Sediment *E. coli*, Phase I: Colony Enumeration and Sediment Habitat Preferences

A Clean Rivers Program Special Studies Report

Aaron Hoff and Angela Kilpatrick
Trinity River Authority

Figure 1. Collection of post-disturbance water column E. coli sample.

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Introduction

Over the past two decades, fecal bacteria, or *E. coli,* has become a concern for many stakeholders in the Trinity River basin, and throughout the state of Texas in general. According to the 2014 *Texas Integrated Report of Surface Water Quality for the Clean Water Act Sections 305(b) and 303(d)*, there are currently more than 250 waterbody segments in Texas listed as impaired for recreational use due to elevated levels of fecal bacteria. These impairments can sometimes be attributed to point sources of bacteria such as malfunctioning human sewage infrastructure, but commonly bacteria impairments arise from nonpoint source pollution that is delivered to the stream as runoff during storm events. However, streams may retain elevated levels of bacteria well after disturbances from storm events have taken place. Current scientific literature indicates that shallow bed sediments can be a significant reservoir of bacteria when resuspended by a disturbance event such as incoming stormwater runoff, floodwater erosion, or other in-stream physical agitation (wildlife, livestock, or human activity). However, the majority of these studies focus on coastal tidal zones, or along beaches of reservoirs or lakes. These are areas where flow velocity slows, where sediment conditions are more likely to be accretive, and where unconsolidated bed sediments are common. A literature review suggests that very little is known about this phenomenon in inland, eroding systems where particle sizes, sediment consolidation conditions, and fluvial geomorphology may differ from those conditions evaluated in preceding studies.

Furthermore, there is indirect acknowledgement that sediments can affect water quality; the Texas Commission on Environmental Quality's (TCEQ) *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods* (RG-415, Revised August 2012) provides guidance that seeks to minimize sediment disturbance when taking water quality samples. However, studies to evaluate sediment influence on water quality are limited.

To further explore the impacts of sediment bacteria on water quality and the bacterial impairment issues in the streams of the Trinity basin, a study was undertaken to identify the extent to which bacteria in sediments may affect water column concentrations.

Background Information and Problem Statement

Existing Standards and Assessment Protocols for Bacteria

In the state of Texas, there are specific uses and numerical criteria associated with a waterbody. One use is recreation. The recreation use consists of five categories, each associated with a certain risk of water ingestion:

- Primary Contact Recreation 1
- Primary Contact Recreation 2
- Secondary Contact Recreation 1
- Secondary Contact Recreation 2
- Noncontact Recreation

Primary contact recreation is defined as any activity where there is a significant risk of water ingestion. This may include activities such as swimming, tubing, or wading (TCEQ, 2014). Each recreation use category has an associated specific numerical criterion. For example, the geometric mean criterion, or standard, for *E.coli* for Primary Contact Recreation 1 (PCR1) is 126 colony-forming units (CFUs) per 100 mL of water. However, the geometric mean criterion for *E.coli* for Secondary Contact Recreation 1 (SCR1) is 630 CFU/100 mL. *E.coli* is used as an indicator organism that predicts the presence of other pathogenic species present in fecal material, such as norovirus, campylobacter, giardia, and cryptosporidium (Hörman et al., 2004). Assessments of recreational use attainment or non-attainment are based on the enumeration of colony forming units (CFUs) of *E. coli* from water samples collected at monitoring stations in lakes, streams, and estuaries. After a sufficient number of samples have been collected over a period of time, they are analyzed for a geometric mean (geomean) and compared to the recreation use standard (USEPA, 1986). This is the standard used by the state of Texas to indicate a recreational use impairment. It is applied to all freshwater features in the state, unless otherwise noted in the Texas Surface Water Quality Standards, as described in the Texas Administrative Code (TCEQ, 2014).

To be statistically significant, the number of samples must not be less than five, equally spaced over a 30-day period (USEPA, 1986). However, a minimum of 20 samples collected within a seven-year period are required for assessment of use attainment. These samples must be analyzed by a state-recognized laboratory, accredited by the National Environmental Laboratory Accreditation Program (NELAP). Once enough data has been collected and analyzed, this seven-year window will advance to include the most current data for analysis. If the waterbody in question does not maintain an *E. coli* concentration level at or below this geomean requirement, individuals that use that particular waterbody for primary contact recreation are considered to be at an elevated exposure risk to pathogenic organisms related to fecal material. Individual samples used in these assessments must also not be taken during extreme hydrologic conditions, such as heavy flooding or otherwise high flow conditions, both during and for at least 24 hours after the event takes place (TCEQ, 2014).

Texas Surface Water Quality Bacteria Standard - History

The existing standard is based on the results of epidemiological work conducted by the U.S. Environmental Protection Agency (EPA) that was published in 1986 (USEPA, 1986). Prior to this study, fecal coliform was used as the single indicator organism for recreational use analyses in all waterbodies. The 1986 study instead proposed two distinct indicator organisms, one for inland freshwaters, and another for marine waters. For freshwater systems, *E. coli* was chosen for a closer study into its applicability. Two sites were chosen for the freshwater study: Lake Erie, with sampling locations near Erie, Pennsylvania and Keystone Lake, with sampling locations near Tulsa, Oklahoma. Both study locations consisted of several sampling stations, with a primary division between those perceived to be relatively unaffected by human activity and those that were considered to be “barely acceptable.” The combined results from both study locations yielded the geomean value of 126 CFUs/100 mL of water, which equated to an “acceptable risk” level of approximately 8 cases of gastrointestinal distress per 1000 individuals engaging in primary contact recreation activities (USEPA, 1986). Although the focus of this study was on the beaches of lakes where primary contact recreation is common, the standard has since been broadly applied to all fresh water streams and rivers, in addition to lakes.

Since the application of this standard, additional assessments have been developed to ascertain the level of contact recreation occurring in a waterbody, known as recreational use attainability analyses (RUAAs). RUAAs are conducted to determine the recreational use category (as described above in the “Existing Standards and Assessment Protocols for Bacteria” section) that is appropriate for a specific waterbody, such as a stream, river, or lake (USEPA, 2006). While the RUAA process effectively refines the application of the 1986 criteria, questions still remain about the factors influencing the wide range and uncertainty associated with measurement of in-stream *E. coli* concentrations. This project endeavors to be one of many steps toward answering these questions.

Habitat Preferences

A growing body of evidence supports the theory that certain physical properties related to sediment and geology may also dictate the presence and concentration of *E. coli*. In a study conducted in bayous near Houston, researchers found that while there was little correlation between sediment moisture content and *E. coli* counts, there was none with sediment organic matter content. The study also found that while there was little preference for *E. coli* to associate with any particular sediment particle size suspended in the water column, in bottom sediments the organism preferred fine sands (60-250 micrometers, or µm). It is also notable that *E. coli* counts were consistently highest in the top 1 cm of sediments, and that this was consistent for all samples collected during the study. Researchers also noted that there was “a significant order of magnitude or more reduction in [*E. coli*] and [*Enterococci*] concentrations from the top 1 cm to the deeper 15, 30, and 60 cm horizons" (Brinkmeyer et al., 2015). Further, research from a creek in rural Maryland noted that there was a more than twofold decrease in *E. coli* levels in 5 cm increments down to a depth of 15 cm in the bed sediments (Pachepsky and Shelton, 2011). One study intentionally limited sample depth to 10 cm or less, although this is likely an artifact of the sediment collection method (via hand trowel) rather than a question of application, as no explanation is provided (Crabill et al., 1999).

Study Area

Geography

Although several sources of water quality impairments exist in Texas, recreational use impairments due to elevated levels of bacteria are by far the most prevalent, with 255 instances cited in the 2014 Integrated Report (TCEQ, 2015). To address this concern, state agencies have focused significant effort into addressing this particular contaminant in the past decade. According to the 2014 Integrated Report, the majority of the streams impaired for fecal bacteria in the state of Texas are located in the central and eastern extents of the state, with a few notable exceptions. To reflect these circumstances, the study area was chosen to reflect similar climate, ecology, and terrestrial characteristics. Several watersheds within the West Fork Trinity River drainage sub-basin were chosen as the study area. These include the Village Creek, Walnut Creek, and Mountain Creek watersheds (Figure 2). County-level data for the study area characterize the climate as 'humid subtropical,' with hot, humid summers and generally mild to cool winters (Kottek et al., 2006). Average annual precipitation for the area varies from 20-24 inches.

Hydrography

At present, the majority of the investigative literature on the subject of *E. coli* in sediments is focused in coastal areas under tidal influence (Carrillo et al., 1985; Gerba and McLeod, 1976), or in areas near large interior lakes (Anderson et al., 2005; Byappanahalli et al., 2003), or in other climatic regions (Flint, 1987). In these areas, changes in elevation often become less pronounced, and flow velocities decrease as incoming water is arrested by the mass of water that exists in the lake, bay, or ocean. These changes in topography, hydrology, and ultimately, flow velocity cause sediment particles to fall out of the water column and accumulate in the streambed. Over time, these processes form the deltaic planes that are common to most large river systems as they reach the ocean.

In contrast, this study seeks to perform similar investigations within three watersheds that reflect the erosive environments commonly found within inland, upland watersheds. While some sedimentation does occur within the micro-environments present in each stream system due to fluvial geomorphological processes, in general there is less accretion overall when compared to the previously mentioned tidal-influenced or lake-centric systems. It is this difference in deposition potential that is seen as the primary defining factor between the area selected for this study and the areas chosen for previous studies related to the topic of *E. coli* in sediments.

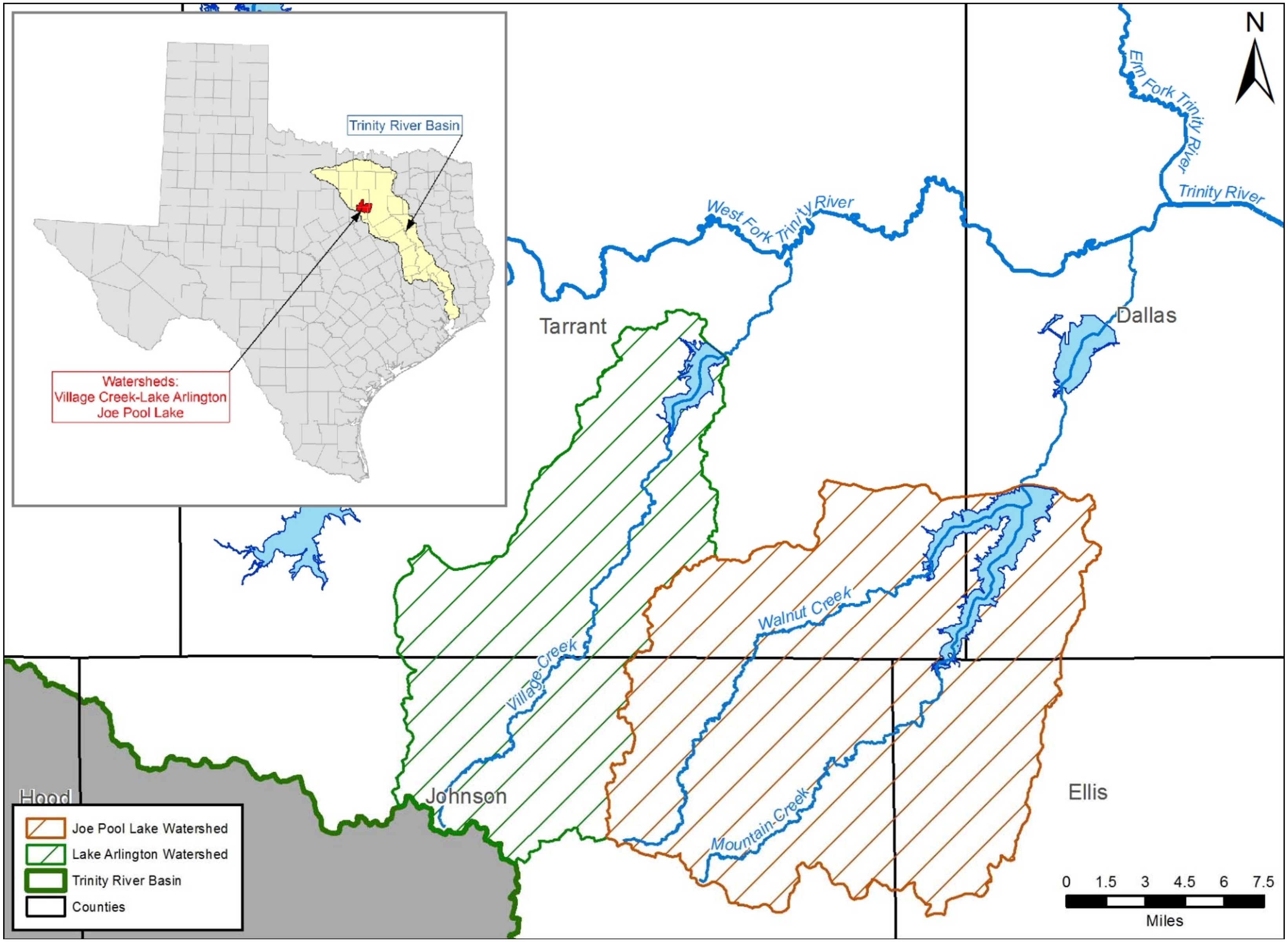


Figure 2: Location of study area watersheds within Tarrant, Dallas, Johnson, and Ellis Counties, Texas. Data source: NHD.

Methods

Based on a review of the available literature, it would appear that the focus of research related to the influence of sediment *E. coli* populations on the water column continues to be geared more towards beaches of lakes and oceans. Where this is not the case, research still tends to remain in coastal or tidally influenced zones where sediment-accretive conditions exist. To further the science into other areas of the state where water quality impairments exist, specifically those in inland or flowing/eroding systems, it is proposed that similar experiments geared toward sediment *E. coli* and their fate and persistence after suspension in the water column by disturbance events be carried out. However, before these more advanced analyses can commence, some fundamental experiments related to habitat preferences, and specifically sediment particle size preference, were proposed as foundational studies. Likewise, little work has been conducted on inland, or flowing/eroding systems in this regard, highlighting the need for this study.

Data Collection

For streams within the study area, an exploration of potential associations between bacterial impairments, sediment particle size, organic matter content, and temperature exhibited in eroding, flowing, or generally non-accretive environments was conducted. Sediment samples were collected from a layer of sediment no deeper than 10 cm, as significant contributions of *E. coli* to the water column from below this sediment depth are not expected under low to normal flow conditions.

Samples were collected every other month at seven sites on four streams: one site on an unnamed tributary to Lake Arlington, one site on Village Creek, one on a tributary of Village Creek, and two sites each on Mountain Creek and Walnut Creek (Figure 4). Bacteria sampling activities consisted of three distinct samples per site: (1) the collection of sediment *E. coli*, (2) water column *E. coli* collected pre-disturbance, and (3) water column *E. coli* after artificial sediment disturbance.

Sediment and pre-disturbance water column *E. coli* data were submitted to TCEQ for review and inclusion in the Surface Water Quality Monitoring Information System (SWQMIS) database. An additional water column sample was collected slightly downstream after sediment had been disturbed and re-suspended. As the post-disturbance water column samples were collected outside of normal Surface Water Quality Monitoring Information (SWQM) sampling procedures, these *E. coli* data are provided in the form of this report rather than submitted to SWQMIS. This data will be used for analysis purposes to determine if sediment disturbances significantly changed the concentration of *E. coli* in the water column.

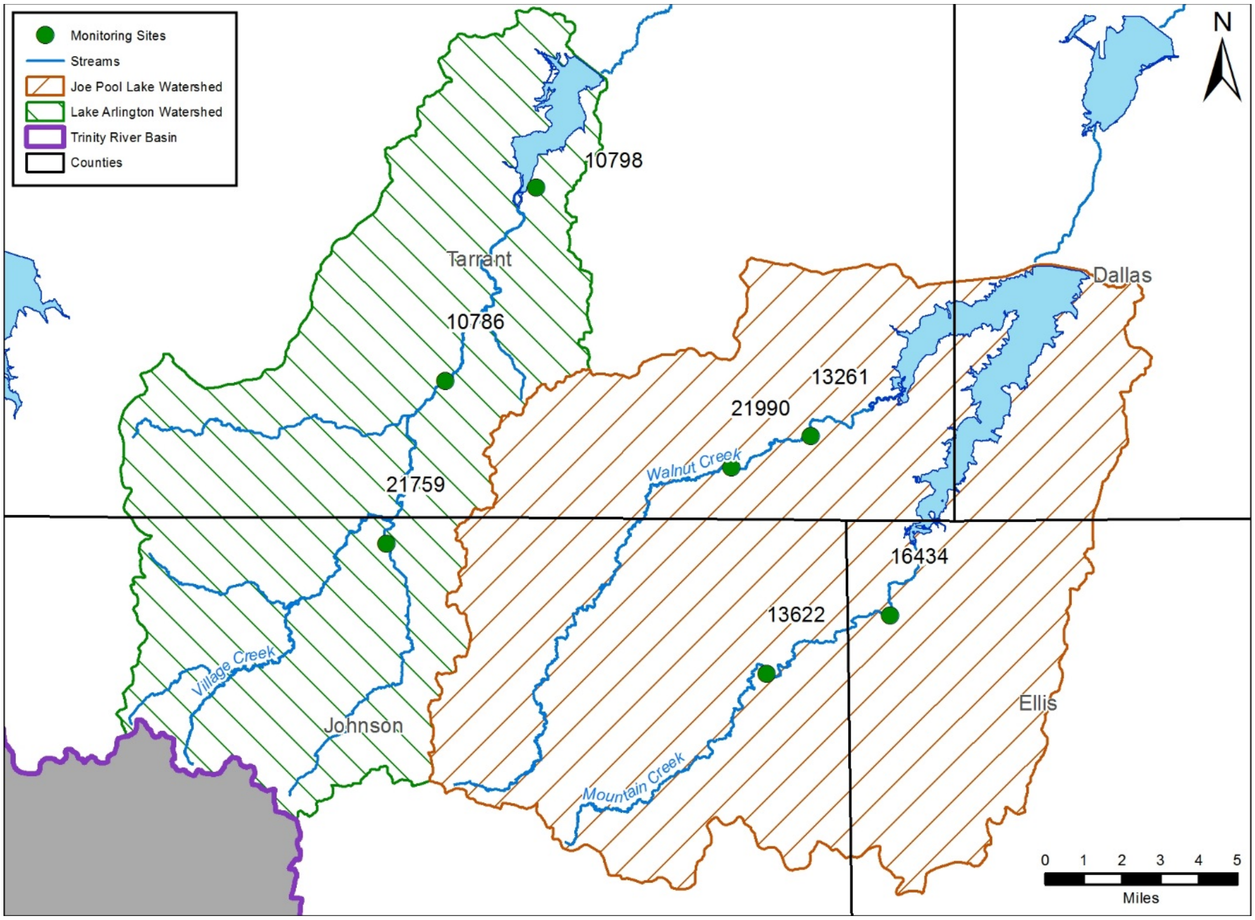


Figure 3. Seven sampling sites utilized for the sediment E. coli study.

To ensure that samples were collected under flow severities of low to normal flow, staff regularly consulted weather data for the region to track recent rainfall, and scheduled sample collection accordingly. It was expected that there would be limited sediment mobility under these flow conditions, which should reduce background influences from sediments in the pre-disturbance samples. Therefore, high and flood flow conditions were not sampled. Additionally, each site was inspected prior to sampling to ensure that bed sediments were not being excessively mobilized at low and normal flows due to outside influences such as animal or human activity and channel disturbances such as construction and erosion. If it was apparent that bed sediments were being resuspended due to these influences, sampling was delayed until a time when the channel had stabilized and there was reduced sediment movement. Avoidance of sampling during or immediately after rainfall events decreased the likelihood of these resuspension events confounding the results of this study.

The following tasks were completed during each sample event:

1) Upon arrival at each site, a distance of 30 seconds downstream of the sediment collection location was determined. To determine this distance, one field staff member introduced a biodegradable neutrally-buoyant item into the stream. A

second field staff member identified the floating item's location after 30 seconds elapsed, and entered the stream at that location.

2) The second field staff member collected a pre-disturbance water column *E. coli* sample.
3) The first field staff member entered the stream and collected a sediment sample for laboratory analysis of *E. coli* and sediment conventionals. These samples were collected with a scoop or dredge depending on the depth of the water at the time of sampling.
4) From their upstream location, the first field staff member adequately disturbed the sediments by kicking up the sediments in a wide zig-zagging pattern across the stream channel. During the sediment disturbance, a second biodegradable neutrally-buoyant item was introduced into the stream.
5) The second field staff member then collected a post-disturbance water column sample once the floating item had reached their location.
6) A sonde was placed upstream of the disturbed area for determination of field parameters.
7) Instantaneous flow was measured at the upstream site and reported under parameter code 00061.
8) Sediment *E. coli* and sediment conventionals samples were delivered to AnaLab for analysis.

Data Analysis

For this initial phase, correlation of sediment *E. coli* populations with water temperature, sediment organic carbon content, and sediment partitioning were studied. The sediment partition is defined as the ratio of each of the six particle size partitions in relation to one another. By considering the entire partition, as opposed to one particle size partition or another (e.g., coarse sand, silt), researchers may be able to learn more about whether sediment-resident *E. coli* favor a specific mix of heterogeneous sediment sizes as ideal habitat, or prefer to colonize within one particular particle size partition. To arrive at these values, a technique known as multiple variable regression was utilized, along with conventional single-variable regressions, where appropriate for comparisons of single sediment partitions to the sediment *E. coli* values. Single-variable regressions for *E. coli* were also explored with water temperature and organic matter content.

Results and Discussion

Dataset Completion

Sampling staff collected six samples at seven sites, including those from scheduled events as well as those from make-up events necessitated by unfavorable sampling conditions. In total, this yielded 42 *E. coli* samples each for (1) pre- and (2) post-disturbance water column samples and (3) sediment *E. coli* samples. Several results within each category contained qualifier flags for less than/greater than (</>) values. For the purposes of this analysis, those samples with '>' flags were deemed appropriate for use, with the flag removed and the value itself used as a conservative estimate within the analysis. Those samples containing a '<' flag were not considered for analysis, nor was the single sediment *E. coli* sample that was analyzed outside of the hold time specified in the analysis method used by the laboratory.

The usable results for each data category, after losses, are as follows:

- Pre-disturbance water column E. coli: 39 results;
- Post-disturbance water column *E. coli*: 40 results; and
- Sediment *E. coli*: 36 results.

In many cases, this yielded only enough data for five paired results per station, limiting the amount of functional statistical analysis that could be carried out at individual stations.

Comparing Pre- and Post-disturbance *E. coli* Enumeration

Pre-disturbance and post-disturbance *E. coli* enumerations were first compared to observe changes. In general, artificial disturbance events led to significant increases in bacteria counts, with mean increases of anywhere between 38% to >5300% at specific stations over the project lifetime, with a particularly notable increase of nearly 26,000% for one sample at site 13261 (Table 1). Still, there were some exceptions, particularly at the Mountain Creek sites, where substrates were notably different due to the higher presence of compacted clays. Here, there were several instances of no increase or even decreases with the post-disturbance samples.

Table 1. Observations of changes in water column E. coli counts after artificial disturbance events.

		% Change in Water Column E. coli Count (MPN/100 mL)		
Station	**Site**	**Max**	**Min**	**Mean**
13622	MC @ FM 157	153.05	-52.94	37.97
16434	MC @ US 287	1414.73	-25.00	461.12
21759	Quil Miller @ CR 532	1018.77	11.76	323.58
10798	Unnamed Trib of Lake Arlington	8658.57	228.81	3031.84
10786	VC at Rendon Rd	531.71	0.00	204.89
13621	WC @ Matlock	25925.00	142.71	5362.71
21990	WC at Katherine Rose	2106.76	5.08	522.71

Multiple-variable Regression

The multiple regression analysis used to determine the amount of influence that the sediment partitioning (e.g., the mix of sediment sizes in the sample) had on *E. coli* abundance in sediments requires at least one more data point than there are independent variables. Therefore, none of the individual sites yielded enough valid data points with which to conduct the analysis, with the six sediment categories necessitating at least 7 data points at each individual site.

Therefore, to provide some useable information for the purposes of this report, individual sites were combined within each of their respective watersheds (Village, Walnut, and Mountain Creeks) and analyzed using a multiple regression analysis (Table 2).

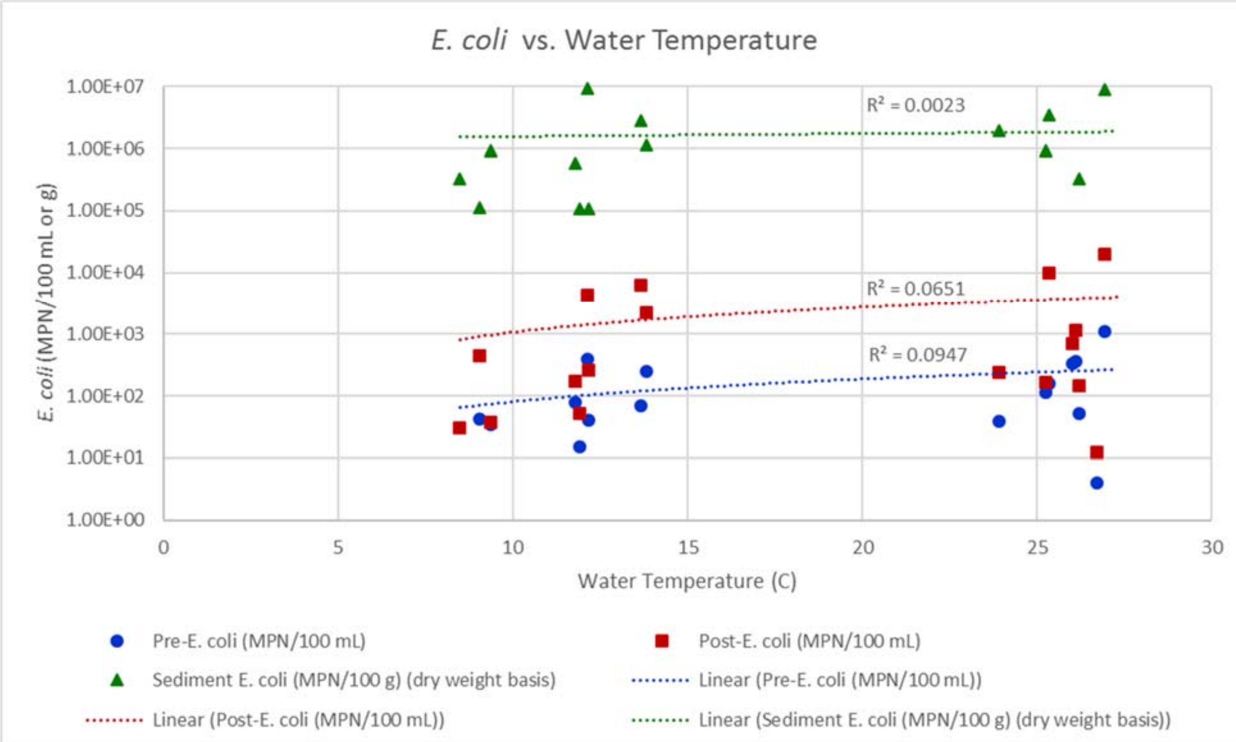
Table 2: Regression statistics for multiple regression analysis within three watersheds.

Watershed	Village Creek	Walnut Creek	Mountain Creek
Multiple R	0.619730849	0.90486253	0.96717667
Multi R Square	0.384066325	0.818776198	0.93543071
Adjusted R Square	-0.143876825	0.546940496	0.741722841
Standard Error	3301538.452	4263316.615	4913378.368
ANOVA P-value	0.642927594	0.152674089	0.181469492
Observations	14	11	9

Within the Village Creek watershed, the resulting multiple R-squared value was 0.38, which was much lower in comparison to the other two watersheds. Within the Walnut Creek watershed, where both stations are located on the main stem of the creek, where the R-squared value was 0.82. Monitoring stations for Mountain Creek were also both located on the main stem, where the R-squared value was 0.93. In both watersheds, it is hypothesized that this phenomenon is the result of having both stations on the main stem, where conditions and *E. coli* growth kinetics might be more similar. This is in contrast to the Village Creek watershed, where only one station is on the main stem, one is within the Quil Miller tributary, and the third is on a separate tributary leading directly to the lake. It is hypothesized that the more heterogeneous nature of the three stations here led to the reduced R-squared value. Likewise, the p-value for the Village Creek data (0.64) is much larger than those of the other two watersheds (0.15 for Walnut Creek and 0.18 for Mountain Creek).

Single-variable Regressions

E. coli counts did not correlate very strongly with either water temperature or organic matter content (Figure 4). As is with the case of the multiple-variable regression above, the dataset may be too small to verify this statistically. Similarly, most single-variable regressions comparing sediment *E. coli* counts with separate sediment particle size partitions also showed weak associations, with the exception being those at the two Walnut Creek stations (Figure 5, Figure 6). R-squared values were significantly higher at these two stations than elsewhere in either Walnut or Village Creek. At Matlock Road, moderate positive associations for R-squared existed for sediment *E. coli* between both clay (0.66) and silt (0.62), with a weaker association in fine sands (0.47). In contrast, strong negative associations existed with medium sand (0.78) and coarse sand (0.84), being much weaker with gravel (0.13). At Katherine Rose Park, similar correlations existed, with moderate positive associations for R-squared values for sediment *E. coli* between clay (0.79), silt (0.75), and fine sands (0.70). Moderate negative associations existed for medium sand (0.63) and gravel (0.89), but was slightly weaker for coarse sand (0.49). With some notable exceptions, R-squared values for these regressions at other sites rarely exceeded 0.1.



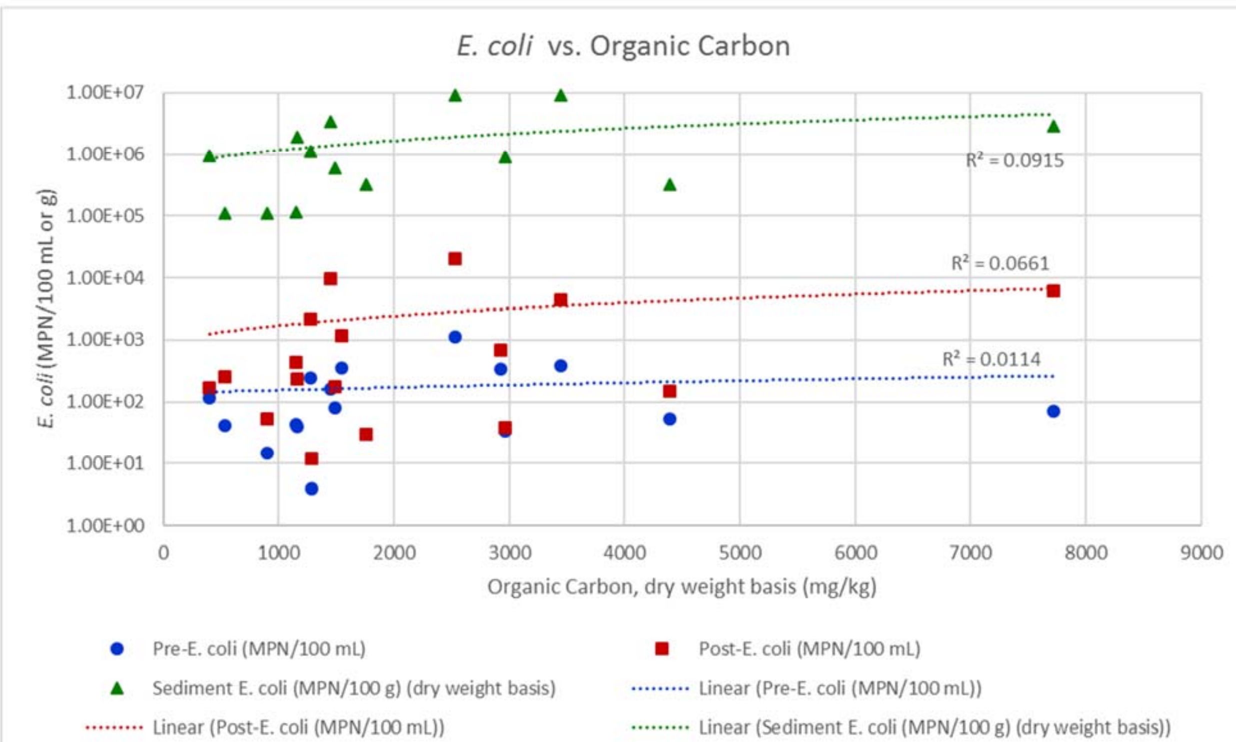


Figure 4. Example regression curves from the Village Creek watershed relating E. coli counts to water temperature (top) and organic matter content (bottom).

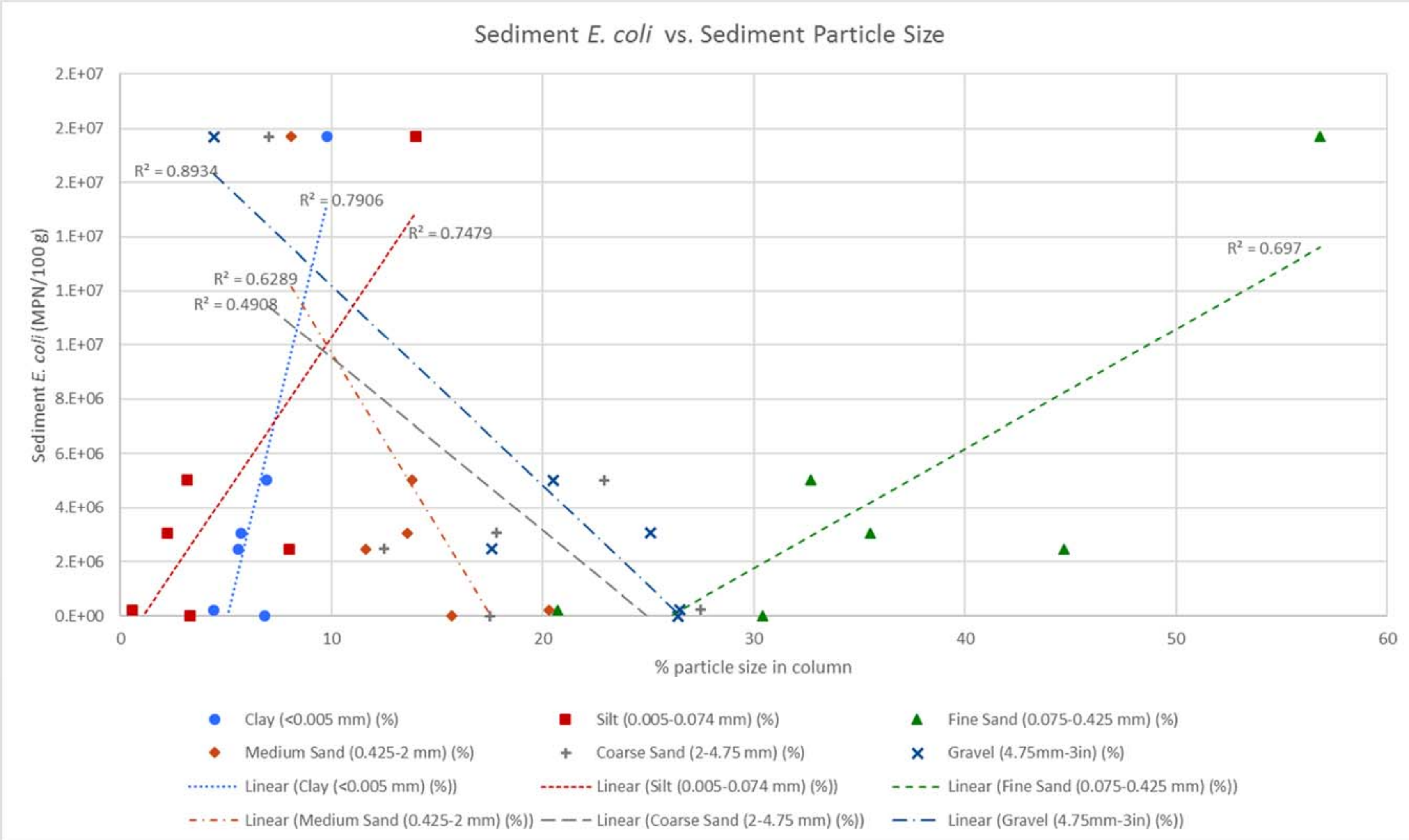


Figure 5. Sediment E. coli colony counts and sediment particle size partition correlations at the Matlock Road site on Walnut Creek.

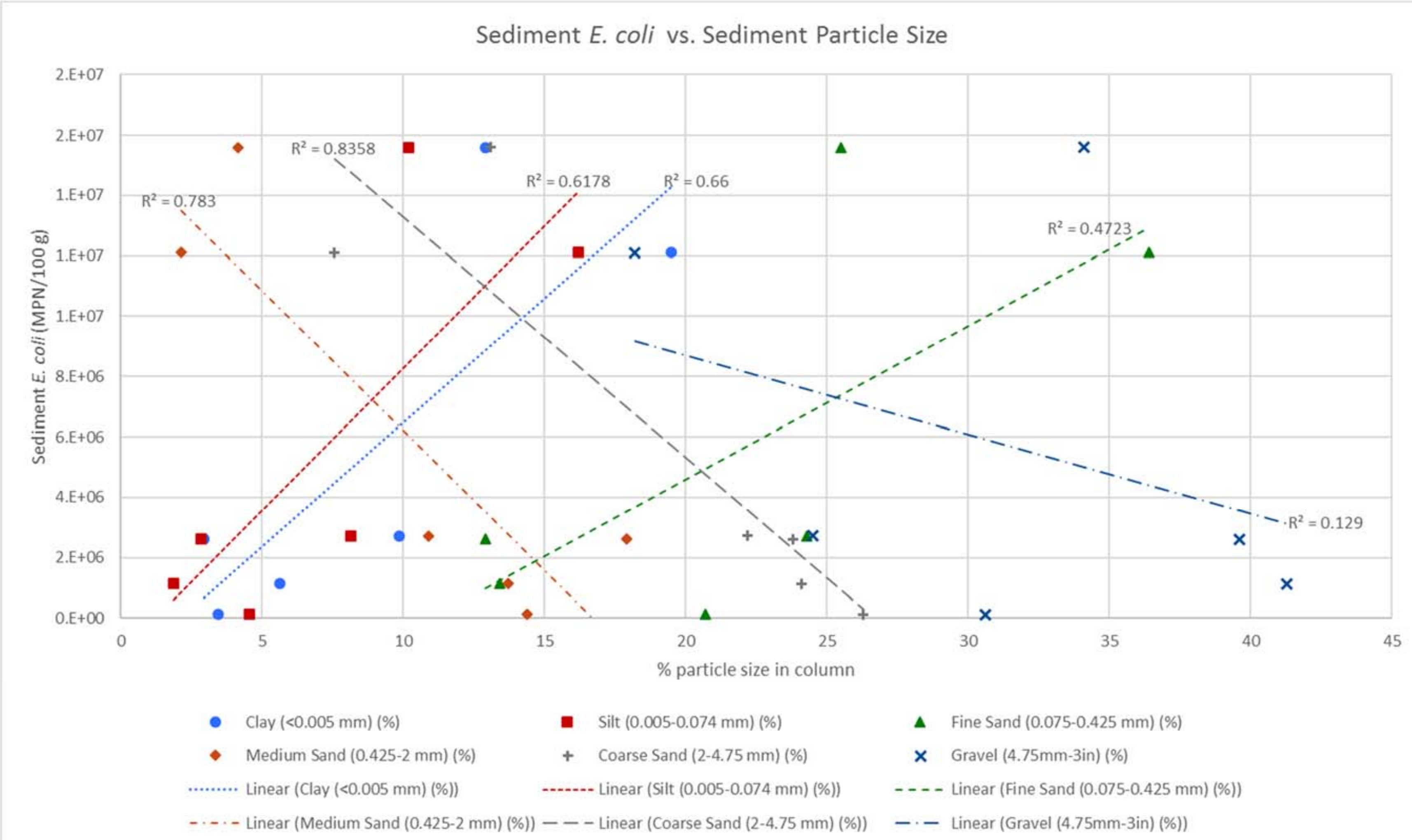


Figure 6. Sediment E. coli colony counts and sediment particle size partition correlations at the Katherine Rose Park site on Walnut Creek.

Conclusions

Core Concept

The primary objective of this study was to determine the impacts of sediment disturbance on the water column. Generally, the artificial disturbance events increased the observed *E. coli* count in the water column, with a few exceptions. Where increases were low or negative, substrates tended to be heavy in compacted clays (e.g., the Mountain Creek sites), and the highest increases tended to be in substrates comprised of coarser particles, including gravel, although additional data collection is needed to ascertain whether a specific coarser sediment particle size in particular (fine sand, coarse sand, gravel) has any outsized influences.

Additional Findings

In the discussion above, it was hypothesized that the heterogeneity between the three stations (in comparison with the two other watersheds) was the primary factor resulting in the lower multiple R-squared value that explained the variance in sediment *E. coli* as a factor of the sediment partitioning, as well as in the differences in p-values between Village Creek and the other two watersheds. Additional samples are needed to verify this hypothesis.

While some interesting associations between sediment *E. coli* and sediment particle size were apparent at the two Mountain Creek sites, sites within the other two watersheds showed no such correlations. If observed separately from the rest of the dataset, samples from the Mountain Creek sites suggest that resident sediment *E. coli* prefer finer particles (clay, silt, fine sand) over coarser particles (medium sand, coarse sand, gravel). Additional samples may also be beneficial to have for further comparison in this analysis, 1) to ensure that the Mountain Creek correlations are valid, and 2) to provide a larger sample size to the other two watersheds to perhaps determine if similar correlations exist.

Future Study Phases

Phase II: Additional habitat preferences investigations

During the FY 2020-2021 Contract period, the TRA staff will continue Phase 1 sampling until a minimum of 12 samples is collected at each site. TRA will also begin Phase 2 of this project during the FY 2020-2021 Contract period. Samples will be collected monthly at one site for a year. Samples will be collected in three areas within the stream reach at the site, which are an area of predominately gravel, an area of predominately sand, and an area of predominately fine sediment.This additional information should provide some further insight into sediment habitat preferences amongst *E. coli* within a single site, thus removing an additional variable seen in the original dataset.

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