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

PREPARED IN COOPERATION WITH THE TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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Introduction

From March 2018 to January 2019, the Trinity River Authority Clean Rivers Program conducted a study to determine the extent to which sediment *E. coli* impacted water column concentrations. At the conclusion of that study, it was determined that additional sampling was needed in order to increase the size of the data set to strengthen the results and conclusions that were reported. The original report for the Sediment *E. coli* Phase 1 project can be found on the Trinity River Authority Basin Planning Reports webpage.

This document serves as an addendum to that report. The monitoring stations and methodology used were as described for the additional sampling that took place from July 2019 to January 2021.

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

With the additional monitoring, sampling staff collected 12 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 84 *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 sediment samples containing a '<' flag for *E. coli* or total organic content 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. For water column *E. coli* and the remaining parameters, any data with a '<' flag was censored to one-half of the value.

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

- Pre-disturbance water column *E. coli*: 84 results;
- Post-disturbance water column *E. coli*: 84 results;
- Sediment *E. coli*: 73 results;

- Total Solids: 84 results;
- Total Organic Carbon: 83 results; and
- Clay, Silt, Fine Sand, Medium Sand, Coarse Sand, Gravel: 84 results each.

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 191% to >5700% at specific stations over the project lifetime, with a particularly notable increase of more than 27,000% for one sample at site 13261 (Table 1**Error! Reference source not found.**).

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	1900.00	-52.94	211.19
16434	MC @ US 287	5090.91	-25.00	699.22
21759	Quil Miller @ CR 532	1018.77	4.87	300.71
10798	Unnamed Trib of Lake Arlington	8658.57	228.81	2290.28
10786	VC at Rendon Rd	1119.08	-35.48	191.38
13621	WC @ Matlock	27420.00	-23.03	5765.98
21990	WC at Katherine Rose	6111.54	5.08	1073.94

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. The two Mountain Creek sites also had very low pre-disturbance *E. coli* concentrations. Perhaps due to the compacted clay system as well. In addition, Village Creek at Rendon Road and Walnut Creek at Matlock had several instances of no increase or decreases. This Village Creek site had the highest percentage of coarse sand and gravel and a predominately bedrock system in the immediate stream bed which may have contributed to low *E. coli* concentrations. It is unknown why the Walnut Creek site at Matlock had low concentrations. There did not seem to be anything remarkable about the site as compared to the other sites in regard to the sediment makeup (Table 2). However, approximately 3.5 kilometers of the stream runs through a golf course immediately upstream of the sampling site; the management practices of which may have impacted the results.

Table 2: Observations of pre- and post-disturbance E. coli and sediment particle sizes.

Station	Site	Pre-Disturbance *E. coli* Geomean (MPN/100 mL)	Post-Disturbance *E. coli* Geomean (MPN/100 mL)	Relative Percent Increase	Sediment *E. coli* Geomean (MPN/100 g)	Average % Clay & Silt (<0.005-0.074 mm)	Average % Fine & Medium Sand (0.075-2 mm)	Average % Coarse Sand & Gravel (2 mm-3 in)
13622	MC @ FM 157	16	25	58%	226,866	52.74	18.89	28.36
13643	MC @ US 287	57	189	229%	58,055	37.56	25.52	37.64
21759	Quil Miller @ CR 532	162	466	188%	154,381	4.88	23.23	71.87
10798	Unnamed Trib of Lake Arlington	257	3,615	1304%	186,832	6.00	25.31	68.72
10786	VC at Rendon Rd	51	100	95%	54,873	4.11	16.35	79.51
13621	WC @ Matlock	103	991	860%	161,673	8.00	37.20	54.77
21990	WC at Katherine Rose	276	1,205	337%	274,386	16.38	30.78	52.74

Multiple-Variable Regression

Multiple-variable regression was 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. It is interesting to note here that the sediment *E. coli* concentration in the Walnut Creek at Matlock site can be almost entirely attributed to the sediment partitioning (Table 3). Sediment *E. coli* concentrations at this site can also be largely attributed to sediment total solids and total organic carbon (Table 4). Similar to the assumption made in the previous section, there was nothing of note about this site to indicate why the relationship between the sediment makeup and *E. coli* levels was so strong. Across all seven sites, the average percent total solids ranged from 44.5 to 85.4% and from 3,359 to 20,056 mg/kg total organic carbon. The values for each of these parameters at Walnut Creek at Matlock were the medians at 74.7% and 4,479 mg/kg, respectively.

The Unnamed Tributary of Lake Arlington had the highest relative percent increase in post-disturbance *E. coli*, as well as, the second highest pre-disturbance and highest post-disturbance *E. coli* concentrations (Table 2). As shown in Table 3 and Table 4, the variation due to sediment partitioning was relatively high while the variation due to total solids and total organic carbon was not as high. However, these relationships were not extremely strong as indicated by the higher ANOVA P-values. This site has been observed to have consistent flow even during extremely dry weather conditions. The watershed is relatively small in comparison to the other sites. Additionally, the stream runs through a densely developed residential area. This seems to indicate that there may have been a constant input of flow containing *E. coli* that supplemented the sediment *E. coli* populations. Sources may include runoff from over-irrigated residential yards with pets, wildlife in the wooded area immediately upstream of the site visiting the stream for food and water, leaking or broken wastewater pipelines, and illicit connections to storm drains.

Table 3: Regression statistics for multiple regression analysis of sediment partitioning.

Station	**Site**	**Multiple R**	**R Square**	**Adjusted R Square**	**Standard Error**	**Observations**	***ANOVA P-Value***
13622	MC @ FM 157	0.6478	0.4196	-1.3216	15462461	9	0.9261
13643	MC @ US 287	0.9088	0.8259	0.3034	1577725	9	0.4367
21759	Quil Miller @ CR 532	0.7224	0.5219	-0.1953	2952298	11	0.6540
10798	Unnamed Trib of Lake Arlington	0.8630	0.7448	0.3620	2206556	11	0.2705
10786	VC at Rendon Rd	0.7853	0.6167	-0.1498	303563	10	0.6260
13621	WC @ Matlock	0.9901	0.9804	0.9510	1170167	11	0.0022
21990	WC at Katherine Rose	0.8437	0.7118	0.3659	4199446	12	0.2228

Table 4: Regression statistics for multiple regression analysis of sediment Total Solids and Total Organic Carbon.

Station	**Site**	**Multiple R**	**R Square**	**Adjusted R Square**	**Standard Error**	**Observations**	***ANOVA P-Value***
13622	MC @ FM 157	0.5409	0.2926	0.0568	9855906	9	0.3540
13643	MC @ US 287	0.4154	0.1725	-0.1033	1985591	9	0.5666
21759	Quil Miller @ CR 532	0.1941	0.0377	-0.2029	2961609	11	0.8576
10798	Unnamed Trib of Lake Arlington	0.5850	0.3422	0.1777	2505036	11	0.1873
10786	VC at Rendon Rd	0.3360	0.1129	-0.1828	325179	9	0.6980
13621	WC @ Matlock	0.6312	0.3984	0.2480	4582938	11	0.1310
21990	WC at Katherine Rose	0.5340	0.2851	0.1262	4929714	12	0.2208

Single-variable Regressions

Sediment *E. coli* counts were indirectly correlated to clay and silt at the two Mountain Creek sites with the correlations being slightly weaker at the upstream station 13622 (Table 5). As stated previously, much of this system appeared to be hard packed clay and may have inhibited the ability of *E. coli* to populate the sediments. The Quil Miller and Unnamed Tributary of Lake Arlington sites had relatively strong indirect correlation between sediment *E. coli* and coarse sand and a weak direct correlation with medium sand at the Village Creek site. The Walnut Creek sites demonstrated direct correlations with fine sand, silt, and clay and indirect correlations with the larger particles. Here again, Walnut Creek at Matlock stands out with the strongest correlations to particle sizes. However, as discussed in previous sections, this site is a bit of an anomaly because there were several samples with post-disturbance *E. coli* levels that were lower than pre-disturbance samples. Overall, correlations to total organic carbon were rather weak.

Table 5: Correlation analysis for sediment E. coli against sediment parameters.

Parameter	13622-MC @ FM 157	16434-MC @ US 287	21759-Quil Miller @ CR 532	10798-Unnamed Trib of Lake Arlington	10786-VC at Rendon Rd	13621-WC @ Matlock	21990-WC at Katherine Rose
Total Solids	0.5384	0.4153	0.1729	-0.4556	0.2122	-0.6106	-0.4987
Total Organic Carbon	-0.4762	-0.2551	-0.0636	-0.1155	-0.3157	0.2239	0.2228
Clay	-0.3585	-0.4777	-0.0403	0.3874	0.1705	0.8821	0.4228
Silt	-0.3601	-0.4919	0.2557	0.2931	-0.3036	0.8955	0.3577
Fine Sand	0.0747	-0.3898	0.3132	0.3066	-0.0104	0.8058	0.2490
Medium Sand	0.1970	0.7018	0.2405	-0.1500	0.4373	-0.3698	-0.3229
Coarse Sand	0.2375	0.2096	-0.5418	-0.5244	0.2296	-0.7674	-0.4218
Gravel	0.4761	0.5260	0.1034	0.0788	-0.3497	-0.7198	-0.1477

Conclusions

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. This illustrates the importance of ensuring that water quality samples are collected in such a way that sediments are not influencing those samples. It also shows that sediment *E. coli* populations can and do contribute to water column *E. coli* concentrations. This will be important to consider in the future when analyzing data. Spikes in *E.*

coli concentrations may not be solely due to runoff from the surrounding and upstream watershed. A portion of the load is very likely to be from sediments that have been stirred up during elevated flow events. Additionally, disturbance of the sediments due to construction, animal activity, or human recreation can contribute *E. coli* to the water column under any flow regime.

Outside of the two Mountain Creek sites which had high levels of clay in the system, there were no consistent patterns observed between finer or coarser sediments and sediment *E. coli* counts. It does appear that the relationship between sediment particle size or organic carbon content and sediment *E. coli* counts may be stream specific as similar patterns can be seen between the upstream and downstream stations on Mountain Creek and Walnut Creek.

It is clear from this data analysis that sediment particle size is not the only, or even most important, factor in sediment *E. coli* concentrations. It seems that there are other factors not included in this study that may have more influence such as sediment nitrogen and phosphorus content that could increase populations. Any number of chemicals that find their way into streams may have an affect on the mortality of bacteria; car washing soaps, antibiotics from agricultural animal waste or septic system drain fields, and chlorine from improper pool draining to name a few. These compounds themselves may have an affinity to particular sediment sizes that may then explain some of the results seen in this report. These ideas certainly warrant further study. However, the main goal of this study was to determine if, and to what extent, sediment *E. coli* populations influence water column *E. coli* concentrations. As a whole, sediment *E. coli* populations do increase water column *E. coli* concentrations. To what extent is more difficult to define and may be stream or site specific or related to factors beyond the scope of this study.