Characterizing Pathogenic Bacterial Regrowth and Impairment Potential along the Rio Grande near Albuquerque

Student Researcher: James Fluke
Department of Civil Engineering
University of New Mexico

Faculty Advisor: Ricardo González-Pinzón, Ph.D.

Problem Definition:

Escherichia coli (E. coli) bacteria have been identified by the United States Environmental Protection Agency (USEPA) as a contaminant of concern in the Rio Grande near Albuquerque (monitored between Angostura and Isleta diversions, ~60 km reach (USEPA, 2010)), where concentrations exceed applicable water quality standards frequently throughout of the year (USEPA, 2010; NMED SWQB, 2005). In fact, nearly 178,000 miles of river and stream in the United States are considered impaired for pathogens, of which 160,000 miles are considered impaired for *E. coli* and fecal coliform bacteria (USEPA, 2016) which are indicators of the presence of pathogens from fecal sources. Particularly dangerous spikes in *E. coli* bacterial concentrations in the Rio Grande have occurred historically in July and August (CDM Smith 2015). Human exposure to *E. coli* from surface waters in the Middle Rio Grande occurs mainly in two ways: 1) through irrigation with impaired Rio Grande water, and 2) via direct contact in recreational (open water sports and fishing) and Native American ceremonial activities. Therefore, it is estimated that ~3000 downstream direct water users (Isleta Pueblo) and other indirect users affected by irrigated crops grown in the area, in addition to the Albuquerque public, are at risk of contracting gastro-intestinal disease following contact with Rio Grande water.

Even though most *E. coli* bacteria are not pathogenic, *E. coli* is considered an indicator of pathogenic fecal coliforms which can cause serious illness in humans and animals that are exposed. The USEPA charges Albuquerque Municipal Separate Stormwater Sewer System (MS4) permittees with mitigation of bacterial contamination in the Rio Grande, to which end ~$20 million were spent on monitoring and best management practices in the urbanized contributing area from 2000-2010 (USEPA, 2010 (Appendix B)). Little reduction in bacterial contamination has been observed during this period and it remains unclear which process and sources are dominant contributors.

Recent studies aimed at determining host-organism and spatial sources of *E. coli* attribute exceedances in this reach (Middle Rio Grande) during the summer months to the first-flush effect, occurring after highly intense monsoon rainfall-runoff events follow dry periods in which contaminants that have accumulated are flushed into the river through the urban drainage network (NMED SWQB, 2005; CDM Smith, 2015). However, the observed occurrence of sustained high bacterial concentrations in the river during the summer does not completely correlate with the inputs of relatively low runoff volumes generated from storm water over short periods of time, which are characteristic of the arid Albuquerque urban watershed, and both studies provide evidence suggesting runoff may not be the main driver of high bacterial concentrations. Analysis of 3 data sources (weekly, monthly, and high flow/low flow/winter condition sampling frequencies) from years 2001-2014 presented in a 2015 study shows a gradual increase in E. coli concentrations along the Middle Rio Grande and shows that E. coli concentrations are much greater during the monsoon season (CDM Smith, 2015), which typically experiences low baseflow and slight increases in river flow due to episodic rainfall events. An earlier 2005 microbial source tracking study conducted on the Middle Rio Grande found that the major host-organism sources of *E. coli* are present under both runoff and non-runoff conditions. This study also found that spatial trends in fecal coliform concentration are similar during runoff and non-runoff conditions, increasing with distance downstream, suggesting that sources of fecal

indicator bacteria (not necessarily magnitudes) are similar during dry and wet weather (NMED SWQB, 2005). Overall this research suggests that high *E. coli* concentrations in the Rio Grande may be the result of loadings from sources other than storm runoff, particularly during dry weather.

Additionally, current EPA loading specifications for entities discharging to the middle Rio Grande consider *E. coli* as a conservative contaminant (USEPA, 2010) even though populations outside of a favorable aqueous environment typically die quickly but can persist and thrive in nutrient rich environments such as water and submerged sediments in irrigation canals, ponds, and transient surface water storage zones (Jamieson et al, 2005; Pachepsky and Shelton, 2011; Sherer et al, 1992). Further complicating our ability to monitor fecal contamination in the Rio Grande is the method of sample analysis for fecal indicator bacteria, which takes 18-24 hours to complete and yields results for surface water that has largely advected downstream and out of the monitored reach (estimated 15-25 hour Rio Grande travel time over 45 miles from Cochiti Dam to Albuquerque, (Langman, 2009)). Considering our lack of understanding of *E. coli* non-point source loadings and transport characteristics, a deeper understanding of the significant contributing sources and in-stream behavior of *E. coli* bacteria is needed to make inferences about the contribution to observed in-stream loadings by point and non-point sources.

Research Question, Hypotheses and Objective:

Research Question: To what degree do streambed sediments represent a source of *E. coli* loadings and what in-stream biochemical processes affect *E. coli* dynamics in the Rio Grande between the Angostura and Isleta diversion structures?

Hypotheses:

(1) In addition to point loadings and storm runoff, *E. coli* loads from streambed sediments contribute to observed exceedances and are introduced to the stream by resuspension episodically following increased discharge and sporadically during normal regulated flows.
(2) The bed morphology of the Rio Grande in this reach provides a favorable habitat (i.e., bacteria can grow or persist) for streambed coliform bacteria, accumulating coliform bacteria during low bed shear conditions and scouring coliform-laden sediments downstream when increased bed shear causes resuspension of fine sediments.

Research Objective: Characterize in-stream sources of *E. coli* fecal indicator bacteria spatially along the Rio Grande and characterize persistence and decay of *E. coli* in streambed sediments.

Methodology

Sampling Methodology: I conducted 2 sampling events with the objective of characterizing *E. coli* concentrations in surface water and streambed sediments spatially along the reach (over 60 km) and within the streambed (across a 210 meter cross-section) (Figure 1). Bacteriological surface water and sediment grab samples were analyzed using Standard Method 1604 for *E. coli* enumeration, which is an EPA approved method for water monitoring. During the second

sampling event I used a continuous bacterial sensor along with surface water and streambed sediment grab-sample measurements at equidistant points across the stream channel. The purpose of using the continuous ultraviolet sensor to is gather data that can be interpreted immediately at a site and allow more efficient bacterial source identification once a relationship between bacterial counts from grab sample analyses and sensor readings is established.

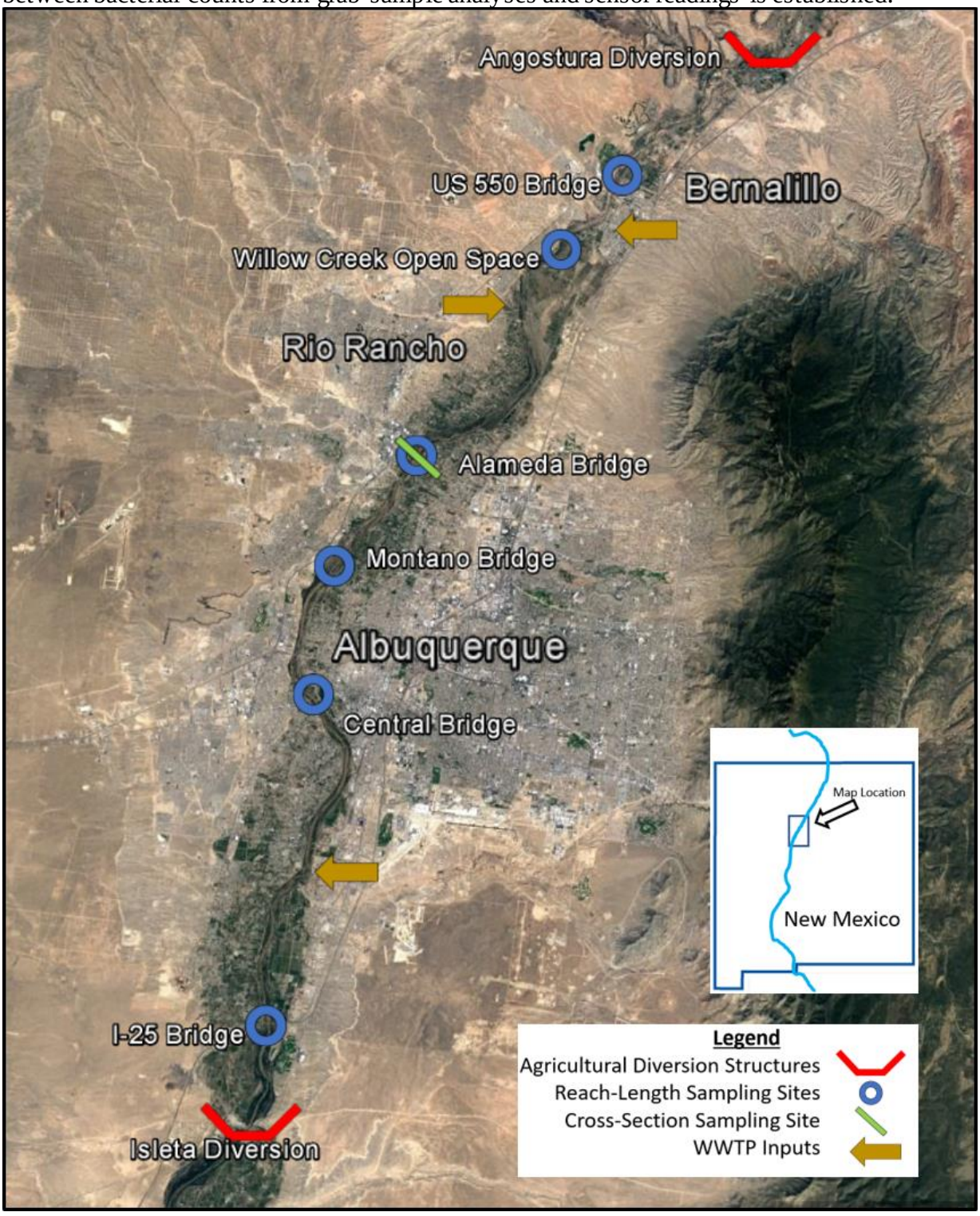

Figure 1: Study location map

Sample Analysis for *E. coli* Concentration: Surface water and sediment samples were analyzed for *E. coli* concentration using the EPA Method 1604 (USEPA Office of Water, 2002) for enumeration of *E. coli* in water using Coliscan Membrane Filtration (MF) (Micrology Laboratories, 2008) chromogenic medium, which is equivalent to the MI agar specified in EPA Method 1604 (Steven Wendelken, USEPA, to Jonathan Roth, Micrology Laboratories, 2010). Samples of water were diluted and vacuum-filtered through 0.45 µm membrane filters, capturing *E. coli* cells which are typically 1 µm in width and 2 µm in length. The filter is then placed on a plate containing the growth medium and incubated for 48 hours in a fume hood under ambient temperature conditions. Colony forming units (cfu's) of fecal coliforms, general coliforms, and *E. coli* form colonies of 0.5-2 mm diameter on the plate (Figure 2). The growth medium contains color-producing substrates that cause colonies of *E. coli* bacteria to appear blue-purple, colonies of the fecal coliform group to appear pink, and colonies of general coliforms to appear colorless. The method has a false positive and false negative rate of 4.3% for *E. coli* according to the USEPA (USEPA Office of Water, 2002). Laboratory equipment was rinsed with deionized (DI) water between analyses and rinsed with 91% isopropyl alcohol solution followed by DI water between every 10 samples and after blanks were run. Blanks were run at a frequency of 10% and consistently returned a non-detect result for *E. coli*, indicating that the lab equipment was not a significant source of contamination.

To analyze sediment concentrations using the MF method, I suspended samples of sediment in water (DI water or overlying river water) by vigorously shaking the sealed glass jars 25 times and removing the aliquot for dilution within 5-10 seconds after shaking. Sample aliquots of were taken using a 100-1000µL pipettor and a 10-100µL pipettor. For the first set of samples I used dilutions of 1/100, 1/50, and 1/40 for surface water sample analysis which correspond to aliquot volumes of 1mL, 2mL, and 2.5mL respectively. For sediment microcosms supernatant dilutions of 1/100, 1/200, 1/400, and 1/1000 were used (3 dilutions were used per sample, depending on fine particle content) which correspond to 1mL, 0.5mL, 0.25mL, and 0.1mL respectively. Clean tips were used for each sample and sample plates were prepared in order from highest dilution (lowest pipette volume) to lowest dilution (highest pipette volume). Pipettor tips were rinsed 3 times with DI water in between uses. The inference in this method is that *E. coli* in sediments would be introduced and entrained into the water by turbulence, remaining suspended if they are free floating cells or attached to small particles and sinking rapidly if attached to sand particles or formed into dense flocs, similarly to the process occurring in the waters of the Rio Grande.

Since *E. coli* concentration in the sediment was measured indirectly in the supernatant from the sediment and water microcosm, the sediment mass and water volume contained in each microcosm was recorded. I used the ratio of sediment mass to water volume to relate the concentration of *E. coli* in the supernatant to the concentration contributed by the sediment, e.g. for a supernatant concentration of 100cfu/100mL, water volume of 300mL, and sediment mass of 50g, the concentration contributed by the sediment is estimated as:

(100cfu/100mL supernatant)*(300mL supernatant/50g sediment)=6 cfu/g sediment.

Figure 2: Sediment and surface water microcosms (left) and incubated MF plate bacteriological results (right)

Reach-Length Sampling – 3/16/2017

To characterize overall spatial trends of *E. coli* in sediment and surface water, I collected surface water and sediment samples from 6 sites along the Middle Rio Grande during early spring. Discharge was ~1500 cubic feet per second (cfs) and water temperature was 51^{O}F as recorded by USGS Stream Gage 08329928 at Alameda Bridge. I took water samples from each of 5 equal width increments at each site using a weighted glass collection container lowered by rope from the sites where bridges allowed full access to the cross section (Figure 3). From the 2 sites without bridge access water samples were taken from as many sections as possible based on the estimated stream width and ability to wade across the stream. Surface water samples were collected in 120mL plastic coliform sampling bottles containing 0.1 g sodium thiosulfate for residual chlorine neutralization as per the sampling method description (USEPA Office of Water, 2002). 2 sediment samples from each site (except the Montano bridge site) were taken from points along the submerged bank and at 2 ft. depth by scooping sediment directly into a submerged glass jar. Surface water and sediment samples were kept on ice until I returned to the lab for sample analysis, which occurred on the same day as sample collection. I enumerated the samples using the EPA approved MF method described above. After the initial sample analysis I used the surface water and sediment samples to create microcosms which were kept in sealed glass jars for 4 days in a dark, refrigerated storage room (45^{O}F) and analyzed again to determine the extent of regrowth or decay of *E. coli* bacteria in the microcosms. The sediment microcosms consisted of 30-80g of sediment (weight as-collected) and 300-400mL of overlying river water kept in sealed glass jars. The jars had little to no head space when they were sealed.

Figure 3: Sampling sediments by wading (left) and sampling surface water from Alameda bridge (right).

Cross-Section Sampling – 5/31/2017

To characterize heterogeneity along the streambed I collected surface water and sediment samples from 10 equal width-increments (as per USGS National Field Manual Chapter A4: Collection of Water Samples) across the Rio Grande at the Alameda Bridge in Albuquerque during late spring (~3900 cfs and water temperature of 60^{O}F as recorded by USGS Stream Gage 08329928 at Alameda Bridge) and analyzed the samples for *E. coli* enumeration using the MF method. Surface water grab samples were collected using a weighted collection container which was rinsed 3 times before sampling at each increment. Streambed sediments were collected using a 25 lb. Ponar grab sampler (Figure 4), which uses a spring loaded mechanism to release its jaws upon impact with the streambed, penetrating the streambed and collecting the top 2-5 cm of sediment. Instruments measuring nitrate (Submersible Ultraviolet Nitrate Analyzer V2 (SUNA)), turbidity, dissolved oxygen, conductivity, and fluorescent dissolved organic matter (YSI Exo Sonde) were deployed at the river bank for the duration of the sampling activity. An instrument measuring tryptophan-like fluorescence (TLF) (Uvilux Tryptophan, Chelsea Technologies Group Ltd.) (Figure 4) in quinine sulfate units (qsu) of fluorescence was used in water samples retrieved from each equal width increment. This instrument is a handheld digital fluorometer which measures the 280 nm wavelength emission generated by UV excitation at a 360 nm wavelength, targeting the amino acid tryptophan which is a known surrogate for bacterial concentration in water (Sorensen et al, 2015). The objective of using this instrument is to build a dataset that can be used to compare bacterial concentrations measured by MF analysis with TLF readings, helping to move bacterial monitoring away from time consuming grab sample collection and analysis and toward instantaneous, continuous monitoring.

I used sediment samples collected in plastic zip-top bags from each equal-width increment to create microcosms consisting of 50±0.5g sediment and 300±10mL DI water in the lab. This contrasts with the previous set of microcosms I created, which included overlying river water and were created at the site resulting in non-standard sediment masses and overlying water content. The revised method of creating microcosms better isolates the sediment bacterial contribution from possible contribution of overlying river water. The microcosms were kept in the dark in sealed glass jars at 70^{O}F and analyzed the day of collection and after 4 days to determine the extent and rate of regrowth or decay of *E. coli* bacteria. Microcosms that showed persistence or regrowth were kept at the same conditions and analyzed again after 13 days.

Figure 4: Uvilux Tryptophan fluorometer used in surface water samples (left) and sediment dredge and washbowl for collecting bottom sediments (right)

Results and Discussion:

3/16/2017 - Reach-Length Data

- Surface water sampling conducted at 6 sites along the reach showed *E. coli* concentrations in surface waters ranging from ≤100 cfu/100mL to 2400 cfu/100mL as shown in Figure 5. The dilutions used in the method were 1/40, 1/50, and 1/100 and resulted in a minimum detection limit of 40 cfu/100mL. Based on these results the method minimum detection limit was lowered to 20 cfu/100mL and dilutions better able to capture a range of orders-of-magnitude (1/20, 1/50, and 1/100) were used for all subsequent analyses. The surface water samples collected during this event do not show a clear spatial trend and indicate significant variability at each site. This phenomenon is corroborated by data review performed by Pachepsky and Shelton in 2003, which found that differences of 2-5 orders of magnitude between *E. coli* measurements of samples from the same site or watershed are not uncommon.

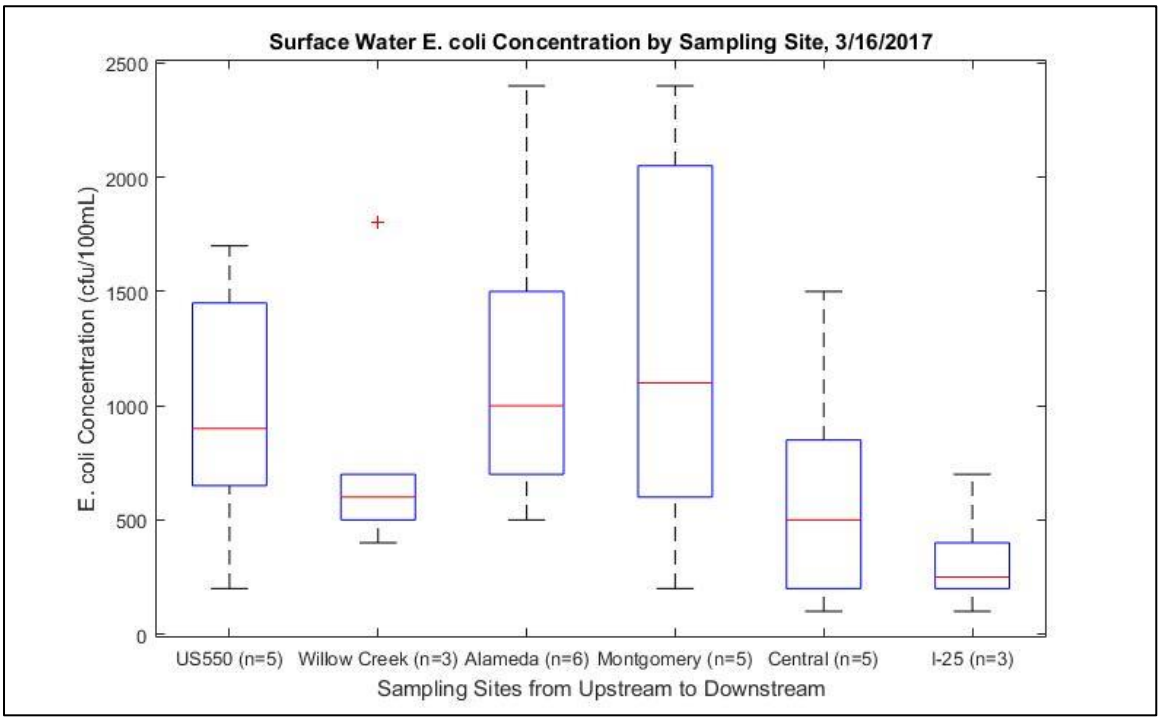

Figure 5: Surface water concentrations resulting from reach-length sampling event

- Surface water and sediment microcosms (Figure 2) were kept in a refrigerated storage room for 4 days and analyzed initially and 4 days after collection. Results from the surface water microcosms showed highest decay between the collection date and the following 4 days in the farthest upstream samples, progressing to lowest decay between days 0 and 4 in the farthest downstream samples (Figure 6), indicating that nutrient and substrate inputs along the reach could be supporting coliform survival. Results from the sediment microcosms showed varying degrees of decay at most sites (Figure 7), indicating that the *E. coli* populations within the microcosms were not given either favorable environmental or nutrient/substrate conditions for regrowth. Blanks were run for 10% of the samples to

evaluate possible contamination from the analysis equipment, which was used repeatedly for all samples, and consistently returned a non-detect for *E. coli.*

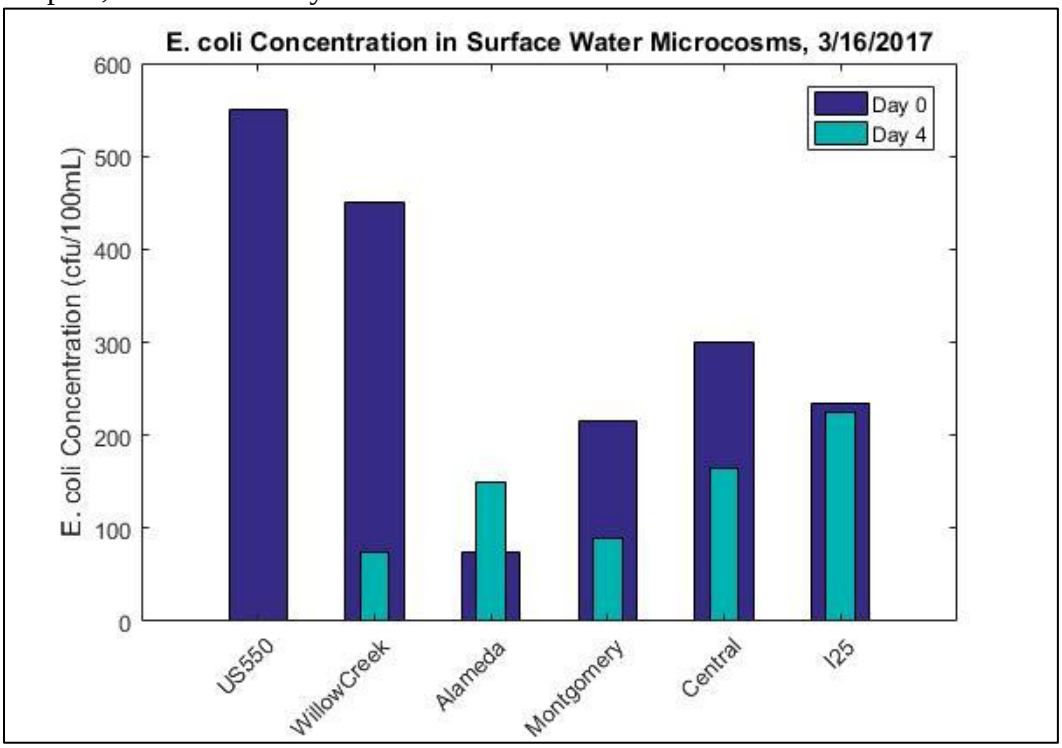

Figure 6: Surface water microcosm results from reach-length sampling event

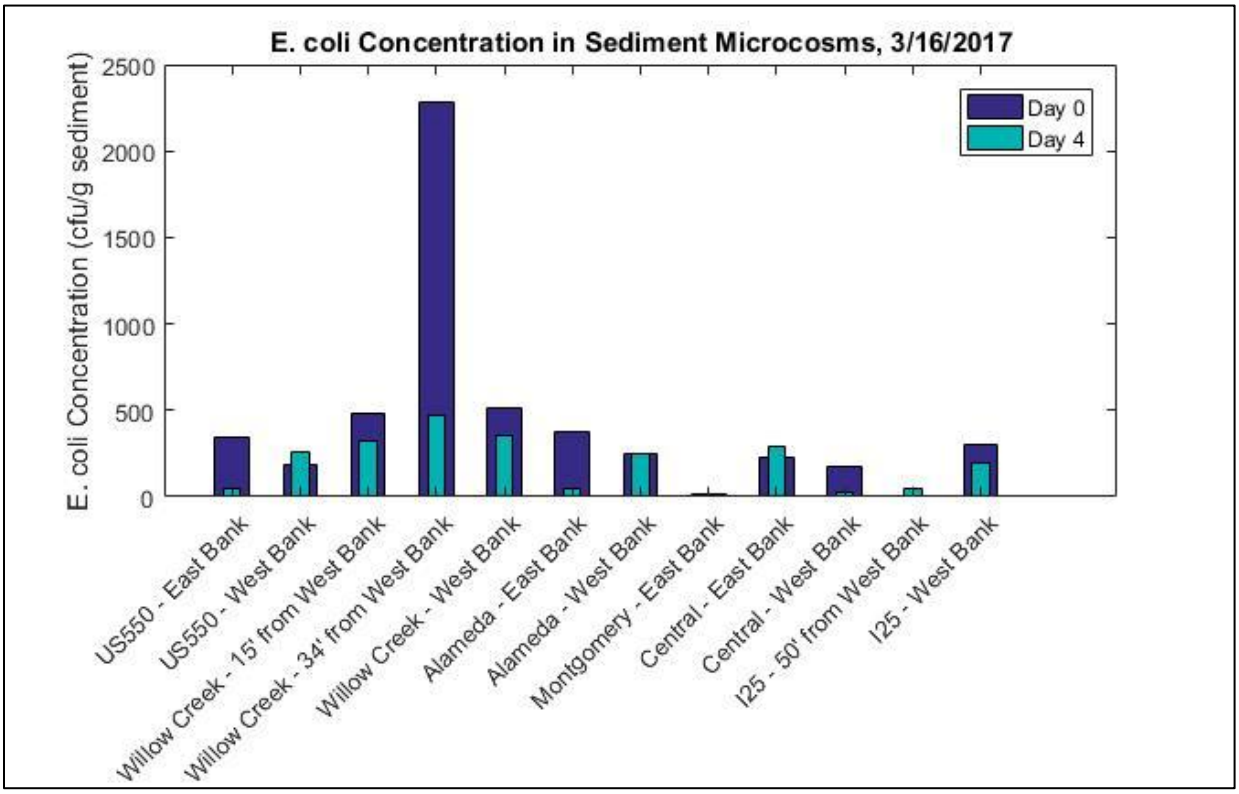

Figure 7: Sediment microcosm results from reach-length sampling event

5/31/2017 Cross Section Data

- Sampling of surface water and streambed sediments at the Alameda Bridge over the Rio Grande in Albuquerque revealed streambed heterogeneity in surface water and sediment *E. coli* concentration. This cross section included a large vegetated sand bar-island which was partially submerged and not part of the main flow in the river, making up a "dead zone" of immobile water and sediment. Surface water samples across the section showed consistently low *E. coli* concentrations, except for surface water samples from the "dead zone" water which showed elevated levels of *E. coli* (Figure 8). Sediment samples showed very low *E. coli* concentrations in increments that were part of the main river flow and consisted of mostly coarse sand, and elevated *E. coli* concentration in the sediments of the "dead zone" increments which consisted of fine clay and organic mud. Regrowth of *E. coli* in the sediments of the "dead zone" was observed (Figure 9), indicating that these sediments can act as an incubator of coliform bacteria under steady-state conditions and as a potential source of *E. coli* through interaction between the "dead zone" and main channel.

- Data collected by the Tryptophan fluorometer and instruments deployed at the river bank are shown in Table 1 below. TLF readings showed no clear trend across the section as did the surface water grab sample analysis other than a tight cluster centered around 16 qsu corresponding to the 115.5 m increment. Readings at all other increments showed variation between 0 and 10 qsu. The difficulty in collecting the surface water samples from the vegetated and shallow "dead zone" may have contributed to inconclusive TLF readings as fine sediments were immediately disturbed by the sampling bucket, introducing high turbidity in the bucket samples which is known to affect readings by fluorescence-measuring instruments. It is also possible that *E. coli* concentrations are not directly related to overall bacterial concentrations, which are targeted by the Tryptophan fluorometer. Further data collection at different conditions is expected to reveal greater information about the relationship between TLF, bacterial density, and *E. coli* presence.

  Data collected by the instruments deployed at the stream bank was steady for all parameters. These data by themselves are not very informative, but will become useful for establishing relationships between water quality parameters and bacterial concentrations once a set of data is generated across different hydraulic and water quality conditions.

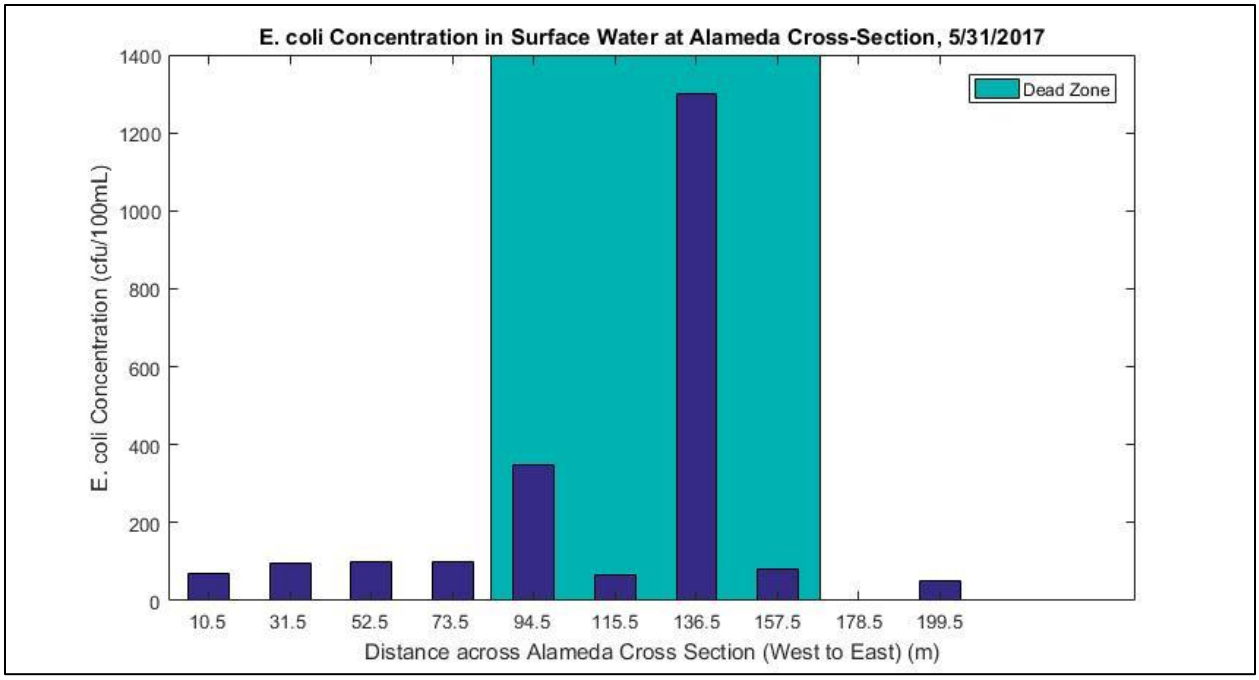

Figure 8: Surface water sample results from cross-section sampling event

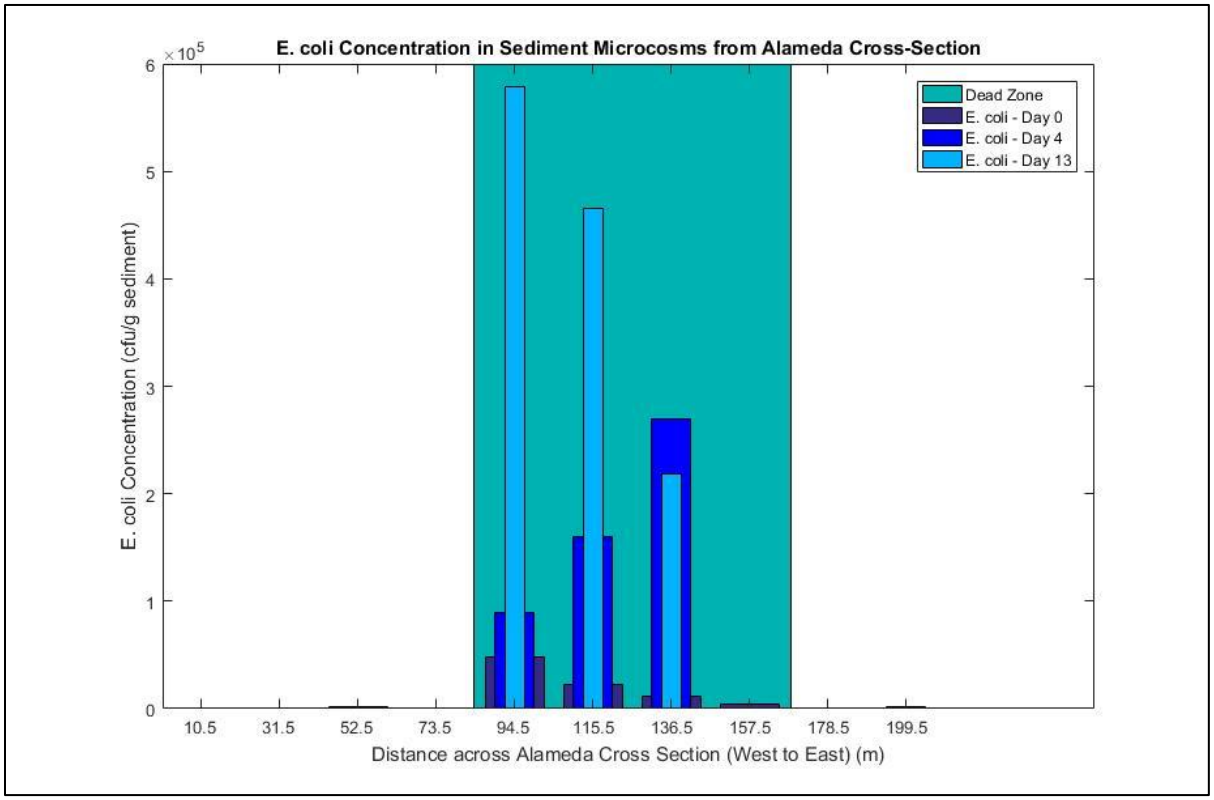

Figure 9: Sediment microcosm results from cross-section sampling event

Water Quality Parameter	Concentration
Tryptophan-like fluorescence (TLF)	0-16
with dissolved oxygen (DO)	9.5 mg/L

specific conductivity	225 µS/cm
Turbidity	55-80 NTU
Total Dissolved Solids (TDS)	150 mg/L
fluorescent Dissolved Organic Matter (fDOM)	40 qsu
Nitrate (NO_3)	≤0.2 mg/L
Total Suspended Solids (TSS)	0 mg/L

Table 1: Water quality parameters measured at cross-section sampling event

Conclusions and Future Work

The results of the data collected to date suggest the following:

- Rio Grande streambed sediments (samples collected near banks and immobile zones) can be favorable environments for *E. coli*, supporting persistence of indicator bacteria over the day to week time scale.
- *E. coli* concentrations in surface water and sediment exhibit variation by orders of magnitude at each site and between sites.
- Sediments from deeper, faster flowing locations tend to contain mostly sand and non-detect to low *E. coli* concentrations, while slower-moving bank and "dead-zone" streambed areas tend to contain more fine material and higher *E. coli* concentrations.
- Surface waters in the dominant flow area return very low *E. coli* concentrations (no samples had non-detection result for *E. coli*) at high flows (5/31/2017 event), while water in "dead-zone" storage areas show higher *E. coli* concentrations.

A deeper understanding of the orders-of-magnitude contributed by point and non-point sources to in-stream loading observed at a given sampling point under varying hydraulic and seasonal conditions is needed. Future efforts will be focused on characterizing surface water and sediment *E. coli* concentrations across hydraulic and seasonal conditions. I expect that further dry-weather monitoring will reveal seasonal and discharge-related characteristics of non-point source loadings (point-source *E. coli* loadings are publicly available and generally stable) while monitoring of pulse loading from specific rainfall events at discharge points and in the Rio Grande will give us greater insight into the magnitude and temporal extent of runoff-driven *E. coli* loading.

Research Beneficiaries

This research benefits public entities responsible for complying with cooperative water quality regulations such as Total Maximum Daily Load (TMDL) documents and Municipal Separate Storm Sewer System (MS4) permits by providing insight into factors (in-stream non-point source loading) not currently considered in the regulatory framework for this watershed. Water users such as recreators, irrigators, and those having primary contact with Rio Grande water also benefit from an improved understanding of sources contributing to water quality exceedances. The data generated in this study are intended to be used with process-integrating computer models created specifically for use in regulatory framework development (e.g. QUAL2k model (Pelletier et al, 2005) developed by USEPA), helping to advance use of modern computational modelling tools in surface water quality regulation.

Acknowledgment of Students and Faculty Members who assisted with the project
Dr. Ricardo Gonzalez-Pinzon, Betsy Schaefer, Luiz Gonzalez-Pinzon, and Jarek Kwiecinski all contributed effort to data collection and instrument operation during the sampling campaigns and to grab sample analysis.

Poster Presentations

- 61st Annual NM Water Confgerence, October 5-7 2016, Silver City, NM
- 14th Annual RMSAWWA/RMWEA Student Water Conference, May 22 2017, Albuquerque, NM

Budget

Funds from the NMWRRI 2016 Student Research Grant award were used to purchase the following items:

- Coliscan Membrane Filtration materials (chromogenic growth medium, membrane filters, petri dishes, etc.) (Micrology Laboratories)
- 120mL coliform sampling bottles containing sodium thiosulfate preservative
- 25 lb. Ponar grab sampler (AMS Samplers)
- Uvilux Tryptophan Fluorometer (Chelsea Technologies Group)

References

CDM Smith, April 2015. "Final Middle Rio Grande *E. coli* Analysis and Research" http://amafca.org/documents/2015_Annual_Report/AMAFCA%202015%20(Jan%20to%20June)%20Annual%20Report%20II.A%20-%20VI.pdf

"Coliscan® MF." Coliscan® MF - Micrology Labs. Accessed February 12, 2017. https://www.micrologylabs.com/page/94/Coliscan-MF

Jamieson, R., D. Joy, H. Lee, R. Kostaschuk, and R. Gordon. "Transport and Deposition of Sediment-associated Escherichia Coli in Natural Streams." *Water Research* 39, no. 12 (2005): 2665-675. doi:10.1016/j.watres.2005.04.040.

Langman, Jeff B. *Traveltime of the Rio Grande in the middle Rio Grande Basin, New Mexico, water years 2003-05*. Reston, VA: U.S. Geological Survey, 2009. https://pubs.usgs.gov/sir/2007/5292/pdf/SIR2007-5292.pdf

Micrology Laboratories, LLC, 2008. "The Coliscan Membrane Filter Method for Escherichia Coli and other Coliforms" https://www.micrologylabs.com/page/127/Directions-for-use

New Mexico Environment Department (NMED) Surface Water Quality Bureau, October 2005. "Middle Rio Grande Microbial Source Tracking Report" https://www.cabq.gov/municipaldevelopment/documents/storm-drainage-documents/storm-drainage-pdfs/MRG-MSTReport.pdf

Pachepsky, Y. A., and D. R. Shelton. "Escherichia Coli and Fecal Coliforms in Freshwater and Estuarine Sediments." *Critical Reviews in Environmental Science and Technology* 41, no. 12 (2011): 1067-110. doi:10.1080/10643380903392718.

Pelletier, G.J., S.C. Chapra, and H. Tao, September 2005. "QUAL2Kw - A framework for modeling water quality in streams and rivers using a genetic algorithm for calibration" <https://fortress.wa.gov/ecy/publications/documents/0503044.pdf>

Sherer, Brett M., J. Ronald Miner, James A. Moore, and John C. Buckhouse. "Indicator Bacterial Survival in Stream Sediments." *Journal of Environment Quality* 21, no. 4 (1992): 591. doi:10.2134/jeq1992.00472425002100040011x.

Steven C. Wendelken, Ph.D. to Jonathan Roth, Ph.D. February 25, 2010. "RE: ATP Case No. D10-0002." In Micrology Labs. Accessed January 18, 2017. https://www.micrologylabs.com/files/EPA_Coliscan_MF_Approval.pdf.

Sorensen, J.p.r., D.j. Lapworth, B.p. Marchant, D.c.w. Nkhuwa, S. Pedley, M.e. Stuart, R.a. Bell, M. Chirwa, J. Kabika, M. Liemisa, and M. Chibesa. "In-situ tryptophan-like fluorescence: A real-time indicator of faecal contamination in drinking water supplies." *Water Research*81 (2015): 38-46. doi:10.1016/j.watres.2015.05.035.

United States Environmental Protection Agency (USEPA), June 2010. "Total Maximum Daily Load (TMDL) For the Middle Rio Grande Watershed" <https://www.epa.gov/sites/production/files/2015-10/documents/middle_rio_grande_nm.pdf>

United States Environmental Protection Agency (USEPA), Office of Water, 2002. *Method 1604: total coliforms and Escherichia coli in water by membrane filtration using a simultaneous detection technique (MI Medium).* Washington, D.C.: nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1002D57.TXT

United States Environmental Protection Agency (USEPA) "National Summary of State Information | Water Quality Assessment and TMDL Information | US EPA," accessed October 2, 2016, https://ofmpub.epa.gov/waters10/attains_nation_cy.control

U.S. Geological Survey, Dept. of the Interior, 2003. “National field manual for the collection of water-quality data” Reston, VA: U.S., https://water.usgs.gov/owq/FieldManual/chapter4/pdf/Chap4_v2.pdf