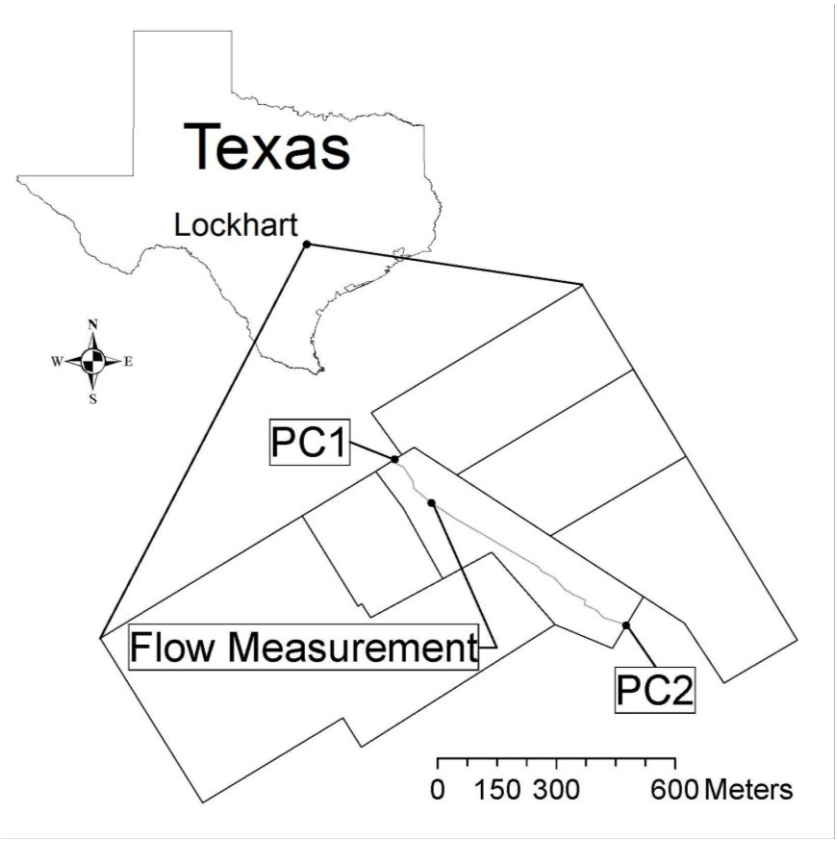
corrected collar data were then combined with sensor data and converted to database files for analysis.

To analyze positional readings collected from the GPS collars, the ArcView (ArcGIS 9, ArcMap Version 9.2, ESRI, Redlands, CA) software package was used. For each collar, the number of positional points in the stream (i.e. within 0.6 m or 2 ft of the midpoint of the stream) and within 4.6 m (15 ft) of the stream were determined using the "Select by Location" function. Percent time spent within each distance from the stream was determined by dividing the number of positional points within each buffer by the total number of positional readings taken. Percent time was then converted to minutes per day.

2.3.4 Instream Sampling Procedures

Two sites located at the inflow and outflow of Clear Fork of Plum Creek to the ranch, PC1 (29°53'35.81"N/97°45'21.06"W) and PC2 (29°53'23.28"N/97°45'2.67"W), respectively, were monitored to assess effectiveness of alternative off-stream water (Figure 2.1). These sites are approximately 0.8 km (0.5 mi) apart. Grab samples were collected and analyzed on a semi-monthly basis at both sampling sites when water was flowing. Water samples were collected directly from the stream, midway in the water column into sterile Whirl-Pak® bags. Bags were held upstream of the sampler and care was exercised to avoid contact with sediment and the surface micro layer of water. After collection, samples were placed on ice for transport to the lab where they were stored at 4^{o}C (39^{o}F) until analysis.

Figure 2.1
Water sample collection and flow measurement sites at 2S Ranch.



2.3.5 Flow Calculation

Flow depth was measured bi-monthly in conjunction with water sample collection. Measurements were made in a 0.9 m (3 ft.) corrugated metal culvert located at a stream crossing 0.16 km (0.1 mi) below PC1 and 0.64 km (0.4 mi) above PC2. Manning's equation (Grant 1991) was used to estimate flow rate for each sampling event. The Manning roughness coefficient (n) was determined from field measurements of flow depth and velocity and compared to published values by Grant (1991) for corrugated metal subdrains. Slope (S) from PC1 to PC2 was determined using field

evaluation of slope as well as elevations on Google Earth® Area (A), and hydraulic radius (R) was obtained from published values (Grant 1991) based on the observed depth (d) in relation to the culvert depth (D).

2.3.6 Analytical Methods

Water sample analysis was conducted within six hours of collection. *E. coli* in water samples were isolated and enumerated using EPA Method 1603 (EPA 2006). If counts were greater than 200 colonies at the highest dilution, the count was reported as too numerous to count (TNTC). Results were reported as colony forming units (cfu) per 100 mL. Finally, an AquaFluor^{TM} Handheld Fluorometer/Turbidimeter (model 8000-010, Turner Designs, Sunnyvale, CA) was obtained in February 2008 allowing measurement of turbidity throughout the remainder of the study. Turbidity measured in water samples was reported in Nephelometric Turbidity Units (NTU).

Additionally, to approximate deposition of *E. coli* in the stream before and after alternative off-stream water was provided, percent time spent by cattle in the stream as determined by the GPS collars was multiplied by published fecal coliform production values (5.4E+09 cfu/AU/day—Metcalf and Eddy 1991) and then converted to *E. coli* concentrations by multiplying the result by 0.63 as EPA suggests (Hamilton et al. 2005).

2.3.7 Evaluation of E. coli Loads

Flow rate at the time each grab sample was assumed to represent the daily average (m^3/s). These flow rates, along with the *E. coli* concentrations, were used to estimate the daily loads for the upstream and downstream sites, PC1 and PC2,

respectively. The daily load contributed by the study watershed was calculated by subtracting the upstream load from the downstream load (PC2 – PC1). This was converted to an AU basis by dividing the daily loads contributed by the study watershed by the number of AUs present in the study watershed during the respective period (i.e. 57 AUs during Year 1 and 76AUs during Year 2).

2.3.8 Statistical Analysis: Water Quality Data

The statistical software, Minitab (Minitab Inc., State College, PA), was used for all statistical calculations. Basic statistics and graphical summaries of each dataset were created to evaluate means, medians, quartiles, confidence intervals, and normality using the Anderson-Darling Normality Test. As a majority of datasets were not normally distributed, they were evaluated with nonparametric statistics. The Mann-Whitney statistical test was used to assess the differences in median (1) minutes cattle spent per day instream and within 4.6 m of the creek; (2) flows; (3) *E. coli* concentrations; (4) *E. coli* loads; and (5) turbidities observed between sites and periods (i.e. with versus without alternative water). An alpha level of 0.05 was used as the level of significance, thus results were considered statistically significant when $p < 0.05$. Regression analysis was used to evaluate the relationship between *E. coli* concentrations at PC1 and PC2, as well as between *E. coli* concentrations and turbidity. Coefficient of determination values were used to evaluate the strength of regression equations for *E. coli* concentrations. Finally, analyses of covariance were developed using the Minitab General Linear Model, specifying the responses as PC2 turbidity, the model as the treatment period (i.e. with

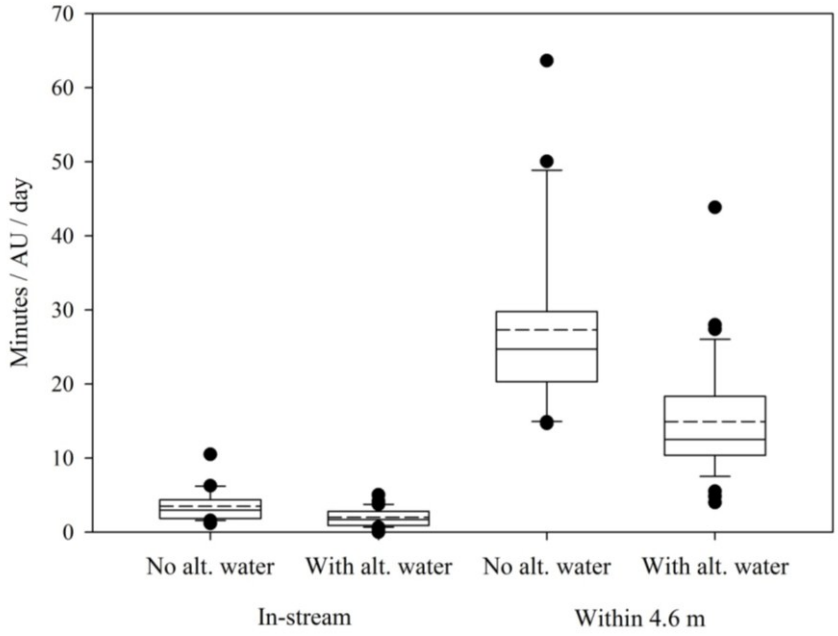
alternative water) or calibration period (without alternative water), and the covariate as PC1 turbidity.

2.4 Results and Discussion

2.4.1 GPS Tracking of Cattle

Comparison of the amount of time cattle spent in and near the creek with and without alternative water available (Appendix A) indicated providing alternative off-stream water reduced the time cattle spent in the stream and within 4.6 m (15 ft.) of the creek (Figure 2.2).

Figure 2.2
Time (min/AU/day) that cattle spent in and near (within 4.6 m) Clear Fork of Plum Creek with and without alternative off-stream water provided. The boundary of the box closest to zero indicates the 25th percentile, the solid line within the box represents the median, the dashed line represents the mean, the boundary of the box farthest from zero indicates the 75th percentile, the whiskers above and below the box indicate the 10th and 90th percentiles, and the circles indicate outliers.



Because shade along the riparian zone was limited (< 5%) and stream depth was not suitable for cooling, it can be assumed that observed reductions were almost solely the result of cattle drinking from the alternative water supply and not the stream. Analysis of the GPS collar data (Table 2.1) indicated providing alternative off-stream water significantly reduced the median amount of time cattle spent in and near the creek ($p < 0.01$).

The amount of time cattle spent within 4.6 m of the creek was reduced 52% from 25 to 12 min/AU/day when provided with off-stream water, compared to the 75% reduction from 15 to 4.25 min/AU/day found by Godwin and Miner (1996) and 51% reduction from 12.7 to 6.2 min/AU/day found by Sheffield et al. (1997). Although the percent reductions from our study were similar to those of Sheffield et al. (1997), the amount of time cattle spent near the stream varied substantially between the studies.

Table 2.1
Descriptive statistics of time (in minutes/day and percent of day) that cattle spent in and near Clear Fork of Plum Creek with and without alternative off-stream water provided.

Distance from creek	Statistic	No Alt. Water min/day (%)	With Alt. Water min/day (%)	Percent Reduction
Instream	Mean	3.5 (0.2%)	2.0 (0.1%)	
	sd	2.2 (0.1%)	1.2 (0.1%)	
	Median*	3.0 (0.2%)a	1.7 (0.1%)b	43%
	Max	10.5 (0.7%)	5.0 (0.3%)	
4.6 m (15 ft.)	Mean	27 (1.9%)	15 (1.0%)	
	sd	12 (0.8%)	8 (0.6%)	
	Median*	25 (1.7%)a	12 (0.8%)b	52%
	Max	64 (4.4%)	44 (3.1%)	

* For each site, medians followed by same letter are not significantly different ($p < 0.05$)

Further, our study found that providing alternative off-stream water reduced stream use from 3.0 to 1.7 min/AU/day, compared to reductions from 25.6 to 1.6 min/cow/day (Miner et al. 1992), 4.7 to 0.7 min/AU/day (Clawson 1993), and 6.7 to 0.7 min/AU/day (Sheffield et al. 1997). Based on the percent time cattle spent in the stream (as determined by the GPS collars), along with published fecal coliform loading rates (Metcalf and Eddy 1991) and the *E. coli* conversion factor suggested by EPA (Hamilton et al. 2005), we estimated the median daily deposition of *E. coli* in the stream was reduced from 1.11E+07 cfu/AU/day to 6.34E+06 cfu/AU/day when alternative water was provided.

The reduction in the percent time cattle spent in the stream observed by this study (43%) was half the reductions of 85 to 94% observed by previous studies (Miner et al. 1992; Clawson 1993; Sheffield et al. 1997). Additionally, the amount of time cattle spent in the stream varied substantially among studies from 3 min per day in our study to almost 26 min per day (Miner et al. 1992) indicating the site specific nature of this measurement. Stream width, depth, accessibility, and adjacent shade play a major role in the amount of time cattle spend in and near streams and thus the percent reductions achievable by providing alternative water. As such, TMDLs and other watershed studies that utilize percent time cattle spend in streams for assessing direct deposition rates would benefit from GPS collars studies to validate models. For example, it was estimated by Orange County, TX, TMDL stakeholders that, on average, cattle drinking water from the bayous spend 10 min per day in the stream during June, July, August, or September, and 5 min per day in March, April, May, October, and November, but did

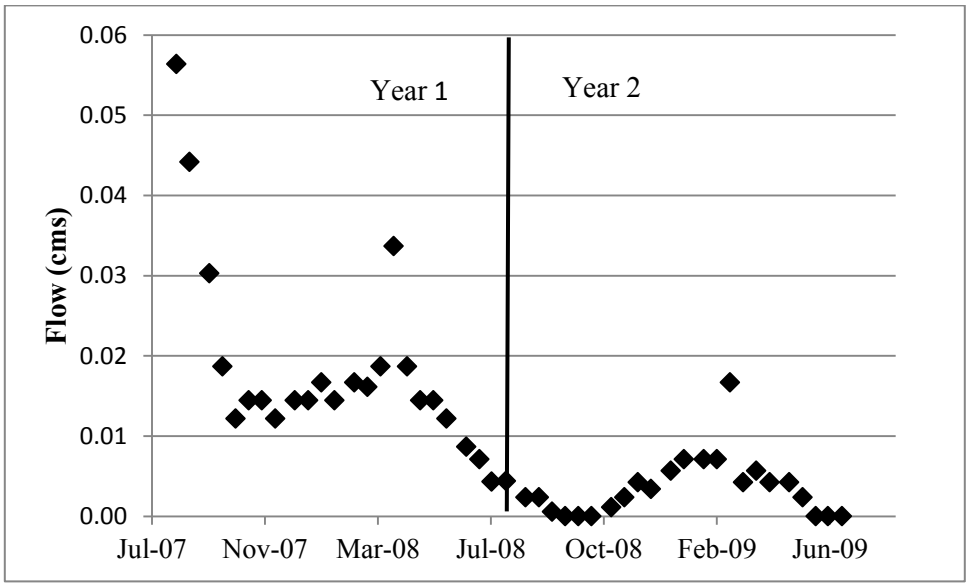
not stand in the bayous to drink from December through February (TCEQ 2007a). Using these assumptions from the TMDL, cattle spend 5.4 minutes/day in the stream on average overall throughout the year. Although this estimate is within the range observed by previous studies, it is 80% higher than the findings of this study, potentially overestimating the bacterial loading allocated to direct deposition from cattle into the creek and resulting in additional unnecessary restrictions. Because of this, evaluation of the time cattle spend in impaired water bodies using GPS collars or other suitable methods is suggested for TMDLs and other watershed planning projects to improve the accuracy of associated water quality models.

2.4.2 Flow

Median streamflow observed during Year 2 [0.003 m^3/s] was significantly lower ($p < 0.001$) than that observed during Year 1 (0.014 m^3/s). In the spring of 2008, the region entered a severe drought that continued throughout the remainder of the study (Figure 2.3). As a result, during the second year of the study when alternative water was provided, flow was reduced 79% compared to that observed during the previous year. Flow ceased in the creek for three months during Year 2 (mid-September through October 2008 and June 2009 through July 2009).

This drought not only impacted flow, but also impacted ranch management decisions (resulting in the increased stocking rate during Year 2), pasture condition (resulting in a decrease from excellent condition at the beginning of the study to poor condition in Year 2), and ultimately instream *E. coli* levels and loading.

Figure 2.3
Discharge (cms) measured in Clear Fork of Plum Creek, July 2007 through July 2009. Discharge measured on July 26, 2007 of 4.38 cms (154.83 cfs) is not shown.



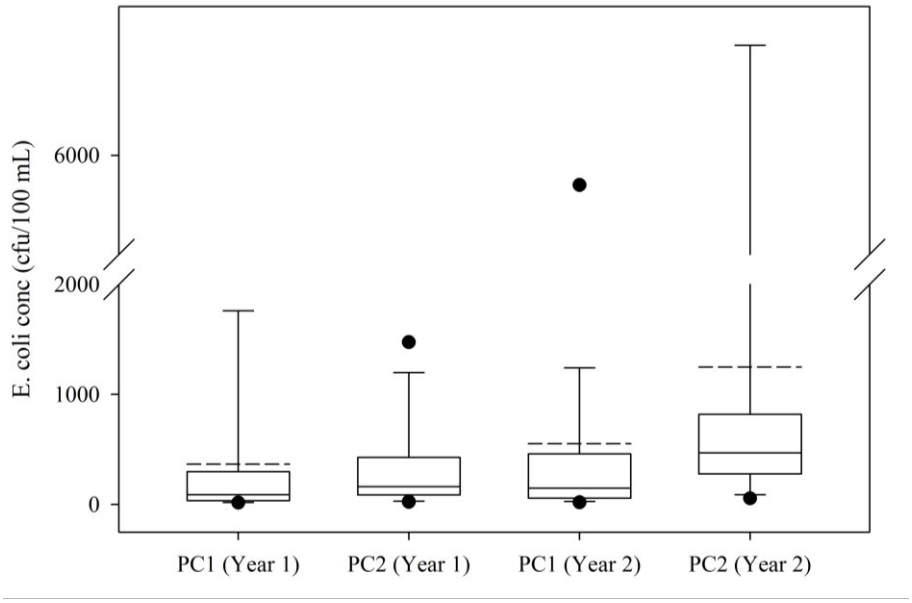
2.4.3 E. coli Levels

A total of 84 samples were collected from the two water quality stations (PC1 and PC2), of which 48 were collected during Year 1 (July 2007 to July 2008) and 36 during Year 2 (July 2008 to July 2009). Fewer samples were collected during Year 2 as a result of periods with no streamflow as previously noted (Appendix B).

E. coli levels at PC2 were correlated with those at PC1 throughout the study ($p < 0.01$) indicating that inflowing *E. coli* levels significantly impacted *E. coli* levels at the downstream site. Further, coefficient of determination values were moderate to high for both Year 1 (r^2=0.58) and Year 2 (r^2=0.83). However, *E. coli* levels increased between PC1 and PC2 during both years (Figure 2.4) indicating that loading from the study area contributed to *E. coli* concentrations at the downstream site (PC2). During Year 1,

median *E. coli* concentrations increased 73 cfu/100 mL ($p = 0.09$) from 88 cfu/100 mL at PC1 to 161 cfu/100 mL at PC2. During Year 2, the increase of 323 cfu/100 mL from 147 cfu/100 mL at PC1 to 470 cfu/100 mL at PC2 was significant ($p = 0.01$).

Figure 2.4
***E. coli* concentrations at PC1 and PC2 in Year 1 (no alternative water provided) and Year 2 (alternative water provided).**



This increase during Year 2 when alternative water was provided was unexpected and inconsistent with the estimated 43% reduction in direct deposition of *E. coli* calculated based on the GPS collar data. The extreme drought that reduced flows by 79% and influenced ranch management decisions to increase stocking rate 34% provide an explanation for much of this increase. With more cattle having access to the creek and less flow to dilute any direct deposition, it would be expected that concentrations would increase, even with the decreased amount of time cattle spent in the stream during Year 2. Based on Year 1 cattle numbers (56.7 AU), median flow (0.014 cms), and estimated

median daily deposition of *E. coli* in the stream (1.11E+07 cfu/AU/day), it was calculated that direct deposition would contribute 52 cfu/100 mL to the median inflowing (PC1) concentration (88 cfu/100 mL) thus, inflowing *E. coli* and direct deposition together (140 cfu/100 mL) represent an estimated 87% of the median *E. coli* concentration observed at PC2 during Year 1 (161 cfu/100 mL). Using the same method for Year 2, it was calculated that direct deposition would contribute 186 cfu/100 mL to the median inflowing (PC1) concentration (147 cfu/100 mL) thus, inflowing *E. coli* and direct deposition (333 cfu/100 mL) represent an estimated 71% of the median *E. coli* concentration observed at PC2 during Year 2 (470 cfu/100 mL).

This evaluation suggests inflowing *E. coli* concentrations, direct deposition by cattle, and reduced dilution resulting from reduced flow all contribute to the *E. coli* concentrations at PC2; however, they do not fully explain the concentrations observed. Approximately 13% of the *E. coli* during Year 1 and 29% during Year 2 are unaccounted for. A portion of the unaccounted *E. coli* likely results from the variability observed in the *E. coli* concentrations. *E. coli* concentrations were highly variable, with standard deviations greatly exceeding mean *E. coli* concentrations. Natural variability in *E. coli* concentrations resulting from the complex nature of bacterial deposition, survival, and transport is likely a significant factor in determining the observed *E. coli* concentrations (Harmel et al. 2010). Due to the drought and resulting increased stocking rate, degraded range condition, and reduced flows during Year 2, significant changes in the fate and transport of *E. coli* occurred making comparisons of the two years difficult.

Measurement uncertainty may have also contributed to data variability. McCarthy et al. (2008) found that combined uncertainty in discrete *E. coli* samples ranged from 15 to 67% and averaged 33%. However, because the field technician, collection methods, lab analyst, and lab methods used were consistent throughout the study, this impact is considered to be consistent across sites and years.

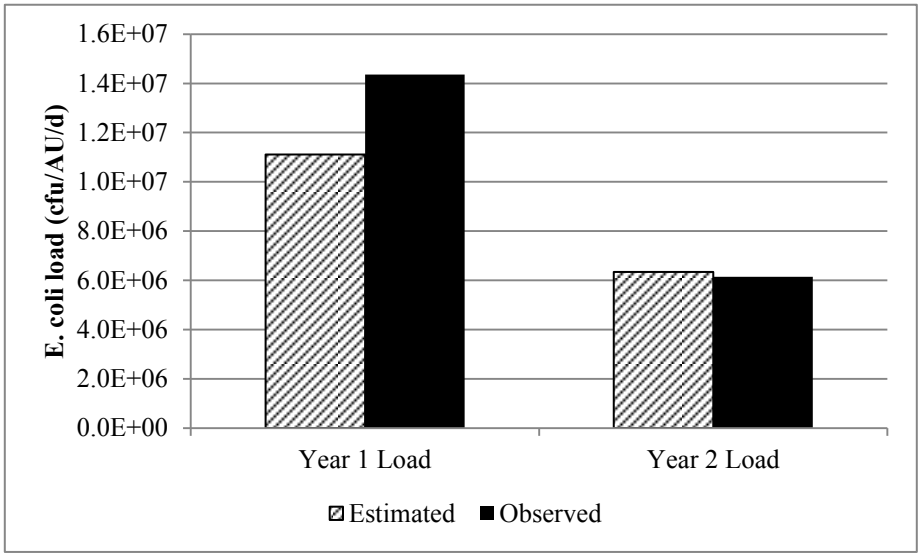
Finally, although not quantified, increased use of the creek by wildlife during the drought could have also impacted *E. coli* concentrations during Year 2. It is logical that wildlife would increasingly use the creek as other water sources in the area were depleted. Thus, even though use of the stream by cattle as documented by the GPS collars decreased significantly when alternative water was provided, increased wildlife use likely contributed to the overall increase in *E. coli* concentrations as well.

2.4.4 E. coli Loading

Contrary to the *E. coli* concentration results, *E. coli* loading (cfu/AU/day) was substantially lower during Year 2 when alternative water was provided (Figure 2.5). The median *E. coli* load in Year 2 (6.15E+06 cfu/AU/day) tended to be 57% lower than that observed during Year 1 (1.44E+07cfu/AU/d); however, the observed difference was not significant ($p = 0.47$). Due to the variability in the loading observed during Year 1, a 99% change in loading or greater would have been required to observe a significant difference in the loadings between years. Despite this, these results are remarkably similar to the estimated Year 1 and 2 *E. coli* deposition in the stream of 1.11E+07 and 6.34E+06 cfu/AU/d, respectively, calculated using the GPS collar data and published fecal coliform data.

Even though observed *E. coli* loading and those estimated using GPS collar data are remarkably similar and both indicated reductions of more than 40%, this study cannot conclusively attribute *E. coli* loading reductions to the alternative water source because of the lack of statistical significance of these observations, the significant decrease in flow observed during Year 2, and the observed increase in *E. coli* levels during Year 2.

Figure 2.5
Estimated and observed instream *E. coli* loading (cfu/AU/day) during Year 1 (no alternative water) and Year 2 (alternative water provided).



2.4.5 Turbidity

Median turbidity levels (Table 2.2) were typically 40% higher at PC1 than at PC2 indicating turbidity generally improved as the creek flowed through the ranch; however, differences were only significant for Year 1 ($p < 0.01$). Turbidity levels flowing into the study watershed played a greater role in determining the levels at PC2

during Year 2. During Year 2, turbidity at PC1 and PC2 were correlated (p = 0.01; r^2=0.36), unlike Year 1 when no correlation between sites was observed (p = 0.98, r^2=0.00). Analysis of covariance between observed turbidities in Years 1 and 2 indicated no significant treatment effect resulted from providing alternative water (p = 0.93).

Table 2.2
Turbidity (NTU) levels measured at PC1 and PC2 during Years 1 and 2.

Period	Statistic	PC1	PC2
Year 1	Mean	35	17
	sd	20	8
	Median*	29a	16b
	Max	62	31
Year 2	Mean	14	12
	sd	11	13
	Median*	10a	6a
	Max	43	47

* For each site, medians followed by same letter are not significantly different (α=0.05)

Turbidity was primarily measured to evaluate its use as a predictor of *E. coli* concentration, as streambed sediment disturbance is suspected to influence *E. coli* levels (Jackson et al. 2011). However, regression analysis results indicated turbidity was not a good predictor of *E. coli* concentrations in Clear Fork of Plum Creek (p = 0.51; r^2=0.01). Similarly, McDonald et al. (2006) did not observe a significant correlation between fecal *enterococci* and turbidity. This differs from the findings of Huey and Meyer (2010) that turbidity is an effective predictor of *E. coli* in the upper Pecos River basin in New Mexico. Also, Collins (2003) developed a statistical model to determine median *E. coli* concentrations based on turbidity that explained 70% of the observed *E. coli* variance.

Similarly, Brady et al. (2009) found that a model based on turbidity and rainfall performed well at predicting *E. coli* levels (81% correct responses) in the Cuyahoga River, Ohio. Thus, turbidity does have utility as a predictor in some watersheds; however, this must be determined on a case-by-case basis and used with caution.

2.5 Summary and Conclusions

Use of GPS collars was found to be a very useful tool, one that would benefit not only future BMP evaluations but also TMDL studies that utilize percent time cattle spend in streams for assessing direct deposition rates. Performing GPS collar studies can enhance water quality models allowing them to more accurately predict *E. coli* loading. In this study, GPS collars indicated the amount of time cattle spent in the stream could be reduced 43% from 3.0 to 1.7 minutes/AU/day by providing alternative off-stream water. As a result of this, direct deposition of *E. coli* into Clear Fork of Plum Creek was estimated to be reduced 4.76E+06 cfu/AU/day from 1.11E+07 cfu/AU/day when no alternative water was provided to 6.34E+06 cfu/AU/day once alternative water was provided. Observed pre- and post-treatment *E. coli* loads suggested similar reductions; however, this study could not conclusively attribute the observed *E. coli* loading reductions to alternative water because of the lack of statistical significance of these observations, the decrease in flow observed during Year 2, and the observed increase in *E. coli* levels during Year 2. A drought during Year 2, which reduced flows by 79% and influenced ranch management decisions to increase the stocking rate by 34%, may explain much of the increase in *E. coli* levels observed. Other probable factors impacting

observed *E. coli* levels include natural variability, changes in fate and transport due to the drought, and potential increased contributions from wildlife.

Although this study did not provide conclusive evidence of reduced *E. coli* levels resulting from providing alternative off-stream water supplies, this practice is still highly recommended due to the significant reductions observed in the time cattle spent in and near the stream, the 51% reduction in fecal coliform documented by Sheffield et al. (1997), and the 85 to 95% decrease in median base flow *E. coli* load found by Byers et al. (2005). These reductions are comparable to those provided by fencing of streams, which reduced *E. coli* 37 to 46% (Meals 2001, 2004) and fecal coliforms 30 to 94% (Brenner et al. 1994; Brenner 1996; Cook 1998; Hagedorn et al. 1999; Lombardo et al. 2000; Meals 2001; Line 2002; Line 2003; Meals 2004). Further, this study supports McIver (2004) who noted alternative water supplies alone would not achieve water quality improvements unless implemented in conjunction with good grazing management (i.e. appropriate stocking rate, evenly distributed grazing, avoiding grazing during vulnerable periods, and providing ample rest after grazing events). As a result of the severe drought, these principles were not adhered to and likely confounded improvements in water quality that could have been due to the provision of alternative water supplies.

Finally, unlike others, this study did not find turbidity to be a good predictor of *E. coli*. Thus, use of turbidity as an indicator must be assessed on a case-by-case basis and used with caution.

CHAPTER III

ASSESSMENT OF CATTLE GRAZING EFFECTS ON *E. COLI* RUNOFF

3.1 Overview

Runoff of *E. coli* and other fecal indicator bacteria from grazing lands has been identified as a significant source of bacterial contamination in need of reductions to improve water quality. Development of best management practices to address these bacterial issues is critical to the success of watershed restoration efforts, where grazing is a substantial contributor to the problem. The effect of grazing management was evaluated to assess its effectiveness as a best management practice. *E. coli* levels in runoff from grazed and ungrazed rangeland, improved pasture, and native prairie sites were monitored from November 2007 through October 2010.

The study found that rotational stocking, if timed appropriately, was a very effective practice for reducing *E. coli* runoff. The impact of grazing timing in relation to runoff events was more significant than the impact of grazing management (i.e. ungrazed, properly stocked or overstocked) or stocking rate. When runoff occurred more than two weeks following grazing, *E. coli* levels in runoff were decreased more than 88%. As a result of these findings, it is recommended that creek pastures and other hydrologically connected pastures be grazed during periods when runoff is less likely (e.g. summer and winter in much of Texas) and upland sites be grazed during rainy seasons when runoff is more likely to occur.

Background levels were relatively consistent among sites, with median levels ranging from 3,500 to 5,500 colony forming units per 100 mL. These substantial background levels should be considered when applying water quality models to develop total maximum daily loads and conducting other water quality assessment or implementation efforts. Impacts of non-domesticated animals on *E. coli* runoff were also significant, being responsible for over 80% of the loading in 2009 at three sites. Finally, although water quality standards are not currently applicable to edge-of-field runoff, it is notable that over 90% of samples exceeded Texas Water Quality Standards for *E. coli* as runoff will significantly increase instream concentrations during storm events. Based on the findings of this study, it is recommended that exemptions from the current standards be made for storm flows and wildlife, or additional research be conducted to accurately define bacterial quality for runoff and establish practical water quality standards.

3.2 Introduction

Livestock grazing on pasture and rangeland is frequently identified as a source of fecal indicator bacteria (i.e. *E. coli*, *Enterococcus*, and fecal coliforms) requiring reductions to improve surface water quality in Texas (TCEQ 2007c, 2008d). Previous studies have documented direct relationships between grazing and increased fecal coliform levels in streams and runoff (Doran and Linn 1979; Doran et al. 1981; Gary et al. 1983; Tiedemann et al. 1987; EPA 2001; Donnison et al. 2004). Contamination of streams can arise through direct deposition of feces, surface runoff, and subsurface flows; however, surface runoff is a key process for delivery of *E. coli* to streams (Collins et al. 2005). As such, best management practices (BMPs) which reduce surface runoff of

bacteria from grazing lands are critical to the success of water resource improvement and protection efforts in impaired water bodies.

The extent to which bacteria from grazing cattle affect water quality is generally a function of the number and size of cattle, the location of fecal deposits in relation to water bodies, characteristics of fecal deposition site, and bacterial survival between time of fecal deposition and runoff events (Larsen et al. 1994). As stocking rate increases, the quantity of manure and bacteria deposited on grazing lands increases (EPA 2003). *E. coli* levels can vary considerably in manure as a result of diet and season (Muirhead et al. 2006; Oliver et al. 2010) with *E. coli* levels in beef cattle feces on grazed pastures ranging from 1.0E+05 to 7.9E+05 colony forming units (cfu) per gram wet weight (McDowell et al. 2008) and 2.2E+05 to 1.3E+07 cfu/g dry weight (Sinton et al. 2007; Van Kessell et al. 2007; McDowell et al. 2008; Oliver et al. 2010). With grazing beef cattle excreting from 28 kg (63 lb) to 32 kg (71 lb) wet weight per animal per day (McDowell et al. 2008), *E. coli* deposition rates may be as high as 2.8E+09 to 4.2E+10 cfu/AU/day. Several studies suggest that instream coliform levels increase with increasing grazing intensity (Larsen et al. 1994; Gary et al. 1983); however, published fecal coliform levels in runoff do not appear to be significantly related to stocking rate (Appendix C).

The location of fecal deposition relative to variable source areas and water courses is an important factor determining potential for *E. coli* in fecal pats to be transported downstream (Tate et al. 2003). Runoff from cattle congregation areas (e.g. near watering sites, fences, gates, and bedding areas) can be a significant source of

bacteria in nearby streams. However, a more important factor is distance from stream with manure deposited 0.6 m (2 ft.) from a stream contributing 83% less bacteria and manure deposited 2.1 m (7 ft.) contributing 95% less than that manure deposited directly into a stream (Larsen et al. 1994).

Variability in bacterial levels in runoff results from differences in deposition site characteristics including soil type, slope, hydrology and drainage patterns, and management (Larsen et al. 1994; FCA 1999; Ferguson et al. 2003) as well as the quantity and intensity of rainfall (Ferguson et al. 2003). However, because bacteria are living organisms and their transport is complex and impacted by adsorption, straining (i.e. filtration), interception, entrapment, and sedimentation, bacterial levels in runoff are difficult to predict.

Further, bacterial levels in runoff and streams are greatly affected by their survival and potential re-growth in the environment. As such, timing of fecal deposition relative to runoff events impacts the potential for *E. coli* to be transported downstream (Tate et al. 2003). Generally, *E. coli* concentrations increase an order of magnitude or greater during the first 6 to 10 days following deposition, followed by a first order decline (Sinton et al. 2007; Van Kessel et al. 2007; Oliver et al. 2010) once moisture levels fall below 70% to 75%. However, their fate is not predictable under complex natural conditions which can result in disparate growth and survival (Van Elsas et al. 2011). If nutrients and energy sources are available and critical abiotic conditions are favorable, *E. coli* can survive and even grow in open environments. Even in the decline phase, bacterial populations in fecal pats remain metabolically active providing the

potential for continued growth should conditions become favorable (Thelin and Gifford 1983). Because fecal pats contain needed nutrients, *E. coli* can persist as long as water and temperature are suitable (Sinton et al. 2007). Fecal pats form a well-defined crust, typically within two days of deposition, which keeps the interior moist allowing *E. coli* to persist. Further, subsequent rainfall can rehydrate the fecal pat and stimulate *E. coli* regrowth (Sinton et al. 2007). As a result, the potential for bacterial contamination of water bodies by rainfall runoff can exist for long periods after cattle are removed from a site (Thelin and Gifford 1983; Larsen et al. 1994). *E. coli* has been observed to survive 30 to 365 days in soil, 10 to 182 days in cattle manure, 99 days in grass, and 35 days in water depending on the chemical, physical, and biological composition of feces, soil, and water it was deposited in (Crane and Moore 1986; University of Wisconsin 2007a, 2007b). Not only can *E. coli* persist in fecal pats for long periods, it can also remain as high as 10^4 cfu/g five months after deposition. Sinton et al. (2007) determined that the time to achieve a 90% decrease (T_{90}) in *E. coli* levels in fecal pats ranged from 38 to 66 days, depending on season with *E. coli* being more persistent during spring, summer, and fall and less persistent in the winter. As a result of this persistence, instream fecal coliform levels may remain elevated for up to nine months following cattle removal (Tiedemann et al. 1988).

Nevertheless, risk of pollution is greatest immediately after deposition of manure. Thelin and Gifford (1983) found fecal coliform levels exceeding 10^6 cfu/100 mL were released from fecal pats less than 5 days old, while 30 day old fecal pats released fecal coliform levels of 4×10^4 cfu/100 mL (96% reduction) indicating

downstream water quality is partially dependent on days since grazing ceased. If runoff occurred the day of fecal deposition, 58 to 90% of the fecal coliform in the manure may be transported in the runoff (Crane et al. 1983, Coyne et al. 1995). Similarly, *E. coli* levels in runoff in pastures actively grazed by sheep may range from 10^5 to 10^6 cfu/100 mL, while levels in runoff occurring 75 days after grazing ranged from 10^3 to 10^4 (Collins et al. 2005). Conversely, if weather conditions are dry and deposition is on well-drained soils, bacterial runoff is greatly reduced (Ogden et al. 2001).

Practices aimed at reducing bacterial runoff from grazing lands generally focus on maintaining adequate ground cover to filter runoff, enhance infiltration, reduce runoff (FCA 1999; Ball et al. 2002; NRCS 2007), and promote soil filtration and the removal of bacteria by sorption, inactivation, and predation (Ferguson et al. 2003). Proper grazing management is essential to maintaining adequate ground cover and as such is critical to addressing bacterial loading from grazing lands (NRCS 2007). Proper grazing management begins with using the correct stocking rate and balancing animal demand with available forage (Redmon 2002; EPA 2003; Fitch et al. 2003; NRCS 2007), and also includes distributing grazing evenly, avoiding grazing during vulnerable periods, and providing ample rest after grazing. With careful planning of grazing, forages can be maintained or improved while also providing water quality benefits (Larsen et al. 1994).

Because grazing lands are the largest land use in Texas, watershed scale *E. coli* runoff data from this land use are needed to support ongoing and future TMDL and watershed planning activities. Further, data are needed on background *E. coli* levels in runoff, *E. coli* levels in runoff from grazed sites with varying levels of grazing

management and grazing intensity, and impacts of best management practices on these levels. Published data on *E. coli* runoff from grazed pastures are sparse (McDowell et al. 2008) with much of the existing data being derived from laboratory experiments and instream monitoring of grazed watersheds. However, laboratory derived data are not sufficient for development of accurate models at the farm and watershed scale (Oliver et al. 2010), and monitoring of streams does not allow the discrimination of surface runoff, subsurface flows, direct deposition, and resuspension of sediment bound *E. coli* (Collins et al. 2005). Further, most existing research was conducted in the Pacific West and Midwest, examined primarily fecal coliform levels, and focused on the detriments of grazing on streams instead of assessing BMPs for abating these effects. Although this information is valuable, it is not universally transferable to Texas and similar climatic regions (Agouridis et al. 2005). The objective of this study was to assess *E. coli* levels in runoff at the small watershed scale from grazed and ungrazed pastures and assess the effect of grazing management (e.g. ungrazed, properly stocked, overstocked), timing of grazing, and stocking rate on *E. coli* levels in runoff.

3.3 Materials and Methods

3.3.1 Site Descriptions

Assessment of the effect of grazing on *E. coli* levels in runoff took place at seven sites distributed among three locations in Texas (Table 3.1).

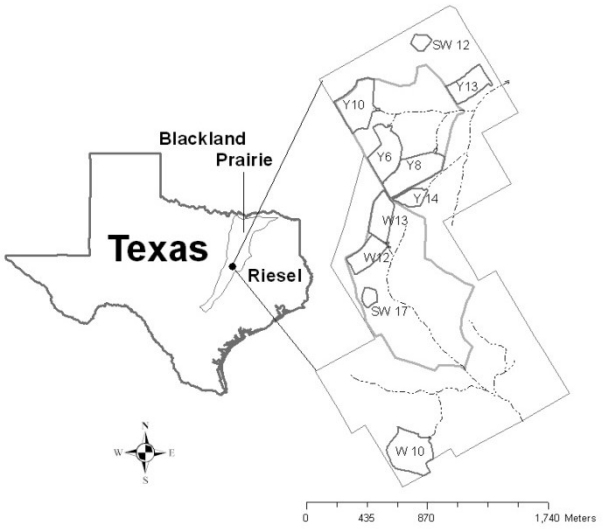
Table 3.1
Locations and characteristics of watershed sites.

Site	Lat/Long	Vegetation	Management
USDA-Agricultural Research Service (ARS) Watersheds, Riesel, TX			
SW12	31° 28’48”N / 96° 52’59”W	Native grassland	Ungrazed
SW17	31° 27’45”N / 96° 53’14”W	Bermudagrass	Properly stocked
Welder Wildlife Foundation, Sinton, TX			
WWR1	28° 6'55.97"N / 97°21'20.82"W	Native rangeland	Ungrazed
WWR3	28° 6'52.60"N / 97°21'13.83"W	Native rangeland	Properly stocked
Beef Cattle Systems Center, College Station, TX			
BB1	30° 31'44.3"N / 96°24'58.3"W	Tifton 85 bermudagrass	Ungrazed
BB2	30° 31'47.5"N / 96°24'57.7"W	Tifton 85 bermudagrass	Properly stocked
BB3	30° 31'47.7"N / 96°24'57.9"W	Tifton 85 bermudagrass	Overstocked

The USDA-ARS Grassland, Soil and Water Research Laboratory in Riesel, TX, was the location of two sites. This research site has been one of the most intensively monitored hydrological research sites in the country since establishment in the 1930s (Harmel et al. 2007). It is located in the Texas Blackland Prairies ecoregion (Omernik 1987) on the border of Falls and McLennan counties (Figure 3.1).

Houston Black clay soils dominate the region. This soil is very slowly permeable when wet; however, preferential flow associated with soil cracks contributes to high infiltration rates when the soil is dry. Mean annual rainfall is approximately 91 cm (36 in). Thirteen runoff stations are operated at the research site to monitor sub-watersheds under both pasture and cropland management. Two 1.2-ha sites were used to evaluate grazing management, SW12 and SW17. The average slope of SW12 is 3.8%, while slope averages 1.8% at SW17.

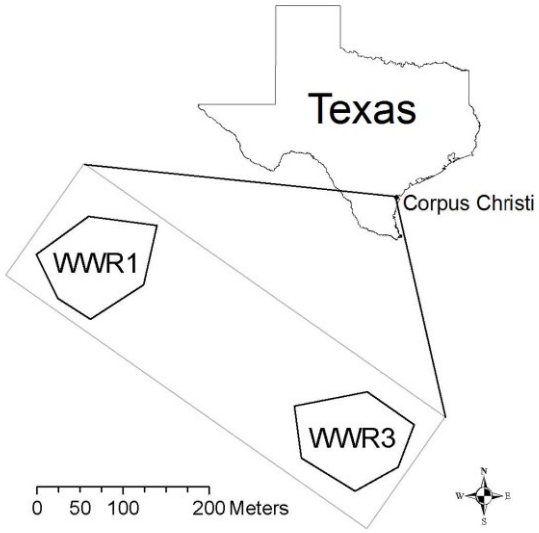
Figure 3.1
Riesel watersheds.



The Rob and Bessie Welder Wildlife Refuge is a 3,156 ha (7,800 ac) native wildlife refuge 13 km (8 mi) north of Sinton, TX, in the Western Gulf Coastal Plain ecoregion of Texas (Omernik 1987) (Figure 3.2). Three 1-ha (2.4 ac) watershed sites were established in 2000 to study the effects of shrub management on water quality and quantity on rangeland. Berm failure on site WWR2 prevented data collection there during the study.

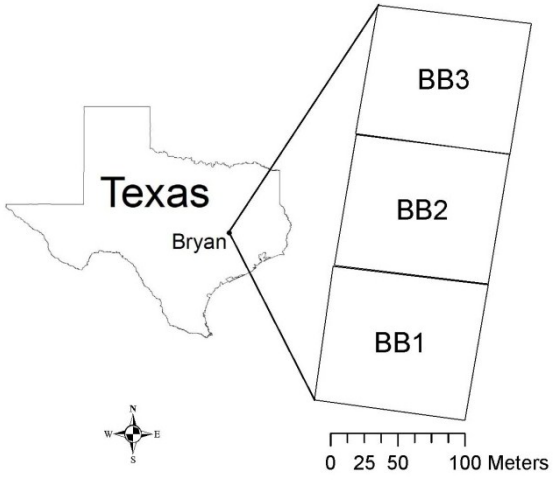
The Welder has never been cultivated and has historically been managed for livestock (Stewart 2003). Precipitation averages 90 cm (36 in) annually. The watershed sites are located on chaparral-mixed grass communities on the east and west sides of Paloma draw, approximately 6 km (4 mi) from the foundation headquarters. Victoria clay (0 to 1% slopes) soils underlay the upper quarter to third of the watershed sites and Monteola clay (5 to 8% slopes) soils underlay the remainder. Both are classified as Hydrologic Soil Group D soils.

Figure 3.2
Welder Wildlife Refuge watersheds.



The final site was the Texas A&M University, Department of Animal Science Beef Cattle Systems Center (BCSC) located west of College Station on the Brazos River. This site was used primarily for row crop production prior to initiation of this study. In October 2007, berms were constructed around three 1-ha watershed sites (Figure 3.3), and the slopes modified so each site would drain to the watershed outlet. Following berm construction, all sites were established to Tifton 85 bermudagrass. These sites are located in the East Central Texas Plains ecoregion (Omernik 1987). Annual precipitation averages 102 cm (40 in). Soils within the study area are classified as Belk clay, a heavier-textured alluvial soil (Hydrologic Soil Group D) found along the Brazos River. Measured slope averages 0.2%.

Figure 3.3
Beef Cattle Systems Center watersheds.



3.3.2 Pasture Management

All sites were fenced so that cattle grazing could be controlled. Three sites were ungrazed (SW12, WWR1, BB1), three were properly stocked (SW17, WWR3, BB2), and one site, BB3, had double the stocking rate of the properly stocked BB2. Site SW12 is notable in that this ungrazed native prairie reference site has not been stocked with cattle or other livestock since the Riesel Watersheds were established in 1937 (Harmel et al. 2006a). Stocked sites were not grazed continuously; instead, six to seven grazing events occurred at each site during the study (Table 3.2). Grass height was monitored monthly to determine timing of grazing. Properly stocked sites were generally stocked once grass height exceeded 6 in, and cattle were removed once grass height was reduced to 3 in as recommended by the USDA Natural Resources Conservation Service Conservation Practice General Specifications for Prescribed Grazing (Practice Standard No. 528).

Table 3.2
Grazing events.

Locale	Site	Start	End	Stocking Rate (AU/ha)*	Stocking Rate (AUD/ha)†	Stocking Rate ha/AUY (ac/AUY)‡
Riesel	SW17	9/12/07	11/14/07	1.1	70	2.6 (6.4)
	SW17	2/25/08	6/2/08	1.1	109	1.7 (4.1)
	SW17	11/5/08	4/21/09	1.1	185	1.6 (3.9)
	SW17	5/1/09	6/3/09	1.1	37	1.7 (4.1)
	SW17	7/15/09	11/6/09	1.1	126	1.1 (2.6)
	SW17	5/3/10	5/24/10	1.1	23	2.3 (5.6)
	SW17	7/19/10	8/27/10	1.1	43	2.5 (6.2)
Welder	WWR3	12/1/07	2/13/08	0.4	31	11.6 (28.7)
	WWR3	4/18/08	4/28/08	2.6	26	6.4 (15.7)
	WWR3	10/20/08	10/25/08	2.9	15	5.1 (12.5)
	WWR3	4/27/09	5/1/09	3.4	14	11.7 (29.0)
	WWR3	6/21/10	6/22/10	2.6	3	140.9 (348.0)
	WWR3	9/1/10	9/11/10	2.6	26	12.8 (31.6)
BCSC	BB2	1/12/09	1/16/09	4.0	16	22.8 (56.3)
	BB2	5/22/09	6/5/09	6.1	79	3.8 (9.5)
	BB2	8/7/09	8/8/09	6.4	6	3.6 (8.9)
	BB2	8/12/09	8/19/09	6.4	46	2.5 (6.1)
	BB2	11/12/09	11/17/09	18.4	90	1.5 (3.8)
	BB2§	2/1/10	2/8/10	2.5	17	1.5 (3.8)
	BB2	6/21/10	7/2/10	17.7	194	1.7 (4.3)
	BB3	1/12/09	1/16/09	8.0	32	11.4 (28.2)
	BB3	5/22/09	6/5/09	13.4	175	1.8 (4.4)
	BB3	8/7/09	8/8/09	12.8	13	1.7 (4.1)
	BB3	8/12/09	8/19/09	12.8	92	1.2 (2.9)
	BB3	11/12/09	11/17/09	36.8	180	0.7 (1.8)
	BB3§	2/1/10	2/8/10	2.5	17	0.8 (1.9)
	BB3	6/21/10	7/2/10	31.7	346	1.0 (2.5)

* Animal units per hectare
† Animal unit days per hectare
‡ Annual stocking rate in hectares per animal unit and acres per animal unit
§ Electric fences failed allowing short-term cattle access

These grazing events allowed evaluation of the impact of a wide range of stocking rates on *E. coli* runoff. It should be noted that electric fences failed on several

occasions at the Beef Cattle Systems Center sites. At BB2 and BB3, electric fences failed on February 1 to 8, 2010; however, minimal grazing occurred on the sites during this time. At BB1, the electric fence failed on March 11, 2009, November 13, 2009 and February 1, 2010 allowing limited cattle access to the site ranging from one to seven days per failure. To the extent possible, fecal pats were removed from BB1 once the fence was restored. However, from February 1 to 8, 2010, several runoff events occurred while cattle had access to the site and are noted in the analysis.

3.3.3 Edge of Field Sampling Procedures

As recommended by Harmel et al. (2006b), flow-weighted composite edge-of-field runoff samples from the seven watershed sites were collected using ISCO 6712 (ISCO Inc., Lincoln, NE) full-size portable samplers with single bottle configuration into surface disinfected polyethylene 15 L (4 gal) round bottles. Flow from each watershed site was measured with ISCO 730 Module bubble flow meters. Flow data were downloaded at least monthly using an ISCO 581 Rapid Transfer Device (RTD). Sites BB1, BB2, BB3, WWR1, and WWR3 were equipped with berms and 90^{o} v-notch weirs to aid in collection and measurement of runoff, while SW12 and SW17 were monitored using 0.9 m (3 ft) H-flumes. Runoff was monitored for three years, from November 2007 through October 2010, at WWR1, WWR3, SW12, and SW17. Runoff at sites BB1, BB2, and BB3 was monitored for two years, from November 2008 through October 2010. The ISCO samplers at sites WWR1, WWR3, BB2, and BB3 enabled when the water level exceeded 6 mm (0.02 ft) and then collected 50 mL for every 4.2 m^3 (150 ft^3) of runoff. The ISCO sampler at BB1 enabled when the water level exceeded 6 mm (0.02 ft) and

then collected 25 mL for every 2.1 m^3 (75 ft^3) of runoff. The ISCO samplers at SW12 and SW17 enabled at 60 mm (0.20 ft) and sampled at 16 m^3 (566 ft^3) intervals. All ISCO samplers were programmed to rinse sample tubing with ambient water prior to collection of each sample. Following each event, samples were retrieved as soon as possible (typically within 24 hr) from the ISCO samplers and placed on ice in a cooler for transport to the lab where they were stored at 4^{o}C (39^{o}F) until analysis.

3.3.4 Analytical Methods

Analysis of water samples for *E. coli* was conducted within 6 hr of retrieval. *E. coli* were enumerated using EPA Method 1603 (EPA 2006). If counts were greater than 200 colonies at the highest dilution, the count was reported as too numerous to count (TNTC). Results were reported as cfu (colony forming units)/100 mL.

3.3.5 Statistical Analysis

The statistical software, Minitab (Minitab Inc., State College, PA), was used for all statistical calculations. Basic statistics and graphical summaries of each dataset were created to evaluate means, medians, percentiles (10th, 25 th, 75 th, and 90 th), and normality (using Anderson-Darling Normality Test). As a majority of datasets were not normally distributed, all were evaluated with nonparametric statistics. The Mann-Whitney statistical test was used to assess differences in median concentrations and loads observed between sites, cattle presence, years and stocking rate. Regression analysis was also used to evaluate *E. coli* decline rates in runoff. An alpha level of 0.05

was accepted as a minimum level of significance; thus, results were considered statistically significant when $p < 0.05$.

3.4 Results and Discussion

3.4.1 Comparison of E. coli Concentrations among Sites

E. coli concentrations were measured in 127 water samples collected from the seven sites during the 3 yr study (Appendix D). Observations varied greatly with standard deviations exceeding mean values at most sites (Table 3.3). When *E. coli* concentration data from all years at each site were combined and evaluated, potential grazing effects on median *E. coli* levels in runoff were only detected at Riesel. Median *E. coli* concentrations at the ungrazed SW12 were 67% lower than those observed at the properly grazed SW17 ($p = 0.03$). No significant differences were observed in median *E. coli* concentrations between sites at either the BCSC or Welder Wildlife Refuge. However, the mean *E. coli* concentration of the overstocked BB3 greatly exceeded those observed at the properly stocked BB2 or ungrazed BB1 at BCSC.

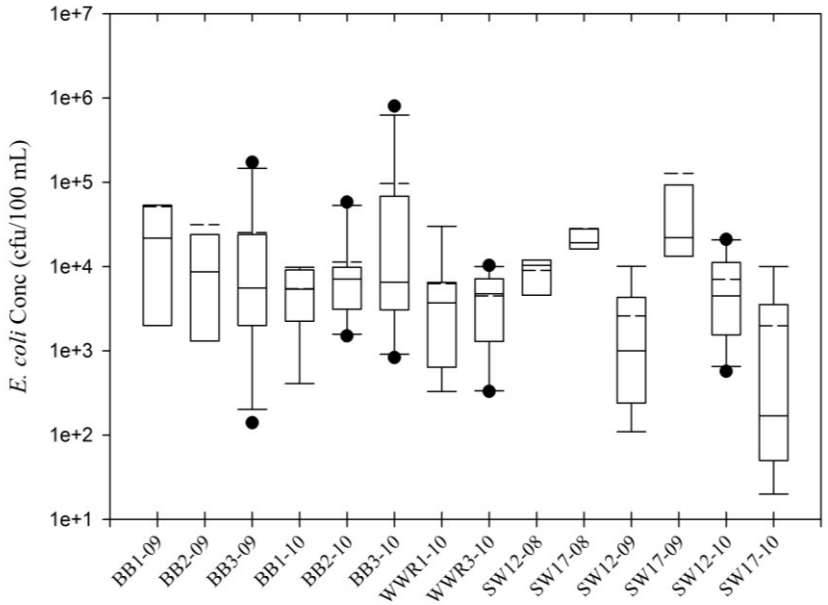
Table 3.3
Summary statistics for *E. coli* concentration data (cfu/100 mL).

Locale	Site	Grazing	Mean	sd	Min.	Q1	Median*	Q3	Max.
BCSC	BB1	Ungrazed	27,083	62,494	410	2,250	7,600ab	22,900	261,000
	BB2	Proper	20,210	42,379	980	2,281	7,100ab	15,107	181,000
	BB3	Overstock	62,469	170,689	140	2,100	5,591ab	24,000	800,000
Welder	WWR1	Ungrazed	6,286	9,241	330	640	3,700ab	6,480	30,000
	WWR3	Proper	4,475	3,288	330	1,298	4,750a	7,145	10,300
Riesel	SW12	Ungrazed	5,932	5,737	110	1,200	4,450a	9,775	21,000
	SW17	Proper	51,548	161,587	20	1,003	13,500b	27,750	800,000

* For all sites pooled together, medians followed by same letter are not significantly different (α=0.05).

To better assess the effects of grazing, annual statistics for each site were evaluated (Figure 3.4). This assessment revealed considerable spatial and temporal variability in the median annual *E. coli* concentrations at both grazed and ungrazed sites. However, as with the combined data, only the Riesel sites (SW12 and SW17) exhibited significantly different median annual *E. coli* concentrations. Interestingly, the highest and lowest median annual concentrations were observed at the properly grazed SW17. The median *E. coli* concentration at the ungrazed SW12 was 46% lower than the concentration at the grazed SW17 during 2008 ($p = 0.01$) and 95% lower in 2009 ($p < 0.01$). Conversely, the median annual *E. coli* concentration at the grazed SW17 was significantly (96%) lower than those at SW12 in 2010 ($p = 0.03$). This demonstrated the extreme temporal variability observed with *E. coli* concentrations.

Figure 3.4
***E. coli* concentrations in runoff from Beef Cattle Systems Center sites (BB1, BB2, BB3), Welder Wildlife Refuge sites (WWR1, WWR3), and Riesel sites (SW12, SW17) in 2008 (-08), 2009 (-09), and 2010 (-10).**



3.4.2 Impact of Non-Domesticated Animals

It is noteworthy that the second highest median annual *E. coli* concentration was observed at the ungrazed BB1 during 2009. *E. coli* levels at BB1 were inexplicably high in 2009. This was the case at all BCSC sites during October 2009 (Table 3.4). At the ungrazed BB1, median *E. coli* concentrations increased an order of magnitude and the maximum observed increased almost three orders of magnitude over levels observed throughout the rest of the study. At the two grazed sites, median concentrations increased half an order of magnitude, and maximum concentrations were approximately an order of magnitude higher than those observed during similar destocked periods throughout the rest of the study. There was no grazing during this period, and there had not been any since two months prior (i.e. last grazed in early-August 2009). Because *E. coli* concentrations in the first runoff event in October at both grazed sites were comparable to those observed during other ungrazed periods and because maximum concentrations were not observed until the fourth runoff event in October, it does not appear the increase could be attributed to the August 2009 grazing treatment. This was confirmed by qPCR analysis of bovine-associated *Bacteroides*. Thus, this suggests sources other than grazing livestock can have a tremendous impact on *E. coli* runoff from grazing lands. Although wildlife and other non-domesticated animal activity were not documented at the sites during this period, their presence offers the only plausible explanation. Possible sources include feral hogs, which are common along the Brazos River floodplain and frequently seen at the BCSC, and migratory birds.

Table 3.4
Comparison of median and maximum *E. coli* concentrations (cfu/100 mL) at BCSC sites during October 2009 to those observed during other ungrazed periods.

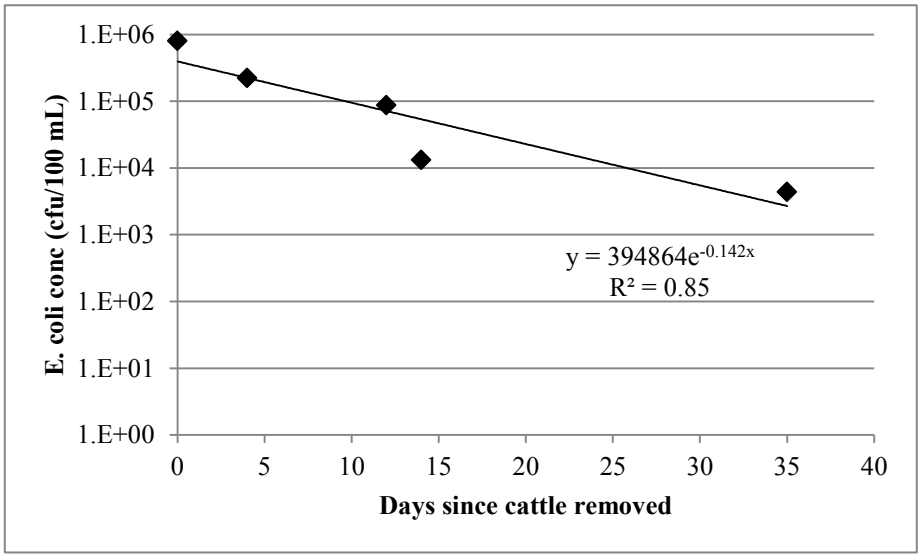
Site	Stat	October 2009	Excluding Oct 2009 & grazed periods
BB1	Median	49,926a	4,400b
	Max	261,000	9,800
BB2	Median*	23,935a	4,150b
	Max	181,000	12,200
BB3	Median*	15,000a	3,500b
	Max	172,500	24,000

* Medians at each site followed by same letter are not significantly different (α=0.05).

3.4.3 Impact of Cattle Presence during Rainfall Events

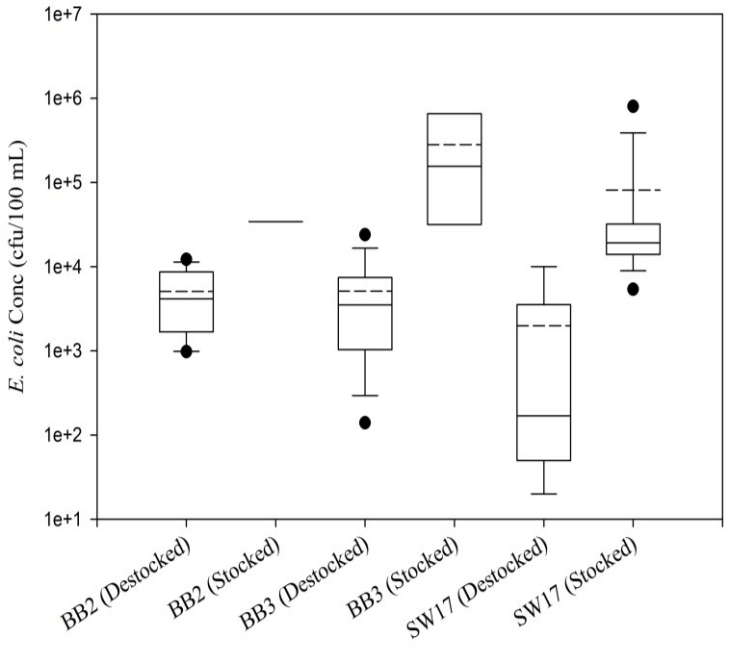
Throughout the study, all sites were rotationally stocked, meaning cattle did not graze the sites continuously. This study revealed runoff events occurring while cattle were actively grazing and within two weeks of the cattle being removed (period referred to as "stocked") generally resulted in the highest *E. coli* levels in runoff from the grazed sites. By two weeks following removal of cattle from a site, *E. coli* levels in runoff decreased substantially and after approximately 30 days, *E. coli* values had declined to background levels. This decline was best observed at BB3 (Figure 3.5) where *E. coli* levels declined from 800,000 cfu/100 mL when cattle were actively grazing to 4,400 cfu/100 ml 35 days after cattle were removed, and similar observations were made at BB2 and SW17. Estimated die-off rates ranged from 0.039/day to 0.142/day and averaged 0.090/day. At these rates, the time to achieve a 90% decrease (T_{90}) in *E. coli* levels ranged from 16 to 59 days and averaged 26 days.

Figure 3.5
***E. coli* levels at BB3 during and following grazing from November 12 to 17, 2009.**



Median *E. coli* concentrations were significantly higher when BB2 ($p = 0.03$), BB3 ($p < 0.01$), and SW17 ($p < 0.01$) were stocked (Figure 3.6) than when they were destocked. At only WWR3 was this relationship not observed ($p = 0.06$). However, this can be easily explained by the very limited stocking that occurred prior to the three runoff events at WWR3 while it was stocked. Due to a very severe drought during 2008 and 2009, this site had not been stocked for over a year and following this drought, it was only lightly stocked for a very short duration (i.e. three AU days (AUD) prior to the first event and 33 AUD prior to the other two events). As such, WWR3 was not included in Figure 3.6 or subsequent analysis.

Figure 3.6
Comparison of *E. coli* levels at grazed sites when runoff occurred during or within two weeks of grazing (Stocked) and more than two weeks after grazing (Destocked). Data from October 2009 at BB2 and BB3 were not included in analysis.



Results from SW17 are particularly noteworthy. Coincidentally, during 2008 and 2009, every runoff event occurred while cattle were actively grazing the site except for one which occurred less than two weeks after cattle were rotated to another pasture. In 2010, again by coincidence, every runoff event occurred while the site was destocked. This resulted in the median *E. coli* concentrations at SW17 being significantly higher than concentrations observed at the ungrazed SW12 in 2008 and 2009 and SW17 levels being significantly lower than SW12 in 2010 as shown in Figure 3.4. This was not planned, but it was fortuitous as it offered insight into the effectiveness of rotational stocking as a management strategy for reducing *E. coli* runoff; and more importantly the significance of timing of grazing in relation to runoff events.

Reductions in *E. coli* concentrations of 88% at BB2, 98% at BB3, and 99% at SW17 were observed when sites were destocked when runoff occurred, thus demonstrating the importance of timing of grazing on bacterial levels. Similarly, Lewis et al. (2010) found that fecal coliform concentrations and loads in runoff occurring more than two weeks after application of dairy manure were 80% lower than those occurring within two weeks of application. Meals and Braun (2006) found that *E. coli* levels in runoff from plots receiving 30-day-old dairy manure were 97% lower than those receiving fresh manure. Gary et al. (1983) and Tiedemann et al. (1987) also found that presence of livestock significantly affected instream bacteria levels. Tiedemann et al. (1988) found instream fecal coliform levels were 88% lower when cattle were not present than when they were present.

This finding suggests creek pastures and other hydrologically critical areas would benefit from rotational stocking with grazing being deferred on such pastures during rainy periods in preference of upland less hydrologically connected sites. More data are needed to confirm this finding and evaluate the impacts of this practice on a watershed scale. Additionally, work is needed to assess the impacts of continuous stocking on runoff. Previous work by Sovell et al. (2000) has shown that in stream fecal coliform levels were consistently higher at continuously stocked sites than at rotationally stocked sites in southeastern Minnesota.

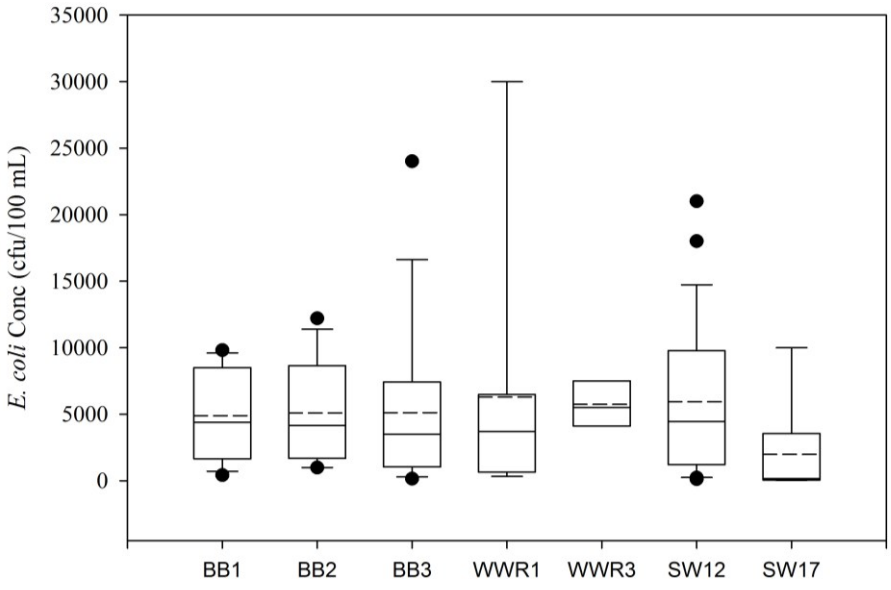
3.4.4 Assessment of Background E. coli Levels

As referenced in the previous section, a relatively consistent “background” level of *E. coli* in runoff was observed at all sites. Once those *E. coli* concentrations observed

during October 2009 at BCSC (attributed to non-domesticated animals) and those attributed to grazing events were removed, the remaining concentrations at all sites were remarkably similar (Figure 3.7) with means ranging from 5,000 to 6,000 cfu/100 mL and median values ranging from 3,500 to 5,500 cfu/100 mL. With the exception of SW17, median background *E. coli* concentrations were not significantly different among sites.

There are a range of possible sources for this background *E. coli*, including rodents, birds, and other wildlife populations in addition to indigenous *E. coli* populations residing in the soil. For example, Oliver et al. (2010) detected *E. coli* levels ranging from below detection to 10^6 cfu/g dry soil between fecal pats on grazed sites which served as an additional chronic source of *E. coli* in runoff.

Figure 3.7
Background *E. coli* levels observed at each site. Concentrations from October 2009 at BCSC sites and those attributed to grazing events were removed.



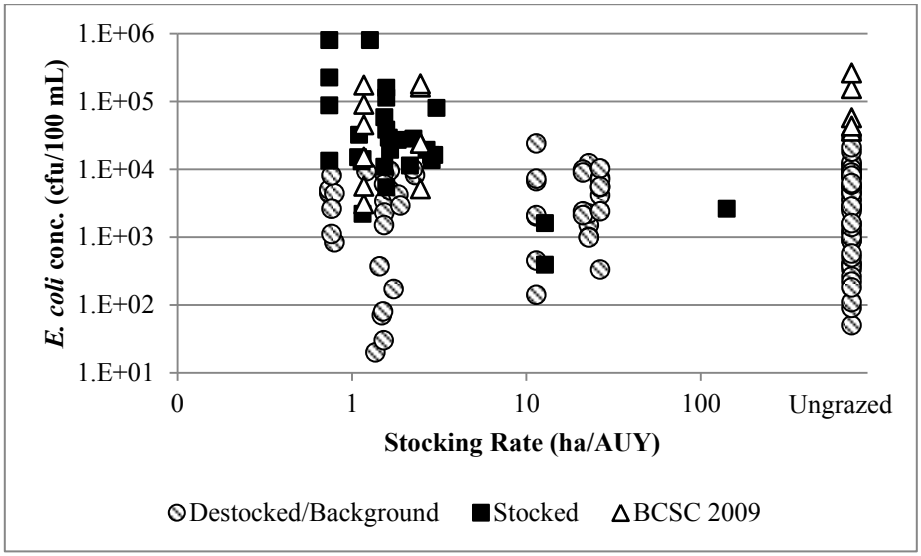
Regardless, background *E. coli* concentrations should be considered when developing TMDLs and watershed plans, conducting modeling to support these activities, and most importantly, when applying water quality standards to storm flow (Harmel et al. 2010). Current models used to assess bacterial loads often do not account for background levels, attributing *E. coli* loads to a relatively limited list of possible sources (e.g. human, livestock, wildlife). As such, they potentially over-allocate loads and load reductions to these categories without consideration of background levels.

3.4.5 Comparison of Stocking Rate (SR) and E. coli Concentration

The annual stocking rate was compared to the *E. coli* concentration for each sampling event (Figure 3.8) as previous studies indicated fecal coliform levels were dependent on livestock density (Tiedemann et al. 1987). Annual stocking rate (i.e. the average stocking rate for the twelve months immediately preceding the runoff event) was selected for this comparison as it is most commonly used in grazing recommendations as well as modeling exercises to evaluate *E. coli* loadings. Stocking rates varied substantially at each of the grazed sites throughout the study (Table 3.2).

When data are evaluated in the context of cattle presence during runoff event, background levels, and non-domesticated animal dominated events observed at BCSC in 2009 (Figure 3.8); it is apparent that stocking rate had some effect. However, presence of cattle on the site during or immediately preceding a runoff event had a much greater impact.

Figure 3.8
Comparison of annual stocking rate to *E. coli* levels associated with destocked/ background, stocked or recently stocked, and non-domesticated animal (BCSC 2009) dominated conditions.



In general, highest *E. coli* concentrations were observed when the annual stocking rate was heavier than 1.3 hectares per AU year (ha/AUY) (3.2 ac/AUY). In comparison, Gary et al. found that instream fecal coliform levels were significantly higher when stocking rate was 3.4 ha/AUY (8.4 ac/AUY) or heavier (1983). No significant differences in median concentrations were observed between stocking rates of 0.7 to 3.1 ha/AUY (1.7 to 7.7 ac/AUY). However, when the stocking rate was 11 ha/AUY (27 ac/AUY) or lighter, median *E. coli* levels were significantly lower than levels observed at stocking rates of 0.7 to 3.1 ha/AUY (1.7 to 7.7 ac/AUY) and did not exceed background levels (based on three observations). Similarly, Gary et al. (1983) observed that instream fecal coliform levels did not exceed background levels when stocking rates were 6.1 ha/AUY (15.1 ac/AUY) or 13.5 ha/AUY (33.4 ac/AUY).

Further, Buckhouse and Gifford (1976) found no significant differences in total or fecal coliform concentrations in runoff from ungrazed sites and sites grazed at 2 ha/AUM (i.e. 24 ha/AUY or 59.3 ac/AUY). Thus, based on these data, pastures stocked heavier than 3.1 ha/AUY (7.7 ac/AUY) should be the focus of initial implementation efforts when addressing bacterial impairments.

Overall, our results, which failed to find a strong link between *E. coli* concentrations and stocking rate, agree with what is observed in published literature (Appendix C). This is likely because at the watershed scale, watershed characteristics play a greater role in determining bacterial levels in runoff than number of animals or stocking rate (Tiedemann et al. 1987, 1988).

3.4.6 E. coli Loading

Annual *E. coli* loading from ungrazed sites generally ranged from 4.0 to 8.0E+10 cfu/ha while annual loading from actively grazed sites generally ranged from 8.0 to 40.0E+10 cfu/ha (Table 3.5). Exceptions were observed at SW17 in 2010, when no runoff occurred while cattle were grazing, and the BCSC sites in 2009 – likely due to the effects of non-domesticated animals. The effects of non-domesticated animals on the annual loadings at the BCSC in 2009 are conspicuous (contributing 80 to 99% of the load in 2009), especially when compared to the stocking rate and corresponding loading values in 2010.

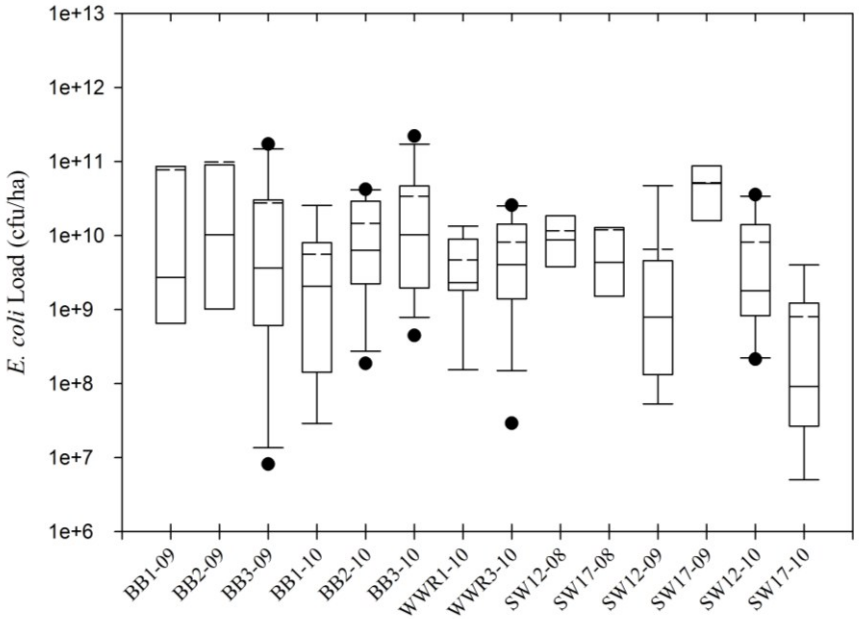
Table 3.5
Comparison of annual *E. coli* loading and stocking rate (AU day/ha).

		AUD/ha			*E. coli* load (cfu/ha)		
Location	**Site**	2008	2009	2010	2008	2009	2010
BCSC	BB1	—	0	17*	—	6.17E+11	5.02E+10
	BB2	—	148	302	—	7.89E+11	1.46E+11
	BB3	—	311	543	—	3.02E+11	4.08E+11
Welder	WWR1	0	0	0	—	—	4.19E+10
	WWR3	83	16	33	—	—	8.14E+10
Riesel	SW12	0	0	0	6.96E+10	5.89E+10	8.14E+10
	SW17	128	354	75	8.35E+10	4.14E+11	7.22E+09

* Electric fences failed allowing short-term cattle access.

Assessment of median event *E. coli* loads at each site in 2008, 2009, and 2010 reveals considerable spatial and temporal variability with significant differences in median event loads only being observed at the Beef Cattle Systems Center in 2010 and Riesel in 2008 and 2010 (Figure 3.9). Median event loads at the ungrazed BB1 were 80% lower in 2010 than those observed at the overstocked BB3 ($p = 0.04$) and 67% lower than levels at the properly stocked BB2 ($p = 0.09$). However, no significant difference was observed between the median event loads at BB2 and BB3 in 2010. Loading at Riesel was interesting in that in 2008, there was no significant difference observed between SW12 and SW17; in 2009, loading at SW17 was significantly greater than loading at SW12 ($p < 0.01$); and finally, in 2010, loading at the ungrazed SW12 was significantly greater than loading at the grazed SW17 ($p = 0.01$). Only at the Welder Wildlife Refuge were no significant differences observed in median *E. coli* loads.

Figure 3.9
***E. coli* loads on a runoff event basis at Beef Cattle Systems Center sites (BB1, BB2, BB3), Welder Wildlife Refuge sites (WWR1, WWR3), and Riesel sites (SW12, SW17) in 2008 (-08), 2009 (-09), and 2010 (-10).**



3.4.7 Comparison of E. coli Levels and Loads to Texas Water Quality Standards

E. coli levels in 114 of the 127 samples collected (90%) exceeded the single sample maximum for *E. coli* in water (394 cfu/100 mL) listed in the Texas Water Quality Standards (TWQS). Further, *E. coli* levels at all sites exceeded the geometric mean (126 cfu/100 mL) listed in the TWQS by over an order of magnitude. These results are similar to Edwards et al. (1997) who found fecal coliform levels in runoff from four pastures in northwest Arkansas exceeded water quality standards 70 to 89% of the time.

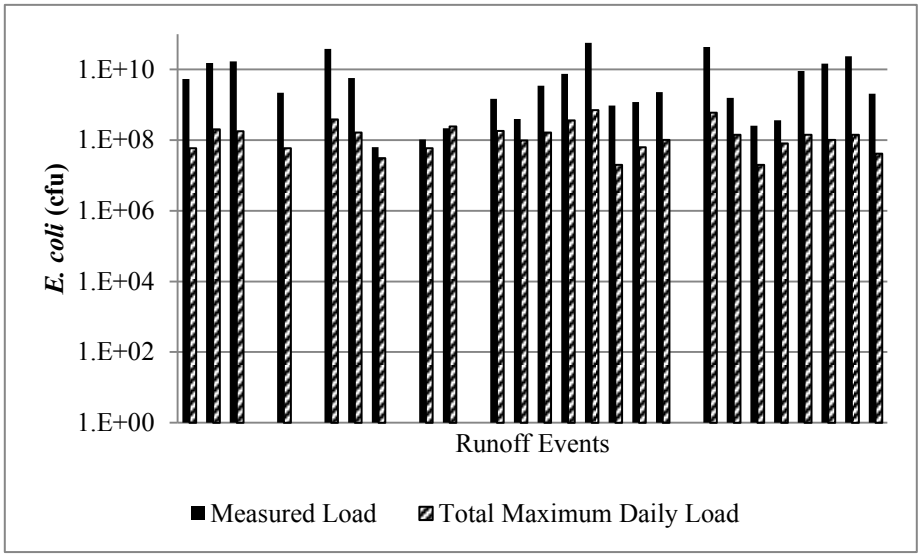
It is especially noteworthy that runoff from the ungrazed BB1, WWR1, and SW12 exceeded the single sample maximum in 88 to 100% of the samples. This is not

uncommon as Doran and Linn (1979) also found bacteria (total and fecal coliforms) in runoff from ungrazed pastures in eastern Nebraska exceeded water quality standards.

Although these water quality standards apply only to surface waters (e.g. streams and lakes) and not edge-of-field runoff as described here, this study does expose the impracticality of applying existing water quality standards to runoff events, especially as it pertains to individual farms or fields. Water quality in streams draining rural watersheds are often cited as exceeding bacterial water quality criteria at some frequency, even when agricultural activities are at a minimum and BMPs are not needed. Further, many studies have found runoff may not achieve water quality standards even after BMP implementation (Dickey and Vanderholm 1981; Clausen and Meals 1989; Walker et al. 1990; Coyne et al. 1995; Fajardo et al. 2001). Thus, it is recommended that exemptions from current standards be made for runoff or storm flows or, as Dickey and Vanderholm (1981) recommended, additional research be conducted to accurately define bacterial quality for runoff and establish practical water quality storm water standards.

Re-evaluation of water quality standards is of utmost importance as TMDLs are being established in hundreds of watersheds across Texas and thousands across the U.S. at great expense. Assessment of the loading reduction needed at the ungrazed native prairie site (SW12) showed a 98% load reduction would be required for that watershed to decrease *E. coli* loads the almost two orders of magnitude needed to achieve existing water quality standards (Figure 3.10). Assessment of the other six sites yielded similar results (data not shown). Attempting to achieve current standards during storm events is an insurmountable goal and not a judicious use of resources.

Figure 3.10
Comparison of measured *E. coli* loads with maximum loads meeting Texas Water Quality Standards in runoff at an ungrazed native prairie reference site (SW12), November 2007 through October 2008.



3.5 Summary and Conclusions

This study found rotational stocking to be an effective practice for reducing *E. coli* runoff. When runoff occurred more than two weeks following grazing, *E. coli* levels in runoff were decreased from 88 to 99% as compared to runoff when the sites were being actively grazed. As a result of these findings, it is recommended that creek pastures and other hydrologically connected pastures be stocked during periods when runoff is less likely (e.g. summer and winter in much of Texas) and upland sites be grazed during rainy seasons when runoff is more likely. Further research is recommended to evaluate the impact of this practice on a watershed scale.

The timing of stocking in relation to subsequent runoff events was much more significant than the impact of grazing management (i.e. ungrazed, properly stocked or

overstocked) or stocking rate. No significant differences were observed between *E. coli* runoff from properly or over-stocked pastures, although very high stocking rates did exhibit the potential to produce the highest *E. coli* concentrations. Highest *E. coli* concentrations were generally observed when runoff occurred within two weeks of grazing and the annual stocking rate was heavier than 1.3 ha/AUY (3.2 ac/AUY); however, no significant differences were observed between stocking rates of 0.7 to 3.1 ha/AUY (1.7 to 7.7 ac/AUY) for these events. When the stocking rate was 11 ha/AUY (27 ac/AUY) or lighter (and runoff occurred within two weeks of them being grazed), *E. coli* levels were significantly lower and did not exceed background levels. Additional research is needed to evaluate runoff from severely overgrazed sites as well as sites that are continuously grazed since runoff conditions from these may be significantly different than those observed by this study.

Background levels were considerable and relatively consistent across all sites, with median levels typically ranging from 3,500 to 5,500 cfu/100 mL. Most existing water quality models and thus total maximum daily loads and other watershed plans do not take background *E. coli* levels into account. Background levels should be considered when applying these models in order to prevent over-allocating loads and loading reductions to other sources.

This study also suggested the potential impact of non-domesticated animals on *E. coli* runoff from grazing lands. As observed at all Beef Cattle Systems Center sites in October 2009, median concentrations increased approximately an order of magnitude presumably due to non-domesticated animals (i.e. feral hogs or migratory birds).

Loading from these sources during this period was responsible for 80% to 99% of the total loading in 2009.

Finally, these results support the need to revise water quality standards as they apply to storm flow conditions. Ninety percent of runoff samples exceeded Texas Water Quality Standards, even at ungrazed sites. Although these water quality standards apply only to surface waters (e.g. streams and lakes) and not edge-of-field runoff as described here, this study does expose the impracticality of applying the existing water quality standards to runoff events, especially in runoff dominated streams. Background levels need to be considered as well as the significant impacts of non-domesticated animals. As such, it is recommended that exemptions from the current standards be made for storm flows and wildlife or additional research be conducted to accurately define bacterial quality for runoff and establish practical stream water quality standards.

CHAPTER IV

EVALUATION OF *BACTEROIDES* QPCR FOR ASSESSING CATTLE FECAL CONTRIBUTIONS IN RUNOFF FROM GRAZING LANDS

4.1 Overview

Excessive levels of fecal indicator bacteria (e.g. *E. coli*, *Enterococcus*, and fecal coliforms) are a major cause of water quality impairment. Better analytical methods are needed to quantify the proportion of bacterial loading contributed by the various sources of bacteria so appropriate restoration goals can be established and restoration efforts targeted. This study evaluated (1) the ability of quantitative polymerase chain reaction (qPCR) analysis of the bovine-associated *Bacteroides* marker, BoBac, to accurately assess the percentage of bovine-associated fecal contamination at the small watershed scale and (2) the relationship between the total *Bacteroides* marker, AllBac, and *E. coli* levels and its relevance as a fecal indicator.

Data suggest the AllBac and BoBac markers are good indicators of recent fecal contamination from cattle. However, although elevated BoBac/AllBac ratios generally aligned well with the presence of cattle, the ratio appeared to underestimate the percentage of bovine-associated fecal contamination. Correlations between *E. coli* and the AllBac and BoBac markers indicated significant geographic variability may exist with these markers. However, significant correlations were observed at stocked sites where local feces were used to generate gene copy curves. This suggests that feces for development of gene copy curves for future studies should be collected from the

watershed being assessed to reduce potential errors resulting from geographic variability in *Bacteroides* populations.

These markers appear to be useful tools for identifying sources of fecal contamination; however, more work is needed to improve their ability to accurately quantify total and source-specific bacterial loading before implementation at the watershed scale.

4.2 Introduction

Excessive levels of fecal indicator bacteria (e.g. *E. coli*, *Enterococcus*, and fecal coliforms) are a major cause of water quality impairment in Texas (TCEQ 2008a) and other regions (Weidhaas et al. 2011). Total Maximum Daily Loads (TMDLs), TMDL Implementation Plans, and other watershed-based plans are being developed to address these impairments. However, watersheds can be affected by microbial pollution from a wide variety of sources (TCEQ 2008d; Weidhaas et al. 2011). Nevertheless, livestock are increasingly under scrutiny (Weidhaas et al. 2011). Grazing cattle are often the most abundant species of livestock in impaired watersheds in Texas and are frequently identified as a source needing reductions (TCEQ 2008b, 2008c). Because of the potential regulatory implications of TMDLs, it is critical to accurately differentiate the potential bacterial contributions of livestock from those of wildlife or humans (TCEQ 2007c).

Computer models are frequently used to assess bacterial sources in watersheds; however, current models do not adequately evaluate wildlife contributions due to insufficient data on populations, distribution, and species-specific fecal loading data. There are also many bacterial source tracking (BST) methods available and more under

development (EPA 2002; Okabe et al. 2007; Jones et al. 2009; Dick et al. 2010); however, to date, no single method has been identified as superior (EPA 2005; Jones et al. 2009), and no standard method has been adopted (Meays et al. 2004).

Library-dependent BST methods are frequently used; however, these methods are burdensome, time-consuming, and costly (EPA 2002; Jones et al. 2009). Further, the lack of understanding of the geographic and temporal stability of BST libraries as well as the potential for regrowth and survival of fecal organisms in the environment, such as *E. coli* on which many of these assays are based, has raised questions about their use. Finally, results of library-dependent classifications are conservatively seen as semi-quantitative and may not provide the level of specificity needed for TMDL-related activities.

To address these issues and assist in the TMDL process, investigation and refinement of library independent BST methods has been proposed. There have been significant developments in library-independent BST methods in recent years. Library independent methods are comparatively more cost-effective, rapid, and potentially more specific and accurate than library dependent methods (Jones et al. 2009; Weidhaas et al. 2011). A number of BST methods have been developed using polymerase chain reaction (PCR) assays targeting members of the *Bacteroidales* order (*Bacteroides* species) to identify and quantify the sources of bacteria (Okabe et al. 2007; Haugland et al. 2010). *Bacteroides* are non-spore forming, anaerobic bacteria found in high concentrations in intestinal tracts, and thus feces (i.e. $> 1 \times 10^{10}$ cells per gram of feces), of warm-blooded animals (Bitton 2005; Dick et al. 2010). Because they are strict anaerobes, *Bacteroides*

do not survive long in the environment, thus their presence provides a good indicator of recent fecal contamination of a water body (Bitton 2005; Layton et al. 2006; Balleste and Blanch 2010). *Bacteroides* also exhibit a high degree of host specificity (Field et al. 2003; Layton et al. 2006) and moderate sensitivity (Field et al. 2003). *Bacteroidales*, like *E. coli*, exhibits a longer persistence with lower temperatures; however, unlike *E. coli*; it is more persistent with higher salinity. Further, predation is also a controlling factor of *Bacteroidales* decay, as it is for *E. coli* (Balleste and Blanch 2010; Dick et al. 2010).

Previous BST studies have used *Bacteroides* markers to detect the presence or absence of *Bacteroides* and *Prevotella* spp. fecal bacteria specific to humans, ruminants (including cattle and deer), pigs, and horses by traditional PCR (Bernhard and Field 2000; Dick et al. 2005). In 2006, a bovine-associated quantitative PCR (qPCR) assay targeting *Bacteroides* was developed by Layton et al. to simultaneously identify and quantify *Bacteroides* markers in water samples and estimate total, human, and bovine fecal pollution in water (i.e. AllBac, HuBac, BoBac, respectively). Layton et al. (2006) found the AllBac assay was a suitable estimator of total fecal contamination and *E. coli* concentrations in water and the BoBac assay was a reliable indicator of bovine fecal contamination, thus confirming qPCR assays as a useful tool for quantifying fecal concentrations and sources of fecal contamination in watersheds. Similarly, Gentry et al. (2007) found AllBac and *E. coli* load rates were highly correlated at baseflow or near baseflow conditions. In addition, Savichtcheva et al. (2007) found total and human-specific *Bacteroides* markers displayed significant predictive capability for the

occurrence of *E. coli* O157, *Salmonella*, heat-labile enterotoxin of enterotoxigenic *E. coli* (ETEC), and heat-stabile enterotoxin for human of ETEC.

Dick et al. (2010) recently found human feces-associated (HF) *Bacteroidales* markers to be a conservative predictor of human-associated *E. coli*; however, AllBac was not found to be a suitable alternate indicator of health risk in place of *E. coli*. Further, Dick et al. (2010) found the HF marker in ratio with AllBac was not a suitable estimator of human contributions due to the heterogeneity in the AllBac marker data resulting from differing degradation rates compared with *E. coli* or other markers. Other issues with *Bacteroides* remain regarding differing abundance among species, as well as continued uncertainties regarding possible geographic influences (Lamendella et al. 2009).

To date, the ability of these methods to reliably and correctly determine fecal contributions from cattle has not been thoroughly tested in environmental water samples. Thus, the primary goal of this study was to determine the ability of the BoBac marker to assess the quantity of *E. coli* loading originating from cattle at the small watershed scale. Additionally, this research further evaluated the relationship between total *Bacteroides* (AllBac) and *E. coli* to assess its relevance as a fecal indicator.

4.3 Materials and Methods

4.3.1 Site Descriptions

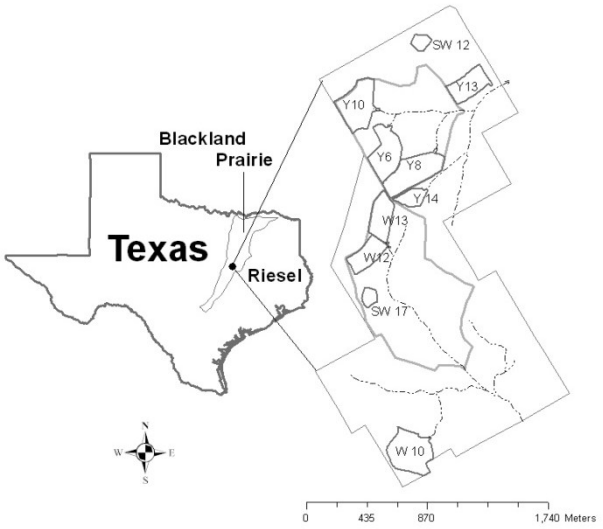
E. coli and the *Bacteroides* AllBac and BoBac markers were determined for each runoff event at seven sites at three Texas locations (Table 4.1).

Table 4.1
Watershed site locations and characteristics.

Site	Latitude, Longitude	Vegetation	Management
Riesel			
SW12	31°28'48" N, 96° 52'59" W	Native grassland	Ungrazed
SW17	31°27'45" N, 96° 53'14" W	Bermudagrass	Properly stocked
Welder Wildlife Refuge			
WWR1	28°6'55.97" N, 97°21'20.82" W	Native rangeland	Ungrazed
WWR3	28°6'52.60" N, 97°21'13.83" W	Native rangeland	Properly stocked
Beef Cattle Systems Center			
BB1	30°31'44.3" N, 96°24'58.3" W	Tifton 85 bermudagrass	Ungrazed
BB2	30°31'47.5" N, 96°24'57.7" W	Tifton 85 bermudagrass	Properly stocked
BB3	30°31'47.7" N, 96°24'57.9" W	Tifton 85 bermudagrass	Overstocked

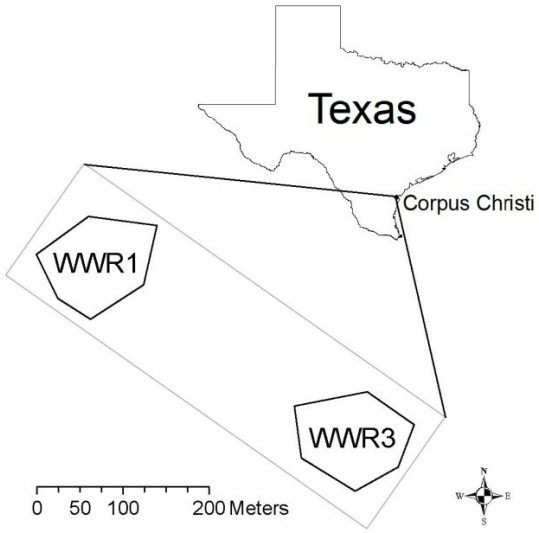
Two sites were located at the USDA-ARS Grassland, Soil and Water Research Laboratory in Riesel, TX, which has been one of the most intensively monitored hydrological research sites in the country since establishment in the 1930s (Harmel et al. 2007). It is located in the Blackland Prairies ecoregion on the border of Falls and McLennan counties (Omernik 1987) (Figure 4.1). Houston Black clay soils dominate the region. This soil is very slowly permeable when wet; however, preferential flow associated with soil cracks contributes to high infiltration rates when the soil is dry. Mean annual rainfall is approximately 91 cm (36 in). Thirteen runoff stations are operated at the research site to monitor sub-watersheds under both pasture and cropland management. Two 1.2-ha sites were used to evaluate grazing management, SW12 and SW17. The average slope of SW12 is 3.8%, while slope averages 1.8% at SW17.

Figure 4.1
Riesel watersheds.



Two sites were located at the Rob and Bessie Welder Wildlife Refuge, a 3,156 ha (7,800 ac) native wildlife refuge 13 km (8 mi.) north of Sinton, TX, in the Western Gulf Coastal Plain ecoregion of Texas (Omernik 1987) (Figure 4.2). Three 1-ha (2.4 ac) sites had previously been established to monitor runoff in 2000 to study the effects of shrub management on water quality and quantity on rangeland. Berm failure on site WWR2 prevented data collection there during the study. The Welder has never been cultivated and has historically been managed for livestock (Stewart 2003). Precipitation averages 90 cm (36 in) annually. The watershed sites are located on chaparral-mixed grass communities on the east and west sides of Paloma draw, approximately 6 km (4 miles) from the foundation headquarters. Victoria clay (0 to 1% slopes) soils underlay the upper quarter to third of the watershed sites and Monteola clay (5 to 8% slopes) soils underlay the remainder. Both soils are classified as Hydrologic Soil Group D soils.

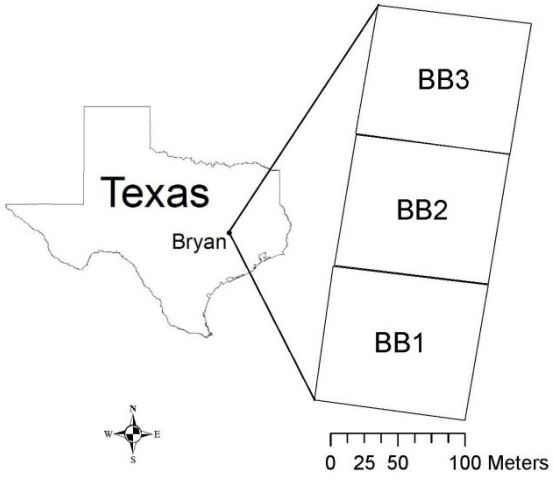
Figure 4.2
Welder Wildlife Refuge watersheds.



The final location for the study was the Texas A&M University, Department of Animal Science Beef Cattle Systems Center (BCSC) located west of College Station on the Brazos River. This site was used primarily for row crop production prior to initiation of this study. In October 2007, berms were constructed around three 1-ha watershed sites (Figure 4.3), and slope modified so each site would drain to the watershed outlet. Following berm construction, all sites were established to Tifton 85 bermudagrass.

The BCSC watershed sites are located in the East Central Texas Plains ecoregion (Omernik 1987). Annual precipitation averages 102 cm (40 in). Soils within the study area are comprised of Belk clay, a heavier-textured alluvial soil (Hydrologic Soil Group D) found along the Brazos River. Measured slope averaged 0.2%.

Figure 4.3
Beef Cattle Systems Center watersheds.



4.3.2 Pasture Management

All sites were fenced so that cattle grazing could be controlled. Three sites were ungrazed (SW12, WWR1, BB1), three were properly stocked (SW17, WWR3, BB2), and one site, BB3, had double the stocking rate of the properly stocked BB2. Site SW12 is notable in that this ungrazed native prairie reference site has not been stocked with cattle or other livestock since the Riesel Research Center was established in 1937 (Harmel et al. 2006a). Stocked sites were not grazed continuously; instead, over the course of the study, six to seven grazing events occurred at each site (Table 4.2). Grass height was monitored monthly to determine timing of grazing. Properly stocked sites were generally stocked once grass height exceeded 6 in, and cattle were removed once grass height was reduced to 3 in as recommended by the USDA Natural Resources Conservation Service Conservation Practice General Specifications for Prescribed Grazing (Practice Standard No. 528).

Table 4.2
Grazing treatments.

Locale	Site	Start	End	Stocking Rate (AU/ha)*	Stocking Rate (AUD/ha)†	Stocking Rate ha/AUY (ac/AUY)‡
Riesel	SW17	9/12/07	11/14/07	1.1	70	2.6 (6.4)
	SW17	2/25/08	6/2/08	1.1	109	1.7 (4.1)
	SW17	11/5/08	4/21/09	1.1	185	1.6 (3.9)
	SW17	5/1/09	6/3/09	1.1	37	1.7 (4.1)
	SW17	7/15/09	11/6/09	1.1	126	1.1 (2.6)
	SW17	5/3/10	5/24/10	1.1	23	2.3 (5.6)
	SW17	7/19/10	8/27/10	1.1	43	2.5 (6.2)
Welder	WWR3	12/1/07	2/13/08	0.4	31	11.6 (28.7)
	WWR3	4/18/08	4/28/08	2.6	26	6.4 (15.7)
	WWR3	10/20/08	10/25/08	2.9	15	5.1 (12.5)
	WWR3	4/27/09	5/1/09	3.4	14	11.7 (29.0)
	WWR3	6/21/10	6/22/10	2.6	3	140.9 (348.0)
	WWR3	9/1/10	9/11/10	2.6	26	12.8 (31.6)
BCSC	BB2	1/12/09	1/16/09	4.0	16	22.8 (56.3)
	BB2	5/22/09	6/5/09	6.1	79	3.8 (9.5)
	BB2	8/7/09	8/8/09	6.4	6	3.6 (8.9)
	BB2	8/12/09	8/19/09	6.4	46	2.5 (6.1)
	BB2	11/12/09	11/17/09	18.4	90	1.5 (3.8)
	BB2§	2/1/10	2/8/10	2.5	17	1.5 (3.8)
	BB2	6/21/10	7/2/10	17.7	194	1.7 (4.3)
	BB3	1/12/09	1/16/09	8.0	32	11.4 (28.2)
	BB3	5/22/09	6/5/09	13.4	175	1.8 (4.4)
	BB3	8/7/09	8/8/09	12.8	13	1.7 (4.1)
	BB3	8/12/09	8/19/09	12.8	92	1.2 (2.9)
	BB3	11/12/09	11/17/09	36.8	180	0.7 (1.8)
	BB3§	2/1/10	2/8/10	2.5	17	0.8 (1.9)
	BB3	6/21/10	7/2/10	31.7	346	1.0 (2.5)

* Animal units per hectare
† Animal unit days per hectare
‡ Annual stocking rate in hectares per animal unit and acres per animal unit
§ Electric fences failed allowing short-term cattle access

These grazing events allowed evaluation of the impact of a wide range of stocking rates on *E. coli* runoff. It should be noted that electric fences failed on several

occasions at the Beef Cattle Systems Center sites. At BB2 and BB3, electric fences failed on February 1 to 8, 2010; however, minimal grazing occurred on the sites during this time. At BB1, the electric fence failed on March 11, 2009, November 13, 2009 and February 1, 2010 allowing cattle limited access to the site ranging from one to seven days per failure. To the extent possible, fecal pats were removed from the site once the fence was restored. However, February 1 to 8, 2010, several runoff events occurred and are noted in the analysis.

4.3.3 Edge of Field Sampling Procedures

As recommended by Harmel et al. (2006b) flow-weighted composite edge of field runoff samples from the seven watershed sites were collected using ISCO 6712 (ISCO Inc., Lincoln, NE) full-size portable samplers with single bottle configuration into surface disinfected polyethylene 15 L (4 gal.) round bottles. Flow from each watershed site was measured with ISCO 730 Module bubble flow meters. Flow data were downloaded at least monthly using an ISCO 581 Rapid Transfer Device (RTD). BB1, BB2, BB3, WWR1, and WWR3 were equipped with berms and 90^{o} v-notch weirs to aid in collection and measurement of runoff, while SW12 and SW17 were monitored using 0.9 m (3 ft.) H-flumes. Runoff was monitored for a period of two years at SW12 and SW17 (November 2007 – October 2009) and BB1, BB2, and BB3 (November 2008 – October 2010), and due to a severe drought, only one year at WWR1 and WWR3 (November 2009 – October 2010). The ISCO samplers at sites WWR1, WWR3, BB2, and BB3 enabled when the water level exceeded 6 mm (0.02 ft) and then collected 50 mL for every 4.2 m^3 (150 ft^3) of runoff. The ISCO sampler at BB1 enabled when the

water level exceeded 6 mm (0.02 ft) and then collected 25 mL for every 2.1 m^3 (75 ft^3) of runoff. The ISCO samplers at SW12 and SW17 enabled at 60 mm (0.20 ft.) and sampled at 16 m^3 (566 ft^3) intervals. All ISCO samplers were programmed to rinse sample tubing with ambient water prior to collection of each sample. Following each event, samples were retrieved as soon as possible (typically within 24 hr) from the ISCO samplers and placed on ice in a cooler for transport to the lab where they were stored at 4°C (39°F) until analysis.

4.3.4 E. coli Analysis

Analysis of all water samples for *E. coli* was conducted within six hours of retrieval from ISCO samplers. *E. coli* in water samples were enumerated using EPA Method 1603 (EPA 2006). If counts were greater than 200 colonies at the highest dilution, the count was reported as too numerous to count (TNTC). Results were reported as cfu (colony forming units)/100 mL.

4.3.5 Bacteroides Analysis

Within 6 hr of retrieval, water samples were also filtered through a sterile Supor-200, 0.2 µM pore size filter (Pall Corporation, Ann Arbor, MI). Filter volumes averaged 30 ml, but varied from 10 to 100 mL depending on the quantity of suspended solids in the sample (i.e. how much could be passed before the filter clogged). Filters were placed in sterile, Whirl-Pak® bags containing 500 µl of guanidine isothiocyanate (GITC) lysis buffer (Walters and Field, 2009) and stored at -80°C until DNA extraction.

The DNA was extracted directly from the filters using QIAamp DNA mini kits (QIAGEN, Valencia, CA). Total DNA from corresponding filters was eluted in 100 µl of 0.01X TE (0.1 mM Tris-EDTA buffer, pH 8.0) into a sterile tube. To remove any residual alcohol, eluted samples were concentrated at 60°C to a volume of 10 to 20 µl using an Eppendorf Vacufuge Plus (Westbury, NY), and their volumes were brought back to 100 µl with 0.01X TE. The DNA was quantified using both Quant-It™ Picogreen® assay (Invitrogen) and a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE). The DNA extracts were stored at –80°C until analyzed by qPCR.

4.3.6 Construction of Bacteroides 16S rRNA Gene Copy Standards

Bovine feces were collected aseptically from a pastured cow at the Texas A&M University, O. D. Butler, Jr. Animal Science Teaching, Research, and Extension Complex (College Station, TX) using a sterile spatula, into a sterile, screw-cap polypropylene specimen tube. The fecal sample was immediately returned to the lab and mixed in a volume of sterile distilled water equal to the weight of the feces. The DNA was extracted from the bovine feces using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) and purified using illustra MicroSpin S-400 HR Columns (GE Healthcare, UK), according to the manufacturers' recommendations.

Bacteroides-specific 16S rRNA genes (32F/708R), containing both the AllBac and BoBac regions, were amplified from fecal DNA extracts (Field et al. 2003). The 25 µL reactions contained 1X Failsafe Buffer A (Epicentre Biotechnologies, Madison, WI), 15 pmol of forward and reverse primers (32F and 708R), 1.25 units of AmpliTaq Gold

DNA Polymerase (Applied Biosystems, Foster City, CA), and 1 µL of fecal community DNA. Thermocycling was conducted in an Eppendorf Mastercycler (Hamburg, Germany) under the following conditions: 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 s, 53°C for 1 min, and 72°C for 1 min; and a final extension of 72°C for 10 min (Field et al. 2003). The PCR product was confirmed on an agarose gel stained with ethidium bromide, gel purified using QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA) and finally cloned into a pDrive Cloning Vector (Qiagen, Valencia, CA). Inserts in presumptive clones were extracted using PerfectPrep Spin Mini Kit (5 Prime, Gaithersburg, MD) and verified by amplification with the AllBac and BoBac primer sets (Layton et al. 2006). These 25 µL reactions contained 1X Failsafe Buffer A (Epicentre), 15 pmol of forward and reverse primers, 1.25 units of AmpliTaq Gold DNA Polymerase, and 1 µL of plasmid template. Thermocycling was conducted in an Eppendorf Mastercycler under the following conditions: 50°C for 2 min, followed by 95°C for 10 min and 50 cycles of 95°C for 30 s, 57°C (BoBac assay) or 60°C (AllBac assay) for 45 s, and final extension at 72°C for 1 min (Layton et al. 2006). The PCR product was confirmed on an agarose gel stained with ethidium bromide. Plasmids were extracted using PerfectPrep Spin Mini Kit (5 Prime, Gaithersburg, MD). The DNA was quantified using Quant-It™ Picogreen® assay (Invitrogen) and normalized to 1 ng/µl with subsequent standards made using 10-fold dilutions in DNA-grade water to 10^{-7}.

4.3.7 Construction of Bacteroides Fecal Curve

Bovine feces were collected aseptically from a pastured cow at the Beef Cattle Systems Center using a sterile spatula, into a sterile, screw-cap polypropylene specimen

tube. The feces were immediately returned to the lab and 1 g was mixed into 100 ml of sterile PCR-grade water producing a fecal concentration of 10,000 mg of feces/L of water. Standards were made using 10-fold dilutions to a concentration of 0.1 mg/L.

4.3.8 Quantitative PCR Assays

Extracted DNA from runoff samples was tested for total (AllBac) and bovine-associated (BoBac) fecal markers as described by Layton et al. (2006). Gene targets as well as the probe and primer sequences and amplicon size for the two qPCR assays used in this study are summarized in Layton et al. (2006). Oligonucleotide primers and 6-carboxyfluorescein (FAM)-BHQ probes were obtained from Integrated DNA Technologies, Inc. (Coralville, IA).

The qPCR was performed in 25 µl reactions containing 12.5 µl QuantiTect Probe PCR Master Mix (QIAGEN, Valencia, CA), 5 µl template, 15 pmol (1 µl) each of forward and reverse primers, 5 pmol (0.5 µl) probe, 1 µl 0.01X TE or spike (2E+05 gene copies), and 4 µl PCR-grade water. Reactions were set up using a CAS-1200™ Precision Liquid Handling System (Corbett Life Science, Australia). The PCR amplification and detection of the fluorescent signal was performed using the Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Science) under the following conditions: 50°C for 2 min, followed by 95°C for 10 min and 50 cycles of 95°C for 30 s, 57°C (BoBac assay) or 60°C (AllBac assay) for 45 s, and 72°C for 60 s (Layton et al. 2006).

For all qPCR runs, standards, negative controls (no DNA), samples and spiked samples were run in triplicate. Each assay contained two types of standard curves, a gene copy standard curve and a fecal dilution standard curve. For the AllBac and BoBac

assays, bovine fecal standards ranging in concentration from 1.0 to 1,000 mg/L were used for calculating the concentration of total and bovine-associated feces in each sample. Similarly, gene copy standards ranging in concentration from 2E+01 to 2E+06 copies per reaction were used for calculating the concentration of total and bovine-associated *Bacteroides* gene copies in each sample. Gene copies or fecal concentrations were calculated from standard curves ($\log_{10}$ concentrations vs. the qPCR threshold cycle). Linear correlations were determined using Microsoft Excel. Results were expressed as gene copies per L of water or as a fecal concentration in mg/L. Percent cattle contribution was estimated by dividing the bovine-specific *Bacteroides* (BoBac) results by the total *Bacteroides* (AllBac) results for each runoff sample.

Sterile 0.01X TE buffer was used as a negative control. The potential for PCR inhibition was measured by spiking samples with 2E+05 copies of plasmid DNA. The amount of PCR inhibition was measured by determining the recovery of the copies in the presence of the runoff sample as calculated from the plasmid DNA standard curve [percent recovery = (measured copies in runoff sample spiked with 2E+05 plasmid copies - measured copies in unspiked runoff sample)/(measured copies in blank sample spiked with 2E+05 plasmid copies) x 100]. The percentage of plasmid recovery was measured in each runoff sample was then determined.

4.3.9 Statistical Analysis

The statistical software, Minitab (Minitab Inc., State College, PA) was used for determining descriptive statistics (means, standard deviations, maximums, medians, etc.), calculating Pearson product moment correlation coefficients and conducting linear

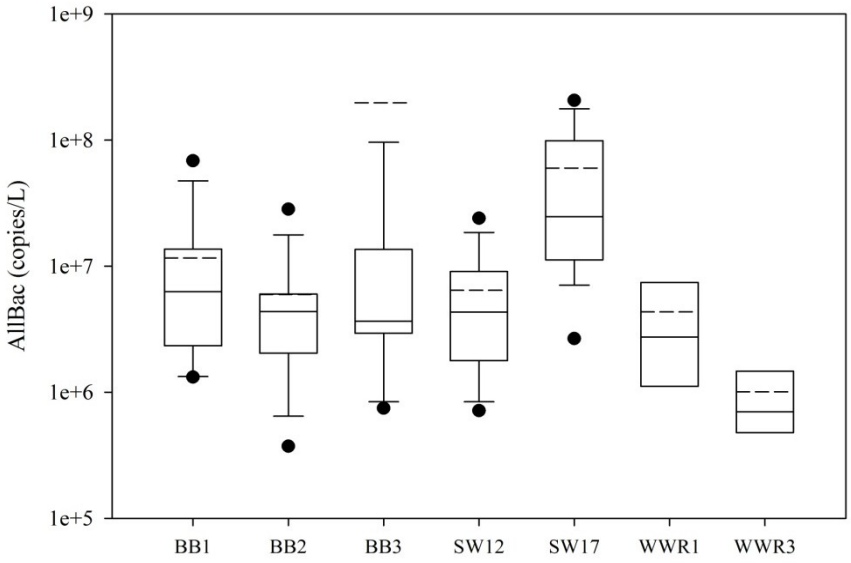
regression analyses on $\log_{10}$ transformed bacterial concentrations, and assessing differences in median concentrations between sites and treatments using the Mann-Whitney test. Regression analysis was used to assess relationships of AllBac and BoBac gene copy and fecal concentrations to *E. coli* levels. An alpha level of 0.05 was accepted as a minimum level of significance; thus results were considered statistically significant when $p < 0.05$.

4.4 Results and Discussion

4.4.1 Comparison of AllBac to SR and Grazing Management

AllBac was detected in all samples and ranged in concentration from 3.5E+05 to 3.5E+09 gene copies/L (Appendix E). The AllBac concentrations were not correlated with grazing management ($p = 0.83$) or stocking rate ($p = 0.53$). With the exception of sites SW17 and WWR3, no significant differences in median AllBac concentrations were observed between sites (Figure 4.4). Significantly higher median AllBac concentrations were observed at SW17, while significantly lower median AllBac gene copies were observed at WWR3. The lower concentrations at WWR3 likely resulted from the minimal grazing that took place the year before the runoff events occurred as a result of a major two year drought during the study. As such, WWR3 was more representative of an ungrazed site than a stocked site during the period samples were collected. In contrast, SW17 was the most extensively grazed site throughout the study, being grazed for over 14 months (out of 24 months), though at a lighter stocking rate than the other sites.

Figure 4.4
Box plot depicting AllBac concentrations (copies/L) at each site. The boundary of the box closest to zero indicates the 25th percentile, the solid line within the box represents the median, the dashed line represents the mean, the boundary of the box farthest from zero indicates the 75th percentile, the whiskers above and below the box indicate the 10th and 90th percentiles, and the circles indicate outliers.



To further evaluate the data, annual AllBac concentrations were assessed (Table 4.3). Comparison of median annual AllBac concentrations among sites also showed no correlation with either grazing management ($p = 0.58$) or annual stocking rate (AUD/ha) ($p = 0.62$). Because AllBac would also detect other fecal sources including wildlife, significant correlations with grazing management and stocking rate would not necessarily be expected.

However, a significant correlation was observed between median annual AllBac concentrations and percentage of runoff events occurring while sites were stocked or had been stocked less than 2 weeks prior to runoff ($p < 0.01$; $r^2=0.52$). This indicates AllBac concentrations are significantly impacted by recent fecal contamination from cattle.

Table 4.3
Comparison of AllBac levels (copies/L) with grazing management, stocking rate (AUD/ha), and cattle presence during runoff events.

Site-Yr§	Mean	sd	Median	Max.	Grazing Management	Annual AUD/ ha	Cattle on site during runoff-%*
BB1-09	1.52E+07	2.22E+07	9.49E+06	6.84E+07	Ungrazed	0	No-0%
BB2-09	4.13E+06	3.25E+06	4.30E+06	1.07E+07	Properly stock	147	No-0%
BB3-09	3.58E+06	3.04E+06	3.30E+06	1.17E+07	Overstocked	312	No-0%
BB1-10	5.88E+06	4.80E+06	3.58E+06	1.41E+07	Ungrazed	17	Yes†-20%
BB2-10	8.72E+06	9.83E+06	4.74E+06	2.82E+07	Properly stock	301	Yes-67%
BB3-10	4.64E+08	1.23E+09	1.45E+07	3.52E+09	Overstocked	543	Yes-75%
SW12-08	8.13E+06	4.87E+06	7.61E+06	1.62E+07	Ungrazed	0	No-0%
SW17-08	6.48E+07	5.38E+07	5.22E+07	1.58E+08	Properly stock	124	Yes-100%
SW12-09	5.95E+06	7.16E+06	4.18E+06	2.39E+07	Ungrazed	0	No-0%
SW17-09	6.21E+07	7.49E+07	1.58E+07	2.06E+08	Properly stock	341	Yes-100%
WWR1-10	4.34E+06	4.66E+06	2.74E+06	1.32E+07	Ungrazed	0	No-0%
WWR3-10	1.01E+06	8.73E+05	6.99E+05	2.72E+06	Propery stock‡	0	No-0%

* Percent of samples collected while site actively or recently stocked (< 2 weeks).
† Electric fences failed February 1 to 8, 2010 allowing cattle access to site.
‡ Although managed at a proper stocking level, a severe drought prevented grazing the year immediately preceding all runoff events; therefore, data from these events are more representative of an ungrazed site.
§ Year, November 1 to October 31.

Other factors (e.g. persistence) and sources (e.g. wildlife) may impact AllBac concentrations as well since AllBac is a measure of total *Bacteroides* and differing persistence between fecal sources may exist between human and ruminant *Bacteroidales* markers (Walters and Field 2009).

4.4.2 Comparison of BoBac to SR and Grazing Management

As with AllBac, BoBac was detected in all samples as well and ranged in concentration from 2.9E+02 to 5.9E+08 gene copies/L (Appendix F). Comparison of BoBac concentrations among sites showed that ungrazed sites generally had lowest

BoBac levels and that stocked sites generally had highest levels (Table 4.4), but median BoBac gene copy concentrations were not correlated with either annual stocking rate (AUD/ha) ($p = 0.10$) or grazing management ($p = 0.21$). However, median BoBac concentrations ($\log_{10}$ transformed) were strongly correlated with the percent of runoff events that occurred while sites were stocked or had been stocked less than 2 weeks prior to runoff ($p < 0.001$; r^2=0.88).

Table 4.4
Comparison of BoBac levels (copies/L) with grazing management, stocking rate (AUD/ha), and cattle presence during runoff events.

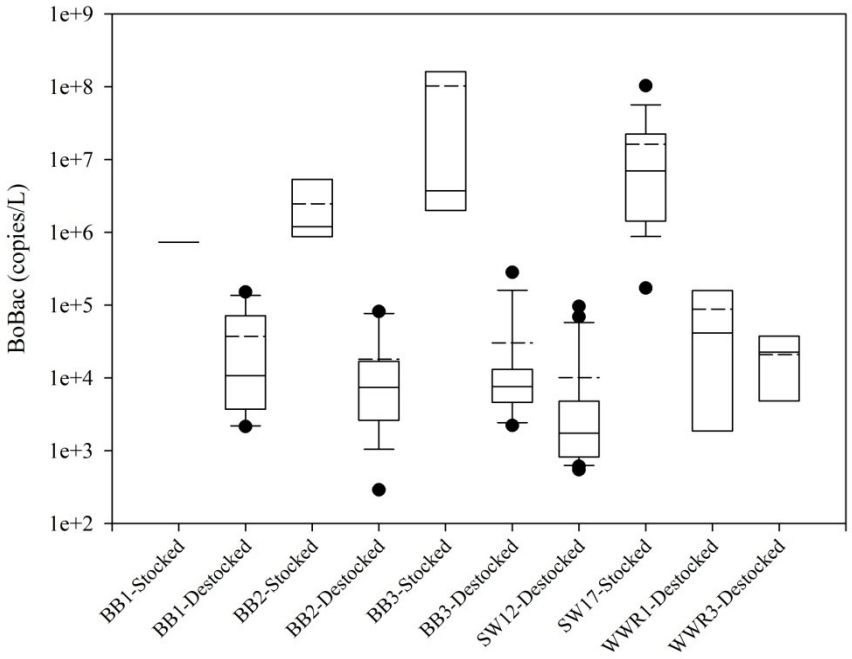
Site-Yr§	Mean	sd	Median	Max.	Grazing Management	Annual AUD/ ha	Cattle on site during runoff-%*
BB1-09	9.10E+03	7.45E+03	6.18E+03	2.31E+04	Ungrazed	0	No-0%
BB2-09	6.56E+03	5.56E+03	4.59E+03	1.66E+04	Properly stock	147	No-0%
BB3-09	6.61E+03	3.75E+03	6.13E+03	1.32E+04	Overstocked	312	No-0%
BB1-10	2.28E+05	2.84E+05	1.12E+05	7.32E+05	Ungrazed	17	Yes†-20%
BB2-10	1.67E+06	2.48E+06	8.87E+05	6.60E+06	Properly stock	301	Yes-67%
BB3-10	7.69E+07	2.05E+08	2.90E+06	5.85E+08	Overstocked	543	Yes-75%
SW12-08	1.26E+04	2.77E+04	1.51E+03	6.90E+04	Ungrazed	0	No-0%
SW17-08	1.09E+07	1.23E+07	5.45E+06	3.30E+07	Properly stock	124	Yes-100%
SW12-09	1.40E+04	3.08E+04	2.17E+03	9.57E+04	Ungrazed	0	No-0%
SW17-09	2.60E+07	3.75E+07	6.95E+06	1.03E+08	Properly stock	341	Yes-100%
WWR1-10	1.02E+05	1.12E+05	7.93E+04	2.92E+05	Ungrazed	0	No-0%
WWR3-10	2.05E+04	1.88E+04	1.73E+04	4.46E+04	Properly stock‡	0	No-0%

* Percent of samples collected while site actively or recently stocked (< 2 weeks).
† Electric fences failed February 1 to 8, 2010 allowing cattle access to site.
‡ Although managed at a proper stocking level, a severe drought prevented grazing the year immediately preceding all runoff events; therefore, data from these events are more representative of an ungrazed site.
§ Year, November 1 to October 31.

Further, BoBac concentrations were significantly higher, exceeding 10^5 copies/L, in runoff when cattle were actively grazing or had grazed the sites within 2 weeks of the runoff event (Figure 4.5) and generally 10^5 copies/L or less when sites were destocked.

The BoBac concentrations were not significantly different among the sites when they were stocked. Similarly, with the exception of SW12, which exhibited the lowest BoBac concentrations, BoBac levels were also not significantly different among the sites when they were destocked. This analysis appears to validate BoBac as a suitable indicator of recent fecal contamination from cattle.

Figure 4.5
BoBac concentrations (copies/L) in runoff from grazing lands at each site when stocked and destocked.



4.4.3 Comparison of AllBac and BoBac Gene Copy Concentrations to E. coli Levels

In order for the AllBac marker to be able to relate to existing water quality standards, there needs to be a strong correlation between AllBac and *E. coli* concentrations. Overall, a significant correlation ($p < 0.001$) was observed between AllBac gene copy and *E. coli* concentrations when data from all sites were compared;

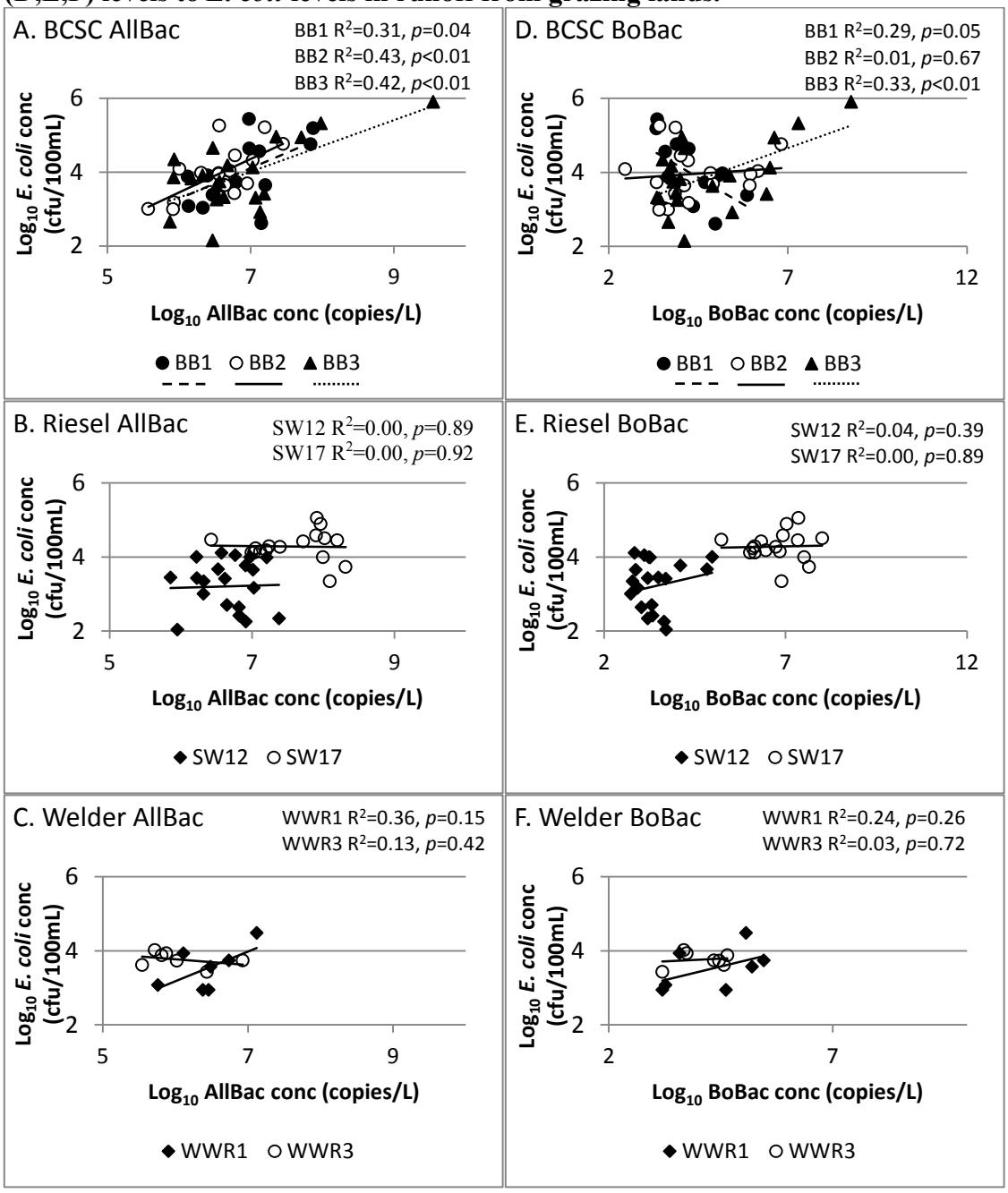
however, the coefficient of determination value was low (r^2=0.21). In comparison, Okabe et al. (2007) found a moderate level of correlation between *Bacteroides-Prevotella* group specific 16S rRNA gene markers and fecal coliforms (r^2=0.49). Correlations of *Bacteroides* markers and fecal indicator bacteria such as *E. coli* are impacted by many factors, particularly the differences in detection methods (culture vs. molecular based) and differences in survivability of each in the environment. Quantitative PCR methods detect DNA from both culturable and unculturable or dead organisms, whereas the *E. coli* methods used detect only culturable cells (Okabe et al. 2007, Dick et al. 2010). This relationship is further complicated by the survivability of the organisms, particularly since data regarding the persistence of *Bacteroides* genes are scarce (Bell et al. 2009), while *E. coli* has been shown to reproduce in the environment under some conditions. However, recent research has demonstrated that little difference exists in the decay rates of fecal *Bacteroides* 16S RNA genes in human, cattle, and pigs using both host-specific and general assays (Bell et al. 2009). Further, recent studies have found several *Bacteroides* markers, such as the HF-human fecal marker, decayed at similar or significantly faster rates than *E. coli* thus making them conservative predictors of *E. coli* (Dick et al. 2010). But, Dick et al. (2010) suggested that a reservoir of AllBac markers may persist and thus may not be useful as an alternate indicator for *E. coli*.

Further, when this relationship was evaluated at each site (Figure 4.6), potential geographic variability in the AllBac marker was indicated. The AllBac gene copy and *E. coli* concentrations were not correlated at Riesel (SW12, SW17) or Welder Wildlife Refuge (WWR1, WWR3) sites. In contrast, a moderate, statistically significant

correlation was observed between AllBac gene copy concentrations and *E. coli* at all BCSC sites (BB1, BB2, BB3). This correlation between *E. coli* and AllBac gene copies at the BCSC sites was perhaps due to the fact that the fecal matter used to generate the gene copy curves was collected from the TAMU Animal Science facility located directly adjacent to the BCSC. Although EPA (2005) and Layton et al. (2006) suggest *Bacteroides* spp. have broad geographic stability, these results and other studies appear to indicate otherwise. For instance, Lamendella et al. (2009) noted differences in the geographic stability of swine-associated *Bacteroidales* assays potentially due to differences in animal management practices among different locations.

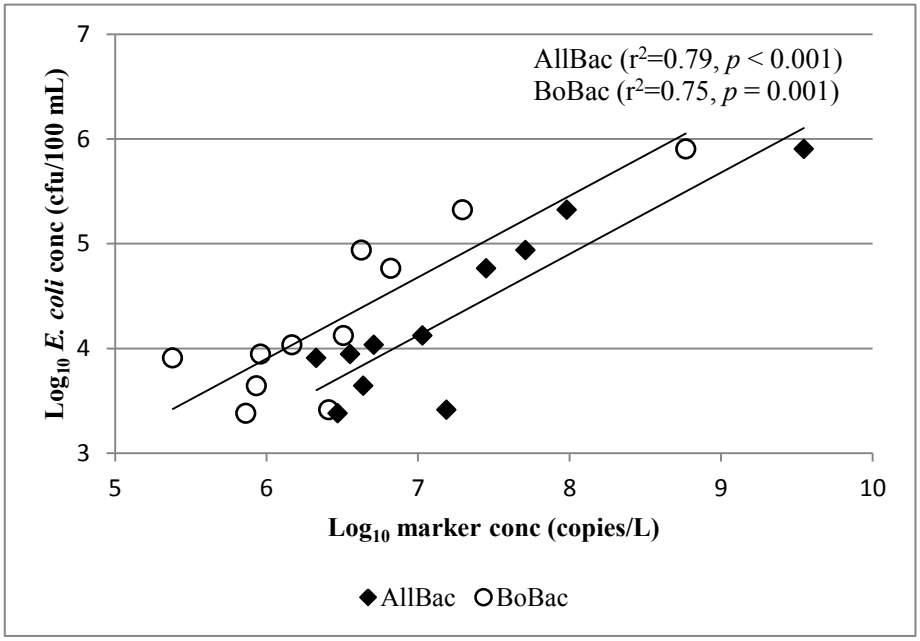
When BoBac gene copy and *E. coli* concentrations from all sites were compared, a statistically significant correlation was observed (r^2=0.16, $p < 0.001$). Similar correlations have been observed with other markers as demonstrated by Weidhaas et al. (2011), who found a comparable correlation to our study between the LA35 poultry marker and *E. coli* levels (r^2=0.37, $p = 0.001$). As would be expected, positive correlations were not observed at the ungrazed SW12, WWR1, and BB1 (negative relationship observed) and significant correlations were observed at the overstocked BB3 and properly stocked SW17 (Figure 4.6). However, no correlation was observed at the properly stocked BB2 and WWR3. This likely resulted from no runoff events occurring at either of these sites while they were actively stocked unlike BB3 and SW17.

Figure 4.6
Linear regression analysis showing correlation of AllBac (A,B,C) and BoBac (D,E,F) levels to *E. coli* levels in runoff from grazing lands.



Finally, the correlation of *E. coli* to AllBac and BoBac when sites were stocked was evaluated. At BCSC sites, *E. coli* was significantly correlated with both AllBac and BoBac when the sites were stocked (Figure 4.7). Conversely, at SW17, where 100% of samples were collected while it was stocked, *E. coli* was not correlated with AllBac (Figure 4.6B) or BoBac (Figure 4.6E) when stocked. This further confirms the geographic variability of these markers suggesting feces for development of gene copy curves should be collected from the watershed being assessed to reduce potential errors resulting from this variability in *Bacteroides* populations.

Figure 4.7
Linear regression analysis showing correlation of AllBac and BoBac concentrations to *E. coli* concentrations in runoff from BCSC sites when stocked.



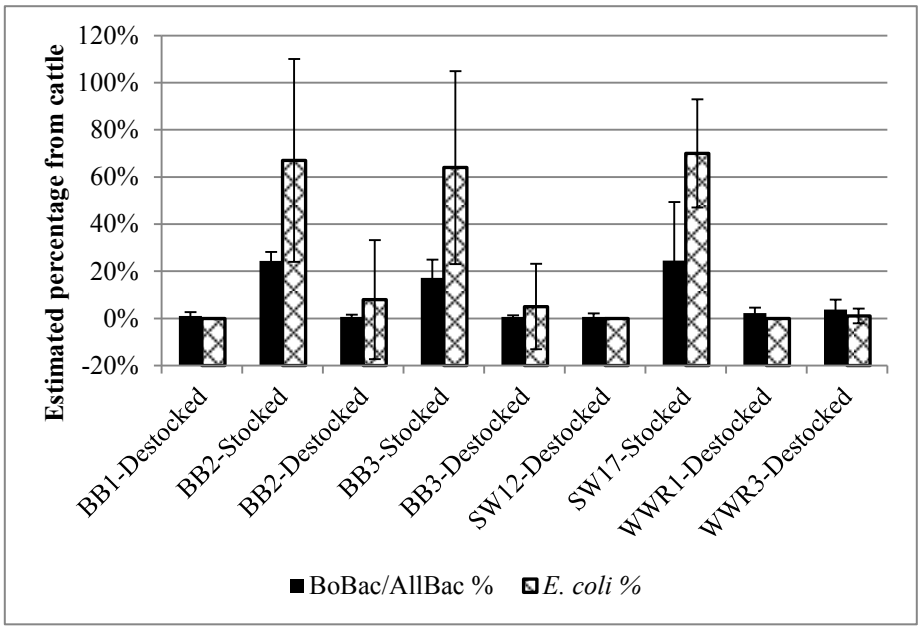
4.4.4 Evaluation of Cattle Contributions Using BoBac/AllBac

The BoBac/AllBac ratio was used to assess the percentage of bovine-associated fecal contamination for each runoff event. To validate this ratio, it was compared to the percentage of bovine-associated contamination estimated using *E. coli* levels determined as described below. For each location (i.e. Welder, BCSC, Riesel), if the *E. coli* level at the grazed site was equal to or less than the average concentration observed at the ungrazed control site, then the percentage of *E. coli* from cattle was considered 0%. If the *E. coli* level at the grazed site exceeded the average concentration observed at the ungrazed control site, then the percent of *E. coli* attributed to cattle was considered to be:

$$\left[\frac{\textit{E. coli}\text{ conc. at grazed site} - \text{average }\textit{E. coli}\text{ conc. at ungrazed control site}}{\textit{E. coli}\text{ conc. at grazed site}}\right] \text{X } 100$$

As would be expected, when sites were destocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac was low averaging ~1% (Figure 4.8). Furthermore, when sites were stocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac averaged 23%. In comparison, *E. coli* analysis estimated that 6% of the bacterial loading resulted from cattle and other ruminants at ungrazed and destocked sites, while an average of 67% of the bacterial loading was estimated to result from cattle and other ruminants when sites were stocked. Thus, although elevated BoBac/AllBac ratios generally aligned well with cattle presence, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac was significantly lower than that estimated by *E. coli* and thus appears to underestimate cattle contributions.

Figure 4.8
Comparison of average percentage of bacterial loading attributed to cattle in runoff from grazing land as estimated using *E. coli* concentrations and BoBac/AllBac gene copy concentrations. The error bars on each bar indicate one standard deviation.



Co-extraction of substances can completely or partially inhibit PCR amplification of target markers in environmental samples (Haugland et al. 2010) and result in underestimation of marker levels as observed using the BoBac/AllBac gene copy percentages. However, percent recoveries for both AllBac and BoBac gene copies in this study generally ranged from 70 to 130% indicating inhibition was likely not a major factor. Similar underestimations have been observed with other markers such as the swine PF163 primer, which did not match well with a majority of environmental water sequences (Lamendella et al. 2009). Potential diversity in *Bacteroides* spp., as observed by Lamendella et al. (2009) with swine markers, complicates development of inclusive host-specific assays. Further, Dick et al. (2010) suggested a reservoir of AllBac markers

may persist in recreational waters and thus may not be useful for estimating the source contributions in ratio with other markers. Similarly, van der Wielen and Medema (2010) questioned whether PCR methods detected only fecal *Bacteroidales* species and suggested environmental sources of *Bacteroidales* of nonfecal origin (including from the hindguts of insects) also occurred. More work is needed to assess the ability of species-specific marker (e.g. BoBac) to total *Bacteroides* (e.g. AllBac) ratios in order to accurately quantify contamination for specific sources (Haugland et al. 2010).

4.4.5 Evaluation of AllBac and BoBac Fecal Concentrations

In addition to measuring AllBac and BoBac gene copy concentrations, AllBac and BoBac fecal concentrations were also measured as described by Layton et al. (2006). In general, the relationship between both AllBac and BoBac fecal concentrations were similar to those observed between the gene copies for these markers. However, fecal concentrations found by this study were substantially lower than those found by Layton et al. (2006) in high flow samples. The AllBac fecal concentrations observed in edge-of-field runoff in this study, which averaged 1.96 mg/L, were approximately two orders of magnitude lower than the levels found in high flow samples by Layton et al. (2006), which ranged from 100 to 452 mg/L and averaged 276 mg/L.

To further evaluate the reasonableness of the fecal concentrations determined by AllBac and BoBac, fecal concentrations were also estimated using *E. coli* concentrations by dividing the observed *E. coli* concentrations (cfu/100 mL) by *E. coli* levels found in beef cattle manure (5.9 $\log_{10}$ cfu/g—McDowell et al. 2008) and converting the concentration to mg/L for comparison. Using this method, fecal concentrations estimated

using *E. coli* levels were comparable to those reported by Layton, averaging 361 mg/L, but were again, approximately 2 orders of magnitude higher than those determined using AllBac and BoBac.

These analyses suggest the fecal concentrations determined by this study using AllBac and BoBac do not accurately represent fecal loadings. The method used by this study contained several divergences from that used by Layton et al. (2006), which may help explain this observed difference. Primarily, runoff samples for this study were filtered and DNA extracted from the filters prior to qPCR analysis while Layton's environmental samples were not filtered and were directly analyzed using qPCR without DNA extraction. Although filtration would be expected to concentrate and increase the measurable levels of *Bacteroides* instead of decrease them, loss of DNA can occur during the extraction process as a result of incomplete cell lysis, DNA sorption to soil or other surfaces, coextraction of enzymatic inhibitors, and loss, degradation, or damage of DNA (Miller et al. 1999). Further, PCR inhibition could have further reduced observable fecal concentrations. However, PCR inhibition was not considered to be a major factor as percent recoveries generally ranged from 70 to 130%.

It was also postulated that *Bacteroides* levels in the fecal sample used to develop the standard curve for this study could possibly be different from those in Layton et al. (2006) resulting in the significantly lower levels. The fecal curves used for this study were based on a single fecal sample from one individual animal. It is unclear whether the Layton et al. (2006) fecal curve was based on one individual animal or a slurry from multiple individuals. However, because *Bacteroides* levels can vary between individuals,

it was anticipated this may have been a factor. Further, considerable variability in *Bacteroides* DNA yield and PCR inhibitor presence may result from intra-specimen variability as well, as feces are very heterogeneous biological materials (Nechvatal et al. 2008). However, when threshold cycle (Ct) values and fecal concentrations from both studies were compared, the fecal concentration standard curves from this study were found to be comparable to Layton et al. (2006). Finally, geographic instability of the markers, as previously noted, may have contributed as well to the lower levels observed. Nevertheless, our study did not find the use of AllBac and BoBac fecal concentrations beneficial as it did not provide additional information and results were suspect due to the significantly lower levels observed.

4.5 Summary and Conclusions

Using host-specific markers can provide important information about sources of fecal pollution to impaired waters (Weidhaas et al. 2011). However, uncertainties remain regarding the geographical stability of markers and the difficulty of interpreting results in relation to regulatory water quality standards and quantifying source contributions (Jones et al. 2009). The goals of this study were to determine the ability of the BoBac marker to accurately assess the percentage of bovine-associated fecal contamination at the small watershed scale and to further evaluate the relationship between total *Bacteroides* (AllBac) and *E. coli* levels and its relevance as a fecal indicator.

Data indicate neither AllBac and BoBac concentrations were correlated with either grazing management or annual stocking rate. However, both markers were significantly correlated with the percentage of runoff events that occurred while sites

were stocked or had been stocked less than two weeks prior to runoff suggesting that they provide a good indicator of recent fecal contamination from cattle. In addition, BoBac concentrations were significantly higher in runoff when cattle were actively grazing or had grazed the sites within two weeks of the runoff event validating BoBac as a suitable indicator of recent fecal contamination from cattle. Further, when sites were destocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac averaged 1% as would be expected. However, when sites were stocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac was lower than expected, averaging only 23% instead of 67% as estimated using *E. coli* concentrations. Thus, although elevated BoBac/AllBac ratios generally aligned well with stocking, it is unclear whether they can accurately estimate the percentage of bovine-associated fecal contamination. More work is needed to assess the ability of species-specific markers (e.g. BoBac) to total *Bacteroides* (e.g. AllBac) ratios to accurately quantify contamination for specific sources.

Overall, a significant, but weak, correlation was observed between AllBac and BoBac gene copy and *E. coli* concentrations when data from all sites were compared; however, results varied by location with best results being obtained at sites where feces was collected for development of gene copy curves. Further, when sites where feces was collected for development of the gene copy curves were stocked, strong correlations between both AllBac and BoBac and *E. coli* were observed. These results are encouraging in that the AllBac and BoBac primers and probes developed and validated based primarily upon samples from eastern Tennessee worked well for some of the

watersheds in Texas; however, the differing results in the various watersheds, apparently due to geographic variability in the AllBac and BoBac markers, suggests that feces for development of gene copy curves for future studies should be collected from each specific watershed being assessed. Using watershed-specific standard curves may help to reduce potential errors resulting from geographic variability in *Bacteroides* populations.

In conclusion, these markers appear to be potentially useful tools for identifying sources of fecal contamination; however, more work is needed to improve their ability to accurately quantify total and source-specific bacterial loading before implementation at the watershed scale.

CHAPTER V

SUMMARY

5.1 Evaluation of BMPs to Reduce Bacterial Pollution from Grazing

This study evaluated two BMPs, alternative water supplies and prescribed grazing, for reducing bacterial contamination of surface waters from grazing cattle. Results of this study indicated the amount of time cattle spent in the stream could be reduced 43% from 3.0 to 1.7 minutes/AU/day by providing alternative off-stream water. As a result of this, direct deposition of *E. coli* into Clear Fork of Plum Creek was estimated to be reduced 4.76E+06 cfu/AU/day from 1.11E+07 cfu/AU/day when no alternative water was provided to 6.34E+06 cfu/AU/day once alternative water was provided. Observed pre- and post-treatment *E. coli* loads suggested similar reductions; however, this study could not conclusively attribute the observed *E. coli* loading reductions to alternative water because of the lack of statistical significance of these observations, the decrease in flow observed during Year 2, and the observed increase in *E. coli* levels during Year 2. A drought during Year 2, which reduced flows by 79% and influenced ranch management decisions to increase the stocking rate by 34%, may explain much of the increase in *E. coli* levels observed. Other probable factors impacting observed *E. coli* levels include natural variability, changes in fate and transport due to the drought, and potential increased contributions from wildlife. Although this study did not provide conclusive evidence of reduced *E. coli* levels resulting from providing alternative off-stream water supplies, this practice is still highly recommended due to the

significant reductions observed in the time cattle spent in and near the stream, the 51% reduction in fecal coliform documented by Sheffield et al. (1997), and the 85 to 95% decrease in median base flow *E. coli* load found by Byers et al. (2005). These reductions are comparable to those provided by fencing of streams (Brenner et al. 1994; Brenner 1996; Cook 1998; Hagedorn et al. 1999; Lombardo et al. 2000; Meals 2001; Line 2002; Line 2003; Meals 2004). Further, this study supports McIver (2004) who noted alternative water supplies alone would not achieve water quality improvements unless implemented in conjunction with good grazing management (i.e. appropriate stocking rate, evenly distributed grazing, avoiding grazing during vulnerable periods, and providing ample rest after grazing events). As a result of the severe drought, these principles were not adhered to and likely confounded improvements in water quality that could have been due to the provision of alternative water supplies.

Results also suggest that rotational stocking is an effective practice for reducing *E. coli* runoff. When runoff occurred more than two weeks following grazing, *E. coli* levels in runoff were decreased from 88 to 99% as compared to runoff when the sites were being actively grazed. This effect of timing of stocking in relation to subsequent runoff events was much more significant than the impact of grazing management (i.e. ungrazed, properly stocked or overstocked) or stocking rate. No significant differences were observed between *E. coli* runoff from properly or over-stocked pastures, although very high stocking rates did exhibit the potential to produce the highest *E. coli* concentrations. Highest *E. coli* concentrations were generally observed when runoff occurred within two weeks of grazing and the annual stocking rate was heavier than 1.3

ha/AUY (3.2 ac/AUY); however, no significant differences were observed between stocking rates of 0.7 to 3.1 ha/AUY (1.7 to 7.7 ac/AUY) for these events. When the stocking rate was 11 ha/AUY (27 ac/AUY) or lighter (and runoff occurred within two weeks of them being grazed), *E. coli* levels were significantly lower and did not exceed background levels. As a result of these findings, it is recommended that creek pastures and other hydrologically connected pastures be stocked during periods when runoff is less likely (e.g. summer and winter in much of Texas) and upland sites be grazed during rainy seasons when runoff is more likely. Further research is recommended to evaluate the impact of rotational stocking of creek pastures on a watershed scale. Additional research is also needed to evaluate runoff from severely overgrazed sites as well as sites that are continuously grazed since runoff conditions from these may be significantly different than those observed by this study.

Although not a primary objective of the original study, a significant finding of this research was that background *E. coli* levels were considerable and relatively consistent across all sites, with median levels typically ranging from 3,500 to 5,500 cfu/100 mL. Most existing water quality models and thus total maximum daily loads and other watershed plans do not take background *E. coli* levels into account. Background levels should be considered when applying these models in order to prevent over-allocating loads and loading reductions to other sources. In addition to this, this study suggests the potential impact of non-domesticated animals on *E. coli* runoff from grazing lands can be considerable. As observed at all Beef Cattle Systems Center sites in October 2009, median concentrations increased approximately an order of magnitude

presumably due to non-domesticated animals (i.e. feral hogs or migratory birds). Loading from these sources during this period was responsible for 80% to 99% of the total loading in 2009. These results support the need to revise water quality standards as they apply to storm flow conditions. Ninety percent of runoff samples exceeded Texas Water Quality Standards, even at ungrazed sites. Although these water quality standards apply only to surface waters (e.g. streams and lakes) and not edge-of-field runoff as described here, this study does expose the impracticality of applying the existing water quality standards to runoff events, especially in runoff dominated streams. Background levels need to be considered as well as the significant impacts of non-domesticated animals. As such, it is recommended that exemptions from the current standards be made for storm flows and wildlife or additional research be conducted to accurately define bacterial quality for runoff and establish practical stream water quality standards.

5.2 Evaluation of qPCR to Assess Bacterial Pollution from Grazing

Finally, this study evaluated the ability of qPCR analysis of the bovine-associated *Bacteroides* marker, BoBac, to accurately assess the percentage of bovine-associated fecal contamination at the small watershed scale and the relevance the total *Bacteroides* marker, AllBac, as a fecal indicator. Data indicate neither AllBac nor BoBac concentrations were correlated with either grazing management or annual stocking rate. However, both markers were significantly correlated with the percentage of runoff events that occurred while sites were stocked or had been stocked less than two weeks prior to runoff suggesting that they provide a good indicator of recent fecal contamination from cattle. In addition, BoBac concentrations were significantly higher

in runoff when cattle were actively grazing or had grazed the sites within two weeks of the runoff event validating BoBac as a suitable indicator of recent fecal contamination from cattle. Further, when sites were destocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac averaged 1% as would be expected. However, when sites were stocked, the percentage of bovine-associated fecal contamination determined using BoBac/AllBac was lower than expected, averaging only 23% instead of 67% as estimated using *E. coli* concentrations. Thus, although elevated BoBac/AllBac ratios generally aligned well with stocking, it is unclear whether they can accurately estimate the percentage of bovine-associated fecal contamination. More work is needed to assess the ability of species-specific markers (e.g. BoBac) to total *Bacteroides* (e.g. AllBac) ratios to accurately quantify contamination for specific sources.

Overall, a significant, but weak, correlation was observed between AllBac and BoBac gene copy and *E. coli* concentrations when data from all sites were compared; however, results varied by location with best results being obtained at sites where feces was collected for development of gene copy curves. Further, when sites where feces was collected for development of the gene copy curves were stocked, strong correlations between both AllBac and BoBac and *E. coli* were observed. These results are encouraging in that the AllBac and BoBac primers and probes developed and validated based primarily upon samples from eastern Tennessee worked well for some of the watersheds in Texas; however, the differing results in the various watersheds, apparently due to geographic variability in the AllBac and BoBac markers, suggests that feces for

development of gene copy curves for future studies should be collected from each specific watershed being assessed. Using watershed-specific standard curves may help to reduce potential errors resulting from geographic variability in *Bacteroides* populations.

Although these markers appear to be potentially useful tools for identifying sources of fecal contamination, more work is needed to improve their ability to accurately quantify total and source-specific bacterial loading before implementation at the watershed scale.

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APPENDIX A

GPS MONITORING DATA

GPS monitoring dates.

Start Date	End Date	Treatment
7/4/07	7/25/07	No trough
10/3/07	10/25/07	No trough
1/11/08	2/2/08	Trough
4/4/08	4/26/08	No trough
9/19/08	10/9/08	Trough
11/7/08	11/29/08	Trough
2/5/09	2/27/09	Trough
4/10/09	5/2/09	Trough

2S GPS Collar Data

GPS	Sample	Total	Corrected	Points in each buffer					% time within each buffer					
Number	Period	Points	Tot Pts	In-stream	15 ft	25 ft	35 ft	50 ft	In-stream	15 ft	25 ft	35 ft	50 ft	BMP
1276	Jul-07	6336	6252	13	93	148	266	433	0.2%	1.5%	2.4%	4.3%	6.9%	None
1909	Jul-07	6336	6183	8	63	122	205	356	0.1%	1.0%	2.0%	3.3%	5.8%	None
1910	Jul-07	6335	6211	8	88	168	260	412	0.1%	1.4%	2.7%	4.2%	6.6%	None
380	Jul-07	6336	6242	8	70	146	217	308	0.1%	1.1%	2.3%	3.5%	4.9%	None
88	Jul-07	6336	6314	46	279	424	506	649	0.7%	4.4%	6.7%	8.0%	10.3%	None
Mean	**Jul-07**	**6336**		**17**	**119**	**202**	**291**	**432**	**0.3%**	**1.9%**	**3.2%**	**4.6%**	**6.9%**	
1276	Oct-07	6446	6327	7	89	145	211	307	0.1%	1.4%	2.3%	3.3%	4.9%	None
1909	Oct-07	6446	6321	11	128	212	287	383	0.2%	2.0%	3.4%	4.5%	6.1%	None
1910	Oct-07	6446	6294	15	103	191	288	440	0.2%	1.6%	3.0%	4.6%	7.0%	None
1951	Oct-07	6446	6267	15	112	195	297	458	0.2%	1.8%	3.1%	4.7%	7.3%	None
1952	Oct-07	6446	6326	9	106	199	289	421	0.1%	1.7%	3.1%	4.6%	6.7%	None
380	Oct-07	6446	6388	13	112	186	256	354	0.2%	1.8%	2.9%	4.0%	5.5%	None
88	Oct-07	6446	6351	26	120	183	265	369	0.4%	1.9%	2.9%	4.2%	5.8%	None
Mean	**Oct-07**	**6446**		**14**	**110**	**187**	**270**	**390**	**0.2%**	**1.7%**	**3.0%**	**4.3%**	**6.2%**	
1909	Jan-08	6621	6538	4	25	39	60	88	0.1%	0.4%	0.6%	0.9%	1.3%	Trough
1910	Jan-08	6624	6482	3	18	27	40	53	0.0%	0.3%	0.4%	0.6%	0.8%	Trough
1951	Jan-08	6624	6541	4	51	74	108	173	0.1%	0.8%	1.1%	1.7%	2.6%	Trough
1952	Jan-08	6624	6555	3	22	43	66	107	0.0%	0.3%	0.7%	1.0%	1.6%	Trough
87	Jan-08	6624	4448	4	41	70	89	126	0.1%	0.9%	1.6%	2.0%	2.8%	Trough
Mean	**Jan-08**	**6623**		**4**	**31**	**51**	**73**	**109**	**0.1%**	**0.5%**	**0.9%**	**1.2%**	**1.9%**	

2S GPS Collar Data

GPS Number	Sample Period	Total Points	Corrected Tot Pts	Points in each buffer					% time within each buffer					BMP
				In-stream	15 ft	25 ft	35 ft	50 ft	In-stream	15 ft	25 ft	35 ft	50 ft	
1276	Apr-08	6624	6460	13	101	179	274	487	0.2%	1.6%	2.8%	4.2%	7.5%	None
1909	Apr-08	6621	6422	25	159	263	373	572	0.4%	2.5%	4.1%	5.8%	8.9%	None
1910	Apr-08	6620	6197	5	64	115	205	343	0.1%	1.0%	1.9%	3.3%	5.5%	None
1951	Apr-08	6624	6434	7	69	121	196	334	0.1%	1.1%	1.9%	3.0%	5.2%	None
1952	Apr-08	6624	6485	15	135	254	380	590	0.2%	2.1%	3.9%	5.9%	9.1%	None
380	Apr-08	6624	6454	21	172	249	360	505	0.3%	2.7%	3.9%	5.6%	7.8%	None
87	Apr-08	6623	4836	21	168	280	409	552	0.4%	3.5%	5.8%	8.5%	11.4%	None
88	Apr-08	6621	6460	15	122	217	370	636	0.2%	1.9%	3.4%	5.7%	9.8%	None
Mean	**Apr-08**	**6623**		**15**	**124**	**210**	**321**	**502**	**0.3%**	**2.0%**	**3.4%**	**5.3%**	**8.2%**	
1276	Sep-08	6048	6011	21	183	271	357	507	0.3%	3.0%	4.5%	5.9%	8.4%	Trough
1909	Sep-08	6048	5958	7	43	69	106	154	0.1%	0.7%	1.2%	1.8%	2.6%	Trough
1910	Sep-08	6048	5839	15	111	161	233	310	0.3%	1.9%	2.8%	4.0%	5.3%	Trough
1951	Sep-08	6048	5906	12	98	164	246	330	0.2%	1.7%	2.8%	4.2%	5.6%	Trough
1952	Sep-08	6048	5975	7	84	138	202	295	0.1%	1.4%	2.3%	3.4%	4.9%	Trough
380	Sep-08	6048	5992	5	43	67	98	169	0.1%	0.7%	1.1%	1.6%	2.8%	Trough
87	Sep-08	6048	4639	8	38	86	129	196	0.2%	0.8%	1.9%	2.8%	4.2%	Trough
88	Sep-08	6048	5940	5	38	71	97	144	0.1%	0.6%	1.2%	1.6%	2.4%	Trough
Mean	**Sep-08**	**6048**		**10**	**80**	**128**	**184**	**263**	**0.2%**	**1.4%**	**2.2%**	**3.2%**	**4.5%**	
1276	Nov-08	6624	6576	8	50	86	115	174	0.1%	0.8%	1.3%	1.7%	2.6%	Trough
1909	Nov-08	6624	6562	2	52	85	121	176	0.0%	0.8%	1.3%	1.8%	2.7%	Trough
1910	Nov-08	6624	6481	12	71	128	165	227	0.2%	1.1%	2.0%	2.5%	3.5%	Trough
1951	Nov-08	1622	1613	0	9	18	27	40	0.0%	0.6%	1.1%	1.7%	2.5%	Trough
1952	Nov-08	6624	6578	4	37	56	80	133	0.1%	0.6%	0.9%	1.2%	2.0%	Trough
380	Nov-08	6624	6583	7	52	85	128	203	0.1%	0.8%	1.3%	1.9%	3.1%	Trough
87	Nov-08	6624	4831	3	43	87	127	216	0.1%	0.9%	1.8%	2.6%	4.5%	Trough
88	Nov-08	6625	6576	8	57	85	112	146	0.1%	0.9%	1.3%	1.7%	2.2%	Trough
Mean	**Nov-08**	**5999**		**6**	**46**	**79**	**109**	**164**	**0.1%**	**0.8%**	**1.4%**	**1.9%**	**2.9%**	

2S GPS Collar Data

GPS	Sample	Total	Corrected	Points in each buffer					% time within each buffer					
Number	Period	Points	Tot Pts	In-stream	15 ft	25 ft	35 ft	50 ft	In-stream	15 ft	25 ft	35 ft	50 ft	BMP
1276	Feb-09	6624	6599	11	59	98	128	160	0.2%	0.9%	1.5%	1.9%	2.4%	Trough
1909	Feb-09	6624	6585	7	55	76	90	109	0.1%	0.8%	1.2%	1.4%	1.7%	Trough
1910	Feb-09	6624	6534	14	81	149	217	309	0.2%	1.2%	2.3%	3.3%	4.7%	Trough
1951	Feb-09	6624	6597	4	52	80	97	131	0.1%	0.8%	1.2%	1.5%	2.0%	Trough
1952	Feb-09	6624	6603	9	76	109	133	158	0.1%	1.2%	1.7%	2.0%	2.4%	Trough
380	Feb-09	6624	6593	10	51	78	104	155	0.2%	0.8%	1.2%	1.6%	2.4%	Trough
87	Feb-09	6624	4721	9	42	60	75	87	0.2%	0.9%	1.3%	1.6%	1.8%	Trough
88	Feb-09	6624	6607	7	40	63	70	91	0.1%	0.6%	1.0%	1.1%	1.4%	Trough
Mean	**Feb-09**	**6624**		**9**	**57**	**89**	**114**	**150**	**0.1%**	**0.9%**	**1.4%**	**1.8%**	**2.3%**	
1276	Apr-09	6624	6507	4	45	90	153	247	0.1%	0.7%	1.4%	2.4%	3.8%	Trough
1909	Apr-09	6624	6547	19	103	161	234	346	0.3%	1.6%	2.5%	3.6%	5.3%	Trough
1910	Apr-09	6624	6401	6	77	139	220	327	0.1%	1.2%	2.2%	3.4%	5.1%	Trough
1951	Apr-09	6624	6509	16	85	155	251	407	0.2%	1.3%	2.4%	3.9%	6.3%	Trough
1952	Apr-09	2773	2694	7	32	50	75	117	0.3%	1.2%	1.9%	2.8%	4.3%	Trough
380	Apr-09	6624	6538	13	127	187	256	359	0.2%	1.9%	2.9%	3.9%	5.5%	Trough
87	Apr-09	6624	4986	13	89	148	212	287	0.3%	1.8%	3.0%	4.3%	5.8%	Trough
88	Apr-09	6624	6516	11	100	176	250	379	0.2%	1.5%	2.7%	3.8%	5.8%	Trough
Mean	**Apr-09**	**6143**		**11**	**82**	**138**	**206**	**309**	**0.2%**	**1.4%**	**2.3%**	**3.5%**	**5.2%**	

APPENDIX B

ALTERNATIVE WATER STUDY — *E. COLI,* FLOW, TURBIDITY AND LOADING DATA

2S Data (Year 1)

Date	Flow (cfs)	Days Since rain	PC1			PC2			2S *E. coli* Load (cfu/d)	# of AU	2S *E. coli* Load (cfu/AU/d)
			E. coli Conc (cfu/100mL)	Turb. (NTU)	*E. coli* Load (cfu/d)	*E. coli* Conc (cfu/100mL)	Turb. (NTU)	*E. coli* Load (cfu/d)			
7/26/07	154.83	0	1220		4.62E+12	920		3.48E+12	-1.14E+12	57	-2.00E+10
8/2/07	1.99	4	50		2.43E+09	70		3.41E+09	9.74E+08	57	1.72E+07
8/16/07	1.56	0	2300		8.78E+10	3300		1.26E+11	3.82E+10	57	6.73E+08
9/6/07	1.07	2	320		8.38E+09	880		2.30E+10	1.47E+10	57	2.59E+08
9/20/07	0.66	1	40		6.46E+08	110		1.78E+09	1.13E+09	57	1.99E+07
10/4/07	0.43	9	17		1.75E+08	287		3.02E+09	2.84E+09	57	5.01E+07
10/18/07	0.51	10	80		9.98E+08	27		3.33E+08	-6.65E+08	57	-1.17E+07
11/1/07	0.51	10	30		3.74E+08	55		6.86E+08	3.12E+08	57	5.50E+06
11/15/07	0.43	24	213		2.24E+09	162		1.70E+09	-5.37E+08	57	-9.46E+06
12/6/07	0.51	10	73		9.05E+08	200		2.50E+09	1.59E+09	57	2.81E+07
12/20/07	0.51	5	31		3.87E+08	35		4.37E+08	4.99E+07	57	8.80E+05
1/3/08	0.59	19	17		2.45E+08	104		1.50E+09	1.26E+09	57	2.21E+07
1/17/08	0.51	2	87		1.09E+09	111		1.39E+09	2.99E+08	57	5.28E+06
2/7/08	0.59	13	21	5	3.03E+08	23	5	3.32E+08	2.89E+07	57	5.09E+05
2/21/08	0.57	5	230	19	3.21E+09	420	16	5.86E+09	2.65E+09	57	4.67E+07
3/6/08	0.66	0	35	23	5.57E+08	125	12	2.02E+09	1.46E+09	57	2.58E+07
3/20/08	1.19	2	340	21	9.89E+09	295	29	8.58E+09	-1.31E+09	57	-2.31E+07
4/3/08	0.66	4	81	35	1.31E+09	103	24	1.66E+09	3.55E+08	57	6.27E+06
4/17/08	0.51	14	170	34	2.12E+09	1475	19	1.84E+10	1.63E+10	57	2.87E+08
5/1/08	0.51	5	210	60	2.62E+09	370	31	4.62E+09	2.00E+09	57	3.52E+07
5/15/08	0.43	1	182	18	1.91E+09	83	21	8.68E+08	-1.05E+09	57	-1.85E+07
6/5/08	0.31	23	90	61	6.75E+08	430	9	3.23E+09	2.55E+09	57	4.50E+07
6/19/08	0.25	13	2600	57	1.60E+10	160	12	9.82E+08	-1.50E+10	57	-2.64E+08
7/2/08	0.15	30	325	62	1.22E+09	500	8	1.87E+09	6.55E+08	57	1.16E+07

2S Data (Year 2)

Date	Flow (cfs)	Days since rain	PC1			PC2			2S *E. coli* Load (cfu/d)	# of AU	2S *E. coli* Load (cfu/AU/d)
			E. coli Conc (cfu/100mL)	Turb. (NTU)	*E. coli* Load (cfu/d)	*E. coli* Conc (cfu/100mL)	Turb. (NTU)	*E. coli* Load (cfu/d)			
7/17/08	0.16	9	410	23	1.57E+09	465	16	1.78E+09	2.10E+08	76	2.77E+06
8/7/08	0.08	14	320	17	6.58E+08	400	12	8.22E+08	1.64E+08	76	2.16E+06
8/21/08	0.08	2	490	30	1.01E+09	1075	6	2.21E+09	1.20E+09	76	1.58E+07
9/4/08	0.02	7	745	27	3.65E+08	93	6	4.55E+07	-3.19E+08	76	-4.20E+06
9/18/08	0.00	5			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00
10/2/08	0.00	21			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00
10/16/08	0.00	1			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00
11/6/08	0.04	0	150	8	1.47E+08	845	5	8.27E+08	6.80E+08	76	8.95E+06
11/20/08	0.08	16	145	4	2.97E+08	450	3	9.25E+08	6.28E+08	76	8.26E+06
12/4/08	0.15	32	43	4	1.58E+08	475	2	1.74E+09	1.59E+09	76	2.09E+07
12/18/08	0.12	10	400	3	1.17E+09	120	2	3.52E+08	-8.22E+08	76	-1.08E+07
1/8/09	0.20	2	106	4	5.19E+08	56	2	2.74E+08	-2.45E+08	76	-3.22E+06
1/22/09	0.25	16	30	2	1.81E+08	530	1	3.25E+09	3.07E+09	76	4.04E+07
2/12/09	0.25	2	450	9	2.76E+09	500	7	3.07E+09	3.07E+08	76	4.04E+06
2/26/09	0.25	16	62	10	3.77E+08	810	8	4.97E+09	4.59E+09	76	6.04E+07
3/12/09	0.59	0	5700	18	8.23E+10	8100	14	1.17E+11	3.46E+10	76	4.56E+08
3/26/09	0.15	12	20	10	7.34E+07	268	13	9.84E+08	9.10E+08	76	1.20E+07
4/9/09	0.20	7	36	5	1.76E+08	280	5	1.37E+09	1.19E+09	76	1.57E+07
4/23/09	0.15	5	660	18	2.42E+09	7000	31	2.57E+10	2.33E+10	76	3.06E+08
5/14/09	0.15	17	78	21	2.86E+08	600	47	2.20E+09	1.92E+09	76	2.52E+07
5/28/09	0.08	4	100	43	2.06E+08	410	34	8.43E+08	6.37E+08	76	8.38E+06
6/11/09	0.00	8			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00
6/24/09	0.00	24			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00
7/9/09	0.00	2			0.00E+00			0.00E+00	0.00E+00	76	0.00E+00

APPENDIX C

PUBLISHED FECAL COLIFORM CONCENTRATIONS IN RUNOFF FROM UNGRAZED AND GRAZED PASTURES

Geometric mean values for fecal coliform concentrations (cfu/100 mL) in runoff from ungrazed and grazed pastures and corresponding annual stocking rates and grazing days (in AU days per hectare—AUD/ha and AU days per acre—AUD/ac).

Grazing	Reference	Stocking Rate* ha/AUY (ac/AUY)	Grazing – AUD/ha (AUD/ac)	Fecal coliform (cfu/100 mL)
Ungrazed	Doran et al. 1981	0.0	0.0	6.6E+03
Ungrazed	Robbins et al. 1972	0.0	0.0	1.0E+04
Grazed	Edwards et al. 1997	3.1 (7.7)	117 (48)	8.7E+03
Grazed	Edwards et al. 1997	1.2 (3.0)	300 (121)	5.5E+04
Grazed	Doran et al. 1981	1.2 (2.9)	308 (124)	5.7E+04
Grazed	Edwards et al. 1997	0.9 (2.3)	386 (156)	3.7E+03
Grazed	Edwards et al. 1997	0.9 (2.3)	386 (156)	2.7E+04
Grazed	Robbins et al. 1972	0.5 (1.2)	773 (313)	3.0E+04

* Stocking rate in hectares per AU year and acres per AU year

APPENDIX D

RUNOFF DATA FROM WELDER WILDLIFE REFUGE, RIESEL, AND BEEF CATTLE SYSTEMS CENTER WATERSHEDS

Welder Wildlife Refuge *E. coli* concentration, flow, and *E. coli* loading data

Date	WWR1 - Ungrazed			WWR3 - Properly Stocked		
	***E. coli* Conc. (cfu/100 ml)**	**Flow (cf)**	***E. coli* Load (cfu/ha)**	***E. coli* Conc. (cfu/100 ml)**	**Flow (cf)**	***E. coli* Load (cfu/ha)**
11/20/09	3700	6355	6.66E+09	4100	8910	1.03E+10
11/21/09	5500	8617	1.34E+10	7500	12081	2.57E+10
12/1/09	30000	228	1.94E+09	5400	808	1.23E+09
12/17/09			0.00E+00	330	311	2.90E+07
1/15/10	7460	5299	1.12E+10	7027	6351	1.26E+10
2/5/10	880	618	1.54E+08	10300	1480	4.32E+09
2/11/10	5500	1200	1.87E+09	5500	2415	3.76E+09
7/1/10	400	20407	2.31E+09	2600	26248	1.93E+10
9/19/10	330	18920	1.77E+09	390	24064	2.66E+09
9/23/10	2800	3312	2.63E+09	1600	3196	1.45E+09

Riesel 2008 and 2009 *E. coli* concentration, flow, and *E. coli* loading data

Date	*SW12 - Ungrazed*				*SW17 – Properly Stocked*			
	E. coli* Conc. *(cfu/100 ml)	***Flow (cf)***	***E. coli Load (cfu)***	***E. coli Load (cfu/ha)***	***E. coli* Conc. *(cfu/100 ml)***	***Flow (cf)***	***E. coli Load (cfu)***	***E. coli Load (cfu/ha)***
3/3/08			0.00E+00	0.00E+00	80000	52	1.18E+09	9.83E+08
3/6/08	11250	1666	5.31E+09	4.42E+09	16200	1136	5.21E+09	4.34E+09
3/11/08	9450	5666	1.52E+10	1.26E+10	16250	1696	7.80E+09	6.50E+09
3/18/08	11750	5066	1.69E+10	1.40E+10	19150	2826	1.53E+10	1.28E+10
4/10/08			0.00E+00	0.00E+00	28000	505	4.00E+09	3.33E+09
4/10/08	4600	1666	2.17E+09	1.81E+09			0.00E+00	0.00E+00
4/17/08			0.00E+00	0.00E+00	11300	566	1.81E+09	1.51E+09
5/14/08	12550	10766	3.83E+10	3.19E+10	27000	8486	6.49E+10	5.41E+10
5/15/08	4450	4566	5.75E+09	4.79E+09			0.00E+00	0.00E+00
3/13/09	260	865	6.37E+07	5.31E+07	5400	1311	2.00E+09	1.67E+09
4/17/09			0.00E+00	0.00E+00	113000	3396	1.09E+11	9.06E+10
4/18/09	220	1666	1.04E+08	8.65E+07			0.00E+00	0.00E+00
4/28/09	110	6866	2.14E+08	1.78E+08	29000	5096	4.18E+10	3.49E+10
9/13/09			0.00E+00	0.00E+00	800000	566	1.28E+11	1.07E+11
10/9/09	1000	5166	1.46E+09	1.22E+09	14000	3966	1.57E+10	1.31E+10
10/11/09	500	2766	3.92E+08	3.26E+08			0.00E+00	0.00E+00
10/13/09	2700	4566	3.49E+09	2.91E+09	13000	7916	2.91E+10	2.43E+10
10/22/09	2600	10166	7.48E+09	6.24E+09	32000	10186	9.23E+10	7.69E+10
10/26/09	10100	19766	5.65E+10	4.71E+10	15000	18676	7.93E+10	6.61E+10
10/30/09	5900	566	9.46E+08	7.88E+08			0.00E+00	0.00E+00

Riesel 2010 *E. coli* concentration, flow, and *E. coli* loading data

Date	*SW12 - Ungrazed*				*SW17 – Properly Stocked*			
	E. coli* Conc. *(cfu/100 ml)	***Flow (cf)***	***E. coli Load (cfu)***	***E. coli Load (cfu/ha)***	***E. coli* Conc. *(cfu/100 ml)***	***Flow (cf)***	***E. coli Load (cfu)***	***E. coli Load (cfu/ha)***
11/21/09	2400	1766	1.20E+09	1.00E+09			0.00E+00	0.00E+00
1/16/10	2800	2865	2.27E+09	1.89E+09			0.00E+00	0.00E+00
1/16/10			0.00E+00	0.00E+00	20	1058	5.99E+06	4.99E+06
1/29/10	8900	16966	4.28E+10	3.56E+10	370	14706	1.54E+09	1.28E+09
2/5/10	1400	3966	1.57E+09	1.31E+09	70	3966	7.86E+07	6.55E+07
2/9/10	1600	566	2.56E+08	2.14E+08	80	1126	2.55E+07	2.13E+07
2/11/10	570	2266	3.66E+08	3.05E+08	30	4526	3.84E+07	3.20E+07
3/9/10	8000	3966	8.98E+09	7.49E+09	170	2266	1.09E+08	9.09E+07
3/21/10	18000	2866	1.46E+10	1.22E+10	4200	566	6.73E+08	5.61E+08
3/25/10	21000	3966	2.36E+10	1.97E+10	2900	1696	1.39E+09	1.16E+09
4/24/10	6200	1166	2.05E+09	1.71E+09	10000	1696	4.80E+09	4.00E+09

Beef Cattle Systems Center *E. coli* concentration, flow, and *E. coli* loading data

Date	*BB1 - Ungrazed*			*BB2 - Properly Stocked*			*BB3 - Overstocked*		
	E. coli Conc. (cfu/100 ml)	***Flow (cf)***	***E. coli load (cfu/ha)***	***E. coli conc. (cfu/100 ml)***	***Flow (cf)***	***E. coli load (cfu/ha)***	***E. coli conc. (cfu/100 ml)***	***Flow (cf)***	***E. coli load (cfu/ha)***
3/13/09							140	205	8.14E+06
3/25/09	1200	210	7.15E+07						
3/26/09				1000	429	1.21E+08	7200	1,703	3.47E+09
3/27/09							2000	62	3.52E+07
4/17/09	1155	1342	4.39E+08	980	3,529	9.79E+08	450	4,781	6.09E+08
4/18/09	4400	1755	2.19E+09	2225	4,214	2.65E+09	2100	5,460	3.25E+09
4/28/09	7600	597	1.28E+09	12200	5,173	1.79E+10	24000	7,710	5.24E+10
10/4/09	57000	200	3.23E+09	5114	781	1.13E+09	3065	4,173	3.62E+09
10/9/09	36000	472	4.82E+09	24043	3,085	2.10E+10	15000	7,134	3.03E+10
10/13/09	42851	9,347	1.13E+11	23826	16,796	1.13E+11	5591	17,952	2.84E+10
10/22/09							172500	149	7.28E+09
10/26/09	261000	6,649	4.91E+11	181000	12,325	6.32E+11	45000	13,513	1.72E+11
11/16/09					-		800000	257	5.82E+10
11/21/09	9300	267	7.03E+08	58000	2,153	3.54E+10	223750	3,474	2.20E+11
11/29/09							87000	323	7.95E+09
12/1/09	8100	3,477	7.98E+09	10800	7,210	2.20E+10	13300	9,300	3.50E+10
12/22/09	2800	86	6.82E+07	3400	1,093	1.05E+09	4400	2,585	3.22E+09
12/30/09				2300	287	1.87E+08	5000	1,113	1.58E+09
1/16/10	410	249	2.89E+07	4900	1,888	2.62E+09	830	1,893	4.45E+08
1/29/10	5400	5,264	8.05E+09	9500	10,028	2.70E+10	4400	11,233	1.40E+10
2/4/10	2400	319	2.17E+08	8800	1,893	4.72E+09	2600	2,378	1.75E+09
2/8/10	9800	1,992	5.53E+09	6000	4,653	7.91E+09	8100	5,448	1.25E+10
2/11/10	2100	3,456	2.06E+09	1500	7,059	3.00E+09	1100	8,204	2.56E+09
6/9/10	8900	10,141	2.56E+10	8200	18,112	4.21E+10	9250	19,258	5.04E+10

APPENDIX E

TOTAL *BACTEROIDES* (ALLBAC) DATA FROM WELDER WILDLIFE REFUGE, RIESEL, AND BEEF CATTLE SYSTEMS CENTER WATERSHEDS

Site	Event Date	Filter vol.(mL)	Vol. eluted (uL)	Template vol. (uL)	AllBac (copies/rxn)	AllBac (copies/L)	AllBac %R	qPCRAllBac fecal conc	AllBac fecal conc (mg/L)
BB1	3/25/09	10	100	5	6.76E+02	1.35E+06	102%	6.47E+01	6.47E-01
BB1	4/17/09	20	100	5	2.15E+03	2.15E+06	81%	1.34E+02	6.68E-01
BB1	4/18/09	30	100	5	2.41E+04	1.61E+07	86%	6.46E+02	2.15E+00
BB1	4/28/09	20	100	5	1.32E+03	1.32E+06	85%	9.83E+01	4.91E-01
BB1	10/4/09	10	100	5	3.42E+04	6.84E+07	106%	6.50E+02	6.50E+00
BB1	10/9/09	30	100	5	1.98E+04	1.32E+07	101%	4.76E+02	1.59E+00
BB1	10/13/09	30	100	5	1.43E+04	9.53E+06	98%	3.72E+02	1.24E+00
BB1	10/26/09	30	100	5	1.11E+05	7.43E+07	98%	5.49E+02	1.83E+00
BB1	10/26/09	30	100	5	1.42E+04	9.44E+06	100%	3.76E+02	1.25E+00
BB1	11/21/09	20	100	5	3.58E+03	3.58E+06	108%	1.80E+02	9.01E-01
BB1	12/1/09	20	100	5	2.52E+03	2.52E+06	98%	1.18E+02	5.90E-01
BB1	1/16/10	25	100	5	1.76E+04	1.41E+07	98%	4.43E+02	1.77E+00
BB1	1/29/10	25	100	5	7.83E+03	6.27E+06	104%	2.11E+02	8.43E-01
BB1	2/4/10	20	200	5	1.48E+03	2.96E+06	100%	1.76E+01	1.76E-01
BB2	3/25/09	10	100	5	4.25E+02	8.50E+05	95%	4.82E+01	4.82E-01
BB2	3/25/09	10	100	5	1.86E+02	3.72E+05	97%	2.88E+01	2.88E-01
BB2	4/17/09	20	100	5	8.27E+02	8.27E+05	103%	7.48E+01	3.74E-01
BB2	4/18/09	30	100	5	8.93E+03	5.95E+06	93%	3.42E+02	1.14E+00
BB2	4/28/09	20	100	5	1.02E+03	1.02E+06	94%	8.34E+01	4.17E-01
BB2	10/4/09	20	100	5	4.37E+03	4.37E+06	99%	2.01E+02	1.01E+00
BB2	10/4/09	30	100	5	6.45E+03	4.30E+06	105%	2.52E+02	8.40E-01
BB2	10/9/09	30	100	5	1.60E+04	1.07E+07	103%	4.01E+02	1.34E+00
BB2	10/13/09	30	100	5	9.01E+03	6.01E+06	89%	2.74E+02	9.14E-01
BB2	10/26/09	30	100	5	2.36E+04	1.58E+07	100%	1.82E+02	6.07E-01
BB2	10/26/09	30	100	5	5.45E+03	3.63E+06	98%	2.21E+02	7.37E-01
BB2	11/21/09	30	100	5	4.23E+04	2.82E+07	99%	7.33E+02	2.44E+00

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	AllBac (copies/rxn)	AllBac (copies/L)	AllBac %R	qPCR AllBac fecal conc	AllBac fecal conc (mg/L)
BB2	12/1/09	20	100	5	5.12E+03	5.12E+06	97%	1.89E+02	9.44E-01
BB2	1/16/10	25	50	1	4.50E+03	9.00E+06	74%	2.98E+02	5.95E-01
BB2	1/29/10	25	100	5	2.55E+03	2.04E+06	96%	1.16E+02	4.66E-01
BB2	2/4/10	20	200	5	1.79E+03	3.57E+06	99%	2.11E+01	2.11E-01
BB2	2/8/10	10	50	1	8.73E+02	4.36E+06	97%	8.06E+01	4.03E-01
BB3	3/13/09	20	100	5	2.93E+03	2.93E+06	101%	1.62E+02	8.11E-01
BB3	3/25/09	10	100	5	7.47E+02	1.49E+06	97%	3.27E+01	3.27E-01
BB3	3/25/09	10	100	5	4.20E+02	8.40E+05	96%	4.80E+01	4.80E-01
BB3	3/27/09	20	100	5	1.17E+04	1.17E+07	86%	3.88E+02	1.94E+00
BB3	4/17/09	20	100	5	7.46E+02	7.46E+05	103%	7.00E+01	3.50E-01
BB3	4/18/09	30	100	5	6.33E+03	4.22E+06	97%	2.75E+02	9.15E-01
BB3	4/28/09	20	100	5	8.59E+02	8.59E+05	90%	7.51E+01	3.76E-01
BB3	10/4/09	40	100	5	6.71E+03	3.35E+06	99%	2.58E+02	6.44E-01
BB3	10/4/09	25	100	5	4.13E+03	3.30E+06	103%	1.95E+02	7.81E-01
BB3	10/9/09	30	100	5	7.14E+03	4.76E+06	100%	2.57E+02	8.56E-01
BB3	10/13/09	30	100	5	5.49E+03	3.66E+06	95%	1.98E+02	6.59E-01
BB3	10/26/09	30	100	5	3.42E+04	2.28E+07	97%	2.37E+02	7.91E-01
BB3	10/26/09	30	100	5	4.45E+03	2.97E+06	98%	1.98E+02	6.59E-01
BB3	11/16/09	20	100	5	3.52E+06	3.52E+09	-59%	1.41E+04	7.06E+01
BB3	11/21/09	30	100	5	1.44E+05	9.60E+07	85%	1.47E+03	4.91E+00
BB3	11/29/09	20	100	5	5.12E+04	5.12E+07	105%	5.68E+02	2.84E+00
BB3	12/1/09	20	100	5	1.07E+04	1.07E+07	96%	3.07E+02	1.54E+00
BB3	1/16/10	25	100	5	1.69E+04	1.35E+07	112%	3.16E+02	1.27E+00
BB3	1/29/10	25	100	5	4.16E+03	3.33E+06	98%	1.51E+02	6.03E-01
BB3	2/4/10	15	50	1	4.62E+03	1.54E+07	94%	3.04E+02	1.01E+00
BB3	2/8/10	15	50	1	6.40E+02	2.13E+06	100%	6.29E+01	2.10E-01

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	AllBac (copies/rxn)	AllBac (copies/L)	AllBac %R	qPCR AllBac fecal conc	AllBac fecal conc (mg/L)
SW12	3/3/08	50	200	5	8.21E+03	6.57E+06	89%	7.98E+01	3.19E-01
SW12	3/6/08	50	100	5	2.35E+04	9.38E+06	113%	1.90E+02	3.80E-01
SW12	3/10/08	50	200	5	2.62E+03	2.10E+06	90%	3.88E+01	1.55E-01
SW12	3/10/08	50	100	5	4.04E+04	1.62E+07	104%	1.68E+02	3.36E-01
SW12	3/18/08	50	100	5	1.46E+04	5.84E+06	92%	1.33E+02	2.66E-01
SW12	4/10/08	50	100	5	2.65E+04	1.06E+07	97%	3.69E+02	7.38E-01
SW12	4/10/08	50	100	5	8.31E+03	3.32E+06	97%	3.27E+02	6.54E-01
SW12	5/14/08	100	100	5	1.87E+04	3.74E+06	93%	5.49E+02	5.49E-01
SW12	5/15/08	99.75	200	5	2.57E+04	1.03E+07	107%	2.69E+02	5.39E-01
SW12	3/13/09	50	100	5	1.65E+04	6.61E+06	104%	2.68E+02	5.36E-01
SW12	4/17/09	40	100	5	4.78E+04	2.39E+07	91%	1.00E+03	2.50E+00
SW12	4/18/09	100	100	5	1.76E+04	3.52E+06	93%	2.80E+02	2.80E-01
SW12	4/28/09	50	200	5	1.12E+03	8.92E+05	77%	1.13E+01	4.53E-02
SW12	10/9/09	25	100	5	1.02E+04	8.17E+06	99%	1.00E+02	4.02E-01
SW12	10/9/09	25	100	5	2.61E+03	2.09E+06	99%	1.47E+02	5.88E-01
SW12	10/11/09	25	100	5	5.56E+03	4.45E+06	113%	1.16E+02	4.64E-01
SW12	10/13/09	25	100	5	2.09E+03	1.68E+06	108%	3.71E+01	1.48E-01
SW12	10/22/09	25	50	5	1.04E+04	4.18E+06	97%		
SW12	10/26/09	25	50	5	4.15E+03	1.66E+06	84%		
SW12	10/30/09	25	50	5	2.02E+04	8.09E+06	116%		
SW12	1/16/10	25	100	5	8.92E+02	7.13E+05	98%	8.08E+01	3.23E-01
SW17	3/3/08	50	200	5	1.15E+05	9.16E+07	87%	1.05E+03	4.20E+00
SW17	3/6/08	50	200	5	3.09E+04	2.47E+07	94%	2.91E+02	1.17E+00
SW17	3/10/08	50	100	5	2.46E+04	9.82E+06	94%	6.55E+02	1.31E+00
SW17	3/10/08	50	200	5	1.40E+04	1.12E+07	96%	1.34E+02	5.38E-01
SW17	3/18/08	50	100	5	4.34E+04	1.74E+07	90%	2.71E+02	5.41E-01

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	AllBac (copies/rxn)	AllBac (copies/L)	AllBac %R	qPCR AllBac fecal conc	AllBac fecal conc (mg/L)
SW17	4/10/08	50	100	5	1.31E+05	5.22E+07	96%	6.14E+02	1.23E+00
SW17	4/18/08	30	100	5	1.48E+05	9.88E+07	92%	2.06E+03	6.87E+00
SW17	5/14/08	30	200	5	1.18E+05	1.58E+08	98%	1.15E+03	7.68E+00
SW17	3/13/09	50	200	5	2.58E+05	2.06E+08	97%	2.32E+03	9.28E+00
SW17	4/17/09	40	100	5	1.63E+05	8.16E+07	89%	2.19E+03	5.48E+00
SW17	4/18/09	40	100	5	1.59E+05	7.96E+07	85%	1.25E+03	3.11E+00
SW17	4/28/09	49	100	5	6.52E+03	2.66E+06	88%	2.80E+02	5.71E-01
SW17	10/9/09	25	100	5	1.54E+05	1.23E+08	106%	6.92E+02	2.77E+00
SW17	10/9/09	25	100	5	1.62E+04	1.30E+07	95%	4.04E+02	1.62E+00
SW17	10/13/09	25	100	5	1.34E+04	1.07E+07	93%	3.23E+02	1.29E+00
SW17	10/22/09	25	250	5	5.25E+04	1.05E+08	75%	1.59E+03	1.59E+01
SW17	10/26/09	50	200	5	1.98E+04	1.58E+07	99%	2.09E+02	8.36E-01
SW17	1/16/10	25	100	5	1.25E+04	9.99E+06	121%	1.15E+02	4.61E-01
WWR1	10/26/09	30	100	5	4.32E+03	2.88E+06	82%	5.46E+01	1.82E-01
WWR1	11/20/09	30	100	5	4.60E+03	3.07E+06	98%	1.76E+02	5.87E-01
WWR1	11/21/09	30	100	5	8.26E+03	5.50E+06	116%	2.90E+02	9.66E-01
WWR1	12/1/09	20	100	5	1.32E+04	1.32E+07	82%	2.78E+02	1.39E+00
WWR1	1/15/10	25	200	5	8.07E+02	1.29E+06	80%	9.87E+00	7.90E-02
WWR1	1/16/10	25	200	5	3.58E+02	5.73E+05	85%	4.55E+00	3.64E-02
WWR1	2/3/10	30	100	5	3.61E+03	2.41E+06	84%	7.00E+01	2.33E-01
WWR3	10/26/09	30	100	5	1.28E+04	8.52E+06	82%	1.18E+02	3.93E-01
WWR3	11/20/09	30	100	5	5.22E+02	3.48E+05	78%	5.40E+01	1.80E-01
WWR3	11/21/09	30	100	5	9.68E+02	6.45E+05	94%	6.29E+01	2.10E-01
WWR3	12/1/09	30	200	5	7.88E+02	1.05E+06	75%	9.65E+00	6.44E-02
WWR3	1/15/10	25	200	5	4.71E+02	7.53E+05	79%	7.74E+00	6.19E-02
WWR3	1/16/10	25	200	5	1.70E+03	2.72E+06	83%	2.01E+01	1.61E-01

APPENDIX F

BOVINE-ASSOCIATED *BACTEROIDES* (BOBAC) DATA FROM WELDER WILDLIFE REFUGE, RIESEL, AND BEEF CATTLE SYSTEMS CENTER WATERSHEDS

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	BoBac (copies/rxn)	BoBac (copies/L)	BoBac %R	qPCR BoBac fecal conc	BoBac fecal conc (mg/L)
BB1	3/25/09	10	100	5	1.15E+01	2.31E+04	94%	7.57E-01	7.57E-03
BB1	4/17/09	20	100	5	3.65E+00	3.65E+03	71%	2.23E-01	1.11E-03
BB1	4/18/09	30	100	5	1.60E+01	1.07E+04	80%	1.93E+00	6.45E-03
BB1	4/28/09	20	100	5	4.51E+00	4.51E+03	78%	2.95E-01	1.47E-03
BB1	10/4/09	10	100	5	3.93E+00	7.85E+03	103%	5.29E-01	5.29E-03
BB1	10/9/09	30	100	5	5.63E+00	3.75E+03	87%	7.38E-01	2.46E-03
BB1	10/13/09	30	100	5	2.56E+01	1.71E+04	93%	1.95E+00	6.50E-03
BB1	10/26/09	30	100	5	3.22E+00	2.15E+03	84%	2.37E-01	7.89E-04
BB1	10/26/09	30	100	5	3.38E+00	2.26E+03	100%	4.58E-01	1.53E-03
BB1	11/21/09	20	100	5	1.51E+02	1.51E+05	92%	1.01E+01	5.03E-02
BB1	12/1/09	20	100	5	1.12E+02	1.12E+05	87%	7.61E+00	3.81E-02
BB1	1/16/10	25	100	5	1.18E+02	9.44E+04	92%	3.18E+01	1.27E-01
BB1	1/29/10	25	100	5	6.09E+01	4.87E+04	97%	9.49E+00	3.79E-02
BB1	2/4/10	20	200	5	7.32E+02	7.32E+05	86%	6.66E+01	3.33E-01
BB2	3/25/09	10	100	5	8.61E+00	1.72E+04	83%	5.59E-01	5.59E-03
BB2	3/25/09	10	100	5	2.30E+00	4.59E+03	87%	1.38E-01	1.38E-03
BB2	4/17/09	20	100	5	2.59E+00	2.59E+03	90%	3.49E-01	1.75E-03
BB2	4/18/09	30	100	5	1.06E+01	7.04E+03	91%	1.31E+00	4.37E-03
BB2	4/28/09	20	100	5	2.89E-01	2.89E+02	90%	1.55E-02	7.75E-05
BB2	10/4/09	20	100	5	2.18E+00	2.18E+03	115%	3.09E-01	1.55E-03
BB2	10/4/09	30	100	5	1.93E+01	1.29E+04	101%	2.28E+00	7.60E-03
BB2	10/9/09	30	100	5	2.49E+01	1.66E+04	97%	2.86E+00	9.52E-03
BB2	10/13/09	30	100	5	1.53E+01	1.02E+04	82%	1.22E+00	4.05E-03
BB2	10/26/09	30	100	5	1.11E+01	7.38E+03	91%	8.50E-01	2.83E-03
BB2	10/26/09	30	100	5	3.96E+00	2.64E+03	77%	2.19E+00	7.31E-03
BB2	11/21/09	30	100	5	9.91E+03	6.60E+06	110%	6.79E+02	2.26E+00

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	BoBac (copies/rxn)	BoBac (copies/L)	BoBac %R	qPCR BoBac fecal conc	BoBac fecal conc (mg/L)
BB2	12/1/09	20	100	5	1.47E+03	1.47E+06	87%	8.19E+01	4.10E-01
BB2	1/16/10	25	50	1	1.02E+02	8.16E+04	223%	1.74E+01	6.98E-02
BB2	1/29/10	25	100	5	8.72E+01	6.98E+04	98%	1.33E+01	5.33E-02
BB2	2/4/10	20	200	5	9.14E+02	9.14E+05	86%	8.30E+01	4.15E-01
BB2	2/8/10	10	50	1	4.30E+02	8.60E+05	96%	2.68E+01	2.68E-01
BB3	3/13/09	20	100	5	1.31E+01	1.31E+04	130%	8.57E-01	4.28E-03
BB3	3/25/09	10	100	5	4.93E+00	9.87E+03	118%	3.65E-01	3.65E-03
BB3	3/25/09	10	100	5	3.06E+00	6.13E+03	79%	1.90E-01	1.90E-03
BB3	3/27/09	20	100	5	2.57E+00	2.57E+03	71%	1.55E-01	7.76E-04
BB3	4/17/09	20	100	5	4.62E+00	4.62E+03	91%	6.04E-01	3.02E-03
BB3	4/18/09	30	100	5	3.32E+00	2.21E+03	107%	4.42E-01	1.47E-03
BB3	4/28/09	20	100	5	3.23E+00	3.23E+03	82%	2.02E-01	1.01E-03
BB3	10/4/09	40	100	5	1.02E+01	8.13E+03	109%	1.26E+00	5.06E-03
BB3	10/4/09	25	100	5	1.52E+01	7.58E+03	107%	1.83E+00	4.57E-03
BB3	10/9/09	30	100	5	8.43E+00	5.62E+03	93%	1.07E+00	3.56E-03
BB3	10/13/09	30	100	5	9.55E+00	6.37E+03	94%	7.87E-01	2.62E-03
BB3	10/26/09	30	100	5	1.64E+01	1.09E+04	82%	2.27E+01	7.57E-02
BB3	10/26/09	30	100	5	1.97E+01	1.32E+04	100%	7.80E+00	2.60E-02
BB3	11/16/09	20	100	5	5.85E+05	5.85E+08	141%	2.56E+04	1.28E+02
BB3	11/21/09	30	100	5	2.95E+04	1.96E+07	112%	1.84E+03	6.12E+00
BB3	11/29/09	20	100	5	4.22E+03	4.22E+06	93%	5.28E+02	2.64E+00
BB3	12/1/09	20	100	5	3.22E+03	3.22E+06	98%	1.69E+02	8.44E-01
BB3	1/16/10	25	100	5	3.52E+02	2.82E+05	105%	5.00E+01	2.00E-01
BB3	1/29/10	25	100	5	9.96E+01	7.97E+04	99%	2.78E+01	1.11E-01
BB3	2/4/10	15	50	1	1.93E+03	2.58E+06	97%	2.86E+02	1.91E+00
BB3	2/8/10	15	50	1	1.80E+02	2.40E+05	122%	1.56E+01	1.04E-01

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	BoBac (copies/rxn)	BoBac (copies/L)	BoBac %R	qPCR BoBac fecal conc	BoBac fecal conc (mg/L)
SW12	3/3/08	50	200	5	2.68E+00	1.07E+03	85%	4.91E-01	9.82E-04
SW12	3/6/08	50	100	5	4.33E+00	1.73E+03	78%	3.37E-01	6.74E-04
SW12	3/10/08	50	200	5	1.52E+00	6.09E+02	88%	2.89E-01	5.77E-04
SW12	3/10/08	50	100	5	4.64E+00	1.86E+03	70%	5.35E-01	1.07E-03
SW12	3/18/08	50	100	5	3.22E+00	1.29E+03	112%	2.51E-01	5.01E-04
SW12	4/10/08	50	100	5	2.11E+00	8.45E+02	106%	1.52E-01	3.04E-04
SW12	4/10/08	50	100	5	1.73E+02	6.90E+04	103%	1.81E+01	3.62E-02
SW12	5/14/08	100	100	5	3.49E+00	6.98E+02	106%	4.03E-01	4.03E-04
SW12	5/15/08	99.75	200	5	3.75E+00	7.52E+02	72%	3.50E-01	3.51E-04
SW12	3/13/09	50	100	5	5.43E+00	2.17E+03	112%	4.03E-01	8.05E-04
SW12	4/17/09	40	100	5	3.22E+00	1.61E+03	86%	4.28E-01	1.07E-03
SW12	4/18/09	100	100	5	3.99E+00	7.98E+02	100%	2.93E-01	2.93E-04
SW12	4/28/09	50	200	5	6.39E+00	5.11E+03	81%	2.82E-01	1.13E-03
SW12	10/9/09	25	100	5	5.65E+00	4.52E+03	103%	2.87E+00	1.15E-02
SW12	10/9/09	25	100	5	6.81E-01	5.45E+02	97%	2.89E-01	1.15E-03
SW12	10/11/09	25	100	5	2.58E+00	2.06E+03	94%	4.73E-01	1.89E-03
SW12	10/13/09	25	100	5	2.01E+00	1.61E+03	98%	3.75E-01	1.50E-03
SW12	10/22/09	25	50	5	2.51E+00	5.02E+03	73%	8.01E-01	8.01E-03
SW12	10/26/09	25	50	5	4.78E+01	9.57E+04	107%	7.86E+00	7.86E-02
SW12	10/30/09	25	50	5	6.32E+00	1.26E+04	93%	1.11E+00	1.11E-02
SW12	1/16/10	25	100	5	3.90E+00	3.12E+03	126%	1.12E+00	4.48E-03
SW17	3/3/08	50	200	5	2.71E+04	1.08E+07	93%	3.05E+03	6.09E+00
SW17	3/6/08	50	200	5	1.36E+04	5.45E+06	92%	1.16E+03	2.33E+00
SW17	3/10/08	50	100	5	2.64E+03	1.06E+06	90%	3.36E+02	6.72E-01
SW17	3/10/08	50	200	5	3.14E+03	1.26E+06	86%	2.83E+02	5.67E-01
SW17	3/18/08	50	100	5	3.53E+03	1.41E+06	115%	3.17E+02	6.34E-01

Site	Event Date	Filter vol. (mL)	Vol. eluted (uL)	Template vol. (uL)	BoBac (copies/rxn)	BoBac (copies/L)	BoBac %R	qPCR BoBac fecal conc	BoBac fecal conc (mg/L)
SW17	4/10/08	50	100	5	5.34E+03	2.14E+06	127%	4.55E+02	9.10E-01
SW17	4/18/08	30	100	5	4.95E+04	3.30E+07	114%	4.02E+03	1.34E+01
SW17	5/14/08	30	200	5	3.29E+04	2.20E+07	86%	2.94E+03	9.80E+00
SW17	3/13/09	50	200	5	1.12E+05	4.48E+07	126%	1.18E+04	2.35E+01
SW17	4/17/09	40	100	5	4.58E+04	2.29E+07	101%	3.43E+03	8.57E+00
SW17	4/18/09	40	100	5	1.72E+04	8.59E+06	101%	1.69E+03	4.22E+00
SW17	4/28/09	49	100	5	4.19E+02	1.71E+05	96%	4.16E+01	8.49E-02
SW17	10/9/09	25	100	5	9.71E+03	7.77E+06	NA	9.36E+02	3.74E+00
SW17	10/9/09	25	100	5	8.69E+03	6.95E+06	87%	4.40E+02	1.76E+00
SW17	10/13/09	25	100	5	1.80E+03	1.44E+06	95%	2.34E+02	9.37E-01
SW17	10/22/09	25	250	5	5.15E+04	1.03E+08	107%	1.67E+03	1.67E+01
SW17	10/26/09	50	200	5	3.58E+03	2.87E+06	92%	2.66E+02	1.07E+00
SW17	1/16/10	25	100	5	6.32E+01	5.05E+04	43%	2.09E+00	8.38E-03
WWR1	10/26/09	30	100	5	2.36E+00	1.57E+03	66%	1.70E-01	5.66E-04
WWR1	11/20/09	30	100	5	2.37E+02	1.58E+05	93%	5.50E+01	1.83E-01
WWR1	11/21/09	30	100	5	4.38E+02	2.92E+05	84%	2.68E+01	8.92E-02
WWR1	12/1/09	20	100	5	1.17E+02	1.17E+05	72%	3.16E+01	1.58E-01
WWR1	1/15/10	25	200	5	2.41E+00	3.85E+03	63%	9.89E-02	7.91E-04
WWR1	1/16/10	25	200	5	1.17E+00	1.87E+03	67%	4.47E-02	3.58E-04
WWR1	2/3/10	30	100	5	6.22E+01	4.14E+04	35%	6.52E+00	2.17E-02
WWR3	10/26/09	30	100	5	3.38E+01	2.25E+04	60%	2.66E+00	8.88E-03
WWR3	11/20/09	30	100	5	5.61E+01	3.74E+04	69%	4.03E+00	1.34E-02
WWR3	11/21/09	30	100	5	6.70E+01	4.46E+04	94%	4.74E+00	1.58E-02
WWR3	12/1/09	30	200	5	2.19E+01	2.92E+04	55%	1.07E+00	7.15E-03
WWR3	1/15/10	25	200	5	3.45E+00	5.52E+03	61%	1.49E-01	1.19E-03
WWR3	1/16/10	25	200	5	9.86E-01	1.58E+03	68%	3.84E-02	3.07E-04

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