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CHARACTERIZING PATTERNS IN E. COLI LEVELS IN RIO GRANDE RIVER WATER AND RIVERBED SEDIMENTS NEAR ALBUQUERQUE, NM

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by

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Characterizing Patterns in E. coli Levels in Rio Grande River Water and Riverbed Sediments near Albuquerque, NM

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ABSTRACT

In this work I examined how Fecal indicator Bacteria (FIB) behave in a large environmental system (Rio Grande near Albuquerque, ~60 km distance). I addressed the questions: How do FIB levels in river water and riverbed sediments of this reach change with distance along the river and throughout one year?

I conducted year-round river water and sediment sampling for concentration of E. coli bacteria, a persistent contaminant in the area. I found that over the year, E. coli loading in river water increased along the 60 km reach and E. coli in the sediments mainly increased near the Albuquerque urban area. Site by site along the reach, relative fluctuations in E. coli loadings and sediment concentrations were seasonally coupled.

This study found high E. coli sediment concentrations during Summer and Fall co-occur with higher Summer and Fall loadings, and higher E. coli sediment concentrations downstream may be related to more frequent exceedances of the Total Maximum Daily Load (TMDL) in the downstream section. However, the net direction of E. coli transfer (river water to sediment or sediment to river water) is unknown at any point and the physical interactions between river water and sediment causing transfer of E. coli cells are not well understood on the reach-scale.

Table of Contents

List of Figures

List of Tables

1) **Introduction**

Surface water impairment due to fecal contamination is a worldwide concern. Waterborne disease (predominantly due to fecal contamination) accounts for 4 billion episodes of illness and 2.2 million deaths yearly[1]. In the US, nearly 178,000 miles of river and stream are considered impaired for pathogens, of which 160,000 miles are considered impaired for *Escherichia coli* (E. coli) and fecal coliform bacteria[2], which are indicators of the contamination from fecal sources. Exposure to pathogens generally occurs through consumption of or contact with contaminated waters, as well as from consumption of crops irrigated with contaminated water. In arid regions, where populations depend on surface water sources such as streams and rivers, waterborne pathogens make stream water dangerous for agricultural uses and as drinking water.

In the US, health risks from waterborne pathogens are mitigated through national environmental water quality standards established under the 1972 Clean Water Act, which requires states to develop stream regulations. Bodies of water that do not meet standards may result in implementation of Total Maximum Daily Loads (TMDLs). TMDLs address water body impairments by establishing allowable loadings to discharging entities and imposing contaminant reduction strategies for sources discharging beyond this allowable loading.

The most common indicator of fecal contamination is the concentration of the bacteria *Escherichia Coli* (E. coli), a gram-negative species of gut bacteria ubiquitous to birds and mammals. Densities of E. coli in fecal matter range from 10^4 to 10^8 colony forming units (cfu) per gram dry weight of feces[3], and current E. coli water quality standards for primary consumption and secondary contact of environmental waters are a

monthly geometric mean of 47 and 126 cfu/100 mL of water, respectively[4]. These values correspond to rates of 4 and 36 occurrences of illness per 1,000 exposures[4,5]. Although most strains of E. coli are not pathogenic, this species is used as an indicator species because it is present where pathogens are present and exists in large numbers compared to pathogens.

Since the US Environmental Protection Agency (USEPA) established the bacteriological water quality criteria in 1986[6], numerous studies have documented growth and survival of fecal indicator bacteria (FIB) including E. coli in surface waters, sediments[7–13] and drinking water distribution systems[14–17], as well as seasonal variability in the fate of E. coli in exposed fecal matter[3,18]. This suggests that current bacteriological criteria for surface waters may have significant flaws as FIB occur naturally and respond differently to local environmental conditions. This response causes widely varying relationships between FIB concentrations and the degree of contamination in environmental waters, making numeric criteria problematic for water quality regulation.

From 2011 to 2012, 28 US states reported 8 outbreaks directly attributable to E. coli in recreational waters resulting in 119 cases of illness and 21 hospitalizations[19], and in 2011 the Center for Disease Control (CDC) determined gastrointestinal illness caused by marine beach exposure resulted in 5 million cases per year and $300 million per year in health expenses[20]. Since fecal contamination remains a widespread and costly problem, we must advance our understanding of surface water fecal contamination to develop new control criteria. However, improving fecal contamination criteria is difficult because FIB are a flawed proxy for waterborne pathogens. Besides enteric bacteria and viruses, there are no known alternative analytes to accurately represent fecal contamination. Because of

widely varying relationships between waterborne illness and FIB between sites and over time[21], high spatial and temporal variation in environmental FIB[7], and natural occurrence and regrowth of FIB[14–16], FIB concentrations may not represent the actual level of human contamination. Thus, research is needed to shed light on the relation between FIB and contamination sources in different watersheds, identify new fecal indicators, and accurately quantify FIB sources and loadings despite high spatial and temporal variability of known fecal indicators[21]. Also, while the key conditions under which FIB cells could persist and grow have been identified in controlled experiments[13,14,22,15], little is known about the effects of FIB persistence and growth in stream systems carrying environmental and waste waters to downstream users.

This work adds to the body of research on this subject by examining spatial and temporal trends and variability in river water and river sediment FIB under a range of human input levels. I studied a ~60 km of reach of the Rio Grande near Albuquerque, New Mexico, which has been consistently classified by the USEPA as impaired by E. coli bacteria. This reach has a range of urbanization levels, from nearly unaffected by human inputs to affected by >1M people, which provided context to understand the role of human activities on E. coli levels.

In 2001, 62 km of the Rio Grande near Albuquerque, New Mexico were assigned a TMDL for E. coli due to excessive bacterial concentrations[5], and despite continued efforts to limit E. coli concentrations totaling ~$20 million worth of investments in the Albuquerque urbanized area[5], the farthest downstream reach is still considered impaired as of the 2018 update of the state list of impaired waters (303d)[23,24]. This suggests that control efforts have not been successful. The anthropogenic sources of E. coli may be

treated wastewater discharges (four present in the study reach) and Municipal Separate Storm Sewer Systems (MS4s) (three present in the study reach)[5] that discharge to the Rio Grande. Measures to address bacterial contamination are daily effluent concentration or loading limits for wastewater treatment plants (WWTPs), variable loading allocations to MS4s based on discharge in the receiving water, construction of stormwater infrastructure, development of Best Management Practices (BMPs), and public education strategies to reduce fecal contamination in the watershed[25]. Since 2001, various studies and data collection efforts by governmental entities and their consultants have concluded that water quality standards are frequently violated[26–28]. The 2010 TMDL for this reach allocates 90-94% of the total maximum daily load to non-point sources and natural background loadings[5]. However, it remains unknown which sources are responsible for exceedances of water quality standards.

In my study, I observed highest E. coli loadings along the reach during the Summer with marginally lower Winter and Fall loadings, while highest concentrations in sediment were observed during Summer and Fall months. The Spring season had both lowest E. coli loadings and, along with Winter, lowest E. coli sediment concentrations. E. coli loadings throughout the year increased approximately linearly as one proceeds down the river while E. coli sediment concentrations increased in the river section affected by urbanization. Seasonally, low E. coli loadings co-occurred with low sediment E. coli concentrations at upstream sites during Fall, Winter, and Spring while downstream sites had relatively high loadings and sediment concentrations for all seasons. Site by site along the reach, relative fluctuations in E. coli loadings and sediment concentrations were seasonally coupled. Downstream sites had elevated E. coli loadings and sediment

concentrations in the Fall, Winter and Spring seasons while upstream sites had loadings and concentrations decrease while progressing from Summer to Spring. E. coli loading data varied from approximately 10^{12}-$10^{13.5}$ cfu/day and E. coli sediment concentration data varied over approximately 5-10^{4} MPN/100g sediment.

2) Literature Review

2.1) *Development of Bacteriological Water Quality Standards*

Epidemiological studies carried out by the US Public Health Service in the 1940s and 1950s established the basis for recreational water quality regulation by relating fecal coliform levels to reported occurrences of illness at recreational beaches in Illinois, Kentucky, and New York[29]. The relationships between waterborne illness (gastrointestinal illness (GI), skin irritations, and respiratory symptoms), recreational bathing, and fecal coliform concentrations were used by the Department of the Interior to propose the first recreational water quality criteria in 1968[6]. After its formation in 1970, the USEPA used improved epidemiological survey methods in 1972 to update these criteria for fecal coliform levels in 1976[30], and later included E. coli concentration as part of the criteria in 1986[31]. The most recent update of the recreational bacteriological water quality criteria was in 2012 and includes single sample maximum and monthly geometric mean values for fecal coliform, E. coli, and Enterococci concentrations for different levels of recreational use[4].

Theory and support for using FIB for water quality determination is described in the seminal work by Geldreich in 1970[32], which describes how fecal coliforms have been shown to be an indicator of fecal pollution in recreational waters. Many studies have been carried out since the establishment of the first recreational criteria by academic and governmental researchers, overall concluding that FIB criteria are effective in identifying health risk from fecal contamination[21]. However, aspects affecting the occurrence, fate and transport of FIB behavior in surface waters have been shown to vary so widely that nearly opposite trends in FIB particle attachment sizes[9,33], seasonal FIB levels in water

and soils[34,35], correlations between FIB and water quality parameters[36,37], and correlations between water and sediment FIB[10,38] have been observed in different watersheds. Generally, FIB are known to increase their growth rate with temperature and thrive in nutrient-rich environments[39], with growth rates of <0.1/d to 2.5/h[40,41]. Die-off rates of environmental FIB have been estimated as 0.006-0.5/d[9,39,42] and have been shown to vary with temperature as well as sediment and water characteristics[7,15,41,43–45]. However, early works in this field acknowledge that extrapolation of findings to other watersheds is often unrealistic, and extensive data is required to understand the influence of individual environmental factors on relative FIB and pathogen persistence[32]. Since environmental responses of FIB can vary so widely, water regulators frequently assume conservative transport of FIB[5] and incorporate all natural, non-point sources and processes into a single factor called the Load Allocation[5,46].

2.2) *Implementation of Bacteriological Water Quality Standards*

In the US, TMDLs are implemented for water bodies that do not meet their designated water quality criteria in order to reduce waste loadings to a sustainable level. Loadings contributing to E. coli levels to surface waters include WWTP effluents, confined animal feeding operations (CAFOs), septic tank seepage, and wildlife inputs and non-point sources such as runoff from agricultural activities and urban stormwater.

The TMDL framework represents a summation of loadings to a water body separated by source type. For rivers and streams, this is represented by the equation:

$$WLA + LA + MOS = TMDL$$

where WLA is the waste load allocation to anthropogenic sources; LA is the load allocation to natural sources; MOS is a margin of safety; and TMDL is the total maximum daily load in the receiving water. All of the previous terms are expressed in units of [cfu/day].

In the Rio Grande, the TMDL for the river is set as the single sample E. coli water quality criterion (47 cfu/100mL) for direct contact multiplied by a static discharge value based on flow regime in the Rio Grande. Rather than calculate different TMDL's for all river flows, 5 flow regimes are defined (low, dry, mid-range, moist, and high) corresponding to percentiles of 0-10%, 10-40%, 40-60%, 60-90%, and 90-100% of days historical Rio Grande discharge was higher than observed discharge. TMDL values for the midpoint flow of each flow regime are calculated. For each flow regime the MOS is estimated and the LA is calculated first considering only point sources (Figure 1 Eqn. 1). WLAs for point sources are set as the single sample water quality criterion multiplied by the design flow of the discharging facility, and WLAs for MS4s are set as a jurisdictional-area determined percentage of the LA

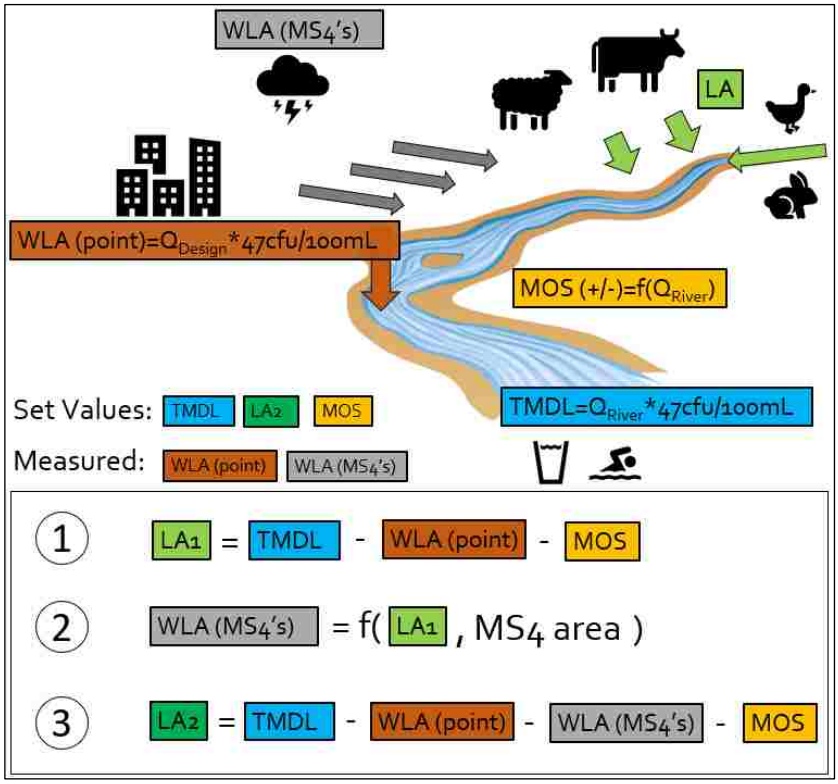

Figure 1: Albuquerque Rio Grande TMDL framework.

calculated considering only point discharges (Figure 1 Eqn. 2). After the WLAs for MS4s are calculated, they are subtracted from the original LA to calculate a new LA considering MS4 discharges (Figure 1 Eqn. 3). For further detail see the 2010 TMDL for the Middle Rio Grande[5]. As a result of this method, both the LA and WLA's for WWTP's for each flow regime are static values. WLA's for WWTP's are ensured using daily effluent samples and loadings from MS4's are inferred from measured loadings in the river. MS4 discharges are highly variable in time and widely distributed in space, and therefore nearly impossible to quantify in isolation.

For compliance, TMDL and WLA values are estimated from point sampling in river water and waste effluents, and estimates of daily discharge. In practice, waste loadings for MS4s are calculated by subtracting point-source WLAs, the MOS and LA from the daily loading in the river estimated by sampling. Since the LA is assumed to be a static value for each flow regime, any loading in excess of the calculated WLAs and LA is assumed to be attributable to MS4s in this framework. This framework implicitly assumes conservative transport of E. coli bacteria (framework is applied to 60 km river distance) and that nonpoint source loadings (LAs) are directly related to discharge (TMDL changes with discharge, WLAs for point sources are constant). Therefore, while the implementation of the TMDL framework represents progress in quantifying and controlling health risk from fecal contamination, significant challenges remain in reliably discerning anthropogenic contamination carrying health risk from non-point FIB sources, which may have different relations to health risk depending on their origin and how they have been affected by the environment.

2.3) *Current Knowledge: Issues and Challenges*

The largest issues in quantifying loads and sources of contamination are centered around the fact that enteric bacteria used as FIB are sensitive to environmental conditions. Temporal variability in environmental FIB levels have been attributed to seasonality, variations in stream mixing and inactivation by sunlight[21], relative rates of growth/die-off, and episodic and sporadic redistribution[7], while spatial variability has been attributed to stream system and anthropogenic forcing heterogeneity[7]. Field studies have shown that FIB are frequently distributed heterogeneously in river system water and sediments[12,13,47], and have highlighted the ability of FIB including E. coli to regrow in water and sediment microcosms[10,13,48]. Studies attempting to quantify FIB loadings in riverbed sediments[12], effects of soil type on FIB persistence[13], relation of FIB presence in soil to waste sources[49], and ability of FIB to grow under environmental conditions[10,36,38] indicate that FIB are difficult to predict and model.

Following establishment of the 2001 TMDL in the middle Rio Grande and high profile spills of untreated waste water to the river, there were two studies conducted to identify the sources of E. coli. The City of Albuquerque, Albuquerque Metropolitan Arroyo Flood Control Authority, the New Mexico Department of Transportation, and the University of New Mexico funded studies published in 2005 and 2015 to identify source types, source locations, and effective measures to reduce measured E. coli levels here[26,28]. The engineering company Parson's Water and Infrastructure sampled Rio Grande water, watershed tributaries, and local animal feces to determine the composition by source of E. coli at different points in the watershed[28]. The study estimated wildlife (primarily avian) sources make up about 46% of observed E. coli. Pets, humans, and livestock were

determined to make up 24%, 16%, and 14%, respectively, of E. coli found in river water samples. Further, the portions contributed by human, avian, and livestock sources increased along the reach, and highest FIB levels were observed following stormwater runoff.

In 2015, engineering company CDM Smith compiled and reviewed existing E. coli data sets generated by the United States Geological Survey (USGS), New Mexico Environment Department (NMED), and Bosque Ecological Monitoring Program (BEMP) in this reach of the Rio Grande[26], finding elevated concentrations of E. coli during Summer months and an overall increase in concentration with downstream distance. Avian flyways, seasonal temperatures, stormwater runoff and tributary flows, wastewater effluents, and persistence of FIB in sand and sediments were cited as likely causes for the elevated Summer levels and increase with downstream distance. The USGS conducted sampling of stormwater outfalls around Albuquerque from 2003-2012 to determine Albuquerque's stormwater quality in terms of various constituents[27], finding that E. coli levels are elevated beyond recreational water quality standards in Albuquerque stormwater, and above levels found in most western US cities. However, the outfall sites sampled experienced an average of 4 to 74 days of flow per year over the study period and contributed an estimated 1.4% of the total annual Rio Grande flow[27], indicating that stormwater likely does not account for sustained high levels of FIB throughout the year. The Albuquerque Metropolitan Arroyo Flood Control Authority, one of several MS4 permittees in the area, funded this study as well as the data review published in 2015. Currently, MS4 permit holders and the NMED collect water quality data in this reach for

NPDES compliance and water quality assessment, and the BEMP is funded to collect water quality data here including E. coli for research and educational purposes[50].

While a large amount of research has examined FIB behavior in surface waters, sediment-water interface soils, and relation of different sources and inputs to observed FIB levels, knowledge gaps still remain in understanding environmental FIB behavior, including quantifying non-point sources, quantifying spatial and temporal variation in concentrations and loadings, and behavior of bacterial populations in waters affected by different watershed types[7]. Knowledge gaps remain in attributing observed FIB loads to their potential sources as well as quantifying loads and sources.

Following federal litigation between the City of Albuquerque and the Isleta Pueblo (located downstream from the Albuquerque wastewater treatment plant) over the 1986 Clean Water Act[51] the Isleta Pueblo was given the authority to enforce water quality standards on river water entering its lands, implying that for some naturally-present contaminants (arsenic, E. coli) that water reaching Isleta Pueblo must be lower in concentration than source water entering the reach. The requirement that Rio Grande water be of sufficient quality for primary contact, including incidental or intentional ingestion of water (E. coli concentration of 47 cfu/100mL)[52] is part of the reason local stakeholders have invested in identifying and reducing FIB sources. The public and agricultural users were warned not to contact river water after spills of untreated sewage to the Rio Grande from WWTP's in this reach in December 2000 (400,000-500,000 gallons of untreated sewage[53]) and February 2015 (6 million gallons of untreated sewage[54]), further heightening awareness of the serious hazards of fecal contamination for downstream consumptive and recreational uses. Both for the sake of public safety and

protection from costly litigation, local water quality managers are invested in better understanding the sources and mechanisms that lead to exceedances of water quality standards in this reach.

3) Proposed Research

Project Objectives:

Identify and quantify seasonal and spatial patterns in E. coli levels in the Rio Grande near Albuquerque.

I hypothesized that E. coli loadings in Rio Grande river water and E. coli concentrations in sediment-water interface sediments are seasonally coupled. More specifically:

Hypothesis 1: E. coli Loading and Concentration Temporal Changes:

E. coli loadings in surface water and E. coli concentrations in sediment-water interface sediments change seasonally as river discharge, stormwater runoff, wildlife inputs, and ambient temperatures affect E. coli survival conditions differently over time. I expected to see the highest loadings in surface water and highest concentrations in sediments corresponding with increased watershed connectivity from Spring to early Fall. Watershed connectivity occurs when portions of the watershed that are not directly adjacent to the stream are hydraulically connected to the stream by flows such as agricultural and stormwater effluents. These flows deliver organic matter, turbidity and fecal matter to the Rio Grande which contribute to favorable conditions for bacteria survival. I expected favorable conditions for E. coli re-growth in riverbed sediments likely results in larger amounts of E. coli readily available for transfer to the overlying water, making the riverbed sediments a net source of E. coli. I expected lowest values for both systems in the Winter when low watershed connectivity and low water temperatures together make the sediment-water interface less favorable for bacteria growth, making riverbed sediments a net sink of E. coli to the system.

Hypothesis 2: E. coli Loading and Concentration Spatial Changes:

I hypothesized that E. coli loadings in Rio Grande surface water and E. coli concentrations in sediment-water interface sediments increase with distance as point and non-point loadings are aggregated along the reach. I expected to see increasing E. coli loadings in surface water and concentrations in sediments with downstream distance as the river progresses from narrow and hard-bottomed to wide and sandy, and aggregated loadings increase favorable survival conditions for FIB.

If these hypotheses are supported, the riverbed sediments would behave both as a source and a sink of E. coli to the river water depending on when and where conditions for E. coli persistence are favorable and stream transport.

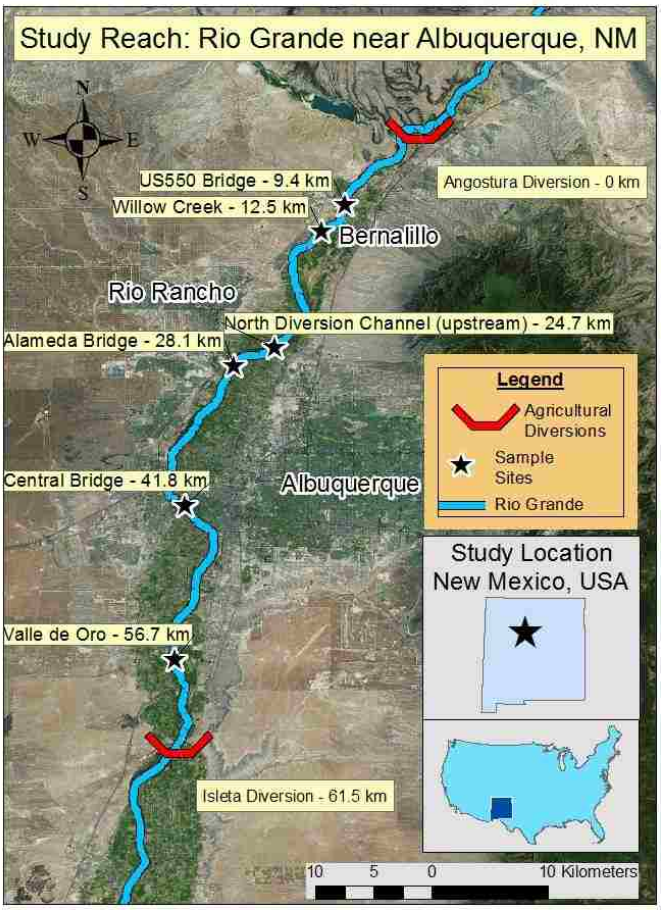

Figure 2: Study reach map.

4) **Methods**

4.1) *Site Description*

I studied a reach of 61.5 km of the Rio Grande near Albuquerque (Figure 2). This reach receives most of its discharge from upstream mountain snowmelt and is highly controlled for irrigation. The study reach has a contributing area watershed of approximately 5180 km^2 and serves as drinking and irrigation water to a population of ~800,000[28,55] along the Albuquerque Metropolitan area, for the City of Albuquerque. Diversion structures on either end of the reach are used during the growing season to route river water into irrigation canals. The US Army Corps of Engineers (USACE) and Middle Rio Grande Conservancy District (MRGCD) release water from impoundments and operate diversion structures to manage Rio Grande water for agricultural irrigation, municipal use, environmental flows, and delivery to downstream users. In 2001, this watershed (contributing area from Cochiti Dam to Isleta Diversion) was approximately 6% developed land, 3% land cultivated for crops, 72% grassland and shrubland, and 18% forest[28]. Peak flows from snowmelt occur typically in May, while peaks from episodic rainfall-runoff events occur July-November[56]. Mean annual discharge, typical turbidity ranges, and ranges of recent publicly available E. coli data are shown in Table 1. The USGS operates several automated discharge gages in this reach, including 2 gages measuring Rio Grande discharge at 15-min intervals (USGS 08329918 at Alameda Bridge and USGS 08330000 at Central Bridge) which were used for this study.

Table 1. Sampling site locations and recent data.

Site No.	Site Name	Lat/Long (N and W)	Mean Annual Flow (m^3/s)♦	Turbidity (NTU)♠	E. coli Average of monthly samples (cfu/100mL)♣	Geometric mean Fecal coliform (cfu/100mL) under non-runoff/runoff conditions♥
1	US550 Bridge	(35.322174, 106.557207)		100-400 (spikes into 1000's)	841	7/354
2	Willow Creek	(35.301619, 106.575356)				12/362
3	North Diversion Channel (upstream)	(35.212027, 106.611886)		150		296/95900
4	Alameda Bridge	(35.197853, 106.643099)	25.7		1182	20/1630
5	Central Bridge	(35.089933, 106.680541)	25.4	200-300	1219	
6	Valle de Oro	(34.971357, 106.688496)	27.6	200-550 (Isleta Dam)	1355	412/4610

♦ USGS information between 2004-2015; ♠ AMAFCA sonde information between 2017 and 2018; ♣ BEMP 2010-2012[50]; ♥ 2005 Microbial Source Tracking Study[28]

4.2) *Use of Historical Data*

Existing E. coli data collected from 2000 to 2015 by the NMED, USGS[57], and Bosque Ecological Monitoring Program (BEMP)[50] were examined for seasonal and spatial trends. The BEMP collected surface water grab samples from 5 sites on a monthly basis from 2010-2012 for analysis at a local water quality lab. This data collection was funded for educational purposes and made publicly available. The NMED and USGS collected monthly samples as part of routine compliance monitoring and analyzed the samples at their respective laboratories. This data was made publicly available via the USGS National Water Information System (NWIS) and the 2010 TMDL for this reach. Examination of these data sets was done by visualizing the data as E. coli loading vs Day

of Year and as E. coli loading vs sampling distance from the upper bound of the reach (See Figure 5).

4.3) *Sampling Design*

To test my hypotheses, I conducted periodic, synoptic sampling campaigns over a year to generate E. coli concentration data from river sediment and surface water grab samples. E. coli concentrations were multiplied by daily discharge reported by the USGS to generate E. coli loading data, which allow comparisons of E. coli between sites and seasons. An extensive measure of E. coli in riverbed sediments using E. coli sediment concentration data was not generated because the volumes of sediment along the river are not known, nor was the concentration profile of E. coli with depth measured.

During each sampling campaign, I collected samples at the 6 sampling sites (Figure 2) on the same day. I repeated these campaigns 17 times over 1 year, with a frequency of 1 campaign every 3 weeks. Due to a logistical difficulty the water sample analyses on the first sample day were not successful, so water sample data from 16 sample days are presented here. Sediment sample collection and analysis was successful for all 17 sample days. Surface water samples were analyzed at the University of New Mexico's Environmental Engineering laboratories using the Membrane Filtration (MF) method. Sediment samples were analyzed by the Albuquerque Bernalillo County Water Utility Authority's (ABC WUA) Water Quality Laboratory (WQL), using a modified version of the Multiple Tube Fermentation (Most-Probable-Number (MPN)) method for E. coli concentration in water. In this method phosphate buffer solution is mixed with the sediment sample for 1-2 minutes in a blender to produce a slurry of sediment and buffer solution. The solid matter is allowed to settle out and the overlying liquid (supernatant) is

analyzed for E. coli concentration, a method commonly used to determine FIB concentration in solid sample material[10–12,36,58,59]. However, the MPN method commonly used for water analysis[11,26,38,60] was used for enumeration of E. coli in the supernatant in contrast to many previous works[10–12,36,58,59] in which the membrane filtration method was used. This avoids complications from filtering sediment particles using the membrane filtration technique and provides a safeguard against laboratory bias influencing observed trends in coupled E. coli water and sediment behavior. This also provides ability to directly compare E. coli values in compliance water samples (frequently collected and analyzed by the ABC WUA WQL) with sediment samples collected in this study.

The sampling sites (Figure 2) were selected to capture the effects of major elements of the system including urbanized areas, WWTP effluents, and large stormwater infrastructure outfalls with the resources available. Site#1 (US550) is upstream of any WWTP effluents and urban stormwater discharges in this reach and is the site least affected by urbanization. The reach between this site and the Cochiti Dam (39 km upstream) has historically had low FIB levels[24,26,28] and communities situated along this reach (San Felipe, Santo Domingo, Pena Blanca) use on-site waste treatment such as septic tanks and total retention ponds for wastewater treatment and do not discharge wastewater to the Rio Grande. Site#2 (Willow Creek) is 1.2 km downstream of the Bernalillo WWTP (design capacity Q=0.035 m^3/s, WLA= 1.43 $x10^9$ cfu/day). Site #3 (North Diversion Channel upstream) is 6.2 km downstream of the Rio Rancho WWTPs (total design capacity Q=0.322 m^3/s, WLA=1.13 $x10^{10}$ cfu/day) and on the southern border of the Sandia Pueblo which has agricultural and livestock operations along the river, representing the combined contribution of Bernalillo and Rio Rancho WWTP

effluents and nonpoint sources from Sandia Pueblo lands. This site is immediately upstream of the largest stormwater outfall in Albuquerque (North Diversion Channel), draining runoff from about 1/3 of the city area. Site #4 (Alameda Bridge) represents the contributions of the North Diversion Channel outfall and sections upstream of the major urban influence of Albuquerque. Between Site #'s 4 and 5 (Central Bridge), numerous agricultural return flows reach the Rio Grande during the growing season (late Spring to Fall), Albuquerque municipal drinking water is withdrawn (average daily Q=2.4 m^3/s from 2016-2018[61]), and residents use extensive recreational areas and trails along the river year-long[62]. Site #6 includes effluent from the Albuquerque WWTP (design capacity Q=3.33 m^3/s, WLA= 1.35 $x10^{11}$ cfu/day) and is the last section of the reach before the Isleta Diversion, which receives input from all upstream sections.

4.4) *Sampling Protocol*

At each site, 2 sediment-water interface grab samples and 1 surface water grab sample were taken. Surface water samples were taken from the bank using pre-sealed, 100mL coliform sampling bottles containing 0.1g sodium thiosulfate to inactivate the effects of any residual chlorine on bacteria during sample storage as described in the Standard Operating Procedure (SOP) for Bacteriological Sampling[63] published by the New Mexico Environment Department (NMED).

Sediment grab samples were taken from a) within the thalweg of the river (when possible) or from a section with relatively deep and fast water flow, and b) near the river bank with low water depth and speed, as agreed upon with NMED staff during development of the SOP S-1 (See Appendix 1 for further detail), which describes sampling riverbed sediments for this project. This sampling scheme was selected to best

represent the typical distribution of E. coli bacteria in the riverbed cross-section observed during exploratory sampling (See Appendices 11 and 12). The sampling locations were selected based on site access over the range of discharges expected during and after runoff events. The selected sites did not present a danger to the sampler, as recommended by the USGS 10-to-1 rule for wading in streams (the product of depth in feet and velocity in ft/s should be less than 10 to safely wade in a stream). At sample points co-located with bridges (US550, Alameda, Central), the sediment grab sample was taken from the bridge when possible to access a deeper location than otherwise possible from the bank. The deeper sample was taken using a Ponar sediment sampler when possible and the shallower sample was taken using a stainless-steel scoop. The sediment sample was placed in a stainless-steel washbowl. Pore water that drained immediately from the sample was removed by tipping the washbowl. A portion of the sample without large rocks or plant matter was placed in a quart-sized zip-top bag and labeled with the sample site, date and time collected. A Chain-of-Custody form provided by the ABC WUA WQL was completed with the identifying information for the sample and submitted along with each sample.

Samples were placed on ice immediately after labelling. Sediment samples were transported to the ABC WUA WQL for analysis within 24 hours of collection. Surface water samples were transported to and analyzed at the UNM laboratory within 8 hours of collection following NMED holding time requirements for bacteriological samples[63].

4.5) *Sample Analysis Protocol*

4.5.1) Surface water samples

Surface water samples were analyzed for E. coli concentration using the USEPA approved Coliscan Membrane Filtration Chromogenic Method[64] (See Appendix 2 for published procedure and Figure 3 for the procedure specific to this study). Briefly, this method requires incubation of the sample water in a nutrient solution containing compounds which produce the colors green and red when the enzymes glucuronidase and galactosidase, respectively, are detected. While glucuronidase (green) identifies E. coli with some certainty, galactosidase (red) identifies coliform bacteria. When both enzymes are detected, the colony will appear blue and is considered a positive result for E. coli. The method has a false positive and false negative rate of 4.3% for E. coli according to the USEPA[65].

Following USEPA recommendations, I took aliquots of 0.1, 1, and 5 mL from the original water sample using plastic-tipped pipettors to properly bracket E. coli concentrations when the order of magnitude of the analyte in the sample was unknown. Pipettor tips were rinsed 3 times with DI water between drawing aliquots and disposed of after the samples for that day were analyzed. Sample aliquots were diluted with DI water to a volume of 40 mL in 100-mL beakers and gently mixed before they were vacuum-filtered through a 0.45 um membrane filter. Following the Coliscan method procedure, the membrane filters were incubated for 72 h at room temperature in the chromogenic nutrient solution using Petri dishes (). The Petri dishes were photographed, disinfected

using a 10% chlorine bleach solution, rinsed, and disposed of in a sealed zip-top bag. Blue colonies were identified and enumerated automatically using publicly available image analysis code segments written into a MATLAB code (See Appendix 4).

The final E. coli concentration was taken as the average of the 2 closest concentration values for each of the 3 plates and stored, along with the raw plate counts, in a Microsoft Excel sheet. Graphical depictions of the colonies counted were automatically generated, displayed, and recorded so that the analyst could verify the automated work coded in MATLAB to count E. coli colonies.

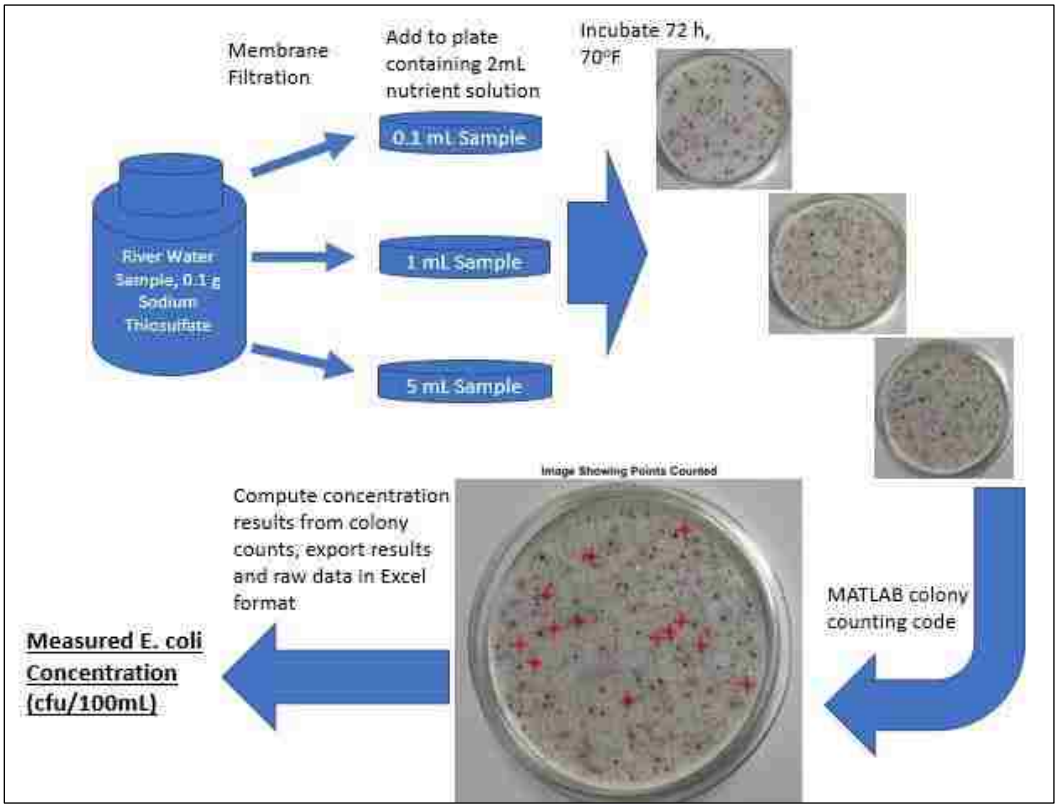

Figure 3: E. coli membrane filtration plate enumeration procedure.

4.5.2) Sediment samples

Sediment samples were analyzed using a variation of the Multiple Tube Fermentation (Standard Methods 9221 C-F) method, which is regularly used by the ABC

WUA WQL for WWTP effluent compliance analyses[66]. The deviation used for sediment samples has also been used by the ABC WUA WQL to analyze samples of WWTP sludge before recycling and for sediment samples from the Albuquerque Bio Park's animal enclosures[67]. Briefly, this method requires incubating aliquots of sample water in a 5x5 array of tubes containing lauryl tryptose medium (A-1 media), which detects the presence of fecal coliforms by the production of gas from lactose[68]. Each row contains a different serial dilution of the sample, generating an array of 5 dilution values, each dilution represented 5 times. If gas production is detected in a tube after 24 h of sample incubation, this is considered a positive result for fecal coliforms and a sterile loop is used to transfer this growth to a tube containing commercial Escherichia Coli (EC) – 4-methylumbelliferyl-β-D-glucuronide (MUG) broth (detects for glucuronidase[69]) and incubated another 24 h. If fluorescence is detected in this second tube, the sample tube is considered positive for E. coli. The analyst then determines which tubes returned a positive result and consults a table returning a "Most Probable Number" of colony forming units for the tray of tubes. To use this method for solids analysis, the solid sample was diluted to 1:10 (solids mass:diluent mass) using stock buffer solution and this resulting slurry was used as the sample. To calculate E. coli concentration by dry mass of the sediment, the moisture contents of sediment masses were calculated as the difference in mass before and after drying in a 110^{O}C oven for 5 h. The final result was back-calculated from the concentration result of the stock slurry, to the corresponding concentration by mass of the wet sediment sample, to the concentration by mass of dry

sediment as shown in the equation:

$$E.\ coli\ Concentration\ in\ Sediment\ \left(\frac{MPN}{g\ dry\ weight}\right) = \left(\frac{MPN}{mL\ supernatant}\right) * Dilution\ \left(\frac{mL\ supernatant}{g\ wet\ sediment}\right) * \frac{1}{\%\ Total\ Solids}\left(\frac{g\ wet}{g\ dry}\right)$$

The concentration of E. coli in sediment obtained through this method are Most Probable Number (MPN) of colony forming units per 100 grams of dry sediment (MPN/100g). Although the units MPN and cfu indicate measurement of the same analyte, the reported units are different because the MPN method is a probabilistic method while the membrane filtration method involves directly counting colonies from a sample water volume. These methods of measuring concentration are not statistically different on the log scale[60] and are considered equivalent for the purposes of this study.

4.6) *Data Analysis*

Surface water E. coli concentrations were multiplied by USGS daily flow data to generate estimates of E. coli loading, in units of cfu/day, 16 times throughout one year. The USGS daily flow data from July 2017 to May 2018 from the USGS Rio Grande at Alameda Bridge (USGS 08329918) and the USGS Rio Grande at Central Bridge (USGS 08330000) gages were used to represent flow for the Rio Grande reaches Angostura to Alameda (New Mexico Standards Section 20.6.4.106) and Alameda to Isleta (New Mexico Standards Section 20.6.4.105), respectively. Sediment samples were successfully collected and analyzed on all of the 17 sample days and water samples were successfully collected and analyzed on the last 16 sample days. The first planned sample day did not produce E. coli water concentration data due to a logistical difficulty that caused the

water samples collected to be held beyond the 8 h allowable sample holding time for this analysis.

To perform statistical calculations on these datasets, several changes were made to the reported results. Results of non-detection for either analysis method were assigned values of the detection limit of the sample run based on the volume of material analyzed. Since loading estimates are based on surface water concentration data, non-detect concentration results were set as the detection limit of 20 cfu/100mL before being multiplied by the USGS daily discharge value. For sediment samples, the detection limit was set as 1 MPN/20g sediment because ~20 g of sediment was mixed with the buffer solution to produce the supernatant analyzed. Results from the MPN procedure indicating less than a certain value were set equal to this value for statistical analysis and display purposes.

Sediment concentration data and E. coli loading data transformed by the logarithm of 10 fit the shape benchmarks for approximate normality ($-1<\text{skewness}<1$, magnitude of kurtosis ~3) The log-transformed E. coli water concentration data has skewness -0.43 and kurtosis 2.39. Log-transformed E. coli loading data has skewness -0.56 and kurtosis 2.65, and the log-transformed E. coli in sediment concentration data has skewness -0.59 and kurtosis 3.20 (Figure 4). Since these conditions are met and the sample size is relatively large (E. coli Load n=96, E. coli Concentration in Sediment n=204), the population is assumed to be approximately normal by the Central Limit Theorem[70]. Confidence intervals for the population were generated using the z-distribution for a population with unknown standard deviation. Confidence intervals for subsets of the population were generated using Students' t-distribution for a small sample

size from an approximately normal distribution with unknown standard deviation. One-way Analysis of Variance (ANOVA) tests were used to determine whether mean sediment concentration and loading values are statistically different when grouped by site and by season. Tukey's Honest Significant Difference (HSD) range test was used to evaluate significant difference between means of individual groupings. All reported values are back-transformed from logarithmic units to arithmetic units of cfu/day or MPN/100g.

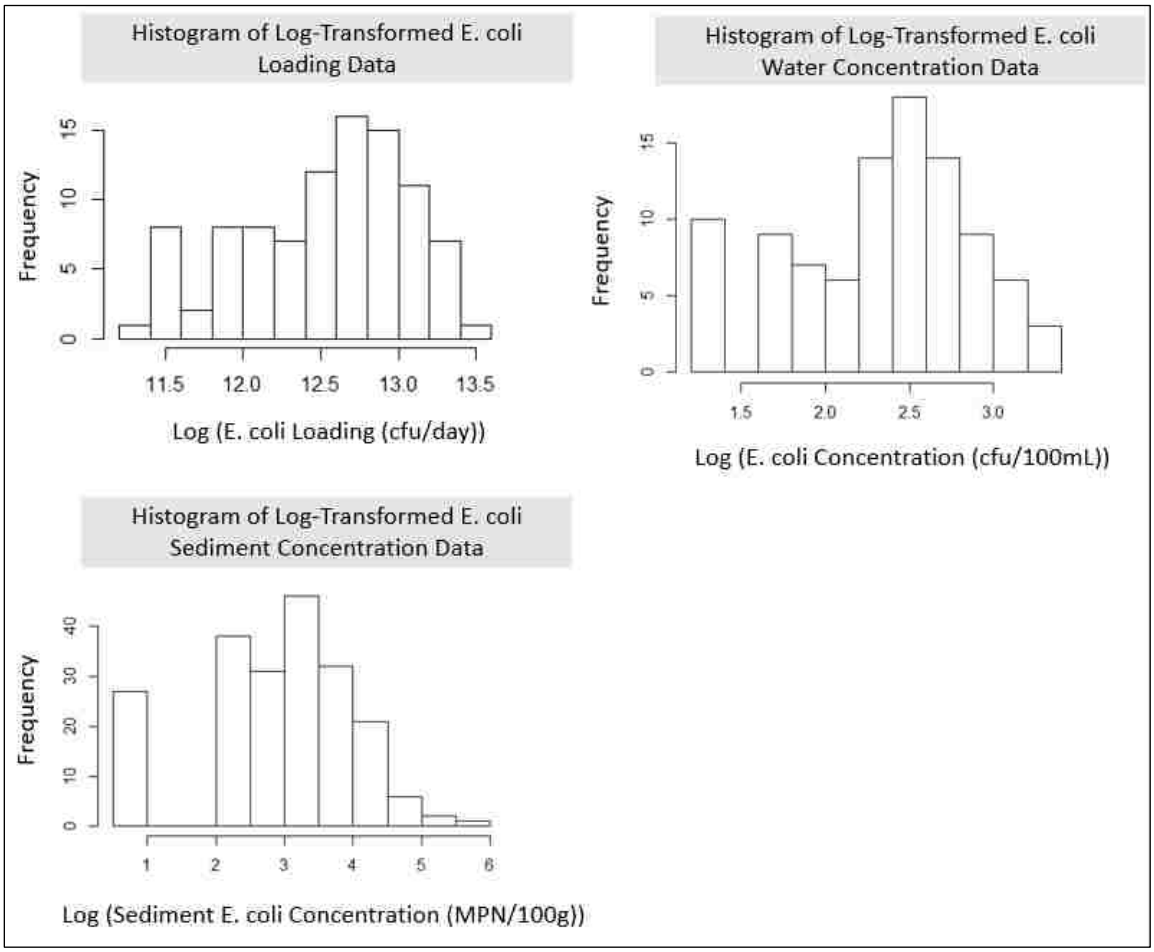

Figure 4: Histograms showing E. coli loading, water concentration, and sediment concentration data sets.

5) **Results**

5.1) *Historical Data*

Analysis of historical data suggested that the highest concentrations of FIB occur in the Summer months, and FIB concentrations generally increase in the downstream direction[26,28]. Visualizations of publicly available E. coli loading data collected by the USGS and NMED from 2001 to 2015 (including the data used in the 2010 TMDL) show highest loadings occurring over days ~190-250 (Summer) (Figure 5b) and loadings overall increasing with downstream distance (Figure 5a). However, much of these data were generated for water quality compliance purposes, which require samples be taken during dry conditions and wet (runoff, soon after precipitation events) conditions. This may have created bias in this dataset as samples representing runoff conditions may be

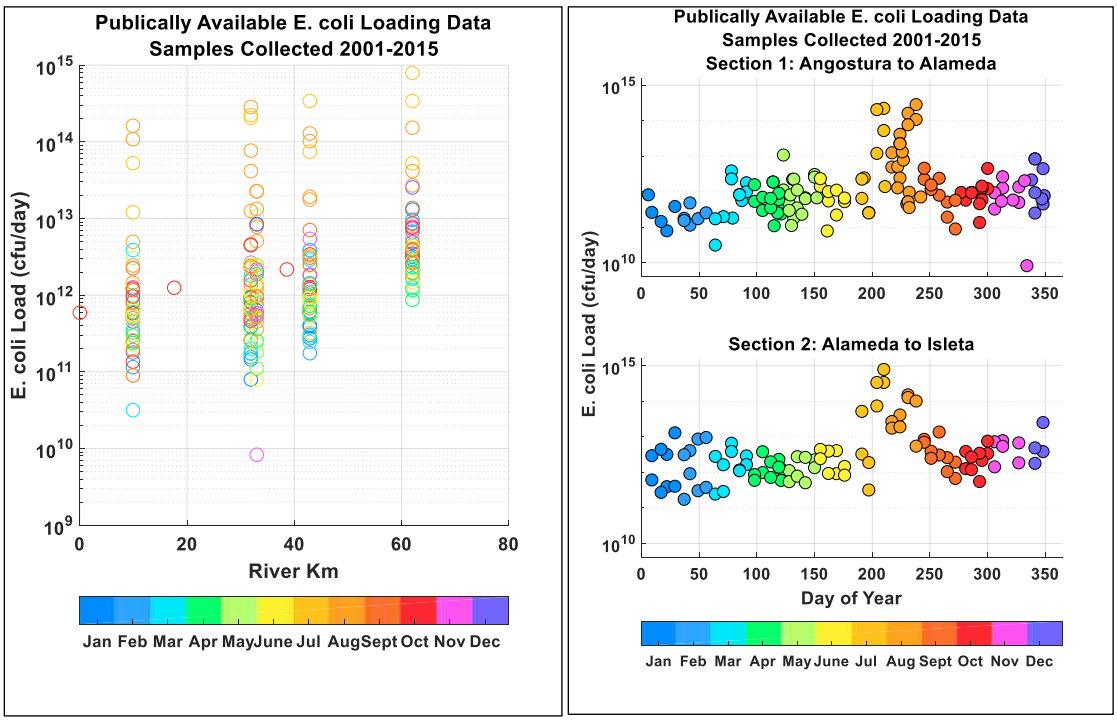

Figure 5: a) Public E. coli data (2001-2015) vs time. b) Public E. coli data (2001-2015) vs space.

overrepresented in terms of how frequently runoff conditions occur throughout the year. Differences in trends between the data generated for this study and data from previous years may be due to sampling bias or inter-annual variation in FIB behavior. Other sources of bias in this dataset could include use of different sampling and analysis methods to determine E. coli concentration.

Presumed (largely unquantified) sources contributing to high loadings mentioned in previous studies are the presence of water fowl and aquatic mammals, leaking septic tank systems along the reach, storm runoff flows washing city surfaces (including the so-called first-flush effect), and regrowth of partially inactivated organisms in WWTP effluent in nutrient rich waters and sediment.

5.2) *E. coli System Changes with Time*

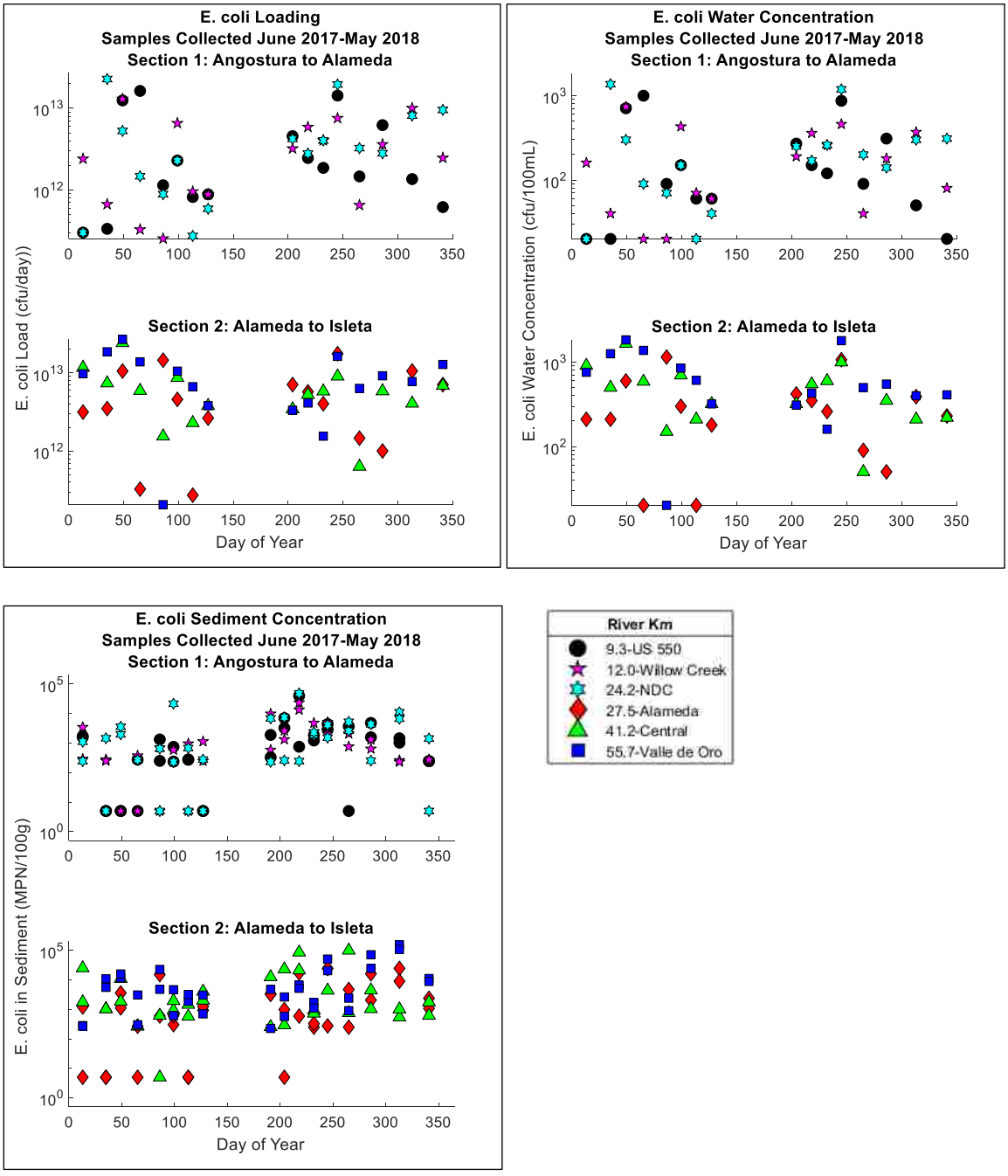

Figure 6: E. coli loading, water concentration, and sediment concentration vs time.

Figure 6 shows the complete sets of E. coli loading and sediment concentration data generated for this study. 95% confidence intervals (CI's) for mean E. coli loading and sediment E. coli concentration for all sites, grouped by season, are shown in Table 2

and Figure 7. Results of the One-Way ANOVA test on the data grouped by season are shown in Table 3. Differences between individual season groups examined using Tukey's HSD method in a multiple comparison test are shown in Table 4 and Figure 8. For all statistical tests, p-values less than 0.05 indicate a significant difference at the 95% condifence level (alpha=0.05).

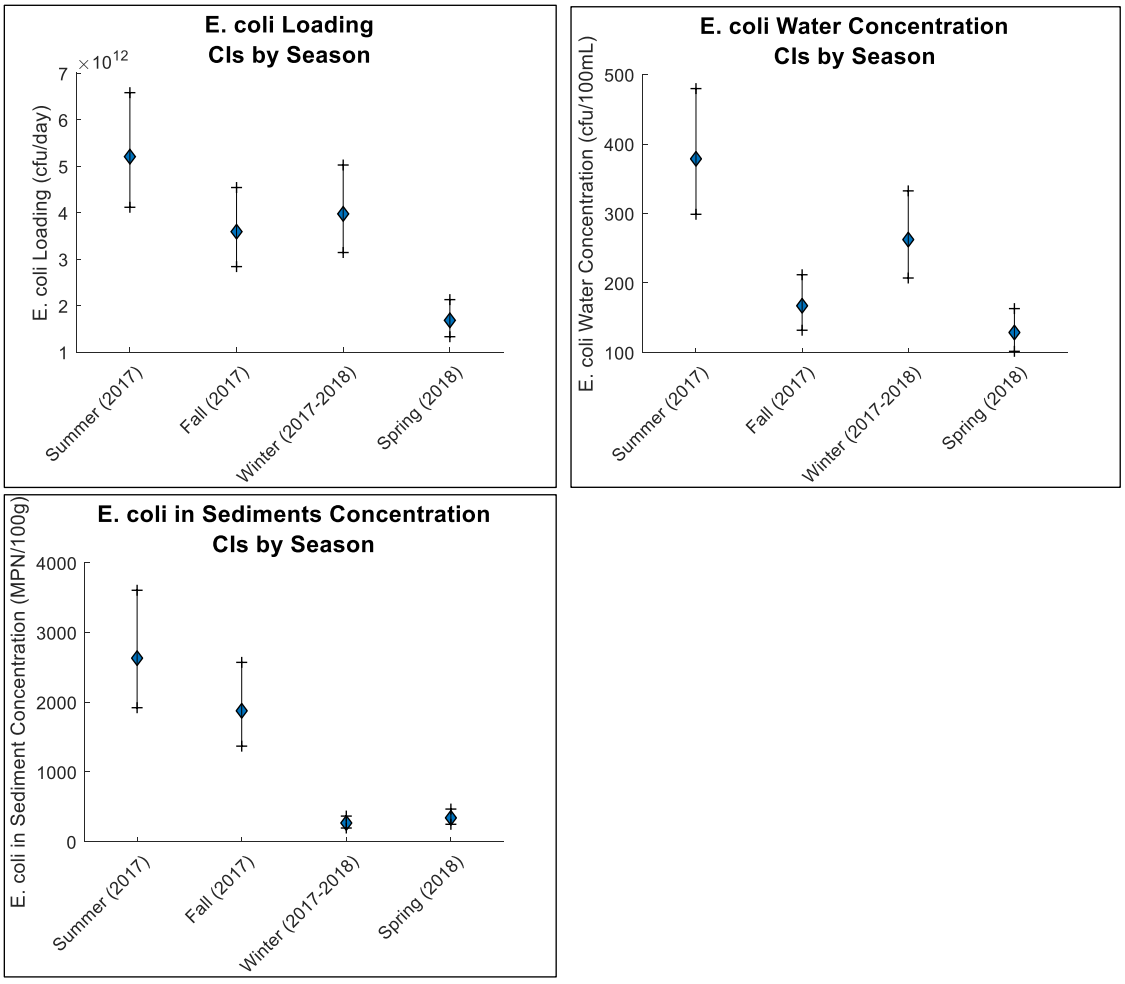

Figure 7: Graphical 95% confidence intervals (CIs) for all sampling sites, grouped by season.

Table 2. Tabular 95% confidence intervals (CI) for all sampling sites, grouped by season.

Water Sample Data				
Season and day of year (DOY)	**CI for E. coli Load (x10^{12} cfu/day) (n=24 per season♦)**		**CI for E. coli Water Concentration (cfu/100mL)**	
	Lower Bound	**Upper Bound**	**Lower Bound**	**Upper Bound**
Summer (DOY 170-260)	4.12	6.59	298.84	479.74
Fall (DOY 260-350)	2.84	4.54	131.96	211.85
Winter (DOY 350-80)	3.14	5.03	207.14	332.54
Spring (DOY 80-170)	1.33	2.13	101.6	163.1
Sediment Sample Data				
Season and day of year (DOY)	**CI for E. coli Sediment Concentration (MPN/100g) (n=48 per season, n=60 for Summer♠)**			
	Lower Bound	**Upper Bound**		
Summer (DOY 170-260)	1920.7	3605.2		
Fall (DOY 260-350)	1369.5	2570.7		
Winter (DOY 350-80)	195.06	366.15		
Spring (DOY 80-170)	248.68	466.79		

♦ 4 sample runs per season at each of 6 sample sites; ♠ 4 sample runs per season (5 for Summer), with 2 samples taken at each of 6 sites

Table 3: Results of ANOVA tests for difference between seasons for all sampling sites.

Dataset	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
Water Sample Data						
E. coli Loading Log (cfu/day)	Between seasons	3.17	3	1.06	4.08	0.0091
	Within seasons	23.88	92	0.26		
	Total	27.05	95			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	3.11	3	1.04	3.92	0.0110
	Within seasons	24.29	92	0.26		
	Total	27.40	95			
Sediment Sample Data						
E. coli Concentration in Sediments Log (MPN/100g)	Between seasons	39.78	3	13.26	13.35	5.58E-08
	Within seasons	198.59	200	0.99		
	Total	238.36	203			

*Shaded values indicate statistically significant difference at the 95% confidence level

Table 4: Results of multiple comparison tests on data from all sampling sites grouped by season.

Dataset	Groups being compared		95% CI Lower Bound for Estimated Difference	Estimated Mean Difference	95% CI Upper Bound for Estimated Difference	p-value*
Water Sample Data						
E. coli Loading Log (cfu/day)	Summer	Fall	-0.22	0.16	0.55	0.6930
	Summer	Winter	-0.27	0.12	0.50	0.8558
	Summer	Spring	0.11	0.49	0.87	0.0067
	Fall	Winter	-0.43	-0.04	0.34	0.9906
	Fall	Spring	-0.06	0.33	0.71	0.1212
	Winter	Spring	-0.01	0.37	0.76	0.0611
E. coli Water Concentration Log (cfu/100mL)	Summer	Fall	-0.03	0.35	0.74	0.0855
	Summer	Winter	-0.23	0.16	0.55	0.7067
	Summer	Spring	0.08	0.47	0.86	0.0113
	Fall	Winter	-0.58	-0.20	0.19	0.5525
	Fall	Spring	-0.27	0.11	0.50	0.8697
	Winter	Spring	-0.08	0.31	0.70	0.1654
Sediment Sample Data						
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-0.35	0.15	0.64	0.8719
	Summer	Winter	0.50	0.99	1.49	0.0000
	Summer	Spring	0.39	0.89	1.38	0.0000
	Fall	Winter	0.32	0.85	1.37	0.0002
	Fall	Spring	0.22	0.74	1.26	0.0015
	Winter	Spring	-0.63	-0.11	0.42	0.9547

*Shaded values indicate statistically significant difference at the 95% confidence level

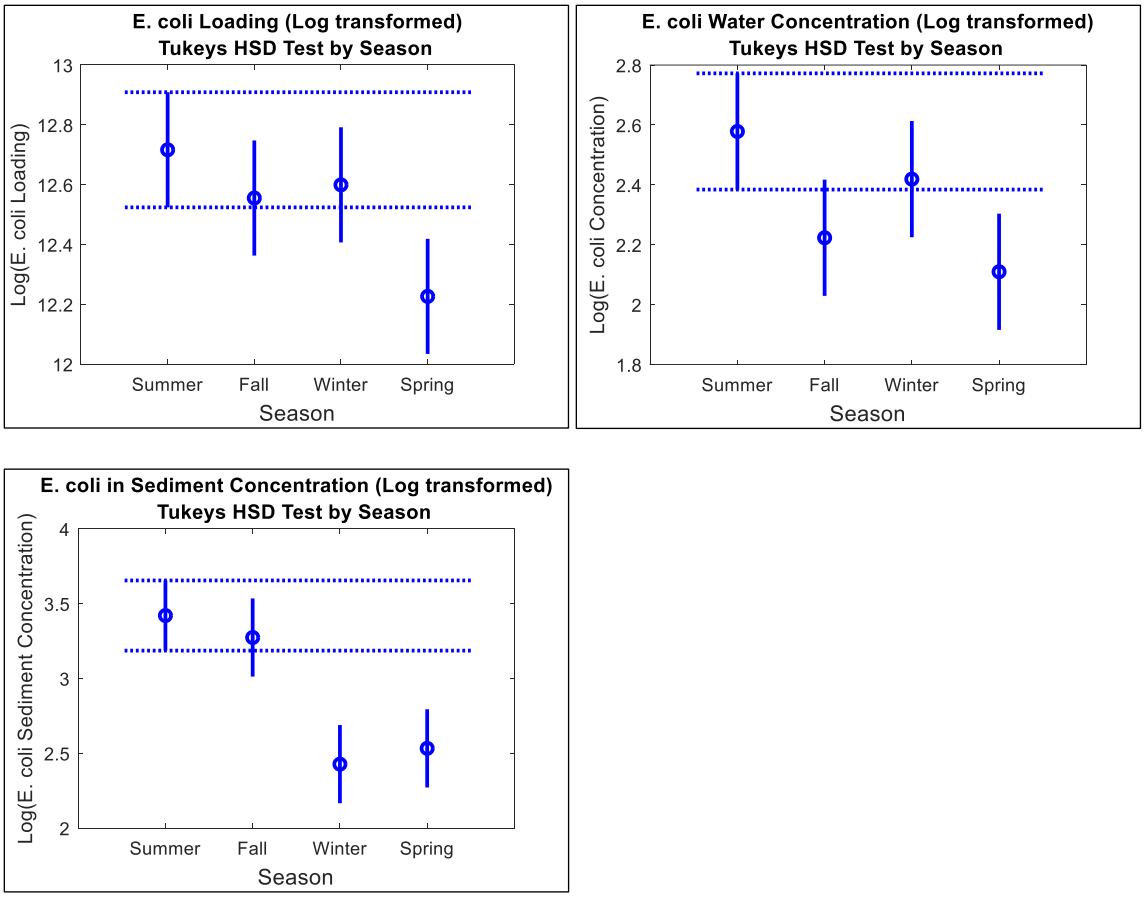

Figure 8: Multiple comparison tests on data from all sites grouped by season (overlap in range indicates no significant difference).

Results of the One-Way ANOVA test show that E. coli loadings grouped by season have significant mean differences at the 95% confidence level. This result supports the hypothesis that E. coli loadings change with season. A multiple comparison test using Tukey's HSD range method shows that Spring loadings are significantly lower than Summer loadings with no other season groupings having statistically different means from one another other (Figure 8, Table 4). I expected to see a peak in the Summer and lowest loadings in the Winter, however lowest loadings were observed in the Spring and no clear Summer peak was captured by the sampling campaigns. Concentrations of

E. coli in the river water followed a similar pattern, with Spring concentrations significantly lower than Summer concentrations.

Regarding E. coli in sediment data, a One-Way ANOVA test shows that there is a significant difference between seasonal group means at the 95% confidence level. A multiple comparison using Tukey's HSD test shows that E. coli concentrations in sediment, grouped by season, show statistical difference between Summer/Fall and Winter/Spring groupings (Figure 8, Table 4). Neither Summer vs Fall nor Winter vs Spring groups are statistically different. This partly supported my hypothesis that the highest concentrations were observed in the late Summer and Fall, although I expected to see high concentrations in Spring despite low temperatures as agricultural irrigation channels delivered sediments, bacteria, and nutrients to the river. Low E. coli concentration in sediment levels were observed in the Winter as expected, and both Winter and Spring estimated mean values are about one order of magnitude below estimated mean Summer and Fall values.

To further understand how the system changes with time along the reach, 95% confidence intervals for the mean seasonal loading at each site (E. coli loading n=16 per site, 4 per season, E. coli Sediment Concentration n=34 per site, 8 per season (Summer n=10)) were calculated and are shown graphically in Figure 9 (See Appendix 6 for complete table of 95% confidence intervals by sampling site). One-Way ANOVA tests for the difference between seasonal groups at each site were performed for loading and sediment concentration (p-values displayed in the figure) (See Appendix 7 for complete table of ANOVA test results).

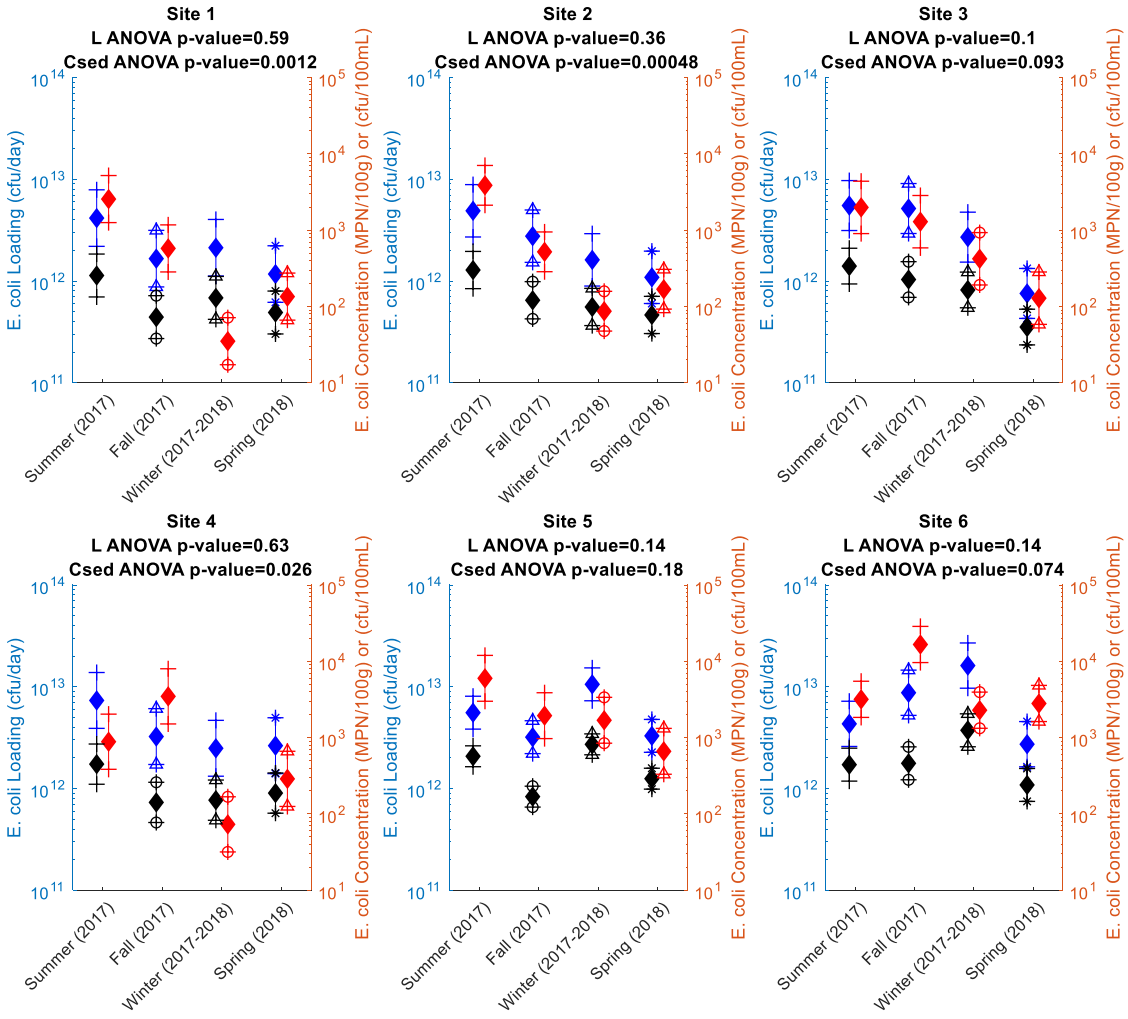

Figure 9: 95% confidence intervals (CI's) for seasonal loading and sediment concentration grouped by site.

Throughout the year at any site, loadings in this dataset do not change significantly with season (Figure 9, Appendix 7). Most sites feature highest loadings in Summer and lowest loadings in Spring, similar to the reach-wide changes with season, although Sites 5 and 6 have highest loadings during the Winter season. E. coli sediment concentrations change significantly with season in sites 1, 2, and 4, which all have lowest concentrations clearly in the Winter. Sites 3, 5, and 6 do not show a significant difference

between seasons at the 95% confidence level. Sediment concentrations generally decrease from Fall/Summer to Winter/Spring seasons except at site 6, which has elevated E. coli sediment concentrations year-round and a peak in the Fall. Relative trends in E. coli loading appear fairly closely coupled with trends in E. coli sediment concentration, with notable deviations at Sites 1, 2, and 4 during the Winter (sediment concentrations lower than during Fall, loadings comparable to Fall loadings) and Sites 4 and 6 during the Fall (sediment concentrations higher than during Summer, loadings comparable to Summer loadings).

5.3) *E. coli System Changes with Location*

shows the complete set of data displayed with respect to distance. 95% CI's for E. coli loading and sediment E. coli concentration data grouped by site are shown in Table 5 and Figure 11. Results of the One-Way ANOVA test on the data grouped by site are shown in Table 6. Differences between individual groups examined using Tukey's HSD method in a multiple comparison test are shown in Table 7 and Figure 12. As above, for all statistical tests p-values less than 0.05 indicate a significant difference at the 95% condifence level (alpha=0.05).

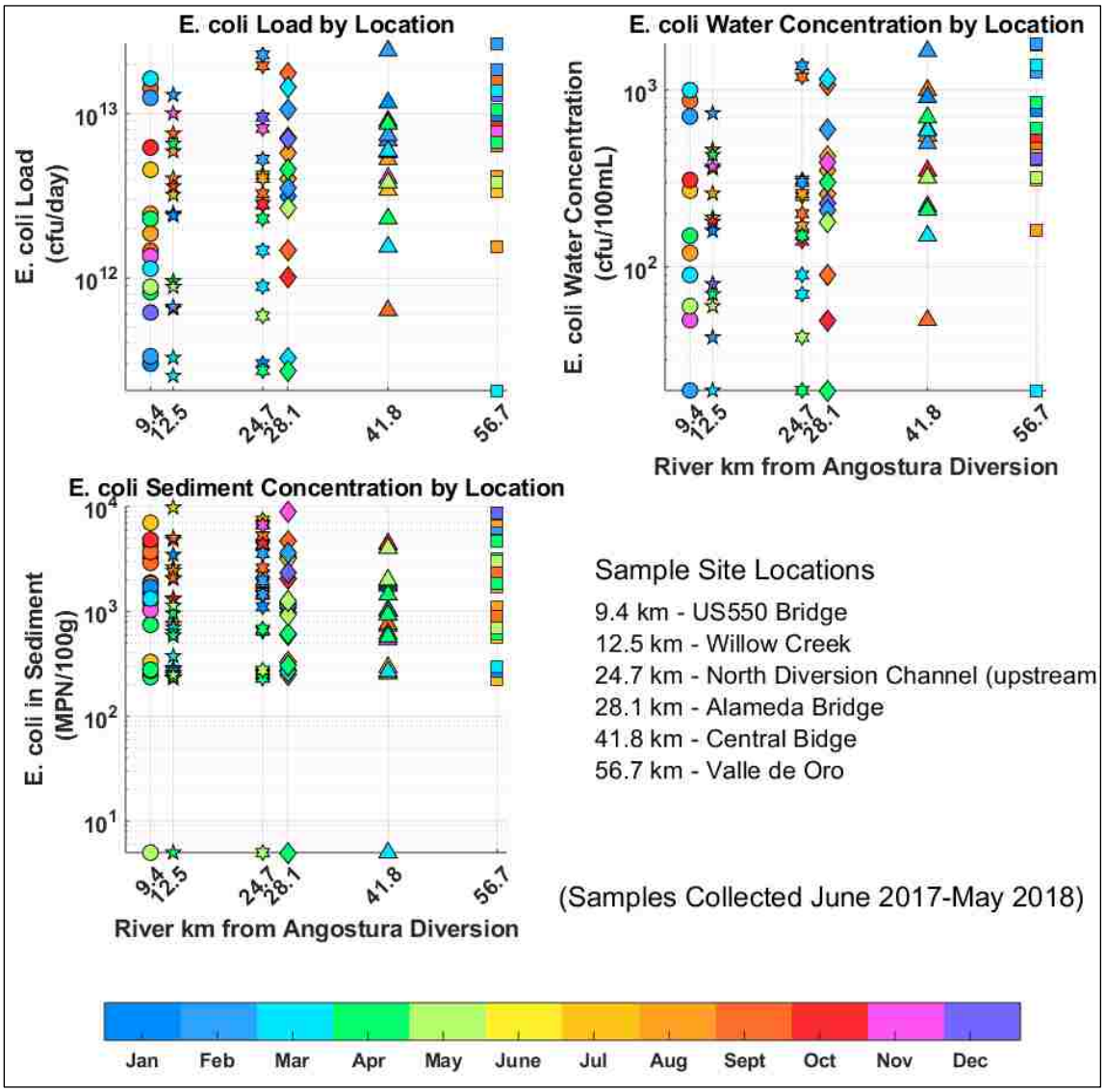

Figure 10: Longitudinal variations of E. coli loading, water concentration, and sediment concentration.

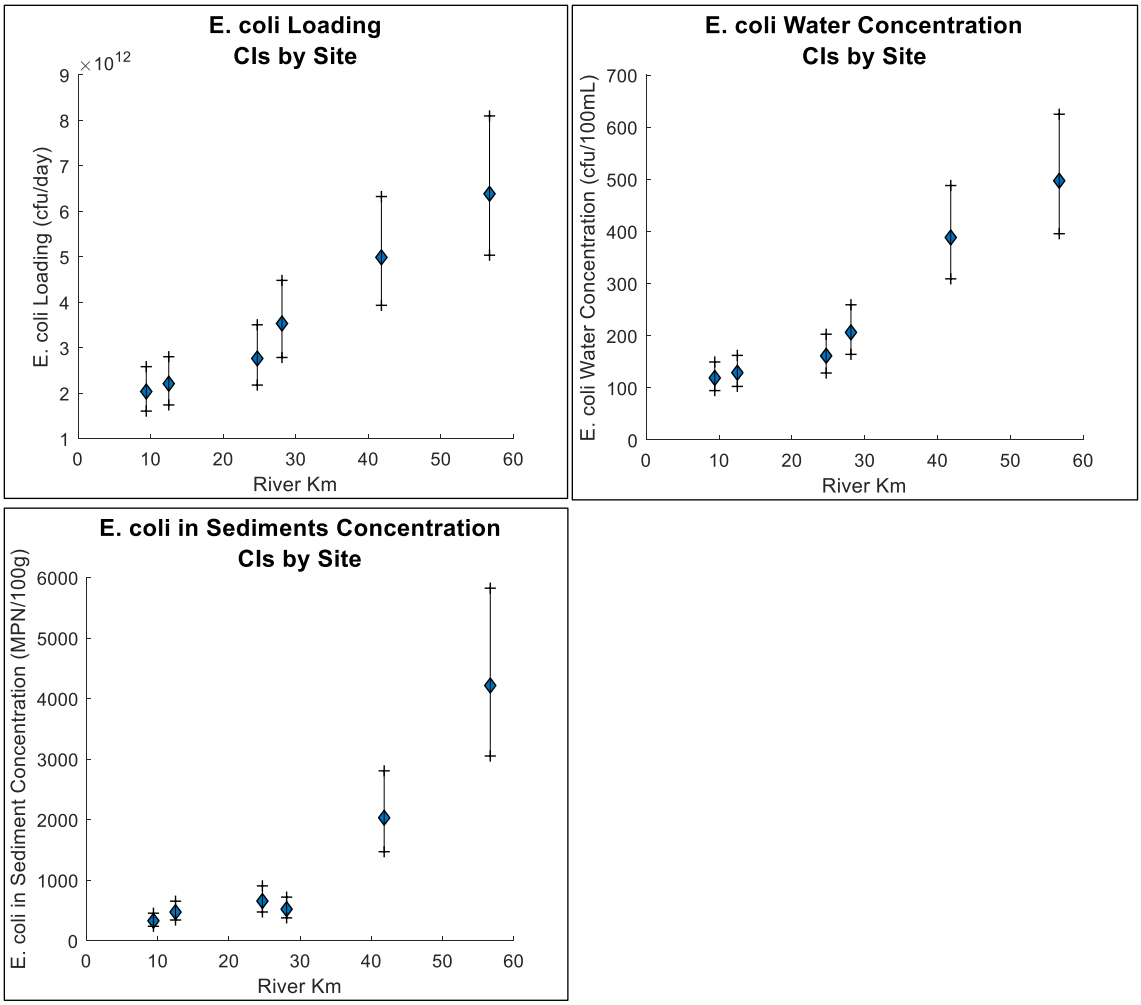

Figure 11: Graphical 95% confidence intervals (CI's) for all seasons, grouped by sampling site.

Table 5. Tabular 95% confidence intervals (CI's for all seasons), grouped by sampling site.

Water Sample Data				
Site#	**CI for E. coli Load (x10^{12} cfu/day) (n=16 per site ♦)**		**CI for E. coli Water Concentration (cfu/100mL)**	
	Lower Bound	**Upper Bound**	**Lower Bound**	**Upper Bound**
1: US550 Bridge	1.61	2.58	94.743	149.6
2: Willow Creek	1.74	2.80	102.75	162.24
3: North Divn. Channel	2.18	3.50	128.47	202.85
4: Alameda Bridge	2.79	4.48	164.26	259.36
5: Central Bridge	3.93	6.32	309.11	488.08
6: Valle de Oro	5.04	8.10	395.72	624.83
Sediment Sample Data				
Site#	**CI for E. coli Sediment Concentration (MPN/100g) (n=34 per site♠)**			
	Lower Bound	**Upper Bound**		
1: US550 Bridge	238.1	454.43		
2: Willow Creek	342.18	653.09		
3: North Divn. Channel	474.49	905.61		
4: Alameda Bridge	377.4	720.3		
5: Central Bridge	1470.4	2806.4		
6: Valle de Oro	3051.6	5824.3		

♦ 16 sample events throughout the year; ♠ 17 sampling events throughout the year, 2 samples per site

Table 6: Results of ANOVA tests for differences between sites.

Dataset	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
Water Sample Data						
E. coli Loading Log (cfu/day)	Between sites	3.12	5	0.62	2.35	0.0473
	Within sites	23.93	90	0.27		
	Total	27.05	95			
E. coli Water Concentration Log (cfu/100mL)	Between sites	5.27	5	1.05	4.29	0.0015
	Within sites	22.13	90	0.25		
	Total	27.40	95			
Sediment Sample Data						
E. coli Sediment Concentration Log (MPN/100g)	Between sites	31.23	5	6.25	5.97	3.62E-05
	Within sites	207.14	198	1.05		
	Total	238.36	203			

*Shaded values indicate statistically significant difference at the 95% confidence level

Table 7: Results of multiple comparison tests on data grouped by season.

Dataset	Sites being compared		95% Confidence Lower Bound for Estimated Difference	Estimated mean difference	95% Confidence Upper Bound for Estimated Difference	p-value*
Water Sample Data						
E. coli Loading Log (cfu/day)	1	2	-0.57	-0.04	0.50	1
	1	3	-0.66	-0.13	0.40	0.9783
	1	4	-0.77	-0.24	0.29	0.7783
	1	5	-0.92	-0.39	0.14	0.2806
	1	6	-1.03	-0.50	0.03	0.0812
	2	3	-0.63	-0.10	0.43	0.9947
	2	4	-0.73	-0.20	0.33	0.8729
	2	5	-0.88	-0.35	0.18	0.3860
	2	6	-0.99	-0.46	0.07	0.1273
	3	4	-0.64	-0.11	0.42	0.9918
	3	5	-0.79	-0.26	0.27	0.7231
	3	6	-0.89	-0.36	0.17	0.3535
	4	5	-0.68	-0.15	0.38	0.9629
	4	6	-0.79	-0.26	0.27	0.7213
	5	6	-0.64	-0.11	0.42	0.9916
E. coli Water Concentration Log (cfu/100mL)	1	2	-0.55	-0.04	0.48	1
	1	3	-0.64	-0.13	0.38	0.9742
	1	4	-0.75	-0.24	0.27	0.7486
	1	5	-1.02	-0.51	0.00	0.0477
	1	6	-1.13	-0.62	-0.11	0.0081
	2	3	-0.61	-0.10	0.41	0.9937
	2	4	-0.71	-0.20	0.31	0.8534
	2	5	-0.99	-0.48	0.03	0.0795
	2	6	-1.10	-0.59	-0.08	0.0150
	3	4	-0.62	-0.11	0.40	0.9902
	3	5	-0.89	-0.38	0.13	0.2596
	3	6	-1.00	-0.49	0.02	0.0688
	4	5	-0.79	-0.27	0.24	0.6226
	4	6	-0.89	-0.38	0.13	0.2581
	5	6	-0.62	-0.11	0.40	0.9899
Sediment Sample Data						
E. coli Sediment Concentration Log (MPN/100g)	1	2	-0.86	-0.16	0.55	0.9884
	1	3	-1.01	-0.30	0.41	0.8336
	1	4	-0.91	-0.20	0.51	0.9665
	1	5	-1.50	-0.79	-0.08	0.0180
	1	6	-1.81	-1.11	-0.40	0.0001
	2	3	-0.85	-0.14	0.56	0.9928
	2	4	-0.75	-0.04	0.66	1.0000
	2	5	-1.34	-0.63	0.07	0.1093
	2	6	-1.66	-0.95	-0.24	0.0018
	3	4	-0.61	0.10	0.81	0.9987
	3	5	-1.20	-0.49	0.22	0.3537

Dataset	Sites being compared		95% Confidence Lower Bound for Estimated Difference	Estimated mean difference	95% Confidence Upper Bound for Estimated Difference	p-value*
	3	6	-1.52	-0.81	-0.10	0.0143
	4	5	-1.30	-0.59	0.12	0.1628
	4	6	-1.61	-0.91	-0.20	0.0034
	5	6	-1.02	-0.32	0.39	0.7971

*Shaded values indicate statistically significant difference at the 95% confidence level

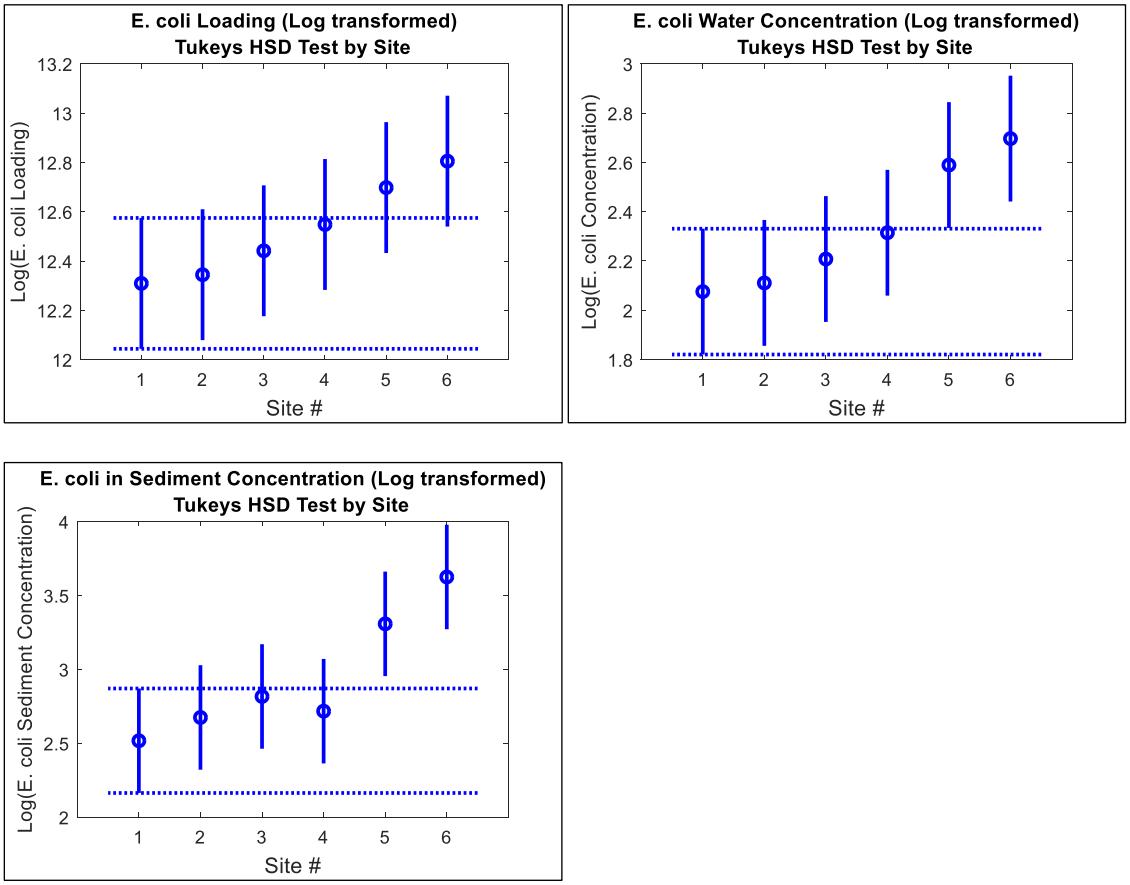

Figure 12: Multiple comparison tests on data grouped by site (overlap in range indicates no significant difference).

I found that estimated mean E. coli loadings gradually increase along the reach, increasing by about one order of magnitude (10^{12} -10^{13} cfu/day) throughout the year. Although results of the One-Way ANOVA test show that E. coli loadings grouped by sampling site show significant difference between sites (Table 6), multiple comparison

tests using Tukey's HSD method show that no groups are statistically different from each other on an individual basis at the 95% confidence level (Figure 12, Table 7). This partly supports my hypothesis as the ANOVA test shows that loadings grouped by site likely do not have equal population means. However, I expected to see a clear increase in load along the reach with loadings at the upstream end significantly different from loadings at the downstream end. This was not shown by the multiple comparison tests. While the mean estimated loading throughout the year increases with site distance, no sample sites show a significantly higher loading throughout the year.

E. coli concentrations in sediment grouped by site show statistical difference between Site#6 and Site #'s 1-4, with Site #5 showing no statistical difference from any other sites by Tukey's HSD test (Figure 12, Table 7). This result supports the hypothesis of increasing E. coli concentration in sediments with downstream distance, with the most significant increase occurring over the urbanized section from Alameda Bridge to Valle de Oro.

To further understand how the system changes along the reach, 95% confidence intervals for the mean loading at each site (E. coli loading n=24 per season, 6 sites per day, 4 sample days per season, E. coli Sediment Concentration n=34 per season, 12 samples per day, 4 sample days per season (5 sample days for Summer)) were calculated for each season and are shown in Figure 13 (See Appendix 9 for complete table of 95% Confidence Intervals by sampling site). One-Way ANOVA tests for the difference between sample site groups during each season were performed for loading and sediment concentration (p-values displayed in figure) (See Appendix 10 for complete table of ANOVA test results).

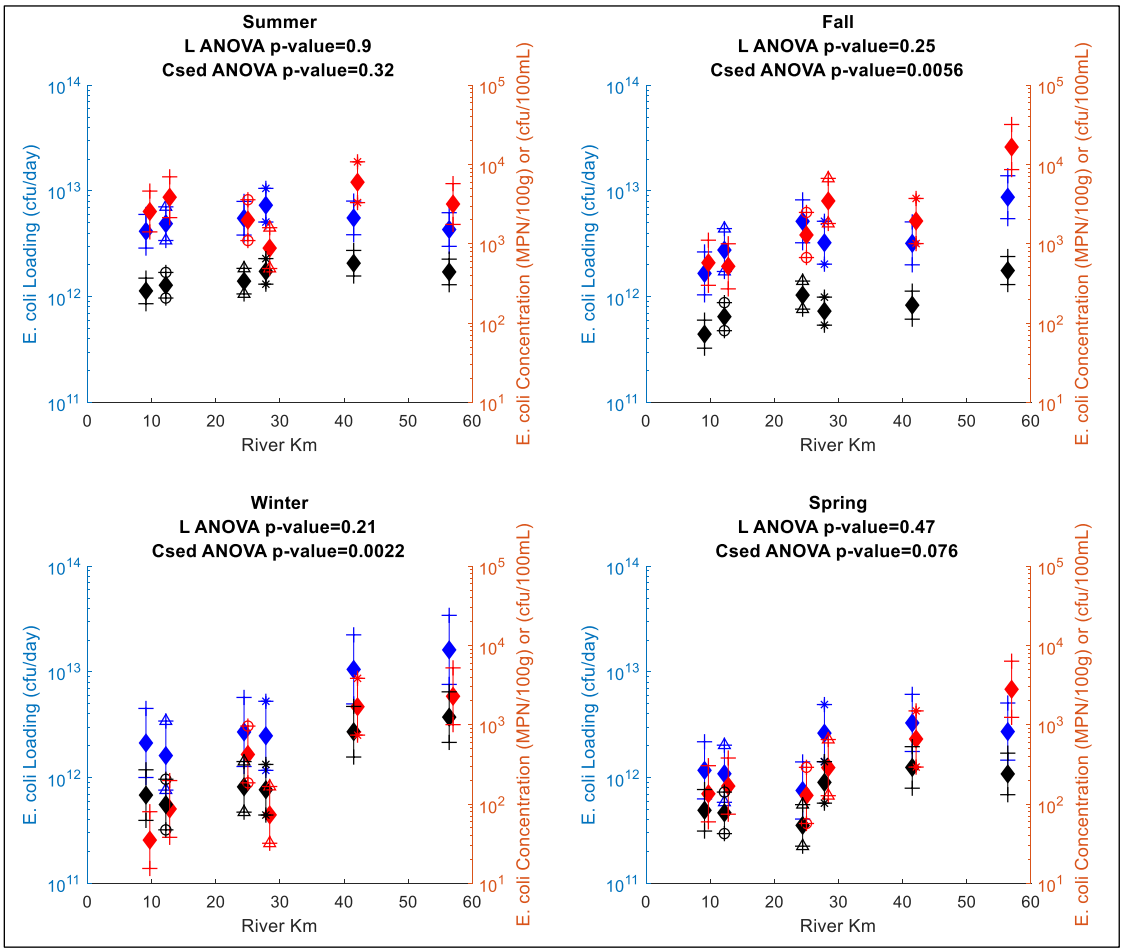

Figure 13: 95% confidence intervals (CI's) for loading and sediment concentration by site, grouped by season.

When data from individual seasons are examined separately, it is clear that different seasons show differing spatial trends for E. coli loading and sediment concentration datasets. For each seasonal group, loadings grouped by sample site do not show significant difference along the reach (Figure 13, Appendix 10). Sediment concentrations show significant difference between sample sites for Fall and Winter seasons, with Summer sediment concentrations fairly constant along the reach and Spring

sediment concentrations increasing marginally but not significantly with distance. During the Fall, sediment concentrations at the farthest downstream site were significantly higher than upstream sites 1 and 2, and during Winter sediment concentrations at sites 5 and 6 were significantly higher than at site 1 (See Appendix 11 for results of multiple comparison test for difference between site means, grouped by season). This shows that seasonal fluctuations in sediment concentrations are greatest at the upstream sites, where E. coli sediment concentrations decrease in Winter and Spring. Concentrations at downstream sites (5 and 6) are comparable between Summer, Winter, and Spring seasons, with Fall concentrations at site #6 representing the highest group mean from the dataset. Loadings appear to follow these trends, with low upstream loadings co-occurring with low upstream sediment concentrations and high downstream loadings co-occurring with high downstream sediment concentrations. Additionally, trends in sediment concentrations and loadings along the reach by season appear to be coupled, with nearly no increase along the reach during Summer, steepest increase along the reach during Winter, and milder increases along the reach during Fall and Spring. While trends in loadings appear to mirror trends in E. coli sediment concentration, the sample number per site and per season is low (n=4), resulting in high standard error between sample sites and no significant effect on seasonal loadings from sample site at the 95% confidence level.

5.4) *E. coli Loading Data with Respect to TMDL*

Figure 14a shows the data used to generate the 2010 TMDL and Figure 14b shows E. coli loading data generated from this study compared with TMLD values. Both figures display E. coli loading vs percentage of days that historical Rio Grande discharge was higher than discharge on the sample day (% of days flow exceeded). These figures

show that the data collected for this study exceed TMDL values in the downstream section. Figure 15 shows loading in excess of the TMDL (exceedance (cfu/day)) vs time, suggesting that exceedances occur more frequently, and consistently in greater magnitude, in the Alameda to Isleta reach.

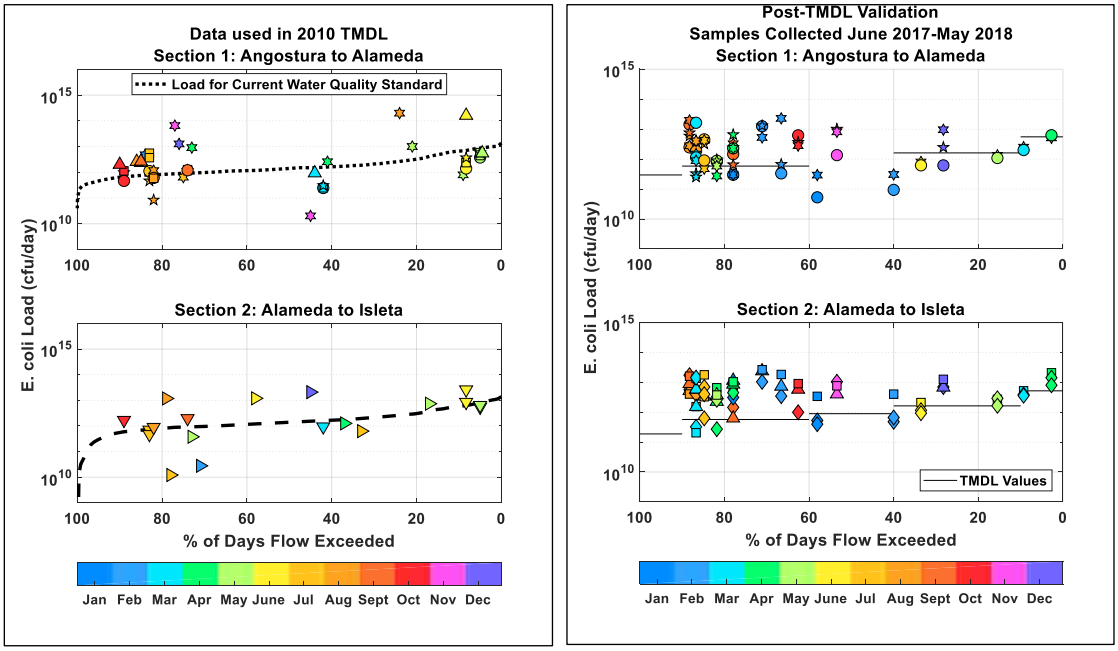

Figure 14: a) E. coli data used to construct TMDL in 2010. b) E. coli data from 2017-2018 compared to TMDL values.

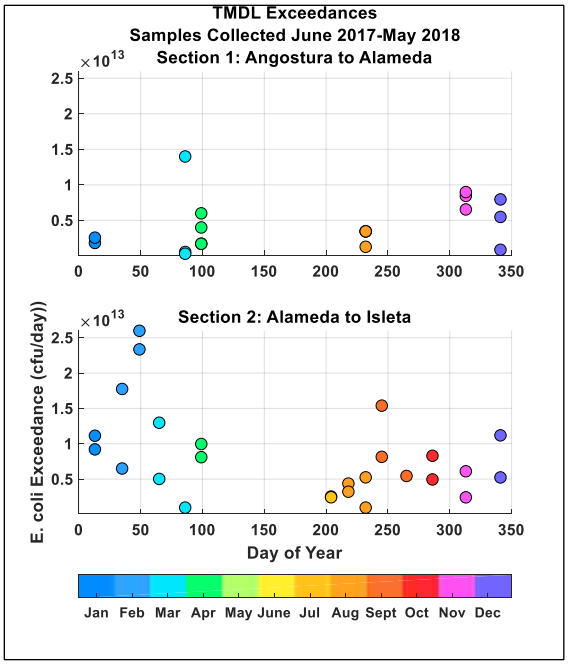

Figure 15: E. coli exceedances vs time.

6) **Discussion and Conclusions**

This work contributes new information about the magnitude and variation in space and time of E. coli levels in sediment-water interface riverbed sediments. I found that E. coli levels in riverbed sediments show strong seasonal differences in concentration between Summer/Fall and Winter/Spring months and are likely supported in greater numbers by downstream river sections. However, fluctuations in sediment E. coli levels over space and time do not appear to be closely coupled with fluctuations in E. coli loadings in the river. These closely-interacting domains show irregular spatial and temporal trends in FIB levels, indicating that factors affecting FIB levels in riverbed sediments and FIB levels in river water are not directly coupled. While both mean E. coli loadings and concentrations in sediments increase with distance, mean loadings increase gradually while mean concentrations in sediments increase more sharply downstream of the Alameda Bridge (Site #4).

6.1) *Spatial variations:*

During the year of sampling, E. coli concentrations in sediments were relatively stable upstream of the Albuquerque urbanized area and increased significantly along the portion affected by the urban area (Site 4-Site 6). This suggests that the effects of urbanization on Rio Grande FIB are more complex than previously realized from observing only river water concentrations and loadings. While there are smaller WWTP discharges, wildlife and agricultural activities along the reach upstream of Albuquerque, it appears that additional factors are related to the increase in sediment E. coli levels in the downstream section of the reach. Literature on environmental FIB frequently cite sandy, finer soils as more likely to contain higher concentrations of E. coli, possibly due

to their negative surface charge, high surface area, and lower shear stresses from overlying flowing water compared to cobble bed channels[7,36,58,59]. A shift in morphology of the Rio Grande from a narrower, cobble-lined channel at the outlet of Cochiti Lake (40 km upstream) to a wider, sandy bed channel in the Albuquerque area has been documented by the USGS[71] and likely contributes to higher temperatures, more favorable habitat for regrowth, or more efficient filtering of cells from river water in downstream sections compared to upstream sections. Other differences in the Albuquerque section could be caused by 1) numerous agricultural returns and drainage channels that carry water laden with sediments and organic matter to the river during the growing season (4 return channels between Sites 4 and 6, 1 return channel between Sites 2 and 3), 2) Albuquerque WWTP effluent containing nutrients and partially-deactivated FIB (3.33 m^3/s, 1.35 $x10^{11}$ cfu/day outfall between Sites 5 and 6), and 3) effects from urban and recreational use such as shedding from bathers and swimmers[18,29] and defecation from homeless populations.

E. coli loadings in this reach increased from upstream to downstream across the year of sampling, with overall loadings along the reach increasing approximately linearly with downstream distance. This shows the increase in loading per distance of river is nearly equal for different segments along the river reach, suggesting that non-point sources dominate the overall E. coli loadings. Large point sources, such as non-compliant WWTP effluents or illegal sewer cross-connections, would be expected to produce a clear increase in load at the same point over the year, which was not observed in this dataset.

Spatial trends between E. coli loadings and sediment concentrations are coupled across seasons, with low sediment concentrations upstream co-occurring with low loadings at upstream sites during Fall, Winter, and Spring. High E. coli sediment concentrations co-occur with high loadings at the downstream sites during Fall and Winter. During Summer, both E. coli loadings and sediment concentrations are similar along the reach, indicating this season likely has high non-point source loadings from upstream to downstream. The Winter season had the greatest increase in both E. coli loadings and sediment concentrations, suggesting upstream sites represent a baseline condition during the Winter. The shift in Rio Grande morphology from the narrow, fast channel with less bed sediment upstream to the wide, sandy, and warmer downstream section likely affects E. coli levels in sediments and how sediment and water column E. coli interact. Both E. coli loading and sediment concentrations at downstream sites decrease during Fall, Winter, and Spring less than at upstream sites.

6.2) *Temporal variations:*

Seasonally, both mean estimated E. coli loadings and concentrations in sediments were highest in Summer months and low in Spring. However, Fall and Winter mean loadings were comparable and only marginally below Summer mean loadings, while Fall mean sediment concentrations were significantly higher than mean Winter concentrations. These results show that the Rio Grande riverbed sediments constitute a variable non-point source or sink of E. coli to the river water, making variable amounts of FIB readily available for entrainment to river water by episodic and sporadic redistribution mechanisms. While this study documents similarities and differences in trends between E. coli loading and sediment concentration which can be indicators of

how these domains interact, determining the net direction of FIB transfer (river water to sediment, or vice versa) cannot be determined from the data collected in this study.

While data from the Summer months had the highest mean loading, it was not significantly higher than Winter or Fall loadings as past data collected in this reach suggested[26–28]. This could be the result of unusually high flows through the reach in 2017 prior to the start of sampling for this study, which likely flushed finer, E. coli laden sediments from depositional zones of the riverbed and floodplain. This may have contributed to lower autochthonous E. coli levels available for redistribution and less favorable organic and fine substrate material in the system to support E. coli survival. Differences between past data and this new dataset could also arise from overrepresented runoff condition sampling in past data compared to the runoff-blind sampling scheme used in this study.

E. coli in sediment concentrations showed clear seasonality along the reach. E. coli sediment concentrations were low in the Winter and Spring, indicating decreased WWTP effluent dilution during low-flow Winter months and early-season agricultural return flows are not closely related to increased E. coli levels in riverbed sediments. The decrease in E. coli concentration from Fall to Winter in riverbed sediments while loading remained fairly constant suggests survival of E. coli in river sediments is affected by seasonal factors such as watershed connectivity and temperature. Seasonal events occurring during the Fall and Summer months such as rainfall runoff, ephemeral and intermittent flows (Jemez River), high temperatures, and late-season agricultural returns may be more closely related to increased E. coli sediment concentrations with Spring and Winter levels representing a baseline condition.

From upstream to downstream, E. coli loadings and sediment concentrations decreased less from Summer 2017 to Spring 2018 at downstream sites compared to upstream sites. Both seasonal E. coli concentrations and loadings at upstream sites (1 and 2) resembled the reach-wide seasonal trends, while downstream sites (5 and 6) had less seasonal variation, showing no significant difference between seasonal groups. This shift in behavior along the reach indicates the downstream section of the reach maintains high concentrations of E. coli in the sediment and loadings throughout the year compared to the upstream section, where both E. coli loadings and sediment concentrations are significantly seasonal. Seasonal trends at upstream sites differ between E. coli loadings and sediment concentrations most at upstream sites and least at downstream sites.

6.3) *TMDL validity:*

In the context of the TMDL framework in this reach, this work shows that riverbed sediments likely release FIB to river water or filter FIB from river water differently depending on seasonal factors and location in the reach. This complexity cannot be accurately described for TMDL purposes simply using flow regime (dry, low, mid-range, moist, and high flows as described in the TMDL for this reach), which is the status quo. Although the direct effects of seasonal and environmental factors on FIB remain unknown, Rio Grande the concentration of E. coli in riverbed sediment fluctuated seasonally and were significantly higher in river sections downstream of the Alameda Bridge (Site 4). E. coli levels in downstream sections and during the Summer and Fall months were approximately one order of magnitude higher than those in upstream sections and during the Winter and Spring seasons, making Summer/Fall seasons and downstream locations most likely to function as a net source of FIB to river water. Net

sink behavior is expected under conditions least favorable for E. coli survival in which cells filtered from the river water and fixed in sediments do not reproduce or return to the river water. This likely occurs during Winter and early Spring as infrequent watershed connectivity and low temperatures contribute to depleted food sources and increased energy requirements for survival of FIB in the river system. Since E. coli sediment concentrations change significantly over time and space, episodic redistribution events driven by runoff or increases in discharge, and sporadic redistribution from shifting riverbed sediments likely transfer different amounts of FIB loadings to river water depending on season and location. This study found high E. coli sediment concentrations during Summer and Fall co-occur with higher Summer and Fall loadings, and higher E. coli sediment concentrations downstream may be related to more frequent exceedances of the TMDL in the downstream section. However, the net direction of E. coli transfer (river water to sediment or sediment to river water) is unknown at any point and the physical interactions between river water and sediment causing transfer of E. coli cells are not well understood on the reach-scale.

6.4) *E. coli presence: Implications*

In terms of public health and water quality compliance, the results of this study indicate that E. coli are harbored in riverbed sediments, and that trends in sediment concentrations and loadings of E. coli are irregular. One option to protect public health during episodic events such as man-made pulse flows or runoff flows is posting a high-flow recreational water use suspension as has been done in eastern and midwestern parts of the US that are subject to Combined Sewer Overflows (CSO's) following precipitation[72]. This would take into account the likelihood that FIB and co-occurring

pathogens in riverbed sediments are transferred from the riverbed to the river water during episodic high flows. Further questions to elucidate how pathogens in river sediments and water interact include determining whether co-occurrence of pathogens with FIB are similar in sediments compared to environmental water, better estimating terms in the mass-balance of FIB in waterways (die-off rate, relative rates of deposition or filtering of FIB to sediments and entrainment rate of FIB from sediments), and generating higher-frequency measurements of FIB and surrogates for fecal contamination.

7) **Appendices**

List of Appendices

1) NMED Sampling SOP (and MOU)

Ciudad Soil and Water Conservation District

Project Title: "Characterization of Pathogenic Bacterial Impairment and Regrowth along the Rio Grande near Albuquerque"

Standard Operating Procedure

for

Streambed Sediment Sampling for *E. coli* Enumeration

Approval Signatures

_____

6/12/2017_____James Fluke, Project Coordinator, CSWCD
Date

_____
_____

Sophie Stauffer, Quality Assurance Officer, SWQB NMED Date

1. Purpose and Scope

This procedure describes the collection of streambed sediment samples from the Rio Grande channel for analysis of *Escherichia coli* (*E. coli*) bacteria enumeration using the Multiple Tube Fermentation Procedure (SM 9221 CF) which is a modified method of Albuquerque WUA WQL SOP 305 "Fecal Coliform by MPN" with Approved Temporary Change Request

2. Personnel Responsibilities

All technical staff and personnel who collect samples for this project will be responsible for implementing this procedure.

The CSWCD Project Coordinator is responsible for keeping an adequate stock of sampling supplies and for maintaining the equipment used for sampling.

3. Background and Precautions

E. coli bacteria are considered an indicator of pathogens and precautions should be taken when sampling ambient waters. Field personnel should avoid accidental ingestion, contact with mucous membranes, eyes and skin to the extent possible, and especially areas with cuts and abrasions. Disposable gloves may be used and a disinfecting hand sanitizer should be used after collecting samples.

4. Definitions

Regrowth: In the context of this project regrowth is the increase in bacterial cells due to cell metabolism.
Ponar Grab Sampler: Sediment sampler widely used in salt and fresh water environments for taking grab samples from hard or sandy bottom water bodies. The sampler uses a Spring loaded pin to release the jaws of the sampler upon impact with the sediment water interface, which penetrate the sediment surface 3-5 cm and scoop a volume of sediment into the sampler when the line is pulled upward.
Pathogen: Pathogenic organisms are organisms capable of causing disease in humans or animals, and are considered likely to be present in waters containing fecal coliforms and *E. coli*.
Equipment blank: A sample consisting of a medium known to be free of the analyte of interest (*E. coli*) which is processed through the sampling equipment in the field and analyzed in the same way as routine samples. Equipment blanks are used to check for contamination from field equipment and procedures.

5. Equipment and Supplies

Field Sampling Kit

- 25 lb. Ponar Grab Sampler with 36 sq. in. sampling area, AMS Samplers Part No. 445.60
- Zip-top plastic bags, heavy duty (x 12 per trip)
- Stainless steel spoon or spatula and washtub
- Cooler with ice
- Field notebook and data sheets, pens and sharpie markers
- De-ionized (DI) water
- Plastic sheeting
- Chest Waders with Rubberized Boots
- Disposable gloves, hand sanitizing solution, and paper towels
- Nylon rope
- Prepared autoclaved sterile sand in zip-top bags for equipment blanks

6. Process Description

- Preparation: Before going in the field, fill out a "Streambed Sediment Record Sheet" (attached) and use this form to record the required information for each sample. Clean sampling equipment and the dedicated storage bin using tap water and laboratory-grade detergent in the UNM Laboratory prior to each sampling run. Prepare small volumes (200g) of standard commercially graded sand (available in UNM Laboratory) by autoclaving and place in zip-top plastic bags for use as equipment blanks. Make sure sufficient zip-top bags and sampling supplies are available and ready a week in advance of the sampling date.

  The process and verification of preparing the equipment blank will take place prior to the first sampling event. This will consist of performing the autoclave procedure on a standardized volume of sand and performing the analysis procedure on the autoclaved sand to verify that this method of blank generation yields a sterile sample.

- Sample Collection:
    1. Prepare a sample handling site by laying out a piece of clean plastic sheeting where the samples will be packaged and labeled.
    2. Rinse the Ponar sampler and stainless steel sampling equipment 3-4 times with ambient water. Place the stainless steel washbowl and scoop on the clean plastic sheeting.
    3. Collect the samples from 2 locations in the stream based on the streambed characteristics of the site. Try to collect one sample from a region of deeper, dominant flow and the other from a bank region with low water depth and speed. For sites without a bridge access the sampling site by wading. Be careful to sample from upstream in order to avoid collecting a disturbed sample. Replicate future sample location as closely as possible to ensure uniformity in sample collection location across sampling dates.
    4. Lower the sampler through the water column until contact is made with the bottom sediments. Allow the Spring mechanism to release before slowly retrieving the sampler containing sediment from the top 3-5 cm of the streambed.
    5. Drain off any excess water in the sampler. Deposit the sediment into the clean stainless steel washtub.
    6. Use the clean, stainless steel spatula to mix the sample and deposit a portion into a clean zip-top plastic bag, avoiding large rocks and pieces of organic matter. Collect at least 200 g of sediment. Replace the remaining sediment that was not collected into the stream.
    7. Rinse the sampler and stainless steel equipment thoroughly in ambient water followed by rinsing with DI water before moving to the next site. Rinse the equipment bin before preparing the equipment for transport and proceeding to the next site.
    8. When generating equipment blanks, pass the prepared autoclaved sediment through the rinsed sampler and washbowl in the same manner as routine samples. Perform this over the plastic sheeting prior to collecting the routine sample at a site.

- Documentation: Label the plastic bag containing the sample with the sample location ID, unique sample ID, sample collection date and time, and place in the cooler, on ice. Record sample information including time and latitude/longitude on the "Streambed Sediment Record Sheet". Record the sample location ID, unique sample ID, sample collection date, sample collection time, analytical method, analyte name, and desired concentration units on the Chain of Custody form before submitting samples to the laboratory. Use a disinfecting hand sanitizer after all samples have been collected from a site.

7. Quality Control and Quality Assurance

QA/QC samples will be sent to the analysis laboratory at the frequencies shown below. Equipment blanks are created by performing the sampling procedure in the field with

prepared sterilized sediment. The sterilized sediment will consist of autoclaved standard commercially-graded sand prepared in the UNM laboratory prior to the sampling day and stored in plastic zip-top bags until use in the field.

Quality Control Sample	Frequency (Sediment and Aqueous matrix samples)	DQI	Measurement Performance Criteria
Equipment blank	1 per sampling event	Contamination – Accuracy	< Method Detection Limit

8. References

1. USEPA, April 2007. Guidance for Preparing Standard Operating Procedures (SOPs) https://www.epa.gov/quality/guidance-preparing-standard-operating-procedures
2. "SOP 8.1 Chemical Sampling – Equipment Cleaning Procedures," New Mexico Environment Department Surface Water Quality Bureau, March 21, 2011. https://www.env.nm.gov/swqb/documents/swqbdocs/MAS/SOP/8.1SOP-ChemicalSampling-EquipmentCleaningProcedures.pdf
3. "SOP 8.2 Chemical Sampling in Lotic Environments – Equipment, Collection Methods, Preservation, and Quality Control," New Mexico Environment Department Surface Water Quality Bureau, April 22, 2016. https://www.env.nm.gov/swqb/SOP/documents/82ChemicalSamplingSOP4-11-2016.pdf
4. NMED, April 2016. "SOP 9.1 Standard Operating Procedures for Bacteriological Sampling and Analysis." < https://www.env.nm.gov/swqb/SOP/>

Streambed Sediment Record Sheet

Field Personnel: _____ Date:

Beginning Mileage: _____ Ending Mileage:

	Location	Time	Sample ID	Comments
Site 1	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			
Site 2	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			
Site 3	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			
Site 4	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			
Site 5	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			
Site 6	Site Latitude and Longitude (decimal degrees): _____ ^{O}N_____ ^{O}W			

2) Coliscan MF Reference

Coliscan® MF/Coliscan® MF Plus Membrane Filter Kit Instructions

For use with Micrology Laboratories filter apparatus only. Read entire instructions before beginning.

Items needed (minimum):

1 Filter Apparatus (with vacuum device)
10 Membrane Filters
10 3 mL Calibrated Droppers (or pipette, any size)
1 Coliscan MF (Plus) bottle
10 50 mm dishes w/ pads

Preparation and setup

1. Thaw the desired number of bottle(s) of Coliscan® MF (Plus) by leaving at room temperature overnight. For rapid same-day thawing, stand in warm water until liquid. All unused bottles should be left in freezer. Collect the water to be tested in the appropriate volume and dilution (see table below). It is best to do this within a couple hours prior to filtering or, if this is not possible, water may be stored in refrigerator for no more than 24 hours.

Water amount to be collected

Water Sources	Amount to collect
Environmental:	
River, lake, pond, stream, ditch	1.0 to 5.0 mL added to sterile dilution water (10 to 90 mL)
Drinking water:	
Well, municipal, bottled	100 mL

2. Open a dropper or pipette and sterilely add 1.75 to 2 mL Coliscan® MF (Plus) to each pad in each dish that is to be used.

3. Filter apparatus setup. The filter apparatus comes in a sterile pack. Open the pack and remove the apparatus. The clear top of the apparatus is the funnel, which is calibrated for 100 and 150 mL samples and is covered with a lid. It fits on the bottom collection container and is sealed with an O-ring. There is a side port with a tip for the attachment of the vacuum syringe. Twist it and it can also be removed. It contains a plug in its tip which can be removed. The contents of the bottom collection container are most easily poured out when the tip is removed. It is easily replaced by twisting back on.

4. To prepare the apparatus for use, remove the funnel and using a clean forceps place a sterile pad on the top grid-work (in the blue circle) of the container.

5. Open a sterile filter envelope and with the clean forceps, carefully remove the membrane filter from the pack. **Be sure to separate the filter from the protective backing and handle the filter carefully so it is not torn or damaged.** Place the filter, grid side up, on top of the sterile pad. Push the funnel down so that it is held and sealed by the O-ring and the filter and pad are held firmly in place. The funnel must be pushed down as far as possible to obtain a good seal.

6. Attach the syringe to the filter apparatus by pushing the end of the hose on to the side port tip of the funnel contained. Be sure that the syringe plunger is not pulled out.

Filtering the water

7. Pour the water sample into the funnel, swirl to mix and create a vacuum by pulling out the plunger of the syringe. The water will be pulled through the filter, depositing any microoganisms present onto the filter surface.

8. When the water sample has been completely passed through the filter, disconnect the syringe, remove the funnel and with the clean forceps remove the filter and place grid side up directly on top of the pad of a dish prepared earlier. Make sure that there are no air spaces (bubbles) between the pad and the membrane filter. Place the lid back on the dish.

9. The filtered water in the collection container should be emptied and the filter apparatus prepared for repeat use. Before the funnel is used again it should cleaned. This may be done by rinsing with alcohol or radiated for 1 minute with germicidal UV if desired. The absorbent pad can generally be reused as it will only contain filtered water (sterile).

Incubation and interpretation

10. Incubate in an incubator or a warm place. If using an incubator, incubate at 35° for 18- 24 hours. If an incubator is not available, find a place that will be warm for a 24 hour period. **DO NOT** place in direct sunlight or over a direct heat source, radiator, furnace duct etc. You may place them near one of these sources or in a warm spot in the kitchen. Allow 24-48 hours for growth to begin. Once growth begins you can incubate another 24 hours for complete growth to take place.

11. Once the incubation period is complete, a count of the colonies can be done. Count all **blue** colonies as ***E. coli* (fecal coliform)** and all **red colonies** as **general coliforms**. The sum of these two is the **total coliform** population.

    Additionally, with Coliscan® MF Plus, verification of *E coli* is accomplished by shining a long wave (366 nanometer) UV light on the back of the dishes (do this in a dark room). If any of the colonies are *E coli*, the area around the colonies will fluoresce a bright bluish color. This fluorescence can also be used as proof for the presence of *E. coli* in a sample, thus making the medium an effective P/A test for *E. coli* if quantitative results are not needed.

If you have any questions, call 1.888.327.9435.

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Phone: 574.533.3351 Fax: 574.533.3370
Call toll free 888.327.9435
Email: info@micrologylabs.com

3) *MATLAB Image Analysis Code (4 files)*

PlateCounter.m

```
clear
clc
close all

jpgFiles = dir('*.jpg');
numfiles = length(jpgFiles);
platecount = cell(1, numfiles);

jpgnames = cellstr(ls('*.jpg'));

%Take sample name from file name
[samplenam1,remain]=strtok(jpgnames,'_');
[samplenam2, remain2]=strtok(remain,'_');
samplenam3=strtok(remain2,'.');
samplenam=strcat(samplenam1,'_',samplenam2);
samplenamefull=jpgnames;
samplenamunique=unique(samplenam);            % Unique sample names


for k = 1:numfiles
  platecount{k} = countfn(jpgFiles(k),samplenamefull(k));
end

close all

Result=[jpgnames';platecount]';
platecountdoub=cell2mat(platecount);

%Take sample id no and aliquot volume from file name
filenamnum=regexp(jpgnames,'\d+(\.)?(\d+)?','match');
filenamdoub=str2double([filenamnum{:}]);
sampleidno = filenamdoub(1:2:end)';          % Odd-Indexed Elements, keep for display
samplesunique=unique(sampleidno);             % Unique sample id no's
idnoindex=(1:length(samplesunique))';
aliquotvol = filenamdoub(2:2:end)';          % Even-Indexed Elements - aliquot volume

%Divide Plate Counts (row) by Aliquot volume and list in column3
conc=(platecountdoub')./aliquotvol;
conc_idno=cat(2,conc,sampleidno);
samplebin=(vertcat(samplesunique,max(sampleidno)+1))-0.5;
rows=discretize(sampleidno,samplebin);     %index # for sample id

%Find dilutions of a single Sample Id No.
%(column) values for each value and list in column4
y = sort(rows(:));
 p = find([true;diff(y)~=0;true]);
 values = y(p(1:end-1));
 instances = diff(p);
 columns=zeros(length(instances),max(instances));

for i=1:length(instances)
     columns(i,1:instances(i))=1:instances(i);
end
nonzeros(columns');
res=cat(2,conc_idno,rows, nonzeros(columns'));

%Find frequency of sample id (# of plates) - should be 3 but may be more or
%less
sampfreq = zeros(size(sampleidno));
for i = 1:length(sampleidno)
sampfreq(i) = sum(sampleidno==sampleidno(i));
end
sampfreq;
```

```
%Construct Matrix of concentration values with one sample per row,
%and concentrations listed in the first n columns
concmatrix=zeros(length(samplesunique),max(instances));
for i=1:length(conc)
    concmatrix(rows(i),res(i,4))=conc(i);
end
concmatrix;

%Take mean of closest 2 consecutive readings (must be at least 2)
%Also compute variance of all readings
concmeanvar=zeros(length(samplesunique),2);
cmatprim=cat(1,concmatrix',zeros(1,length(samplesunique)));
diff=abs(diff(cmatprim));
for i=1:length(samplesunique)
     [r,c]=min(diff(1:(instances(i)-1),i));
     concmeanvar(i,1)=0.5*(cmatprim(c,i)+cmatprim(c+1,i));
     concmeanvar(i,2)=var(cmatprim(1:(instances(i)),i));
end
concmeanvar;

%Final Output
ResultRaw=[jpgnames,num2cell(sampleidno),num2cell(aliquotvol),platecount',num2cell(conc)]
;
labelraw={'FileName','SampleIDNo','AliquotVol','Platecount','Concentration(cfu/mL)'};
resultfinal=[string(samplenamunique),samplesunique,instances,concmeanvar];
labelfinal={'SampleName','SampleIDNo','NumPlates','MeanConc(cfu/mL)','Variance'};
xlswrite('sampleresults.xlsx',[labelraw;ResultRaw],'Raw');
xlswrite('sampleresults.xlsx',[labelfinal;(resultfinal)],'Summary');
```

Countfn.m

```
function [ platecount ] = countfn( jpgFile, jpgname )
%UNTITLED3 Summary of this function goes here
filenam=char(jpgname);
filenami=strrep(filenam,'.',',');
filename=filenami(1:end-4);
filename1=strcat(filename,'.png');
close all
% Read in image into an array.
    [rgbImage, storedColorMap] = imread(jpgFile.name);
    [rows, columns, numberOfColorBands] = size(rgbImage);
    % If it's monochrome (indexed), convert it to color.
    % Check to see if it's an 8-bit image needed later for scaling).
    if strcmpi(class(rgbImage), 'uint8')
        % Flag for 256 gray levels.
        eightBit = true;
    else
        eightBit = false;
    end
    if numberOfColorBands == 1
        if isempty(storedColorMap)
            % Just a simple gray level image, not indexed with a stored color map.
            % Create a 3D true color image where we copy the monochrome image into all 3
(R, G, & B) color planes.
            rgbImage = cat(3, rgbImage, rgbImage, rgbImage);
        else
            % It's an indexed image.
            rgbImage = ind2rgb(rgbImage, storedColorMap);
            % ind2rgb() will convert it to double and normalize it to the range 0-1.
            % Convert back to uint8 in the range 0-255, if needed.
            if eightBit
                rgbImage = uint8(255 * rgbImage);
            end
        end
    end
```

```
    % Convert RGB image to HSV
    hsvImage = rgb2hsv(rgbImage);
    % Extract out the H, S, and V images individually
    hImage = hsvImage(:,:,1);
    sImage = hsvImage(:,:,2);
    vImage = hsvImage(:,:,3);

    % Now select thresholds for the 3 color bands.
    % Assign the low and high thresholds for each color band.

        hueThresholdLow = 0.49;
        hueThresholdHigh = 0.69;
        saturationThresholdLow = 0.13;
        saturationThresholdHigh = 1.0;
        valueThresholdLow = 0.08;
        valueThresholdHigh = 0.63;

    % Interactively and visually set/adjust thresholds using custom thresholding
application.
    % Available on the File Exchange:
http://www.mathworks.com/matlabcentral/fileexchange/29372-thresholding-an-image
%   [hueThresholdLow, hueThresholdHigh] = threshold(hueThresholdLow, hueThresholdHigh,
hImage);
%   [saturationThresholdLow, saturationThresholdHigh] = threshold(saturationThresholdLow,
saturationThresholdHigh, sImage);
%   [valueThresholdLow, valueThresholdHigh] = threshold(valueThresholdLow,
valueThresholdHigh, vImage);

%   % Show the thresholds as vertical magenta bars on the histograms.
%   PlaceThresholdBars(6, hueThresholdLow, hueThresholdHigh);
%   PlaceThresholdBars(7, saturationThresholdLow, saturationThresholdHigh);
%   PlaceThresholdBars(8, valueThresholdLow, valueThresholdHigh);


    % Now apply each color band's particular thresholds to the color band
    hueMask = (hImage >= hueThresholdLow) & (hImage <= hueThresholdHigh);
    saturationMask = (sImage >= saturationThresholdLow) & (sImage <=
saturationThresholdHigh);
    valueMask = (vImage >= valueThresholdLow) & (vImage <= valueThresholdHigh);

%   % Display the thresholded binary images.
%   fontSize = 16;
%   subplot(3, 4, 10);
%   imshow(hueMask, []);
%   title('=   Hue Mask', 'FontSize', fontSize);
%   subplot(3, 4, 11);
%   imshow(saturationMask, []);
%   title('&   Saturation Mask', 'FontSize', fontSize);
%   subplot(3, 4, 12);
%   imshow(valueMask, []);
%   title('&   Value Mask', 'FontSize', fontSize);
    % Combine the masks to find where all 3 are "true."
    % Then we will have the mask of only the red parts of the image.
    coloredObjectsMask = uint8(hueMask & valueMask & saturationMask);
%   subplot(3, 4, 9);
%   imshow(coloredObjectsMask, []);
%   caption = sprintf('Mask of Only Regions\nof The Specified Color');
%   title(caption, 'FontSize', fontSize);

    % Tell user that we're going to filter out small objects.
    smallestAcceptableArea = 90; % Keep areas only if they're bigger than this.

    % Open up a new figure, since the existing one is full.
    figure;
    % Maximize the figure.
    set(gcf, 'units','normalized','outerposition',[0 0 1 1]);
```

```
    % Get rid of small objects.  Note: bwareaopen returns a logical.
    coloredObjectsMask = uint8(bwareaopen(coloredObjectsMask, smallestAcceptableArea));
    %subplot(3, 3, 1);
    %imshow(coloredObjectsMask, []);
    %fontSize = 13;
    %caption = sprintf('bwareaopen() removed objects\nsmaller than %d pixels',
smallestAcceptableArea);
    %title(caption, 'FontSize', fontSize);

    % Smooth the border using a morphological closing operation, imclose().
    structuringElement = strel('disk', 4);
    coloredObjectsMask = imclose(coloredObjectsMask, structuringElement);
    %subplot(3, 3, 2);
    %imshow(coloredObjectsMask, []);
    %fontSize = 16;
    %title('Border smoothed', 'FontSize', fontSize);

    % Fill in any holes in the regions, since they are most likely red also.
    coloredObjectsMask = imfill(logical(coloredObjectsMask), 'holes');
    %subplot(3, 3, 3);
    %imshow(coloredObjectsMask, []);
    %title('Regions Filled', 'FontSize', fontSize);

    % You can only multiply integers if they are of the same type.
    % (coloredObjectsMask is a logical array.)
    % We need to convert the type of coloredObjectsMask to the same data type as hImage.
    coloredObjectsMask = cast(coloredObjectsMask, 'like', rgbImage);
%   coloredObjectsMask = cast(coloredObjectsMask, class(rgbImage));

    % Use the colored object mask to mask out the colored-only portions of the rgb image.
    maskedImageR = coloredObjectsMask .* rgbImage(:,:,1);
    maskedImageG = coloredObjectsMask .* rgbImage(:,:,2);
    maskedImageB = coloredObjectsMask .* rgbImage(:,:,3);
    % Concatenate the masked color bands to form the rgb image.
    maskedRGBImage = cat(3, maskedImageR, maskedImageG, maskedImageB);
    labeledImage = bwlabel(coloredObjectsMask, 8);
    blobMeasurements = regionprops(labeledImage, 'all');
    allBlobCentroids = [blobMeasurements.Centroid];
    centroidsX = allBlobCentroids(1:2:end-1);
    centroidsY = allBlobCentroids(2:2:end);
    % Show the masked off, original image.
    subplot(1,2,1);
    imshow(rgbImage);
    %imshow(maskedRGBImage);
    fontSize = 13;
    caption = sprintf('Original Image');
    title(caption, 'FontSize', fontSize);
    % Show the original image next to it.
    subplot(1,2,2);
    imshow(rgbImage);
    hold on
    plot(centroidsX, centroidsY, 'r+', 'MarkerSize', 20, 'LineWidth', 1.8);
    title('Image Showing Points Counted', 'FontSize', fontSize);
    print(char(filename),'-dpng')

    % Measure the mean HSV and area of all the detected blobs.
    [meanHSV, areas, numberOfBlobs] = MeasureBlobs(coloredObjectsMask, hImage, sImage,
vImage);
    %if numberOfBlobs > 0
        %fprintf(1, '\n-----------------------------------------------\n');
        %fprintf(1, 'Blob #, Area in Pixels, Mean H, Mean S, Mean V\n');
        %fprintf(1, '-----------------------------------------------\n');
    %   for blobNumber = 1 : numberOfBlobs
            %fprintf(1, '#%5d, %14d, %6.2f, %6.2f, %6.2f\n', blobNumber,
areas(blobNumber), ...
            %   meanHSV(blobNumber, 1), meanHSV(blobNumber, 2), meanHSV(blobNumber, 3));
    %   end
```

```
    %else
        % Alert user that no colored blobs were found.
        %message = sprintf('No blobs of the specified color were found in the
image:\n%s', jpgFile);
        %fprintf(1, '\n%s\n', message);
        %uiwait(msgbox(message));
    %end
platecount=numberOfBlobs;
end
```

MeasureBlobs.m

```
function [meanHSV, areas, numberOfBlobs] = MeasureBlobs(maskImage, hImage, sImage,
vImage)
try
    [labeledImage, numberOfBlobs] = bwlabel(maskImage, 8);     % Label each blob so we
can make measurements of it
    if numberOfBlobs == 0
        % Didn't detect any blobs of the specified color in this image.
        meanHSV = [0 0 0];
        areas = 0;
        return;
    end
    % Get all the blob properties.  Can only pass in originalImage in version R2008a and
later.
    blobMeasurementsHue = regionprops(labeledImage, hImage, 'area', 'MeanIntensity');
    blobMeasurementsSat = regionprops(labeledImage, sImage, 'area', 'MeanIntensity');
    blobMeasurementsValue = regionprops(labeledImage, vImage, 'area', 'MeanIntensity');

    meanHSV = zeros(numberOfBlobs, 3);  % One row for each blob.  One column for each
color.
    meanHSV(:,1) = [blobMeasurementsHue.MeanIntensity]';
    meanHSV(:,2) = [blobMeasurementsSat.MeanIntensity]';
    meanHSV(:,3) = [blobMeasurementsValue.MeanIntensity]';

    % Now assign the areas.
    areas = zeros(numberOfBlobs, 3);  % One row for each blob.  One column for each
color.
    areas(:,1) = [blobMeasurementsHue.Area]';
    areas(:,2) = [blobMeasurementsSat.Area]';
    areas(:,3) = [blobMeasurementsValue.Area]';
catch ME
    errorMessage = sprintf('Error in function %s() at line %d.\n\nError Message:\n%s',
...
        ME.stack(1).name, ME.stack(1).line, ME.message);
    fprintf(1, '%s\n', errorMessage);
    uiwait(warndlg(errorMessage));
end
return; % from MeasureBlobs()
```

PlaceThresholdBars.m

```
function PlaceThresholdBars(plotNumber, lowThresh, highThresh)
try
    % Show the thresholds as vertical red bars on the histograms.
    subplot(3, 4, plotNumber);
    hold on;
    yLimits = ylim;
    line([lowThresh, lowThresh], yLimits, 'Color', 'r', 'LineWidth', 3);
    line([highThresh, highThresh], yLimits, 'Color', 'r', 'LineWidth', 3);
    % Place a text label on the bar chart showing the threshold.
```

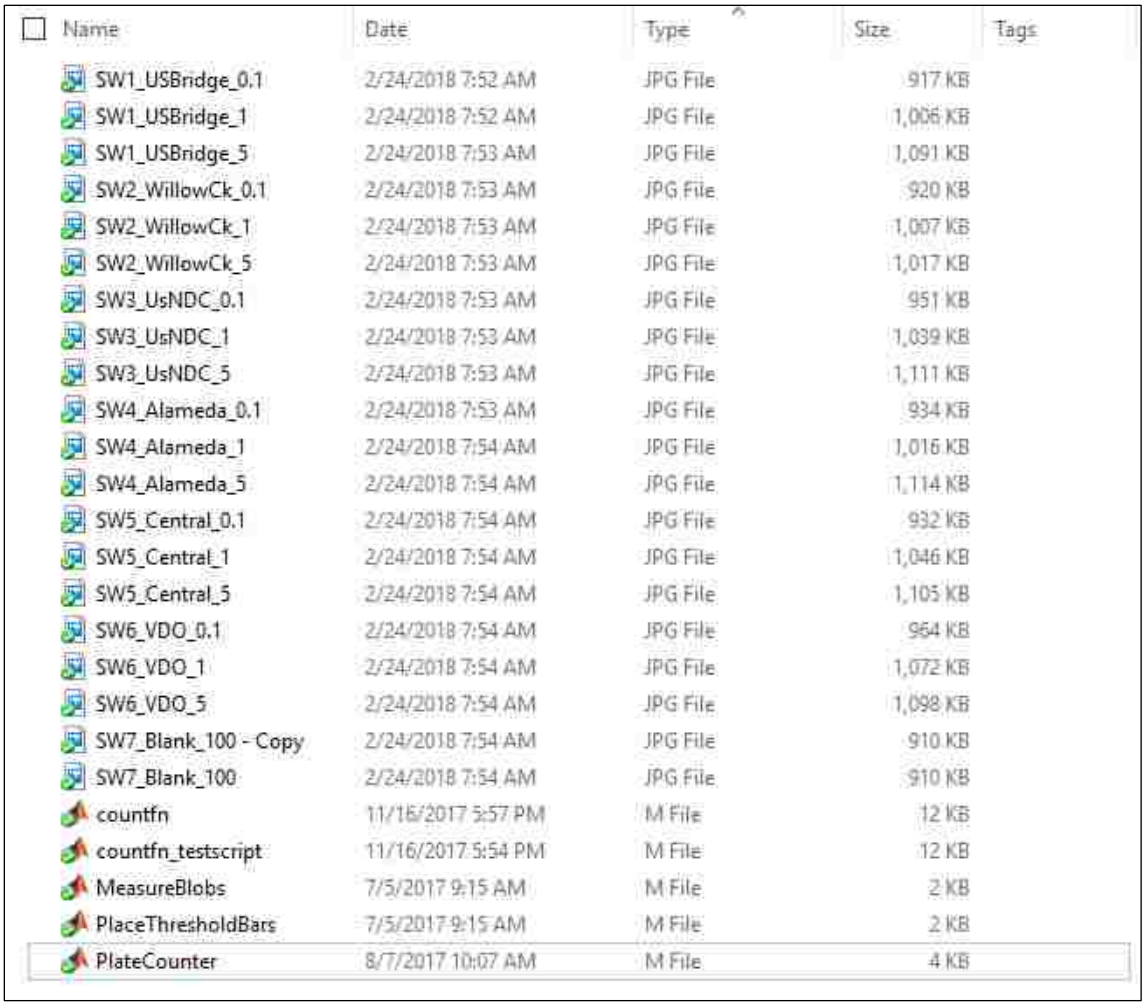
```
    fontSizeThresh = 14;
    annotationTextL = sprintf('%d', lowThresh);
    annotationTextH = sprintf('%d', highThresh);
    % For text(), the x and y need to be of the data class "double" so let's cast both to
double.
    text(double(lowThresh + 5), double(0.85 * yLimits(2)), annotationTextL, 'FontSize',
fontSizeThresh, 'Color', [0 .5 0], 'FontWeight', 'Bold');
    text(double(highThresh + 5), double(0.85 * yLimits(2)), annotationTextH, 'FontSize',
fontSizeThresh, 'Color', [0 .5 0], 'FontWeight', 'Bold');

    % Show the range as arrows.
    % Can't get it to work, with either gca or gcf.
%   annotation(gca, 'arrow', [lowThresh/maxXValue(2) highThresh/maxXValue(2)],[0.7 0.7]);

catch ME
    errorMessage = sprintf('Error in function %s() at line %d.\n\nError Message:\n%s',
...
        ME.stack(1).name, ME.stack(1).line, ME.message);
    fprintf(1, '%s\n', errorMessage);
    uiwait(warndlg(errorMessage));
end
return; % from PlaceThresholdBars()
```

Image Analysis Notes

To run the image analysis code, set up the file folder as shown below. Be sure to make a copy of the blank sample photo (code requires at least 2 photos per sample). Run the file “Plate Counter” and view the results in the Excel sheet produced in the file folder.



Name	Date	Type	Size	Tags
SW1_USBridge_0.1	2/24/2018 7:52 AM	JPG File	917 KB	
SW1_USBridge_1	2/24/2018 7:52 AM	JPG File	1,006 KB	
SW1_USBridge_5	2/24/2018 7:53 AM	JPG File	1,091 KB	
SW2_WillowCk_0.1	2/24/2018 7:53 AM	JPG File	920 KB	
SW2_WillowCk_1	2/24/2018 7:53 AM	JPG File	1,007 KB	
SW2_WillowCk_5	2/24/2018 7:53 AM	JPG File	1,017 KB	
SW3_UsNDC_0.1	2/24/2018 7:53 AM	JPG File	951 KB	
SW3_UsNDC_1	2/24/2018 7:53 AM	JPG File	1,039 KB	
SW3_UsNDC_5	2/24/2018 7:53 AM	JPG File	1,111 KB	
SW4_Alameda_0.1	2/24/2018 7:53 AM	JPG File	934 KB	
SW4_Alameda_1	2/24/2018 7:54 AM	JPG File	1,016 KB	
SW4_Alameda_5	2/24/2018 7:54 AM	JPG File	1,114 KB	
SW5_Central_0.1	2/24/2018 7:54 AM	JPG File	932 KB	
SW5_Central_1	2/24/2018 7:54 AM	JPG File	1,046 KB	
SW5_Central_5	2/24/2018 7:54 AM	JPG File	1,105 KB	
SW6_VDO_0.1	2/24/2018 7:54 AM	JPG File	964 KB	
SW6_VDO_1	2/24/2018 7:54 AM	JPG File	1,072 KB	
SW6_VDO_5	2/24/2018 7:54 AM	JPG File	1,098 KB	
SW7_Blank_100 - Copy	2/24/2018 7:54 AM	JPG File	910 KB	
SW7_Blank_100	2/24/2018 7:54 AM	JPG File	910 KB	
countfn	11/16/2017 5:57 PM	M File	12 KB	
countfn_testscript	11/16/2017 5:54 PM	M File	12 KB	
MeasureBlobs	7/5/2017 9:15 AM	M File	2 KB	
PlaceThresholdBars	7/5/2017 9:15 AM	M File	2 KB	
PlateCounter	8/7/2017 10:07 AM	M File	4 KB	

4) Raw Data

E. coli Water Sample Data:

Date	Discharge Alameda (cfs)	Discharge Central (cfs)	SampleName	km	Mean E. coli Concentration (cfu/100mL)	E. coli Load (cfu/day)
7/12/2017	769	488	SW1_USBern	9.4	0	0
7/12/2017	769	488	SW2_WillowCk	12.5	0	0
7/12/2017	769	488	SW3_UsNDC	24.7	0	0
7/12/2017	769	488	SW4_Alameda	28.1	0	0
7/12/2017	769	488	SW5_Central	41.8	0	0
7/12/2017	769	488	SW6_VDO	56.7	0	0
7/25/2017	691	440	SW1_USBern	9.4	270	4.56458E+12
7/25/2017	691	440	SW2_WillowCk	12.5	190	3.21211E+12
7/25/2017	691	440	SW3_UsNDC	24.7	250	4.22646E+12
7/25/2017	691	440	SW4_Alameda	28.1	420	7.10045E+12
7/25/2017	691	440	SW5_Central	41.8	320	3.44478E+12
7/25/2017	691	440	SW6_VDO	56.7	310	3.33713E+12
8/9/2017	670	392	SW1_USBridge	9.4	150	2.45881E+12
8/9/2017	670	392	SW2_WillowCk	12.5	360	5.90114E+12
8/9/2017	670	392	SW3_UsNDC	24.7	170	2.78665E+12
8/9/2017	670	392	SW4_Alameda	28.1	350	5.73722E+12
8/9/2017	670	392	SW5_Central	41.8	550	5.27482E+12
8/9/2017	670	392	SW6_VDO	56.7	430	4.12395E+12
8/23/2017	635	397	SW1_USBridge	9.4	120	1.86429E+12
8/23/2017	635	397	SW2_WillowCk	12.5	260	4.0393E+12
8/23/2017	635	397	SW3_UsNDC	24.7	260	4.0393E+12
8/23/2017	635	397	SW4_Alameda	28.1	260	4.0393E+12
8/23/2017	635	397	SW5_Central	41.8	600	5.82774E+12
8/23/2017	635	397	SW6_VDO	56.7	160	1.55406E+12
9/6/2017	673	370	SW1_USBridge	9.4	870	1.43249E+13
9/6/2017	673	370	SW2_WillowCk	12.5	460	7.57411E+12
9/6/2017	673	370	SW3_UsNDC	24.7	1190	1.95939E+13
9/6/2017	673	370	SW4_Alameda	28.1	1070	1.7618E+13
9/6/2017	673	370	SW5_Central	41.8	1000	9.05233E+12
9/6/2017	673	370	SW6_VDO	56.7	1800	1.62942E+13
9/26/2017	665	519	SW1_USBridge	9.4	90	1.46428E+12
9/26/2017	665	519	SW2_WillowCk	12.5	40	6.50789E+11
9/26/2017	665	519	SW3_UsNDC	24.7	200	3.25395E+12
9/26/2017	665	519	SW4_Alameda	28.1	90	1.46428E+12

9/26/2017	665	519	SW5_Central	41.8	50	6.34886E+11
9/26/2017	665	519	SW6_VDO	56.7	500	6.34886E+12
10/17/2017	821	684	SW1_USBridge	9.4	310	6.22678E+12
10/17/2017	821	684	SW2_WillowCk	12.5	180	3.61555E+12
10/17/2017	821	684	SW3_UsNDC	24.7	140	2.81209E+12
10/17/2017	821	684	SW4_Alameda	28.1	50	1.00432E+12
10/17/2017	821	684	SW5_Central	41.8	350	5.8571E+12
10/17/2017	821	684	SW6_VDO	56.7	550	9.20402E+12
11/14/2017	1110	793	SW1_USBridge	9.4	50	1.35785E+12
11/14/2017	1110	793	SW2_WillowCk	12.5	370	1.00481E+13
11/14/2017	1110	793	SW3_UsNDC	24.7	300	8.1471E+12
11/14/2017	1110	793	SW4_Alameda	28.1	390	1.05912E+13
11/14/2017	1110	793	SW5_Central	41.8	210	4.07428E+12
11/14/2017	1110	793	SW6_VDO	56.7	400	7.76054E+12
12/12/2017	1260	1280	SW1_USBridge	9.4	20	6.16537E+11
12/12/2017	1260	1280	SW2_WillowCk	12.5	80	2.46615E+12
12/12/2017	1260	1280	SW3_UsNDC	24.7	310	9.55632E+12
12/12/2017	1260	1280	SW4_Alameda	28.1	230	7.09018E+12
12/12/2017	1260	1280	SW5_Central	41.8	220	6.88956E+12
12/12/2017	1260	1280	SW6_VDO	56.7	410	1.28396E+13
1/14/2018	614	526	SW1_USBridge	9.4	20	3.00439E+11
1/14/2018	614	526	SW2_WillowCk	12.5	160	2.40352E+12
1/14/2018	614	526	SW3_UsNDC	24.7	20	3.00439E+11
1/14/2018	614	526	SW4_Alameda	28.1	210	3.15461E+12
1/14/2018	614	526	SW5_Central	41.8	910	1.17108E+13
1/14/2018	614	526	SW6_VDO	56.7	760	9.78043E+12
2/6/2018	682	605	SW1_USBridge	9.4	20	3.33713E+11
2/6/2018	682	605	SW2_WillowCk	12.5	40	6.67426E+11
2/6/2018	682	605	SW3_UsNDC	24.7	1370	2.28593E+13
2/6/2018	682	605	SW4_Alameda	28.1	210	3.50399E+12
2/6/2018	682	605	SW5_Central	41.8	500	7.40089E+12
2/6/2018	682	605	SW6_VDO	56.7	1260	1.86502E+13
2/20/2018	720	597	SW1_USBridge	9.4	710	1.25069E+13
2/20/2018	720	597	SW2_WillowCk	12.5	740	1.30354E+13
2/20/2018	720	597	SW3_UsNDC	24.7	300	5.2846E+12
2/20/2018	720	597	SW4_Alameda	28.1	600	1.05692E+13
2/20/2018	720	597	SW5_Central	41.8	1660	2.42461E+13
2/20/2018	720	597	SW6_VDO	56.7	1840	2.68751E+13
3/6/2018	668	411	SW1_USBridge	9.4	1000	1.63431E+13
3/6/2018	668	411	SW2_WillowCk	12.5	20	3.26862E+11

3/6/2018	668	411	SW3_UsNDC	24.7	90	1.47088E+12
3/6/2018	668	411	SW4_Alameda	28.1	20	3.26862E+11
3/6/2018	668	411	SW5_Central	41.8	590	5.9327E+12
3/6/2018	668	411	SW6_VDO	56.7	1380	1.38765E+13
3/27/2018	518	422	SW1_USBridge	9.4	90	1.14059E+12
3/27/2018	518	422	SW2_WillowCk	12.5	20	2.53465E+11
3/27/2018	518	422	SW3_UsNDC	24.7	70	8.87128E+11
3/27/2018	518	422	SW4_Alameda	28.1	1150	1.45743E+13
3/27/2018	518	422	SW5_Central	41.8	150	1.54868E+12
3/27/2018	518	422	SW6_VDO	56.7	20	2.06491E+11
4/10/2018	625	507	SW1_USBridge	9.4	150	2.29366E+12
4/10/2018	625	507	SW2_WillowCk	12.5	430	6.57517E+12
4/10/2018	625	507	SW3_UsNDC	24.7	150	2.29366E+12
4/10/2018	625	507	SW4_Alameda	28.1	300	4.58733E+12
4/10/2018	625	507	SW5_Central	41.8	700	8.6829E+12
4/10/2018	625	507	SW6_VDO	56.7	850	1.05435E+13
4/24/2018	558	447	SW1_USBridge	9.4	60	8.19113E+11
4/24/2018	558	447	SW2_WillowCk	12.5	70	9.55632E+11
4/24/2018	558	447	SW3_UsNDC	24.7	20	2.73038E+11
4/24/2018	558	447	SW4_Alameda	28.1	20	2.73038E+11
4/24/2018	558	447	SW5_Central	41.8	210	2.2966E+12
4/24/2018	558	447	SW6_VDO	56.7	610	6.67108E+12
5/8/2018	601	486	SW1_USBridge	9.4	60	8.82235E+11
5/8/2018	601	486	SW2_WillowCk	12.5	60	8.82235E+11
5/8/2018	601	486	SW3_UsNDC	24.7	40	5.88157E+11
5/8/2018	601	486	SW4_Alameda	28.1	180	2.64671E+12
5/8/2018	601	486	SW5_Central	41.8	320	3.80491E+12
5/8/2018	601	486	SW6_VDO	56.7	320	3.80491E+12

E. coli in Sediment Sample Data:

Date	SAMPLE_POINT_ID	0	E. coli in Sediment (MPN/g)
7/12/2017	WillowCk_1	12.5	5.85
7/12/2017	WillowCk_2	12.5	98.18
7/12/2017	US550_1	9.4	18.85
7/12/2017	US550_2	9.4	3.29
7/12/2017	UsNDC_1	24.7	2.31
7/12/2017	UsNDC_2	24.7	68.36
7/12/2017	ALAMEDA_1	28.1	33.67
7/12/2017	ALAMEDA_2	28.1	32.12
7/12/2017	Central_1	41.8	2.54

7/12/2017	Central_2	41.8	124.39
7/12/2017	TRIP BLANK	80	0.01
7/12/2017	DsRioBravo_1	56.7	47.45
7/12/2017	DsRioBravo_2	56.7	2.25
7/25/2017	METHOD BLANK	#N/A	0.01
7/25/2017	US550_1	9.4	32.5
7/25/2017	US550_2	9.4	70.1
7/25/2017	WillowCk_1	12.5	26.3
7/25/2017	WillowCk_2	12.5	13.3
7/25/2017	UsNDC_1	24.7	74.5
7/25/2017	UsNDC_2	24.7	2.59
7/25/2017	ALAMEDA_1	28.1	9.83
7/25/2017	ALAMEDA_2	28.1	0.01
7/25/2017	Central_1	41.8	2.94
7/25/2017	Central_2	41.8	229
7/25/2017	TRIP BLANK	80	0.01
7/25/2017	DsRioBravo_1	56.7	5.71
7/25/2017	DsRioBravo_2	56.7	26.2
8/9/2017	US550_1	9.4	7.5
8/9/2017	US550_2	9.4	399
8/9/2017	WillowCk_1	12.5	134
8/9/2017	WillowCk_2	12.5	233
8/9/2017	UsNDC_1	24.7	2.45
8/9/2017	UsNDC_2	24.7	482
8/9/2017	ALAMEDA_1	28.1	5.9
8/9/2017	ALAMEDA_2	28.1	171
8/9/2017	Central_1	41.8	852
8/9/2017	Central_2	41.8	212
8/9/2017	DsRioBravo_1	56.7	66.2
8/9/2017	DsRioBravo_2	56.7	51.5
8/9/2017	TRIP BLANK	80	0.01
8/23/2017	US550_1	9.4	12.19
8/23/2017	US550_2	9.4	18.35
8/23/2017	WillowCk_1	12.5	24.43
8/23/2017	WillowCk_2	12.5	48.1
8/23/2017	UsNDC_1	24.7	18.69
8/23/2017	UsNDC_2	24.7	22.96
8/23/2017	ALAMEDA_1	28.1	2.48
8/23/2017	ALAMEDA_2	28.1	3.29
8/23/2017	Central_1	41.8	9.34

8/23/2017	Central_2	41.8	7.26
8/23/2017	DsRioBravo_1	56.7	16.97
8/23/2017	DsRioBravo_2	56.7	11.07
8/23/2017	TRIP BLANK	80	0.01
9/6/2017	US550_1	9.4	29.1
9/6/2017	US550_2	9.4	43.8
9/6/2017	WillowCk_1	12.5	50.5
9/6/2017	WillowCk_2	12.5	20.4
9/6/2017	UsNDC_1	24.7	15.3
9/6/2017	UsNDC_2	24.7	41.3
9/6/2017	ALAMEDA_1	28.1	2.75
9/6/2017	ALAMEDA_2	28.1	242
9/6/2017	Central_1	41.8	43.6
9/6/2017	Central_2	41.8	5225
9/6/2017	TRIP BLANK	80	0.01
9/6/2017	DsRioBravo_1	56.7	504
9/6/2017	DsRioBravo_2	56.7	204
9/26/2017	WillowCk_1	12.5	20.73
9/26/2017	US550_2	9.4	0.01
9/26/2017	WillowCk_2	12.5	7.58
9/26/2017	US550_1	9.4	36.91
9/26/2017	UsNDC_1	24.7	25.97
9/26/2017	UsNDC_2	24.7	52.5
9/26/2017	ALAMEDA_1	28.1	2.48
9/26/2017	ALAMEDA_2	28.1	46.9
9/26/2017	Central_1	41.8	7.56
9/26/2017	Central_2	41.8	989.76
9/26/2017	TRIP BLANK	80	0.01
9/26/2017	DsRioBravo_1	56.7	23.84
9/26/2017	DsRioBravo_2	56.7	8.98
10/17/2017	US550_2	9.4	15.7
10/17/2017	US550_1	9.4	48.2
10/17/2017	WillowCk_1	12.5	13.1
10/17/2017	WillowCk_2	12.5	6.38
10/17/2017	UsNDC_1	24.7	2.53
10/17/2017	UsNDC_2	24.7	43.3
10/17/2017	ALAMEDA_1	28.1	160
10/17/2017	ALAMEDA_2	28.1	20.6
10/17/2017	Central_1	41.8	44.5
10/17/2017	Central_2	41.8	10.3

10/17/2017	DsRioBravo_1	56.7	242
10/17/2017	DsRioBravo_2	56.7	707
10/17/2017	TRIP BLANK	80	0.01
11/14/2017	US550_2	9.4	10.3
11/14/2017	US550_1	9.4	14.5
11/14/2017	WillowCk_1	12.5	2.6
11/14/2017	WillowCk_2	12.5	2.3
11/14/2017	UsNDC_1	24.7	110.6
11/14/2017	UsNDC_2	24.7	66.5
11/14/2017	ALAMEDA_1	28.1	243.5
11/14/2017	ALAMEDA_2	28.1	89.9
11/14/2017	Central_1	41.8	5.4
11/14/2017	Central_2	41.8	10.1
11/14/2017	DsRioBravo_1	56.7	1538.7
11/14/2017	DsRioBravo_2	56.7	1063.4
11/14/2017	TRIP BLANK	80	0.01
12/12/2017	US550_2	9.4	2.43
12/12/2017	US550_1	9.4	2.44
12/12/2017	WillowCk_1	12.5	2.49
12/12/2017	WillowCk_2	12.5	2.77
12/12/2017	UsNDC_1	24.7	0.01
12/12/2017	UsNDC_2	24.7	14.24
12/12/2017	ALAMEDA_1	28.1	10.75
12/12/2017	ALAMEDA_2	28.1	23.61
12/12/2017	Central_1	41.8	17.53
12/12/2017	Central_2	41.8	6.14
12/12/2017	DsRioBravo_1	56.7	110.74
12/12/2017	DsRioBravo_2	56.7	87.83
12/12/2017	TRIP BLANK	80	0.01
1/14/2018	US550_2	9.4	15.71
1/14/2018	US550_1	9.4	17.1
1/14/2018	WillowCk_1	12.5	2.89
1/14/2018	WillowCk_2	12.5	34.63
1/14/2018	UsNDC_1	24.7	2.44
1/14/2018	UsNDC_2	24.7	11.07
1/14/2018	ALAMEDA_1	28.1	12.05
1/14/2018	ALAMEDA_2	28.1	0.01
1/14/2018	Central_1	41.8	17.62
1/14/2018	Central_2	41.8	249.93
1/14/2018	DsRioBravo_1	56.7	2.81

1/14/2018	DsRioBravo_2	56.7	2.69
1/14/2018	TRIP BLANK	80	0.01
2/6/2018	US550_2	9.4	0.01
2/6/2018	US550_1	9.4	0.01
2/6/2018	WillowCk_1	12.5	2.77
2/6/2018	WillowCk_2	12.5	2.48
2/6/2018	UsNDC_1	24.7	0.01
2/6/2018	UsNDC_2	24.7	14.53
2/6/2018	ALAMEDA_1	28.1	0.01
2/6/2018	ALAMEDA_2	28.1	0.01
2/6/2018	Central_1	41.8	10.32
2/6/2018	Central_2	41.8	10.15
2/6/2018	DsRioBravo_1	56.7	55.62
2/6/2018	DsRioBravo_2	56.7	107.74
2/6/2018	TRIP BLANK	80	0.01
2/20/2018	US550_2	9.4	0.01
2/20/2018	US550_1	9.4	0.01
2/20/2018	WillowCk_1	12.5	0.01
2/20/2018	WillowCk_2	12.5	0.01
2/20/2018	UsNDC_1	24.7	19.67
2/20/2018	UsNDC_2	24.7	35.62
2/20/2018	ALAMEDA_1	28.1	11.2
2/20/2018	ALAMEDA_2	28.1	35.85
2/20/2018	Central_1	41.8	108.99
2/20/2018	Central_2	41.8	18.55
2/20/2018	DsRioBravo_1	56.7	115.37
2/20/2018	DsRioBravo_2	56.7	157.21
2/20/2018	TRIP BLANK	80	0.01
3/6/2018	US550_2	9.4	0.01
3/6/2018	US550_1	9.4	2.75
3/6/2018	WillowCk_1	12.5	3.74
3/6/2018	WillowCk_2	12.5	0.01
3/6/2018	UsNDC_1	24.7	2.71
3/6/2018	UsNDC_2	24.7	2.71
3/6/2018	ALAMEDA_1	28.1	0.01
3/6/2018	ALAMEDA_2	28.1	2.67
3/6/2018	Central_1	41.8	2.66
3/6/2018	Central_2	41.8	2.67
3/6/2018	DsRioBravo_1	56.7	30.61
3/6/2018	DsRioBravo_2	56.7	3

3/6/2018	TRIP BLANK	80	0.01
3/27/2018	US550_2	9.4	13.25
3/27/2018	US550_1	9.4	2.49
3/27/2018	WillowCk_1	12.5	7
3/27/2018	WillowCk_2	12.5	0.01
3/27/2018	UsNDC_1	24.7	0.01
3/27/2018	UsNDC_2	24.7	6.47
3/27/2018	ALAMEDA_1	28.1	150
3/27/2018	ALAMEDA_2	28.1	5.94
3/27/2018	Central_1	41.8	6.14
3/27/2018	Central_2	41.8	0.01
3/27/2018	DsRioBravo_1	56.7	47.54
3/27/2018	DsRioBravo_2	56.7	224.43
3/27/2018	TRIP BLANK	80	0.01
4/10/2018	US550_2	9.4	7.44
4/10/2018	US550_1	9.4	2.36
4/10/2018	WillowCk_1	12.5	5.88
4/10/2018	WillowCk_2	12.5	2.34
4/10/2018	UsNDC_1	24.7	2.3
4/10/2018	UsNDC_2	24.7	215.26
4/10/2018	ALAMEDA_1	28.1	3.03
4/10/2018	ALAMEDA_2	28.1	6.08
4/10/2018	Central_1	41.8	9.28
4/10/2018	Central_2	41.8	19.41
4/10/2018	DsRioBravo_1	56.7	6.1
4/10/2018	DsRioBravo_2	56.7	46.27
4/10/2018	TRIP BLANK	80	0.01
4/24/2018	US550_2	9.4	2.71
4/24/2018	US550_1	9.4	2.78
4/24/2018	WillowCk_1	12.5	9.56
4/24/2018	WillowCk_2	12.5	0.01
4/24/2018	UsNDC_1	24.7	0.01
4/24/2018	UsNDC_2	24.7	6.77
4/24/2018	ALAMEDA_1	28.1	0.01
4/24/2018	ALAMEDA_2	28.1	0.01
4/24/2018	Central_1	41.8	5.73
4/24/2018	Central_2	41.8	14.45
4/24/2018	DsRioBravo_1	56.7	18.6
4/24/2018	DsRioBravo_2	56.7	31.78
4/24/2018	TRIP BLANK	80	0.01

5/8/2018	US550_2	9.4	0.01
5/8/2018	US550_1	9.4	0.01
5/8/2018	WillowCk_1	12.5	2.48
5/8/2018	WillowCk_2	12.5	11.34
5/8/2018	UsNDC_1	24.7	0.01
5/8/2018	UsNDC_2	24.7	2.75
5/8/2018	ALAMEDA_1	28.1	9.26
5/8/2018	ALAMEDA_2	28.1	12.52
5/8/2018	Central_1	41.8	20
5/8/2018	Central_2	41.8	40.17
5/8/2018	DsRioBravo_1	56.7	7.01
5/8/2018	DsRioBravo_2	56.7	30.52
5/8/2018	TRIP BLANK	80	0.01

5) 95% confidence intervals for each season, grouped by site

Site #1: US 550 Bridge	**CI for E. coli Load (Log-units of cfu/day)**		**CI for E. coli Water Concentration (cfu/100mL)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
Summer (DOY 170-260)	12.62	0.28	2.41	0.29
Fall (DOY 260-350)	12.22	0.28	1.86	0.29
Winter (DOY 350-80)	12.33	0.28	2.11	0.29
Spring (DOY 80-170)	12.07	0.28	1.92	0.29
Site #1: US 550 Bridge	**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
Summer (DOY 170-260)	3.41	0.29		
Fall (DOY 260-350)	2.76	0.32		
Winter (DOY 350-80)	1.55	0.32		
Spring (DOY 80-170)	2.13	0.32		

Site #2: Willow Creek	**CI for E. coli Load (Log-units of cfu/day)**		**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
Summer (DOY 170-260)	12.69	0.26	2.48	0.25
Fall (DOY 260-350)	12.44	0.26	2.08	0.25
Winter (DOY 350-80)	12.21	0.26	1.99	0.25
Spring (DOY 80-170)	12.04	0.26	1.89	0.25
Site #1: US 550 Bridge	**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
Summer (DOY 170-260)	3.59	0.25		
Fall (DOY 260-350)	2.72	0.27		
Winter (DOY 350-80)	1.94	0.27		
Spring (DOY 80-170)	2.23	0.27		

Site #3: North Diversion Channel	**CI for E. coli Load (Log-units of cfu/day)**		**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**

Summer (DOY 170-260)	12.74	0.25	2.53	0.24
Fall (DOY 260-350)	12.71	0.25	2.35	0.24
Winter (DOY 350-80)	12.43	0.25	2.22	0.24
Spring (DOY 80-170)	11.88	0.25	1.73	0.24
Site #1: US 550 Bridge	**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
Summer (DOY 170-260)	3.30	0.32		
Fall (DOY 260-350)	3.11	0.36		
Winter (DOY 350-80)	2.63	0.36		
Spring (DOY 80-170)	2.11	0.36		

Site #4: Alameda Bridge	**CI for E. coli Load (Log-units of cfu/day)**		**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
Summer (DOY 170-260)	12.87	0.28	2.65	0.27
Fall (DOY 260-350)	12.51	0.28	2.15	0.27
Winter (DOY 350-80)	12.40	0.28	2.18	0.27
Spring (DOY 80-170)	12.42	0.28	2.27	0.27
Site #1: US 550 Bridge	**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
Summer (DOY 170-260)	2.95	0.34		
Fall (DOY 260-350)	3.54	0.38		
Winter (DOY 350-80)	1.86	0.38		
Spring (DOY 80-170)	2.46	0.38		

Site #5: Central Bridge	**CI for E. coli Load (Log-units of cfu/day)**		**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
Summer (DOY 170-260)	12.75	0.16	2.76	0.14
Fall (DOY 260-350)	12.50	0.16	2.23	0.14
Winter (DOY 350-80)	13.02	0.16	2.91	0.14
Spring (DOY 80-170)	12.52	0.16	2.46	0.14

Site #1: US 550 Bridge	CI for E. coli Sediment Concentration (Log-units of MPN/100g)	
	Estimated Mean	Standard Error (+/-)
Summer (DOY 170-260)	3.78	0.28
Fall (DOY 260-350)	3.29	0.32
Winter (DOY 350-80)	3.23	0.32
Spring (DOY 80-170)	2.82	0.32

Site #6: Valle de Oro	CI for E. coli Load (Log-units of cfu/day)		CI for E. coli Sediment Concentration (Log-units of MPN/100g)	
	Estimated Mean	Standard Error (+/-)	Estimated Mean	Standard Error (+/-)
Summer (DOY 170-260)	12.64	0.23	2.65	0.22
Fall (DOY 260-350)	12.94	0.23	2.66	0.22
Winter (DOY 350-80)	13.21	0.23	3.10	0.22
Spring (DOY 80-170)	12.44	0.23	2.38	0.22
Site #1: US 550 Bridge	**CI for E. coli Sediment Concentration (Log-units of MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
Summer (DOY 170-260)	3.50	0.22		
Fall (DOY 260-350)	4.22	0.25		
Winter (DOY 350-80)	3.36	0.25		
Spring (DOY 80-170)	3.45	0.25		

6) ANOVA tests for difference between seasons, grouped by site

Site #1: US550 Bridge	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	0.65	3.00	0.22	0.67	0.586
	Within seasons	3.86	12.00	0.32		
	Total	4.51	15.00			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	0.72	3	0.24	0.73	0.553
	Within seasons	3.95	12	0.33		
	Total	4.67	15			
E. coli Sediment Concentration Log (MPN/100g)	Between seasons	17.16	3.00	5.72	6.80	0.001
	Within seasons	25.24	30.00	0.84		
	Total	42.40	33.00			

Site #2: Willow Creek	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	0.97	3.00	0.32	1.17	0.361
	Within seasons	3.31	12.00	0.28		
	Total	4.28	15.00			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	0.79	3	0.26	1.07	0.399
	Within seasons	2.97	12	0.25		
	Total	3.76	15			
E. coli Sediment Concentration Log (MPN/100g)	Between seasons	14.30	3.00	4.77	7.94	0.000
	Within seasons	18.01	30.00	0.60		
	Total	32.31	33.00			

Site #3: North Diversion Channel	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	1.93	3.00	0.64	2.56	0.104
	Within seasons	3.01	12.00	0.25		
	Total	4.94	15.00			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	1.41	3	0.47	2.06	0.159
	Within seasons	2.73	12	0.23		
	Total	4.14	15			
	Between seasons	7.32	3.00	2.44	2.34	0.093

E. coli Sediment Concentration Log (MPN/100g)	Within seasons	31.27	30.00	1.04		
	Total	38.58	33.00			

Site #4: Alameda Bridge	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	0.57	3.00	0.19	0.60	0.626
	Within seasons	3.77	12.00	0.31		
	Total	4.33	15.00			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	0.64	3	0.21	0.74	0.548
	Within seasons	3.47	12	0.29		
	Total	4.11	15			
E. coli Sediment Concentration Log (MPN/100g)	Between seasons	12.31	3.00	4.10	3.55	0.026
	Within seasons	34.68	30.00	1.16		
	Total	47.00	33.00			

Site #5: Central Bridge	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	0.71	3.00	0.24	2.19	0.142
	Within seasons	1.31	12.00	0.11		
	Total	2.02	15.00			
E. coli Water Concentration Log (cfu/100mL)	Between seasons	1.12	3	0.37	4.77	0.021
	Within seasons	0.94	12	0.08		
	Total	2.06	15			
E. coli Sediment Concentration Log (MPN/100g)	Between seasons	4.16	3.00	1.39	1.73	0.181
	Within seasons	23.98	30.00	0.80		
	Total	28.14	33.00			

Site #6: Valle de Oro	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between seasons	1.39	3.00	0.46	2.24	0.136
	Within seasons	2.47	12.00	0.21		
	Total	3.86	15.00			

E. coli Water Concentration Log (cfu/100mL)	Between seasons	1.05	3	0.35	1.81	0.198
	Within seasons	2.33	12	0.19		
	Total	3.38	15			
E. coli Sediment Concentration Log (MPN/100g)	Between seasons	3.80	3.00	1.27	2.55	0.074
	Within seasons	14.91	30.00	0.50		
	Total	18.71	33.00			

7) Multiple comparison tests for difference between individual seasons, grouped by site

Site #1: US550 Bridge	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-0.79	0.40	1.59	0.756
	Summer	Winter	-0.90	0.29	1.48	0.885
	Summer	Spring	-0.64	0.55	1.74	0.539
	Fall	Winter	-1.30	-0.11	1.08	0.993
	Fall	Spring	-1.04	0.15	1.34	0.981
	Winter	Spring	-0.93	0.26	1.45	0.915
E. coli Water Concentration Log **(**cfu/100mL)	Summer	Fall	-0.66	0.55	1.75	0.555
	Summer	Winter	-0.91	0.29	1.50	0.886
	Summer	Spring	-0.72	0.48	1.69	0.641
	Fall	Winter	-1.46	-0.25	0.95	0.923
	Fall	Spring	-1.26	-0.06	1.14	0.999
	Winter	Spring	-1.01	0.19	1.40	0.964
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-0.54	0.65	1.83	0.458
	Summer	Winter	0.68	1.86	3.05	0.001
	Summer	Spring	0.10	1.28	2.46	0.030
	Fall	Winter	-0.03	1.22	2.46	0.058
	Fall	Spring	-0.62	0.63	1.88	0.523
	Winter	Spring	-1.83	-0.58	0.66	0.586

Site #2: Willow Creek	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-0.85	0.25	1.35	0.906
	Summer	Winter	-0.62	0.48	1.58	0.581
	Summer	Spring	-0.45	0.65	1.76	0.337
	Fall	Winter	-0.87	0.23	1.34	0.922
	Fall	Spring	-0.70	0.40	1.51	0.702
	Winter	Spring	-0.93	0.17	1.27	0.966
E. coli Water Concentration Log **(**cfu/100mL)	Summer	Fall	-0.65	0.40	1.44	0.681
	Summer	Winter	-0.56	0.48	1.53	0.536
	Summer	Spring	-0.46	0.59	1.63	0.378
	Fall	Winter	-0.96	0.09	1.13	0.994
	Fall	Spring	-0.85	0.19	1.24	0.945
	Winter	Spring	-0.94	0.10	1.15	0.990
	Summer	Fall	-0.13	0.87	1.87	0.105
	Summer	Winter	0.65	1.65	2.65	0.001

E. coli Sediment Concentration Log (MPN/100g)	Summer	Spring	0.36	1.36	2.36	0.005
	Fall	Winter	-0.28	0.78	1.83	0.208
	Fall	Spring	-0.56	0.49	1.54	0.592
	Winter	Spring	-1.34	-0.29	0.77	0.879

Site #3: North Diversion Channel	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-1.02	0.03	1.08	1.000
	Summer	Winter	-0.74	0.31	1.36	0.817
	Summer	Spring	-0.19	0.86	1.92	0.122
	Fall	Winter	-0.77	0.28	1.33	0.856
	Fall	Spring	-0.22	0.83	1.89	0.140
	Winter	Spring	-0.50	0.55	1.61	0.434
E. coli Water Concentration Log **(**cfu/100mL)	Summer	Fall	-0.83	0.18	1.18	0.952
	Summer	Winter	-0.69	0.31	1.31	0.792
	Summer	Spring	-0.20	0.80	1.80	0.137
	Fall	Winter	-0.87	0.14	1.14	0.977
	Fall	Spring	-0.38	0.62	1.62	0.300
	Winter	Spring	-0.52	0.49	1.49	0.500
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-1.13	0.19	1.50	0.980
	Summer	Winter	-0.64	0.67	1.99	0.515
	Summer	Spring	-0.13	1.19	2.51	0.088
	Fall	Winter	-0.90	0.49	1.87	0.777
	Fall	Spring	-0.39	1.00	2.39	0.224
	Winter	Spring	-0.87	0.52	1.90	0.744

Site #4: Alameda Bridge	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-0.82	0.35	1.53	0.807
	Summer	Winter	-0.71	0.47	1.65	0.646
	Summer	Spring	-0.73	0.44	1.62	0.683
	Fall	Winter	-1.06	0.12	1.29	0.991
	Fall	Spring	-1.09	0.09	1.27	0.996
	Winter	Spring	-1.20	-0.03	1.15	1.000
E. coli Water Concentration Log **(**cfu/100mL)	Summer	Fall	-0.63	0.50	1.63	0.569
	Summer	Winter	-0.66	0.47	1.60	0.614
	Summer	Spring	-0.75	0.38	1.51	0.754
	Fall	Winter	-1.16	-0.03	1.10	1.000

	Fall	Spring	-1.25	-0.12	1.01	0.988
	Winter	Spring	-1.22	-0.09	1.04	0.995
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-1.98	-0.59	0.79	0.653
	Summer	Winter	-0.30	1.08	2.47	0.169
	Summer	Spring	-0.90	0.49	1.87	0.776
	Fall	Winter	0.22	1.68	3.14	0.020
	Fall	Spring	-0.38	1.08	2.54	0.207
	Winter	Spring	-2.06	-0.60	0.87	0.687

Site #5: Central Bridge	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-0.45	0.24	0.93	0.734
	Summer	Winter	-0.97	-0.28	0.41	0.642
	Summer	Spring	-0.46	0.23	0.92	0.765
	Fall	Winter	-1.21	-0.52	0.17	0.171
	Fall	Spring	-0.71	-0.01	0.68	1.000
	Winter	Spring	-0.19	0.51	1.20	0.186
E. coli Water Concentration Log **(**cfu/100mL)	Summer	Fall	-0.06	0.53	1.12	0.082
	Summer	Winter	-0.74	-0.16	0.43	0.857
	Summer	Spring	-0.29	0.29	0.88	0.474
	Fall	Winter	-1.27	-0.69	-0.10	0.021
	Fall	Spring	-0.82	-0.24	0.35	0.644
	Winter	Spring	-0.14	0.45	1.04	0.158
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-0.66	0.49	1.64	0.660
	Summer	Winter	-0.60	0.55	1.70	0.572
	Summer	Spring	-0.20	0.96	2.11	0.132
	Fall	Winter	-1.16	0.06	1.28	0.999
	Fall	Spring	-0.75	0.47	1.68	0.725
	Winter	Spring	-0.81	0.41	1.62	0.800

Site #6: Valle de Oro	**Seasons being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	Summer	Fall	-1.26	-0.31	0.65	0.778
	Summer	Winter	-1.53	-0.57	0.38	0.326
	Summer	Spring	-0.75	0.20	1.15	0.923
	Fall	Winter	-1.22	-0.27	0.69	0.838
	Fall	Spring	-0.45	0.51	1.46	0.427
	Winter	Spring	-0.18	0.77	1.73	0.128
	Summer	Fall	-0.94	-0.02	0.91	1.000

E. coli Water Concentration Log (cfu/100mL)	Summer	Winter	-1.37	-0.45	0.47	0.497
	Summer	Spring	-0.66	0.27	1.19	0.828
	Fall	Winter	-1.36	-0.43	0.49	0.528
	Fall	Spring	-0.64	0.28	1.21	0.800
	Winter	Spring	-0.21	0.72	1.64	0.152
E. coli Sediment Concentration Log (MPN/100g)	Summer	Fall	-1.63	-0.72	0.19	0.161
	Summer	Winter	-0.77	0.14	1.05	0.974
	Summer	Spring	-0.85	0.05	0.96	0.998
	Fall	Winter	-0.10	0.86	1.82	0.090
	Fall	Spring	-0.19	0.77	1.73	0.148
	Winter	Spring	-1.05	-0.09	0.87	0.994

8) 95% confidence intervals for each site, grouped by season

Summer (2017)	**CI for E. coli Load (x10^{12} cfu/day)**		**CI for E. coli Water Concentration (cfu/100mL)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
1: US550 Bridge	12.62	0.16	2.41	0.16
2: Willow Creek	12.69	0.16	2.48	0.16
3: North Divn. Channel	12.74	0.16	2.53	0.16
4: Alameda Bridge	12.87	0.16	2.65	0.16
5: Central Bridge	12.75	0.16	2.76	0.16
6: Valle de Oro	12.64	0.16	2.65	0.16
Summer (2017)	**CI for E. coli Sediment Concentration (MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
1: US550 Bridge	3.41	0.26		
2: Willow Creek	3.59	0.26		
3: North Divn. Channel	3.30	0.26		
4: Alameda Bridge	2.95	0.26		
5: Central Bridge	3.78	0.26		
6: Valle de Oro	3.50	0.26		

Fall (2017)	**CI for E. coli Load (x10^{12} cfu/day)**		**CI for E. coli Water Concentration (cfu/100mL)**	
	Estimated Mean	**Standard Error (+/-)**	**Estimated Mean**	**Standard Error (+/-)**
1: US550 Bridge	12.22	0.20	1.86	0.18
2: Willow Creek	12.44	0.20	2.08	0.18
3: North Divn. Channel	12.71	0.20	2.35	0.18
4: Alameda Bridge	12.51	0.20	2.15	0.18
5: Central Bridge	12.50	0.20	2.23	0.18
6: Valle de Oro	12.94	0.20	2.66	0.18
Summer (2017)	**CI for E. coli Sediment Concentration (MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
1: US550 Bridge	2.76	0.28		
2: Willow Creek	2.72	0.28		
3: North Divn. Channel	3.11	0.28		
4: Alameda Bridge	3.54	0.28		
5: Central Bridge	3.29	0.28		
6: Valle de Oro	4.22	0.28		

Winter (2017-2018)	CI for E. coli Load (x10^{12} cfu/day)		CI for E. coli Water Concentration (cfu/100mL)	
	Estimated Mean	Standard Error (+/-)	Estimated Mean	Standard Error (+/-)
1: US550 Bridge	12.33	0.33	2.11	0.32
2: Willow Creek	12.21	0.33	1.99	0.32
3: North Divn. Channel	12.43	0.33	2.22	0.32
4: Alameda Bridge	12.40	0.33	2.18	0.32
5: Central Bridge	13.02	0.33	2.91	0.32
6: Valle de Oro	13.21	0.33	3.10	0.32
Summer (2017)	**CI for E. coli Sediment Concentration (MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
1: US550 Bridge	1.55	0.36		
2: Willow Creek	1.94	0.36		
3: North Divn. Channel	2.63	0.36		
4: Alameda Bridge	1.86	0.36		
5: Central Bridge	3.23	0.36		
6: Valle de Oro	3.36	0.36		

Spring (2018)	CI for E. coli Load (x10^{12} cfu/day)		CI for E. coli Water Concentration (cfu/100mL)	
	Estimated Mean	Standard Error (+/-)	Estimated Mean	Standard Error (+/-)
1: US550 Bridge	12.07	0.27	1.92	0.26
2: Willow Creek	12.04	0.27	1.89	0.26
3: North Divn. Channel	11.88	0.27	1.73	0.26
4: Alameda Bridge	12.42	0.27	2.27	0.26
5: Central Bridge	12.52	0.27	2.46	0.26
6: Valle de Oro	12.44	0.27	2.38	0.26
Summer (2017)	**CI for E. coli Sediment Concentration (MPN/100g)**			
	Estimated Mean	**Standard Error (+/-)**		
1: US550 Bridge	2.13	0.35		
2: Willow Creek	2.23	0.35		
3: North Divn. Channel	2.11	0.35		
4: Alameda Bridge	2.46	0.35		
5: Central Bridge	2.82	0.35		
6: Valle de Oro	3.45	0.35		

9) ANOVA tests for difference between sites, grouped by season

Summer (2017)	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between sites	0.16	5	0.03	0.32	0.895
	Within sites	1.82	18	0.10		
	Total	1.99	23			
E. coli Water Concentration Log (cfu/day)	Between sites	0.33	5	0.07	0.64	0.670
	Within sites	1.87	18	0.10		
	Total	2.21	23			
E. coli Sediment Concentration Log (MPN/100g)	Between sites	4.02	5	0.80	1.21	0.315
	Within sites	35.74	54	0.66		
	Total	39.76	59			

Fall (2017)	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between sites	1.21	5	0.24	1.47	0.247
	Within sites	2.96	18	0.16		
	Total	4.18	23			
E. coli Water Concentration Log (cfu/day)	Between sites	1.47	5	0.29	2.35	0.083
	Within sites	2.25	18	0.13		
	Total	3.72	23			
E. coli Sediment Concentration Log (MPN/100g)	Between sites	12.54	5	2.51	3.87	0.006
	Within sites	27.18	42	0.65		
	Total	39.72	47			

Winter (2017-2018)	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between sites	3.39	5	0.68	1.59	0.214
	Within sites	7.68	18	0.43		
	Total	11.07	23			
E. coli Water Concentration Log (cfu/day)	Between sites	4.29	5	0.86	2.11	0.111
	Within sites	7.33	18	0.41		
	Total	11.62	23			
	Between sites	23.06	5	4.61	4.50	0.002
	Within sites	43.03	42	1.02		

E. coli Sediment Concentration Log (MPN/100g)	Total	66.09	47			

Spring (2018)	**Source of Variance**	**Sum of Squares**	**Degrees of freedom**	**Mean Squared Error**	**F-Statistic**	**p-value***
E. coli Loading Log (cfu/day)	Between sites	1.39	5	0.28	0.95	0.471
	Within sites	5.26	18	0.29		
	Total	6.65	23			
E. coli Water Concentration Log (cfu/day)	Between sites	1.81	5	0.36	1.32	0.301
	Within sites	4.94	18	0.27		
	Total	6.74	23			
E. coli Sediment Concentration Log (MPN/100g)	Between sites	10.88	5	2.18	2.17	0.076
	Within sites	42.14	42	1.00		
	Total	53.02	47			

10) Multiple comparison tests for difference between individual sites, grouped by season

Summer (2017)	**Sites being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	1	2	-0.79	-0.07	0.64	0.999
	1	3	-0.84	-0.12	0.59	0.993
	1	4	-0.96	-0.25	0.47	0.877
	1	5	-0.84	-0.13	0.59	0.992
	1	6	-0.73	-0.02	0.70	1.000
	2	3	-0.77	-0.05	0.66	1.000
	2	4	-0.89	-0.17	0.54	0.968
	2	5	-0.77	-0.05	0.66	1.000
	2	6	-0.66	0.06	0.77	1.000
	3	4	-0.84	-0.12	0.59	0.993
	3	5	-0.72	0.00	0.71	1.000
	3	6	-0.61	0.11	0.82	0.997
	4	5	-0.60	0.12	0.84	0.994
	4	6	-0.49	0.23	0.95	0.905
	5	6	-0.61	0.11	0.83	0.996
E. coli Water Concentration Log **(**cfu/100mL)	1	2	-0.80	-0.07	0.65	1.000
	1	3	-0.85	-0.12	0.60	0.994
	1	4	-0.97	-0.25	0.48	0.883
	1	5	-1.07	-0.35	0.38	0.649
	1	6	-0.96	-0.24	0.49	0.894
	2	3	-0.78	-0.05	0.67	1.000
	2	4	-0.90	-0.17	0.55	0.970
	2	5	-1.00	-0.28	0.45	0.823
	2	6	-0.89	-0.17	0.56	0.975
	3	4	-0.85	-0.12	0.60	0.994
	3	5	-0.95	-0.23	0.50	0.915
	3	6	-0.84	-0.12	0.61	0.995
	4	5	-0.83	-0.10	0.62	0.997
	4	6	-0.72	0.01	0.73	1.000
	5	6	-0.61	0.11	0.83	0.996
E. coli Sediment Concentration Log (MPN/100g)	1	2	-1.25	-0.18	0.89	0.996
	1	3	-0.97	0.11	1.18	1.000
	1	4	-0.61	0.46	1.54	0.800
	1	5	-1.44	-0.37	0.71	0.911
	1	6	-1.17	-0.09	0.98	1.000
	2	3	-0.79	0.29	1.36	0.967
	2	4	-0.43	0.64	1.72	0.497
	2	5	-1.26	-0.19	0.89	0.995

	2	6	-0.99	0.09	1.16	1.000
	3	4	-0.72	0.35	1.43	0.926
	3	5	-1.55	-0.48	0.60	0.776
	3	6	-1.28	-0.20	0.87	0.993
	4	5	-1.91	-0.83	0.24	0.219
	4	6	-1.63	-0.56	0.52	0.648
	5	6	-0.80	0.28	1.35	0.974

Fall (2017)	**Sites being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	1	2	-1.13	-0.22	0.69	0.969
	1	3	-1.40	-0.49	0.42	0.539
	1	4	-1.20	-0.29	0.62	0.908
	1	5	-1.20	-0.28	0.63	0.915
	1	6	-1.63	-0.72	0.19	0.172
	2	3	-1.18	-0.27	0.64	0.928
	2	4	-0.98	-0.07	0.84	1.000
	2	5	-0.98	-0.06	0.85	1.000
	2	6	-1.41	-0.50	0.41	0.524
	3	4	-0.71	0.20	1.11	0.979
	3	5	-0.70	0.21	1.12	0.976
	3	6	-1.14	-0.23	0.68	0.965
	4	5	-0.91	0.01	0.92	1.000
	4	6	-1.34	-0.43	0.48	0.668
	5	6	-1.35	-0.44	0.48	0.656
E. coli Water Concentration Log **(**cfu/100mL)	1	2	-1.02	-0.22	0.57	0.946
	1	3	-1.29	-0.49	0.30	0.396
	1	4	-1.08	-0.29	0.50	0.849
	1	5	-1.16	-0.37	0.43	0.691
	1	6	-1.60	-0.80	-0.01	0.047
	2	3	-1.07	-0.27	0.52	0.880
	2	4	-0.86	-0.07	0.73	1.000
	2	5	-0.94	-0.14	0.65	0.991
	2	6	-1.38	-0.58	0.21	0.235
	3	4	-0.59	0.20	1.00	0.962
	3	5	-0.67	0.13	0.92	0.995
	3	6	-1.10	-0.31	0.49	0.813
	4	5	-0.87	-0.08	0.72	1.000
	4	6	-1.31	-0.51	0.28	0.356
	5	6	-1.23	-0.44	0.36	0.521

E. coli Sediment Concentration Log (MPN/100g)	1	2	-1.16	0.04	1.25	1.000
	1	3	-1.55	-0.35	0.85	0.951
	1	4	-1.98	-0.78	0.42	0.394
	1	5	-1.73	-0.53	0.67	0.779
	1	6	-2.66	-1.46	-0.26	0.009
	2	3	-1.60	-0.40	0.81	0.921
	2	4	-2.03	-0.82	0.38	0.333
	2	5	-1.77	-0.57	0.63	0.715
	2	6	-2.70	-1.50	-0.30	0.007
	3	4	-1.63	-0.43	0.77	0.891
	3	5	-1.38	-0.18	1.02	0.998
	3	6	-2.31	-1.11	0.09	0.085
	4	5	-0.95	0.25	1.45	0.988
	4	6	-1.88	-0.68	0.52	0.546
	5	6	-2.13	-0.93	0.27	0.209

Winter (2017-2018)	**Sites being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	1	2	-1.35	0.12	1.59	1.000
	1	3	-1.57	-0.10	1.36	1.000
	1	4	-1.54	-0.07	1.40	1.000
	1	5	-2.16	-0.70	0.77	0.664
	1	6	-2.35	-0.88	0.59	0.430
	2	3	-1.69	-0.22	1.24	0.996
	2	4	-1.65	-0.19	1.28	0.998
	2	5	-2.28	-0.82	0.65	0.510
	2	6	-2.47	-1.00	0.47	0.301
	3	4	-1.43	0.04	1.50	1.000
	3	5	-2.06	-0.59	0.88	0.791
	3	6	-2.24	-0.78	0.69	0.560
	4	5	-2.10	-0.63	0.84	0.749
	4	6	-2.28	-0.81	0.66	0.513
	5	6	-1.65	-0.18	1.28	0.998
E. coli Water Concentration Log **(**cfu/100mL)	1	2	-1.31	0.12	1.55	1.000
	1	3	-1.54	-0.10	1.33	1.000
	1	4	-1.50	-0.07	1.37	1.000
	1	5	-2.23	-0.80	0.63	0.507
	1	6	-2.42	-0.98	0.45	0.294
	2	3	-1.66	-0.22	1.21	0.996
	2	4	-1.62	-0.19	1.25	0.998
	2	5	-2.35	-0.92	0.52	0.362
	2	6	-2.54	-1.10	0.33	0.193

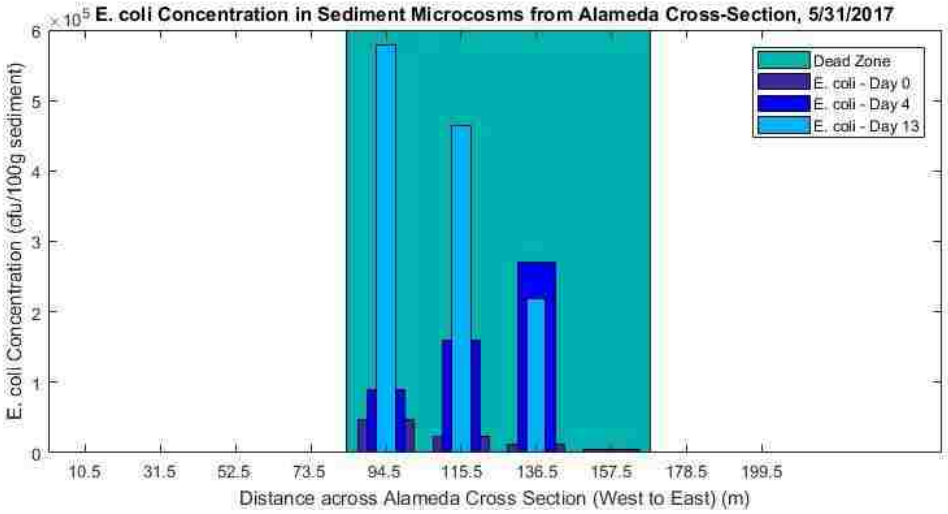
	3	4	-1.40	0.04	1.47	1.000
	3	5	-2.13	-0.69	0.74	0.644
	3	6	-2.31	-0.88	0.55	0.407
	4	5	-2.16	-0.73	0.70	0.596
	4	6	-2.35	-0.92	0.52	0.365
	5	6	-1.62	-0.18	1.25	0.998
E. coli Sediment Concentration Log (MPN/100g)	1	2	-1.90	-0.39	1.12	0.970
	1	3	-2.59	-1.08	0.43	0.291
	1	4	-1.83	-0.32	1.19	0.988
	1	5	-3.19	-1.68	-0.17	0.021
	1	6	-3.32	-1.81	-0.30	0.011
	2	3	-2.20	-0.69	0.82	0.752
	2	4	-1.44	0.07	1.59	1.000
	2	5	-2.80	-1.29	0.22	0.134
	2	6	-2.93	-1.42	0.09	0.076
	3	4	-0.75	0.76	2.27	0.663
	3	5	-2.11	-0.60	0.91	0.839
	3	6	-2.24	-0.73	0.78	0.697
	4	5	-2.87	-1.36	0.15	0.098
	4	6	-3.01	-1.50	0.01	0.054
	5	6	-1.64	-0.13	1.38	1.000

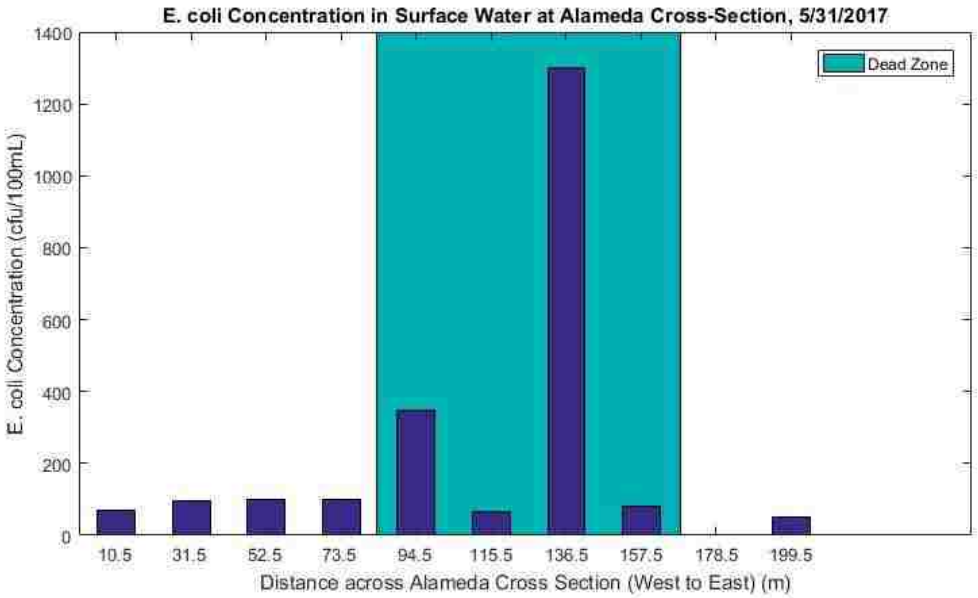
Spring (2018)	**Sites being compared**		**95% CI Lower Bound for Estimated Difference**	**Estimated Mean Difference**	**95% CI Upper Bound for Estimated Difference**	**p-value***
E. coli Loading Log **(**cfu/day)	1	2	-1.18	0.03	1.25	1.000
	1	3	-1.02	0.19	1.40	0.996
	1	4	-1.57	-0.35	0.86	0.936
	1	5	-1.66	-0.45	0.77	0.844
	1	6	-1.58	-0.37	0.85	0.925
	2	3	-1.06	0.16	1.37	0.998
	2	4	-1.60	-0.38	0.83	0.910
	2	5	-1.69	-0.48	0.73	0.803
	2	6	-1.61	-0.40	0.82	0.897
	3	4	-1.76	-0.54	0.67	0.715
	3	5	-1.85	-0.64	0.58	0.565
	3	6	-1.77	-0.56	0.66	0.693
	4	5	-1.31	-0.10	1.12	1.000
	4	6	-1.23	-0.01	1.20	1.000
	5	6	-1.13	0.08	1.30	1.000
E. coli Water Concentration	1	2	-1.14	0.03	1.21	1.000
	1	3	-0.99	0.19	1.37	0.995
	1	4	-1.53	-0.35	0.83	0.928

Log (cfu/100mL)	1	5	-1.72	-0.54	0.64	0.692
	1	6	-1.64	-0.46	0.72	0.813
	2	3	-1.02	0.16	1.34	0.998
	2	4	-1.56	-0.38	0.79	0.899
	2	5	-1.75	-0.57	0.60	0.641
	2	6	-1.67	-0.49	0.69	0.768
	3	4	-1.72	-0.54	0.63	0.689
	3	5	-1.91	-0.73	0.45	0.393
	3	6	-1.83	-0.65	0.53	0.517
	4	5	-1.37	-0.19	0.99	0.995
	4	6	-1.28	-0.11	1.07	1.000
	5	6	-1.09	0.08	1.26	1.000
E. coli Sediment Concentration Log (MPN/100g)	1	2	-1.59	-0.10	1.40	1.000
	1	3	-1.47	0.02	1.52	1.000
	1	4	-1.83	-0.33	1.17	0.985
	1	5	-2.19	-0.69	0.80	0.739
	1	6	-2.81	-1.32	0.18	0.112
	2	3	-1.38	0.12	1.61	1.000
	2	4	-1.73	-0.23	1.26	0.997
	2	5	-2.09	-0.59	0.90	0.841
	2	6	-2.72	-1.22	0.27	0.167
	3	4	-1.85	-0.35	1.14	0.981
	3	5	-2.21	-0.71	0.78	0.715
	3	6	-2.83	-1.34	0.16	0.103
	4	5	-1.86	-0.36	1.13	0.978
	4	6	-2.48	-0.99	0.51	0.375
	5	6	-2.12	-0.63	0.87	0.809

11) Exploratory Cross-Sectional Sampling Data (May 2017)

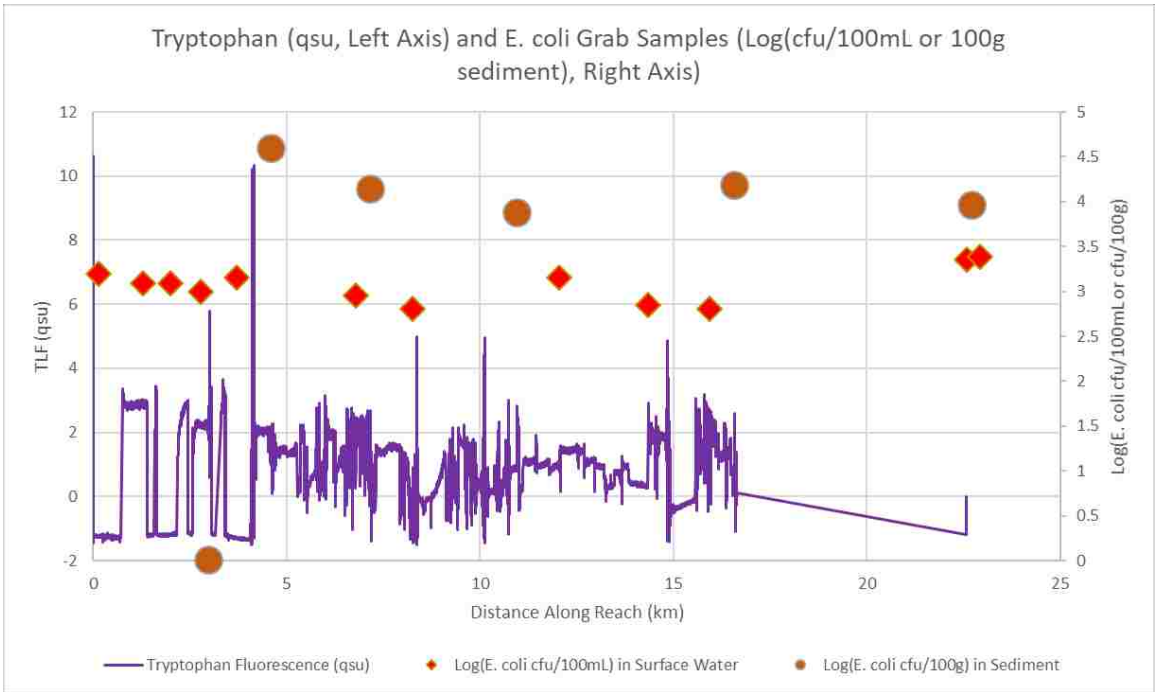
Surface water and sediment samples were taken from the Alameda Bridge and analyzed for E. coli concentration. Sediment samples were kept at room temperature for 13 days and analyzed for E. coli concentration on the day of collection and after 4 and 13 days.





12) Exploratory Reach-Length Sampling (June 2017)

Surface water and sediment samples were collected and tryptophan-like fluorescence (TLF), a surrogate for bacterial concertation, was measured continuously while floating down the Rio Grande in a small boat. Samples were analyzed for E. coli concentration. TLF readings proved difficult to interpret.



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