Effects of Agricultural Management, Land Use, and Watershed Scale on E. coli Concentrations in Runoff and Streamflow

R.D. Harmel, R. Karthikeyan, T. Gentry, R. Srinivasan

ABSTRACT. Fecal contamination of surface waters is a critical water quality concern with serious human health implications. Many states use Escherichia coli as an indicator organism for fecal contamination and support bacterial Total Maximum Daily Loads (TMDLs); however, model applicability is greatly restricted due to the sparse availability of E. coli data for validation and calibration at various scales. Similarly, watershed-scale information on the effects of management practices and land use on E. coli fate and transport is limited. Thus, this study was designed to measure E. coli concentrations in edge-of-field runoff and in streams with various agricultural management practices, land uses, and watershed scales. Results showed that application of dairy compost to pasture, cultivated, and mixed land use sites did not significantly affect E. coli concentrations in runoff at the field scale. In contrast, grazed sites had significantly higher runoff E. coli concentrations than cultivated sites, but the increase cannot be attributed solely to grazing cattle. No significant differences in E. coli concentrations were determined for presumably “impacted” and “unimpacted” rural streams with differing anthropogenic inputs, which highlights the challenges of managing and regulating bacterial contamination. The results also showed that E. coli concentrations consistently decreased as watershed scale increased from field to small watershed to river basin scale. Results from this study highlight the importance of considering all potential sources (including animal feeding operations, wastewater treatment plants and on‐site systems, cultivated and pasture fields, wildlife, and streambed resuspension) to properly assess E. coli contamination in rural watersheds. Results also demonstrated the need for an improved basic scientific understanding of fecal bacteria in the environment to reduce the substantial uncertainty associated with assessing, modeling, managing, and regulating bacterial contamination.

Keywords. Fecal bacteria, Surface water, TMDL, Watershed planning, Water quality.

Pathogens, specifically indicator bacteria, such as fecal coliforms and Escherichia coli, are the leading causes of surface water impairment in the U.S. (USEPA, 2009a). Elevated levels of E. coli, which are indicative of fecal contamination (Byappanahalli et al., 2003), also indicate the potential presence of other enteric pathogens in the impaired water bodies (Bolster et al., 2009). Contamination of streams with E. coli often results from a combination of human and animal fecal material from point and nonpoint sources. Wastewater treatment plants (WWTPs), storm sewers, and other legal and illegal domestic point source discharges can contribute E. coli (Teague et al., 2009). Other point sources of E. coli include direct fecal deposition by wildlife and livestock (Zeckowski et al., 2005; Paul et al., 2006). Nonpoint sources of E. coli include runoff from rural, agricultural, and urban landscapes, and subsurface flow from leaking on‐site wastewater treatment systems (Paul et al., 2006; Teague et al., 2009). Several previous watershed-scale studies have attempted to estimate bacterial contributions from these various sources using empirical or process‐based equations (Jenkins et al., 1984; Wilkinson et al., 1995; Fraser et al., 1998; Wilcock et al., 1999; Jamieson et al., 2002; Nagels et al., 2002; Tian et al., 2002; Crowther et al., 2002; Moyer and Hyer, 2003; Collins and Rutherford, 2004; Paul et al., 2006; Parajuli et al., 2009a, 2009b; Chin et al., 2009; Teague et al., 2009).

In Texas, rural streams are increasingly identified as bacterially impaired and placed on the Texas Water Quality Inventory and 303(d) list. As of January 2006, 197 water bodies in the state of Texas were impaired because they did not meet the state’s bacteria concentration criteria (geometric mean of 126 cfu per 100 mL and a single maximum of 394 cfu per 100 mL for E. coli) designed to protect contact recreation use (TCEQ, 2008; Texas Administrative Code, 2009).

To address bacterial impairments, the Clean Water Act mandates that states develop Total Maximum Daily Loads (TMDLs) to determine the amount of bacteria (or other causes of impairment) that impaired waters can receive from various sources without exceeding water quality standards (USEPA, 1999). The TMDL process is largely predicated...
upon the use of models, such as the Soil and Water Assessment Tool (SWAT) (Arnold et al., 1998), Hydrological Simulation Program - Fortran (HSPF) (Bicknell et al., 1997), or Spatially Explicit Load Enrichment Calculation Tool (SELECT) (Riebschleger, 2008; Teague et al., 2009), to determine the loads contributed by various sources in a watershed. The models or other methods (such as load duration curves; Cleland, 2002) are often applied at different scales from field to river basin to estimate water quality status and the impacts of various land use and management alternatives (Benham et al., 2006).

However, there remains a large degree of uncertainty in simulating *E. coli* fate and transport, which is due to several factors. First, relatively few *E. coli* data sets are available for model calibration and validation. Data collected from watersheds of varying scales and land uses with different management practices are especially rare, which severely limits the ability of models to predict *E. coli* fate and transport from various sources in response to management alternatives. In addition, the uncertainty in measured *E. coli* data also contributes to the uncertainty in bacterial modeling (Harmel et al., 2006; McCarthy et al., 2008). Second, large variations in reported values for *E. coli* persistence in the environment result largely from a lack of understanding of the fundamental processes controlling fate and transport mechanisms. For example, it is unclear what proportion of *E. coli* cells are transported via surface flow as single cells as opposed to attached to soil particles (Muirhead et al., 2006a, 2006b; Oliver et al., 2007; Mankin et al., 2007; Soupir et al., 2008a; Soupir et al., 2010). Similarly, *E. coli* survival kinetics in different environments (Wang et al., 2004; Soupir et al., 2008b), the resuspension of streambed sediment and associated *E. coli* (Rehmann and Soupir, 2009), and the potential for establishment of naturalized populations in soils or sediments (Ishii et al., 2006; Jamieson et al., 2004) are not well understood. Despite numerous laboratory and small-scale studies investigating many of these factors, there is a need for additional studies that compare *E. coli* fate and transport at multiple watershed scales.

This current study contributes to addressing this need by examining *E. coli* concentrations in surface waters at multiple scales, from edge-of-field to small watershed to river basin, under different land uses and management practices. Specifically, *E. coli* concentrations were measured in edge-of-field runoff and in streams with various land uses, agricultural management practices, and watershed scales. Data were collected from 13 sites with drainage areas ranging from 0.003 to 6070 km². The effects of management practices, land use, and watershed scale on *E. coli* concentrations were analyzed and documented for use in watershed model calibration, validation, and enhancement.

**Methods**

**Site Description**

To quantify *E. coli* concentrations for various management, land uses, and watershed scales, 13 sampling stations were established in the Leon River basin in 2004 (fig. 1). These sites ranged from 0.3 ha field-scale sites to 6070 km² basin-scale sites (table 1). Collection of *E. coli* data began at most sites in 2005 and lasted for at least three years.

At the field-scale sites, different fertilization schemes were implemented to assess the impact of common nutrient sources used in the region (inorganic fertilizer and dairy manure compost). On the four field-scale sites (SS1, SS2, SS3, M) with cultivated crop production, commercial fertilizer was applied to the grain sorghum crop in 2005 and the oats crop in 2006. Then a combination of dairy compost (6 to 30 m³ ha⁻¹) and supplemental commercial fertilizer was applied to three sites (SS1, SS3, M) or commercial fertilizer only to site SS2 based roughly on crop requirements for grain sorghum production in 2007 and 2009 and oat production in 2008. On the pasture fields (P1, P2), no fertilizer was applied until the summer of 2006, and then dairy compost (14 to 30 m³ ha⁻¹) was applied to P1 based on recommended nutrient rates. The pasture sites, grazed with varying intensity, received variable-rate direct manure inputs from cattle and horses throughout the study.

In contrast to the field-scale sites, land use was not manipulated for this study on the small watershed (MC1, MC2, RC1, RC2, RC3) and basin-scale sites (LR1, LR2). Mustang Creek (fig. 1) was selected because it was assumed to represent an “unimpacted” rural stream based on cursory assessment activity as part of the USDA Conservation Effects Assessment Project (Harmel et al., 2008). Specifically, no acute anthropogenic inputs (e.g., dairies, WWTPs) were identified, visual indicators of water quality were good

<table>
<thead>
<tr>
<th>Site</th>
<th>Scale</th>
<th>Land Use</th>
<th>Area</th>
<th>Data Collection Period</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Field</td>
<td>Grazed pasture</td>
<td>0.4 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>11</td>
</tr>
<tr>
<td>P2</td>
<td>Field</td>
<td>Grazed pasture</td>
<td>0.3 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>12</td>
</tr>
<tr>
<td>SS1</td>
<td>Field</td>
<td>Cultivated</td>
<td>0.9 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>15</td>
</tr>
<tr>
<td>SS2</td>
<td>Field</td>
<td>Cultivated</td>
<td>0.9 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>15</td>
</tr>
<tr>
<td>SS3</td>
<td>Field</td>
<td>Cultivated</td>
<td>1.2 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>16</td>
</tr>
<tr>
<td>M</td>
<td>Field</td>
<td>Cultivated (80%), Grazed pasture (20%)</td>
<td>18.1 ha</td>
<td>Aug. 2005 - Mar. 2009</td>
<td>14</td>
</tr>
<tr>
<td>Mustang Creek (MC1, MC2)</td>
<td>Small watershed</td>
<td>Mixed rural</td>
<td>14.7 km² (MC1) 55.1 km² (MC2)</td>
<td>Feb. 2005 - Mar. 2009</td>
<td>127</td>
</tr>
<tr>
<td>Resley Creek (RC1, RC2, RC3)</td>
<td>Small watershed</td>
<td>Mixed rural with dairies, irrigated fields, WWTP</td>
<td>57.6 km² (RC1) 128.9 km² (RC2) 217.5 km² (RC3)</td>
<td>Mar. 2006 - Mar. 2009</td>
<td>74</td>
</tr>
<tr>
<td>Leon River (LR1, LR2)</td>
<td>River basin</td>
<td>Mixed rural with dairies, WWTPs, small communities</td>
<td>5200 km² (LR1) 6070 km² (LR2)</td>
<td>Jan. 2005 - Feb. 2009</td>
<td>172</td>
</tr>
</tbody>
</table>
Figure 1. E. coli water quality sampling sites for this study in the Leon River basin.

(e.g., absence of algal masses, presence of sensitive fish species), and no field data were available to contradict the assumption of limited impact. Resley Creek was selected because it represents an “impacted” rural stream based on Texas Commission on Environmental Quality assessment and listing as impaired for primary contact recreation due to excessive bacterial levels (TCEQ, 2008). Land use in both of these small watersheds was dominated by grazed shrubland and grassland and cultivated cropland, but the upper end of Resley Creek also contained numerous dairies. Resley Creek also received city of Dublin WWTP discharge and received subsurface return flow from irrigated fields in the floodplain.

The basin-scale sites were located on the main stem of the Leon River. The Leon River basin (fig. 1) lies within the Western Cross Timbers, Limestone Cut Plain, and Northern Blackland Prairie ecoregions (Griffith et al., 2004). The basin has a subhumid climate characterized by hot summers and mild, dry winters. Occasional high-intensity, short-duration thunderstorms occur during the spring and summer months.

** Sampling Protocol **

At all sites, water samples were collected in routine visits in alternating weeks (approximately) when water was flowing and in each additional maintenance and storm event visit with flowing water. Grab samples were manually collected near the center of flow in 0.71 L (24 oz.) sterile, polyethylene Whirl-Pak bags (NASCO, Inc., Fort Atkinson, Wisc.). The bag was carefully opened to avoid touching the interior and submerged below the water surface pointing upstream. Once the bag was approximately 3/4 full, it was twirled by holding onto the twist tie ends and securely closed. Once closed, possible leakage was checked by gently squeezing the Whirl-Pak bag. Immediately after collection, samples were stored in a cooler on ice and transported back to the laboratory. Samples were stored at 4°C prior to analysis.

At the Resley Creek sites, storm samples were collected manually during runoff events, but at the other sites storm water samples were collected with automated ISCO 6700 samplers. Each sampler was programmed to rinse the sample tubing with ambient water prior to collection of each sample. At each field-scale site, an automated sampler collected frequent flow-interval (1.32 mm volumetric depth)
samples and composited them into a 16 L bottle. At each small watershed site, a sampler collected frequent flow-interval (2.54 mm volumetric depth) samples in 24 one-liter bottles. The volumetric depth, as presented by Harmel et al. (2003), is the depth of runoff from a defined watershed area. At each basin-scale site, a sampler collected time-interval samples (8 h) in 24 one-liter bottles.

Storm event samples were retrieved from the field as soon as possible (usually within 24 hr) following the sample collection. For each sample bottle, a subsample was poured into a 0.71 L (24 oz.) sterile, polyethylene Whirl-Pak (NASCO, Inc., Fort Atkinson, Wisc.) bag. Once the bag was approximately 3/4 full, it was twirled, securely closed, and checked for leaks by gently squeezing. Samples were stored in a cooler on ice and transported back to the laboratory. For some large-magnitude runoff events, only the first, last, and near-peak samples were subsampled for E. coli analysis.

**Enumerating E. coli in Water Samples**

Analysis was initiated within 6 to 24 h of sample collection. The E. coli concentration in each sample was determined by the membrane filter procedure using m-ColiBlue24 broth (Millipore, Billerica, Mass.) and an EPA-approved method developed by Hach (USEPA, 2009b). Multiple dilutions, based on the magnitude of previous values measured at that site, were used for each sample. Filtered samples on m-ColiBlue24 media were incubated for 24 h ±30 min at 35°C. Then within 30 min of the end of the incubation period, the colonies were counted (E. coli bacteria appear as blue) with a 10× to 15× stereoscopic microscope. The colonies were reported as the number of colonies per 100 mL of water. If no blue colonies appeared after the 24 h incubation, a count of 0 was assigned. For graphical and statistical analyses, counts of 0 were assigned the value of 1 cfu/100 mL. Ideally, the total number of colonies on the plates were between 20 and 80; however, if at the highest dilution there were more than 200 colonies or if the colony boundaries were indistinct, then the count was reported as too numerous to count (TNTC). In hindsight, our dilution protocol worked well, as almost all of the data were within 24 hr following the sample collection.

**Experimental Design and Statistical Analysis**

Comparisons of E. coli concentrations were made between field-scale watersheds with and without compost application, between presumably “impacted” and “unimpacted” streams, and between field-scale, small watershed, and basin-scale sites. For each comparison, the experimental unit was the watershed represented by the water quality sampling site. For each comparison, graphical techniques were first used to compare the distribution of E. coli concentrations. Then a Kolmogorov-Smirnov test was used to determine whether the data were normally distributed or not (a priori α level = 0.05). Since almost all of the data were not normally distributed, the Mann-Whitney test was used to determine statistically significant differences in median values between data sets. All statistical analyses were conducted with Minitab software (ver. 12, State College, Pa.: Minitab, Inc.) and according to procedures described by Helsel and Hirsch (1993) or Haan (2002).

**Results and Discussion**

**Overview of Measured E. coli Concentrations**

The distributions of E. coli concentrations measured in this study were skewed, with relatively more low concentration data but with occasional, extremely high concentrations. To determine which distribution best fit the data, an extensive curve fitting analysis with the least squares method and the Kolmogorov-Smirnov goodness-of-fit test compared the normal, log normal, and exponential distributions. For each scale (field, small watershed, and river basin), the log normal distribution was the best fit for the measured E. coli data; however, the best-fit log normal distributions were often significantly different (α level = 0.05) from that of the measured data.

Most of the data exhibited considerable variability or dispersion, as exhibited by the data ranges and variances. With only one exception, standard deviations were higher, and often much higher, than measured mean values (tables 2 through 5). The high variability in E. coli concentrations, which is as noteworthy as results of the subsequent evaluations of land use and scale effects, is attributed to the temporally and spatially dynamic nature of bacterial deposition, survival, and transport.

As with all measured data, the inherent uncertainty associated with determining E. coli concentrations also contributes to variability in the resulting data (Harmel et al., 2009). The only known comprehensive assessment of uncertainty in measured E. coli concentrations was conducted for urban storm runoff in Australia (McCarthy et al., 2008). In that study, mean E. coli concentrations of 621 MPN per 100 mL were reported for light industrial land use, 7500 to 12400 MPN per 100 mL for medium to high density residential, and 31000 MPN per 100 mL for rural residential land use. McCarthy et al. (2008) reported that the uncertainty introduced by storage time averaged ±25% (range ±9% to ±44%), that uncertainty introduced by the Colilert MPN analytical technique averaged ±22% (range ±12% to ±51%), and that the combined uncertainty averaged ±33% (range ±15% to ±67%). In the absence of project-specific uncertainty data, it was assumed that the average uncertainty estimated by McCarthy et al. (2008) adequately represented (for purposes of discussion) the uncertainty of E. coli concentrations in the present study.

**Comparison of Field Scale Sites**

**Effects of Compost Application**

Based on graphical and statistical comparisons of measured water quality data collected from the cultivated, cultivated with grazed pasture, and grazed pasture fields, compost application did not affect E. coli concentrations in runoff (table 2). The measured E. coli concentrations were also much lower than those commonly reported in runoff following fresh (non-composted) manure application (10^4 to 10^6 cfu per 100 mL) (Lewis et al., 2005; Meals and Braun, 2006). Together these results support the conclusion that dairy compost did not add significant E. coli bacteria to the application fields, which is not surprising because proper dairy manure composting procedures are reported to inactivate the vast majority of E. coli present (Shepherd et al., 2007). In general, composted manure should have negligible E. coli concentrations because typical composting procedures generate temperatures of at least 55°C (Storteboom et
Effects of Land Use

Because insignificant differences in runoff E. coli concentrations were observed for sites with and without compost application, data were grouped (pooled) by land use category to evaluate potential land use effects. Visual comparison clearly indicated that mean, median, and extreme high E. coli concentrations were typically lower for the cultivated fields than for the grazed pasture fields or the site with both cultivated land and grazed pasture (fig. 2). The Mann-Whitney test indicated that the median concentration for the cultivated sites was significantly lower than for the other land uses (p < 0.005) (table 3). E. coli concentrations appeared to be similar between the grazed pasture fields and the site with both cultivated land and grazed pasture, and statistical tests indicated that the medians were not significantly different (table 3).

Table 2. Descriptive statistics of E. coli concentrations (cfu per 100 mL) from fields with and without compost application.

<table>
<thead>
<tr>
<th>Site</th>
<th>Statistic</th>
<th>Without Compost</th>
<th>With Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated (SS1, SS2, SS3)</td>
<td>Mean</td>
<td>1277</td>
<td>793</td>
</tr>
<tr>
<td></td>
<td>Median[a]</td>
<td>570 a</td>
<td>280 a</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>3770</td>
<td>6640</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1430</td>
<td>1414</td>
</tr>
<tr>
<td>Cultivated with grazed pasture (M)</td>
<td>Mean</td>
<td>2400[b]</td>
<td>2965</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2400</td>
<td>16000</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>2400</td>
<td>16000</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>--</td>
<td>4321</td>
</tr>
<tr>
<td>Grazed pasture (P1, P2)</td>
<td>Mean</td>
<td>3101</td>
<td>3515</td>
</tr>
<tr>
<td></td>
<td>Median[a]</td>
<td>3020 a</td>
<td>1660 a</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>8080</td>
<td>20000</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2595</td>
<td>6006</td>
</tr>
</tbody>
</table>

[a] Within land use categories, median values followed by the same letter are not significantly different (α = 0.05).

[b] Only one runoff sample was collected at this site prior to compost application.

Table 3. Descriptive statistics of E. coli concentrations (cfu per 100 mL) from cultivated fields, grazed pasture fields, and a site with both cultivated land and grazed pasture.

<table>
<thead>
<tr>
<th>Site</th>
<th>Statistic</th>
<th>Cultivated (SS1, SS2, SS3)</th>
<th>Cultivated with Grazed Pasture (M)</th>
<th>Grazed Pasture (P1, P2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>965</td>
<td>2924</td>
<td>3289</td>
</tr>
<tr>
<td></td>
<td>Median[a]</td>
<td>300 a</td>
<td>1060 b</td>
<td>2465 b</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>6440</td>
<td>10000</td>
<td>20000</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1423</td>
<td>4154</td>
<td>4362</td>
</tr>
</tbody>
</table>

[a] Median values followed by the same letter are not significantly different (α = 0.05).

Figure 2. E. coli concentrations in runoff from cultivated fields (SS1, SS2, SS3), grazed pasture fields (P1, P2), and the site with both cultivated land and grazed pasture (M); data with and without compost application were grouped. The same legend is applicable to all box-and-whisker plots.

one of the few studies that compared E. coli runoff from cultivated and grazed land, McDowell et al. (2006) found very little difference between E. coli levels from grazed pasture and cropland. In contrast to the McDowell et al. (2006) study, cultivated land in the current study was not grazed and thus received no recent fecal inputs from livestock.

Whereas wildlife was the major, and likely the only, E. coli source on the cultivated fields, the grazed pasture received contributions from horses and cattle as well as wildlife. This grazed pasture was used as a horse and bull trap; therefore, portions were heavily grazed at times, resulting in substantial fresh manure inputs. More moderate grazing intensity, as would be expected on well managed pasture, would likely reduce runoff E. coli concentrations. Similarly, the site with both cultivated land (14.4 ha) and grazed pasture (3.4 ha) received contributions from both cattle and wildlife. What is unknown about the sites with grazed pasture is the relative contribution of wildlife versus livestock. It is not appropriate to simply assume that any increase relative to cultivated cropland is directly caused by grazing livestock because of probable differences in wildlife populations and time spent within cultivated and pasture fields. The contributions of livestock along with desirable and undesirable wildlife complicates the issue of managing fecal contamination in rural watersheds, especially as more studies indicate that wildlife is a major source of E. coli in surface waters (Casarez et al., 2007; Parajuli et al., 2009b). This complication can be partially overcome by combining spatially explicit E. coli estimation models such as SELECT (Riebschleger, 2008; Teague et al., 2009) with bacterial source tracking (BST) techniques to identify and quantify potential E. coli sources. Without such methods, arbitrary source control choices must be made with little confidence that water quality will be improved.

Comparison of “Impacted” and “Unimpacted” Streams

When Resley Creek and Mustang Creek were compared graphically, few striking differences in E. coli concentrations were apparent (fig. 3), although the extreme high values were larger for the “impacted” Resley Creek. When all data from each stream were grouped, the Mann-Whitney test indicated (p = 0.056) that the median values are not significantly different (although this p value near α = 0.05 casts doubt on the null hypothesis of no significant difference) (table 4). According to the Texas Administrative Code (2009), if more than 25% of samples exceed the single sample maximum criteria of 394 cfu per 100 mL (in other words, if the 75th percentile value is greater than 394 cfu per 100 mL), then the stream is impaired for its designated contact recreation use. Based on this standard, both streams would be judged as impaired for con-
tact recreation based on these data (fig. 3), which is troubling since the “impacted” stream has numerous anthropogenic sources (dairies and a WWTP), whereas the “unimpacted” stream has none. The similarity of E. coli concentrations, resulting either from weak or misunderstood linkages between bacteria sources and receiving waters, further exemplifies the difficulty of managing and regulating bacterial contamination of surface water.

Based on a visual data comparison, E. coli concentrations typically decreased from upstream to downstream for each stream (fig. 3), but these differences were only significant for Mustang Creek (table 4). If only Resley Creek data were available for this comparison of upstream and downstream concentrations, one would likely conclude that the WWTP and dairies concentrated in the upper portion of the Resley Creek watershed contributed most of the bacteria, since the concentrations tended to decrease downstream. However, the same trend was observed in the data from Mustang Creek, a much more homogeneous watershed without dairy and WWTP contribution. The similarity between streams may be due to the fact the E. coli sources in rural watersheds may include wildlife, streambed resuspension, and failing on-site wastewater treatment systems, as well as animal feeding operations, WWTPs, and cultivated and pasture fields (Riebschleager et al., 2008; Puri et al., 2009; Teague et al., 2009). Thus, all potential E. coli sources, transport and survival processes (e.g., growth, re-growth, and die-off), and watershed characteristics (e.g., source spatial distribution, land uses, watershed scale, and stream geometry) should be considered to properly assess E. coli contamination (Bai and Lung, 2005; Bolster et al., 2009).

The concentrations of E. coli under various flow regimes (generally baseflow as generated by subsurface return flow and WWTP discharge, and storm flow as generated by rainfall runoff) would presumably offer insight into the relative contributions of bacterial sources. These two streams, which differ in land use and anthropogenic flow contribution, are well suited for such an investigation. If the dominant source is nonpoint source runoff from agricultural land, one would expect higher concentrations in rainfall-induced runoff events. In contrast, if the dominant source is subsurface return flow and/or WWTP discharge, one would expect higher concentrations under baseflow conditions. When data for each stream were examined, flow and E. coli concentrations were not related, which indicates the probable presence of numerous, temporally and spatially variable bacterial sources in these watersheds. Runoff from cultivated fields, pastures, and riparian areas all with bacterial contributions from livestock and wildlife, WWTP and septic system discharges and overflows, as well as subsurface return flow as contributed by leaching, irrigation water, and dairy effluent, probably all contribute E. coli to the streams in these two small watersheds.

**INFLUENCE OF SCALE**

In working with these E. coli data collected from scales ranging from edge-of-field to large river basin, it seemed apparent that the scale at which data were taken directly impacted measured concentrations. Scale influences were certainly evident in the comparison of the “impacted” and “unimpacted” streams, as discussed previously. To further explore the influence of scale, all of the monitoring sites were divided into three broad categories based on watershed scale: field (<100 ha), small watershed (100 ha to 250 km²), and river basin (>250 km²). Graphical analysis clearly indicated that E. coli concentrations decreased as watershed scale increased (fig. 4). Statistical analysis confirmed this observation, as significant differences in median concentrations were pres-

![Figure 3. E. coli concentrations measured from Resley Creek and Mustang Creek. For data from individual sites on each stream, data are presented from upstream to downstream (left to right).](image1)

![Figure 4. E. coli concentrations measured at the field, small watershed, and river basin scales.](image2)
ent (table 5). A trend of decreasing high concentrations (e.g., 75th and 90th percentile values) was also observed as scale increased (fig. 4), even though extreme high maximum values occurred at all of the scales (table 5). While it is not appropriate to conclude that increasing scale is the sole or major cause of decreasing E. coli concentrations, this inverse relationship is certainly present.

Although this study cannot definitely determine any specific mechanisms to explain the decrease in E. coli levels with increasing scale, potential reasons may include die-off, settling out and entrapment of particle-associated E. coli, and differences in the relative amounts of baseflow and surface runoff (Bai and Lung, 2005; Oliver et al., 2007; Schultz-Fademrecht et al., 2008). In any case, this spatial trend should be incorporated into watershedscale bacterial transport modeling. To better represent scale effects in basin-scale modeling, E. coli fate and transport at the field scale could be simulated with single-field hydrologic response units (HRU) in SWAT or with other field-scale models and then incorporated progressively at the small watershed and basin scale. Such up-scaling of model results should improve the accuracy of downstream E. coli predictions.

**Implications for Managing and Regulating Bacterial Contamination**

Many of the E. coli analyses in the present study illustrate the need for improved understanding of bacteria in the environment and highlight the difficulties in managing and regulating bacterial contamination of surface water. Specific examples appear subsequently.

First, the influence of watershed scale (observed both within the “impacted” Resley Creek and “unimpacted” Mustang Creek small watersheds and across a wide range of scales from field to river basin) occurred regardless of land use and the presence or absence of substantial anthropogenic or background E. coli sources within the watersheds. This apparent influence supports the need for targeted research to establish clear linkages between bacterial sources and receiving waters and the need to incorporate watershed scale into bacterial modeling applications. Potential initial steps for establishing these linkages could include a combination of BST and modeling to determine relative E. coli contributions from the various watershed sources. Chin (2010) pointed out that the relationship between fecal coliform loading and instream concentrations is essential in determining the terrestrial fecal coliform loading reductions necessary to implement a TMDL. That study used data from 18 impaired streams in urbanized areas and showed that the probability of median instream concentrations exceeding threshold concentrations is independent of the loading rate (fecal coliforms per day) in the watershed; instead, the probability of exceedance was more closely related to the loading intensity (fecal coliforms per km² per day). Thus, the same terrestrial loading on a small watershed will likely create a greater water quality impact than if distributed over a larger watershed, presumably due to the proximity of loadings to streams in smaller watersheds.

Secondly, E. coli concentrations were similar and occurred at levels that indicated bacterial impairment for both “impacted” and “unimpacted” rural streams. The fact that two streams (one a minimally impacted stream with mostly background sources and a similar stream but with numerous anthropogenic sources including dairies and a WWTP) had similar E. coli concentrations and would both be judged as impaired for contact recreation presents challenges for managing and regulating bacterial contamination. It certainly stresses the need to identify, characterize, and quantify all potential E. coli sources in watershed risk assessments, so that effective (in terms of cost and pollutant reduction) practices and remedial strategies are implemented. Similarly, improved understanding of E. coli fate, transport, and survival in terrestrial and aquatic environments is needed, so that modeling linkages can be made between sources and downstream concentrations, where standards attainment is commonly evaluated.

Lastly, in spite of the variability and uncertainty in measured concentrations, the influence of livestock and wildlife contributions was certainly evident at the field scale; however, high E. coli concentrations (relative to the current Texas stream standard and to data collected downstream) were observed at all field-scale sites with or without readily identifiable E. coli sources. This result indicates the need for improved understanding of anthropogenic and background E. coli sources and of bacterial fate, transport, and survival in upland terrestrial environments, so upland and downstream sources can be effectively and efficiently managed and accurately represented in models.

**Conclusions**

Water quality standards are vital components of protecting U.S. waters from fecal contamination and ensuring that contact and noncontact recreation designated uses are maintained. Thus, in light of the difficulties presented, enforcement of appropriate standards should be diligently pursued. Success in this pursuit of setting and enforcing appropriate standards, however, relies on improved understanding related to: (1) the magnitudes and the temporal and spatial distribution of background and anthropogenic sources of bacteria, (2) the survival and transport of bacteria in terrestrial and aquatic environments, and (3) the linkages between bacteria sources and receiving waters. In addition, the likelihood of substantial input of bacteria by wildlife should be carefully considered. This occurrence does not preclude appropriate remedial focus and action on anthropogenic sources of bacteria, but it does substantially complicate regulatory formulation and enforcement where wildlife are shown to be a major source.

While the limited understanding of fundamental processes does not preclude discussion and enhancement of advanced topics (such as BST and bacterial transport modeling), increased attention should be given to the basic science of fecal indicator bacteria in the environment. Only with a sound scientific understanding of fundamental pro-
cesses can the substantial uncertainty associated with bacterial transport assessment and modeling be reduced. Only then can effective and efficient management and regulation of bacterial contamination become a reality.

ACKNOWLEDGEMENTS

This research was a component of the USDA Conservation Effects Assessment Project. Funding for this project was also provided by the Texas State Soil and Water Conservation Board. We recognize Trixie Delisle for her dedication and expertise in performing sample collection. We also want to thank the Melde, Patton, and Schrank families for their wonderful cooperation on this project. The anonymous reviewers, along with Carl Bolster and Ashok Mishra who reviewed previous versions of the manuscript, also deserve recognition for their contribution.

REFERENCES


Muirhead, R. W., R. P. Collins, and P. J. Bremer. 2006b. Numbers and transported state of Escherichia coli in runoff direct from...


Padia, R. 2010. Occurrence and fate of *Escherichia coli* from non-point sources in Cedar Creek watershed, Texas. Unpublished MS thesis. College Station, Tex.: Texas A&M University, Department of Biological and Agricultural Engineering.


