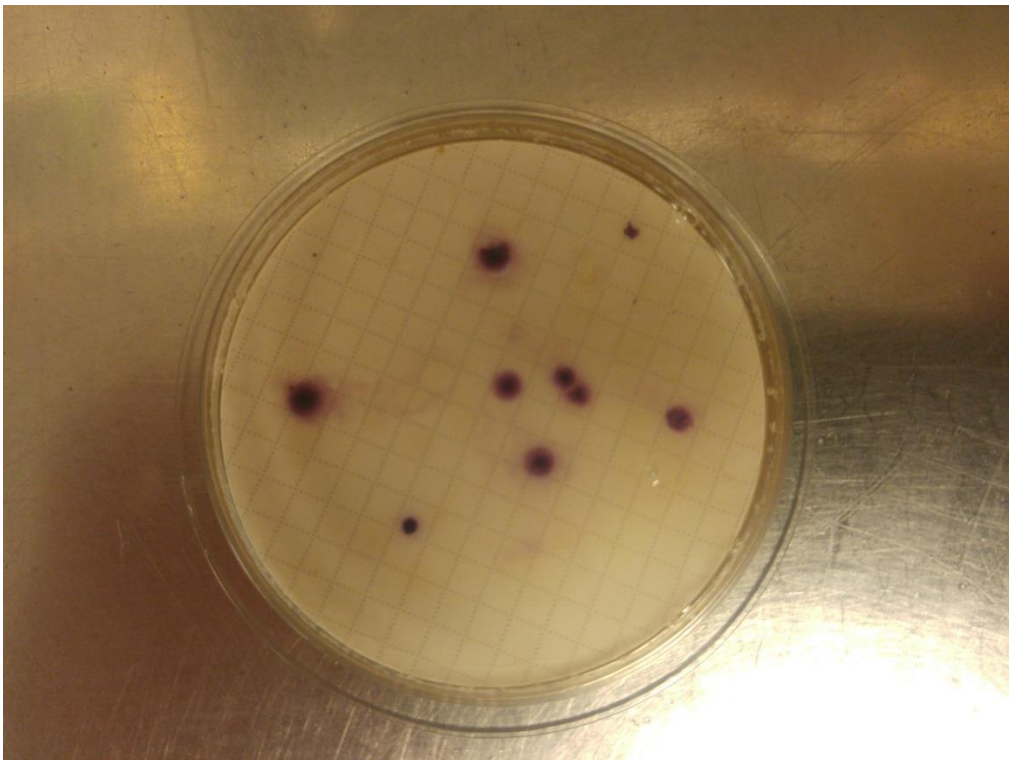
Bacterial Source Tracking to Support the Implementation of the Plum Creek Watershed Protection Plan

Final Report
Contract #TMDL-2016-13003-31056
TSSWCB Project 16-61



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Introduction

Plum Creek rises in Hays County north of Kyle and runs south through Caldwell County, passing Lockhart and Luling, and eventually joins the San Marcos River at their confluence north of Gonzales County. Plum Creek is 52 miles in length and has a drainage area of 389 mi^2. According to the *2008 Texas Water Quality Inventory (TWQI) and 303(d) List*, Plum Creek (Segment 1810) is impaired by elevated bacteria concentrations (category 5c) and exhibits nutrient enrichment concerns for ammonia, nitrate+nitrite nitrogen and total phosphorus. In the *2012 TWQI and 303d List*, the Texas Commission on Environmental Quality (TCEQ) recognized the work being done in the Plum Creek watershed to reduce the pollutant loading and restore the water quality and changed the stream's assessment category to 4b. The most recent 2014 TWQI and 303d List confirms that the original impairments and concerns are still present.

The Texas State Soil and Water Conservation Board (TSSWCB) and Texas AgriLife Extension Service established the Plum Creek Watershed Partnership (PCWP) in April 2006. The PCWP Steering Committee completed the *Plum Creek WPP* in February 2008. Information about the PCWP is available at http://plumcreek.tamu.edu/. Sources of pollutants identified in the Plum Creek WPP include urban storm water runoff, pet waste, failing or inadequate on-site sewage facilities (septic systems), wastewater treatment facilities, livestock, wildlife, invasive species (feral hogs), and oil and gas production.

Originally, the Plum Creek WPP was to be developed using only existing water quality data. However, discussions with stakeholders identified data gaps which would make source identification and establishment of water quality goals difficult. Accurate source identification is key to prioritizing implementation projects for funding. Through TSSWCB project 03-19, *SWQM to Support Plum Creek WPP Development*, the Guadalupe-Blanco River Authority (GBRA) collected water quality data to fill the identified data gaps.

Facilitated by the Plum Creek Watershed Coordinator (TSSWCB Projects 11-07 and 14-10), implementation of the Plum Creek WPP continues. TSSWCB projects 08-07 *Implementing Agricultural Nonpoint Source Components of the Plum Creek WPP* provided technical assistance and financial incentives through the local soil and water conservation districts to agricultural producers in developing and implementing WQMPs. That assistance continues in TSSWCB Project 13-06 *Implementing Agricultural Nonpoint Source Components of the Plum Creek Watershed Protection Plan*. In order to reduce feral hog impacts on the stream, education and technical assistance was provided, through project 08-07, by Texas AgriLife Extension Service to landowners in the watershed on strategies to reduce and manage feral hog populations. Feral hog education and technical assistance is currently available in the Plum Creek Watershed

through TSSWCB projects 12-06 *Statewide Delivery of Lone Star Healthy Streams Feral Hog Component and Providing Technical Assistance on Feral Hog Management in Priority Watersheds*, and 14-12 *Enhancing Feral Hog Management through Statewide Implementation of Lone Star Healthy Streams*. The cities of Kyle and Lockhart received TCEQ CWA §319(h) funding to retrofit detention facilities to improve water quality, educate and stencil storm sewer inlets, map existing storm water facilities, implement a dog waste collection station program, and coordinate city "housekeeping" activities designed to improve water quality (street sweeping, creek cleanup days, etc). Additionally, Lockhart evaluated their existing storm water system, identified and prioritized upgrades to the city's storm water management system, and coordinated creek cleanup days, and household hazardous and electronic waste collection days. An education and outreach campaign was initiated during the watershed planning process that focused on educating watershed residents and landowners on the impacts of specific land use activities, illegal dumping, proper operation and maintenance of OSSFs and proper disposal of pet waste. The City of Kyle implemented a storm water management program that included improvements to storm water retention ponds. The City of Lockhart mapped the storm system. Using these maps, GBRA conducted illicit discharge detection monitoring on the city's storm system in 2015 (*Plum Creek Watershed Protection Plan (WPP) Implementation – Illicit Discharge Monitoring* **(***TCEQ CWA Project No. 582-14-43865))*. Both cities have included public education and outreach in their programs. Monitoring sites downstream of these two cities will collect base flow data as well as flows impacted by storm water.

To demonstrate improvements in water quality, the Plum Creek WPP describes a water quality monitoring program designed to evaluate the effectiveness of BMPs implemented across the watershed and their impacts on in-stream water quality. Bacterial Source Tracking (BST) surface water quality monitoring (SWQM) data will be used as an added component in the adaptive management of the WPP in order to evaluate progress in implementing the Plum Creek WPP and achieving water quality restoration. Sampling locations and frequencies are located so that the effectiveness of BMPs implemented in the watershed can be assessed. Data collected under previous and ongoing SWQM projects (TSSWCB project 03-19, 10-54, 10-07 and 14-11 & 17-58) will be used along with data from this project as background for comparison of data collected after BMPs have been implemented. Additionally, monitoring sites have been located so that other BMPs that are recommended in the PC WPP, such as conversion of septic tanks to public wastewater system collection systems, feral hog control and water quality management plans on agricultural lands within the watershed, can be assessed for their impacts on in-stream water quality as well as their progress in achieving water quality restoration.

Prior to this monitoring, funds have not been directed toward conducting a BST study for the watershed. The Plum Creek WPP is now in its 9th year of implementation and the PCWP Steering Committee believes strongly that a bacterial source tracking (BST) study for the watershed is a critical component at a critical time for the Plum Creek WPP. BST will provide key insight into the effectiveness of current management practices and will allow planners and

cooperating entities in the Plum Creek watershed to develop more targeted strategies to address bacteria loading from nonpoint source pollution.

The purpose of this Technical Report is to clearly delineate the contributions and distribution of the E. coli monitoring results collected during the course of this project. This report may be used by the Plum Creek Watershed Protection Steering Committee to inform decisions regarding the targeting and effectiveness of best management practices (BMPs) and implementation activities in the Plum Creek watershed.

Project Overview

Through this project, GBRA collected Bacterial Source Tracking (BST) surface water quality monitoring (SWQM) data to characterize the Plum Creek watershed. The sampling regime included monthly BST samples to be collected at current routine and targeted SWQM sampling locations throughout the watershed over a one year period. Monitoring data will be used along with data from the ongoing Clean Rivers Program (CRP) & Plum Creek SWQM programs to assess and evaluate the effectiveness of existing and future best management practices (BMPs) implemented in the watershed as a result of the Plum Creek WPP. This data will provide a more complete and representative data set to characterize the Plum Creek watershed, target future BMPs, and document water quality improvements.

GBRA conducted the work performed under this project including technical and financial supervision, preparation of status reports, coordination with local stakeholders, SWQM sample collection and analysis, and data management. GBRA participated in the PCWP, Steering Committee, and Technical Advisory Group in order to communicate project goals, activities and accomplishments to affected parties. GBRA will continue to host and maintain an Internet webpage http://www.gbra.org/plumcreek/ for the dissemination of information.

Currently, routine ambient water quality data is collected monthly at three main stem stations on Plum Creek by GBRA (Site nos. 17406, 12640 and 12647) through CRP. This project allowed GBRA to conduct routine BST monitoring monthly at the 3 CRP stations and at 2 additional TSSWCB PC WPP implementation project targeted monitoring stations (20484 & 12556) in the upper and lower watershed over a 12 month period. When conditions allowed, the BST sampling occurred at the same time as existing monitoring for field, conventional, flow and bacteria parameter groups in order to reduce travel and labor costs. GBRA made an effort to collect samples during both wet and dry conditions. GBRA collected a total of 60 BST samples altogether. GBRA processed and enumerated the samples for shipment using the EPA 1603 Modified mTEC Method of Analysis. Once processed, the stabilized samples were shipped overnight to the Texas A & M AgriLife Research Soil and Aquatic Microbiology Lab (TAMU SAML) for final BST analysis. This BST monitoring complemented the existing routine and targeted ambient monitoring regime conducted by GBRA in the Plum Creek watershed.

GBRA posts monitoring data to the GBRA website quarterly. GBRA will summarize the results and activities of this project through inclusion in GBRA's CRP Basin Highlights Report and/or

Basin Summary Report. Additionally, the results and activities of this project were summarized in quarterly reports to the stakeholders of the PCWP Steering Committee and in revisions to the Plum Creek WPP. GBRA also developed this Technical Data Report to summarize the results from the BST *E. coli* enumeration water quality data collected through Task 3 of the work plan. This report shall, at a minimum, provide an assessment of the water quality results with respect to the concentration and distribution of *E. coli* bacteria throughout the watershed. This data may be used for measuring effectiveness of BMPs implemented and to spur discussion of interim progress in achieving the Plum Creek WPP water quality goals. The technical report will be transferred to existing stakeholder groups within the watershed by GBRA and the Plum Creek Watershed Coordinator (PCWC) to be utilized by stakeholders to revise and refine current management practices to achieve the goals outlined in the Plum Creek WPP.

Methods

Quality Assurance Project Plan

Water quality data was collected under an approved QAPP. The objective of the quality assurance task was to develop and implement data quality objectives (DQOs) and quality assurance/control (QA/QC) activities in order to ensure data of known and acceptable quality are generated through this project. The QAPP was signed by all parties and approved by the TSSWCB in September of 2016. No amendments to the QAPP were made throughout the course of the twelve month sampling period.

Sample Collection Methodology

GBRA field staff collected monthly bacterial source tracking samples from the five stations identified on the map in Appendix A of this document over a one year monitoring period. The GBRA followed the bacteria sample collection procedure specified in the TCEQ *Surface Water Quality Monitoring Procedures Manual, Volume 1: Physical and Chemical Monitoring Methods, RG-415, August 2012*. The GBRA field staff collected a 100 mL sterile bacteria bottle monthly at all five monitoring stations. The bacteria bottle was collected from the centroid of the flow in the stream. The sample bottle was dipped into the water at 0.3 meters of depth with the open mouth of the bottle oriented upstream of the sampler. The collection bottles were immediately placed into an iced cooler and transported back to the GBRA laboratory for subsequent *E. coli* analysis within 8 hours of collection.

Laboratory Analysis Methodology

The GBRA laboratory staff utilized *EPA Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC), December 2009*, for the enumeration of *E. coli* on the samples collected for this project. The portion of the collected water samples was filtered through a 0.45 micron gridded membrane filter, and the filter was subsequently placed on a plate of modified m-TEC agar growth media. The modified m-TEC plate was inverted and incubated at 35.0°C +/- 0.5°C for 2, +/- 0.5 hours and then the plates were transferred into a water tight bag and incubated in a 44.5°C +/- 0.2°C water bath for an additional 22 +/-2 hours. Distinguishable red-magenta colored *E. coli* colonies were identified and counted. The GBRA laboratory standard operating procedure for this method was developed as a part of a previous TSSWCB project *10-07 Surface Water Quality Monitoring Project and Additional Activities to Support the Implementation of the Plum Creek Watershed Protection Plan*. The GBRA laboratory has held TNI accreditation for this parameter through the TCEQ since the second quarter of FY 2013. In addition the quality assurance protocols outlined in the method, maintaining accreditation of this parameter requires biennial audits of the laboratory by the TCEQ, annual internal laboratory audits and biannual blind proficiency testing of unknown standards. Procedures for any other laboratory analysis performed by GBRA as part of the TCEQ GBRA Clean Rivers Program or TSSWCB Implementation monitoring project QAPPs referenced in this document were in accordance with the most recently published or online edition of *Standard Methods for the Examination of Water and Wastewater*, or the most recent version or other reliable procedures acceptable to TCEQ. The GBRA Regional Laboratory analyzed the samples under the associated QAPP in compliance with the TNI Standards and is accredited in accordance with NELAP requirements for matrix, method, parameter combinations listed in Table A7 of the referenced QAPP on the date the samples were processed for analysis. Copies of laboratory SOPs are retained by the GBRA and are available for review by TCEQ. Laboratory SOPs are consistent with EPA requirements as specified in the method.

Transfer of Samples to the TAMU SAML

The colonies on the enumerated E. coli plates were identified by the GBRA laboratory staff and shipped to the Texas A&M Soil and Aquatic Microbiology Laboratory (TAMU SAML) in College Station, Texas. The shipping procedure was outlined in the SAML-12-105 Standard Operating Procedure titled "*Cultivation of E. coli from Samples and Pre-Processing for Isolation and Bacterial Source Tracking*" included in Appendix B of this document. This procedure involved marking each positive *E. coli* colony on the outside of all enumerated media plates with a marker, along with the station identification, number of colonies counted and any dilution factors utilized. Each dilution plate was individually wrapped in Parafilm plastic. The wrapped plates were grouped by monitoring station in water tight, plastic whirl-pack bags. The bags were placed media side up into a cooler with ice to be shipped to the TAMU SAML laboratory within three days of initial processing. The GBRA laboratory bench sheet for the EPA 1603 test

analysis was emailed to the TAMU SAML laboratory to aid in sample plate identification. The TAMU SAML received the sample plates and performed ERIC-PCR, Riboprinting, and *Bacteriodales* PCR on the *E. coli* colonies received in order to determine possible sources of the bacteria colonies, as described in the TSSWCB Project 16-51 *Texas Bacterial Source Tracking Program for FY16-18* QAPP.

Data Transmittal and Information Transfer

As designated in the project work plan, data collected for this project was not transferred to the TCEQ Surface Water Quality Monitoring Information System (SWQMIS) database. All EPA 1603 sample results were transferred from the GBRA laboratory to the TAMU SAML on an electronic copy of the GBRA laboratory analysis bench sheet, via email. No Corrective Actions were generated by GBRA field or laboratory staff during the course of this project. If a problem occurred that threatened to result in a potential data loss, then every effort was made to resample the event in order to avoid a data loss. No data losses were recorded as a part of this project, but the Plum Creek at Plum Creek Road station did not have any detectable colonies during the 09/26/2016 collection event and therefore no colonies from this station event were submitted to the TAMU SAML for additional BST analysis. The June sample collection event was resampled due to a laboratory power outage that would have resulted in a data loss if the sample had not been recollected.

A critical part of the project is to disseminate information on Plum Creek and the project to stakeholders and other interested parties throughout the state. GBRA will include a summary of the results and activities of this project in GBRA's Clean Rivers Program *Basin Summary Report* in fiscal year 2018. Additionally, the results and activities of this project were summarized in quarterly reports to the stakeholders of the PCWP Steering Committee and in updates to the Plum Creek WPP.

Results and Observations

The GBRA conducted monthly bacterial source tracking sample collection events twelve times between the acceptance of the QAPP on 09/06/2017 and the end of the prescribed collection period on 08/31/2017. GBRA attempted to collect an even distribution of sampling events during dry and wet weather ambient conditions. In order to maximize efficiency, the GBRA attempted to collect all BST project samples at the same time as CRP and TSSWCB implementation monitoring project routine and targeted sampling events. *E. coli* bacteria as analyzed by EPA Method 1603 was the only water quality monitoring parameter collected for this TSSWCB 16-61 project. The collection of CRP and TSSWCB implementation monitoring samples on the same days as this project allowed for the additional correlation analysis of the data collected for this project with the traditional IDEXX Colilert 18 Quanti-Tray *E. coli* method analysis data acquired as a part of these other monitoring projects. Rainfall totals from seven days prior to the sample collection events were calculated from the closest NOAA Weather

station at the Austin Bergstrom Airport in order to assist with determining the influence of non-point source runoff resulting from rainfall.

The monthly BST sample collection events for this project did not always coincide with the other routine and targeted monitoring in the watershed or fall in an equal distribution pattern of wet and dry ambient weather conditions due to conflicting work plans and data collection objectives. Clean Rivers Program routine sample collection events at the Plum Creek at CR 135 (Station 12640), Plum Creek at CR 202 (Station 12647), and Plum Creek at Plum Creek Road (Station 17406) monitoring stations were captured at the same time as BST sampling events a total of nine times (83.3% of samples collected) during the course of this project. TSSWCB implementation monitoring routine sample collection events at the Clear Fork at Salt Flat Road (Station 12556) were captured at the same time as BST sampling during eleven events (91.7% of samples collected). The TSSWCB implementation targeted monitoring at the Plum Creek at Heidenreich Lane (Station 20484) occurred at the same time as BST sampling during eight events (66.7% of samples collected). Due to variable weather patterns, monthly BST collection events were not collected in a perfectly even ratio of dry and wet weather ambient weather conditions. A total of seven of the twelve BST collection events fell during wet weather conditions (58.3% of samples collected), while only five events fell under dry weather conditions (41.7% of samples collected). These factors limited the amount of data available for correlation analysis.

Following the BST sampling event collected during the month of June, a lightning strike hit the GBRA laboratory. The laboratory water bath that was being used to incubate the EPA 1603 *E. coli* samples collected on 06/05/17 lost power during the incubation cycle. The laboratory results from these samples were disqualified by the GBRA laboratory and the samples were not transferred to the TAMU SAML laboratory for further analysis. GBRA resampled the June BST samples on 06/12/2017, under similar weather conditions, in order to avoid a data loss, but the *E. coli* samples could no longer be directly correlated with any of the field, flow or conventional data from other monitoring projects collected on 06/05/17.

The TCEQ contact recreation standard for the maximum allowable geometric mean of *E. coli* is 126 cfu/100 mL. The geometric mean of *E. coli* concentrations from every station collected during this project exceeded the standard. The GBRA compared the geometric mean concentrations of the bacteria samples analyzed by EPA method 1603 during this project with other *E. coli* samples that were also collected at the same locations and time, but analyzed with the IDEXX Colilert 18 Quanti-Tray method. The geometric means of the samples analyzed with the IDEXX Quanti-Tray method appeared slightly higher than those analyzed with the EPA 1603 method. This difference in geometric means appeared to be largely due to the limited number of events in the IDEXX Quant-Tray analysis data set, because this parameter was only captured during CRP or TSSWCB implementation monitoring events, outside of the scope of this project. In preparation for a T-test comparison, an F-Test was performed on the data from each station to determine if the variances between the two sample sets were equal. The F test showed that the variances between the EPA 1603 and IDEXX Colilert 18 Quanti-Tray data were not equal at

station 17406 (F statistic = 3.82 & F Critical = 3.02) & station 20484 (F statistic = 4.43 & F Critical = 3.60) because the F statistic was found to be greater than the F critical value at the p=0.05 significance level. The sample means from both E. coli analysis methods collected during concurrent events were compared by a t-test assuming unequal variance for stations 17406 and 20484 and equal variance for all other stations. The T-test showed that the difference between the sample means of each data set at the p=0.05 significance level were not significantly different and therefore these two testing methodologies give essentially similar results for all five monitoring stations.

Table 1. Geometric mean concentrations of *E. coli* analyzed by EPA 1603 and IDEXX Colert-18 Quanti-Tray.

BST Station	Median Streamflow (cfs)	EPA 1603 *E. coli* Geomean (cfu/100 mL)	Total Number of EPA 1603 *E. coli* Samples	IDEXX *E. coli* Geomean (MPN/100 mL)	Total Number of IDEXX *E. coli* Samples	Relative % Difference Between Methods
12640 – Plum Creek at CR 135	45	445	12	447	10	0.45%
12556 – Clear Fork at CR 128	11	579	12	591	11	2.05%
12647 – Plum Creek at CR 202	44	875	12	1241	10	34.59%
17406 – Plum Creek at Plum Creek Road	17	788	12	990	10	22.82%
20484 – Plum Creek at Heidenreich Lane	9.4	1482	12	2598	8	54.71%

All concentrations were greater than the TCEQ contact recreation standard of 126 cfu/100 mL of *E. coli*.

Figure 1. Geometric mean concentrations for the EPA 1603 and IDEXX Quanti-Tray E. coli methods at all five bacterial source tracking monitoring stations.

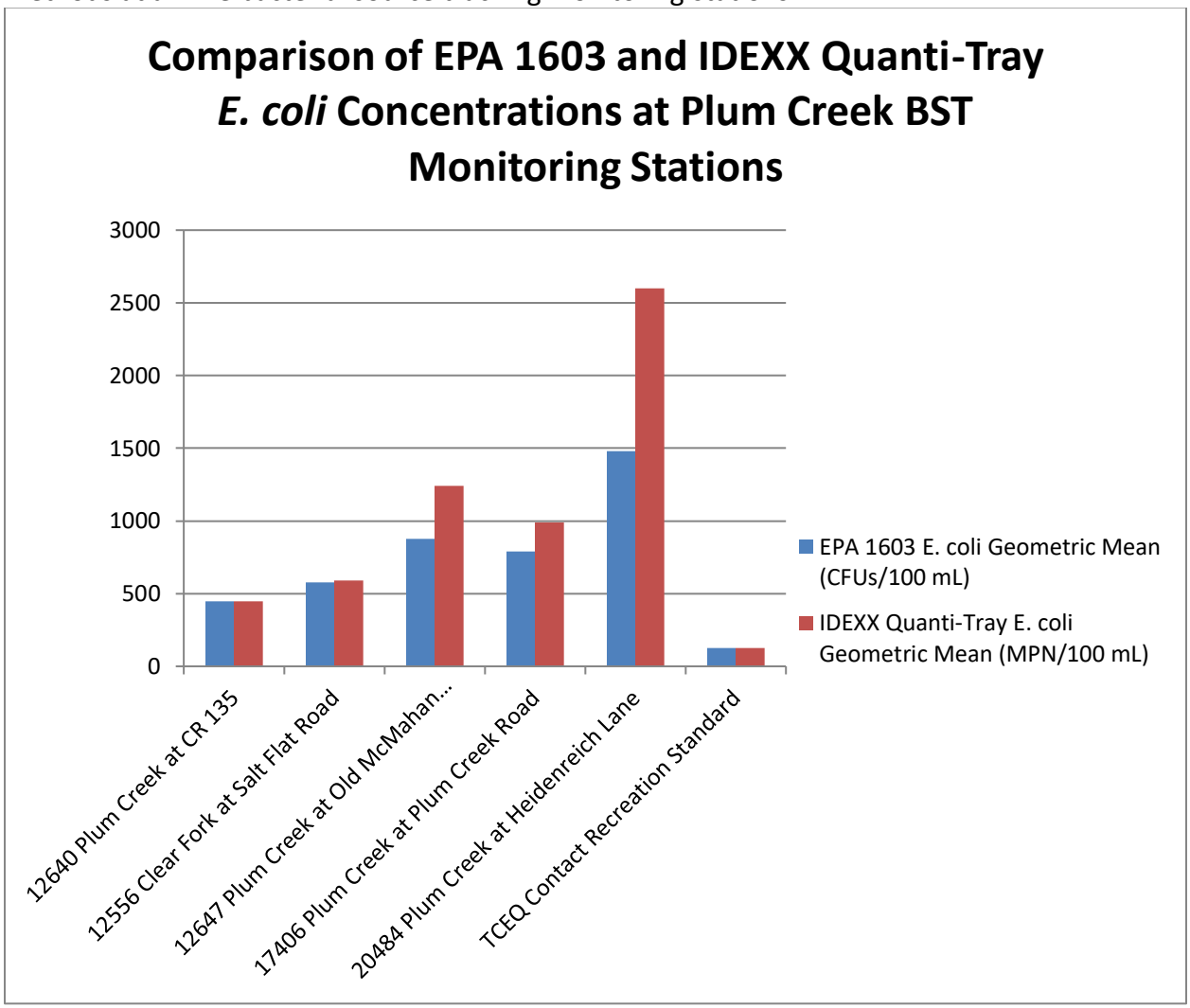


Table 2. Geometric Mean Concentrations of *E. coli* analyzed by EPA 1603 and IDEXX Colert-18 Quanti-Tray during wet weather conditions.

BST Station	Median Streamflow (cfs) – Wet Weather	EPA 1603 *E. coli* Geomean (cfu/100 mL) – Wet Weather	Total Number of EPA 1603 *E. coli* Samples – Wet Weather	IDEXX *E. coli* Geomean (MPN/100 mL) – Wet Weather	Total Number of IDEXX *E. coli* Samples – Wet Weather
12640 – Plum Creek at CR 135	244	784	7	887	6
12556 – Clear Fork at CR 128	29	1332	7	1411	6
12647 – Plum Creek at CR 202	242	3189	7	3501	6
17406 – Plum Creek at Plum Creek Road	46	1423	7	1911	6
20484 – Plum Creek at Heidenreich Lane	34	2336	7	3341	5

All concentrations were greater than the TCEQ contact recreation standard of 126 cfu/100 mL of *E. coli*.

Table 3. Geometric Mean Concentrations of E. coli analyzed by EPA 1603 and IDEXX Colert-18 Quanti-Tray during dry weather conditions.

BST Station	Median Streamflow (cfs) – Dry Weather	EPA 1603 *E. coli* Geomean (cfu/100 mL) – Dry Weather	Total Number of EPA 1603 *E. coli* Samples – Dry Weather	IDEXX *E. coli* Geomean (MPN/100 mL) – Dry Weather	Total Number of IDEXX *E. coli* Samples – Dry Weather
12640 – Plum Creek at CR 135	23	202	5	160	4
12556 – Clear Fork at CR 128	4.4	180	5	208	5
12647 – Plum Creek at CR 202	13	143	5	262	4
17406 – Plum Creek at Plum Creek Road	6.0	387	5	369	4
20484 – Plum Creek at Heidenreich Lane	4.8	784	5	1709	3

All concentrations were greater than the TCEQ contact recreation standard of 126 cfu/100 mL of *E. coli*.

Table 4. Concentrations of all *E. coli* samples analyzed for bacterial source tracking analysis at all sampling stations.

Date	12640 – Plum Creek at CR 135 *E.coli* (CFU/100 mL)	12556 – Clear Fork at CR 128 *E.coli* (CFU/100 mL)	12647 – Plum Creek at CR 202 *E.coli* (CFU/100 mL)	17406 – Plum Creek at Plum Creek Road *E.coli* (CFU/100 mL)	20484 – Plum Creek at Heidenreich Lane *E.coli* (CFU/100 mL)
09/26/2016	1100	1600	2500	<20	2000
10/24/2016	88	110	92	310	1400
11/14/2016	460	260	37	310	360
12/12/2016	360	440	150	350	380
01/30/2017	430	440	230	800	350
02/20/2017	3200	15000	14000	8800	4100
03/13/2017	6800	2800	4800	600	400
04/03/2017	500	700	11000	5000	4300
05/08/2017	160	190	140	710	1300
06/12/2017	200	240	550	1000	590
07/24/2017	120	80	550	160	1300
08/07/2017	220	1500	22000	900	120000

Numbers with a yellow outline indicate that the concentration was greater than the TCEQ contact recreation standard of 126 cfu/100 mL of *E. coli*, while numbers with a green outline indicate concentrations below the standard.

Figure 2. Monthly *E. coli* concentrations with rainfall totals at Plum Creek at CR 135 (Station 12640)

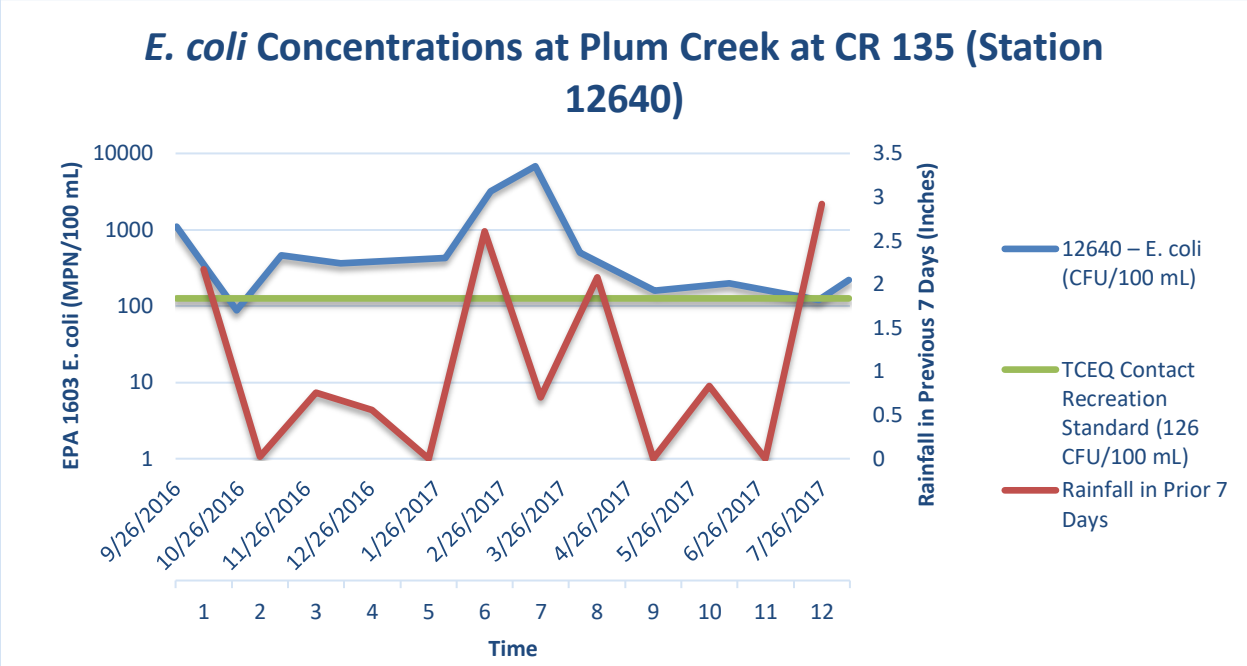


Figure 3. Monthly *E. coli* concentrations with rainfall totals at the Salt Branch at Salt Flat Road (Station 12556)

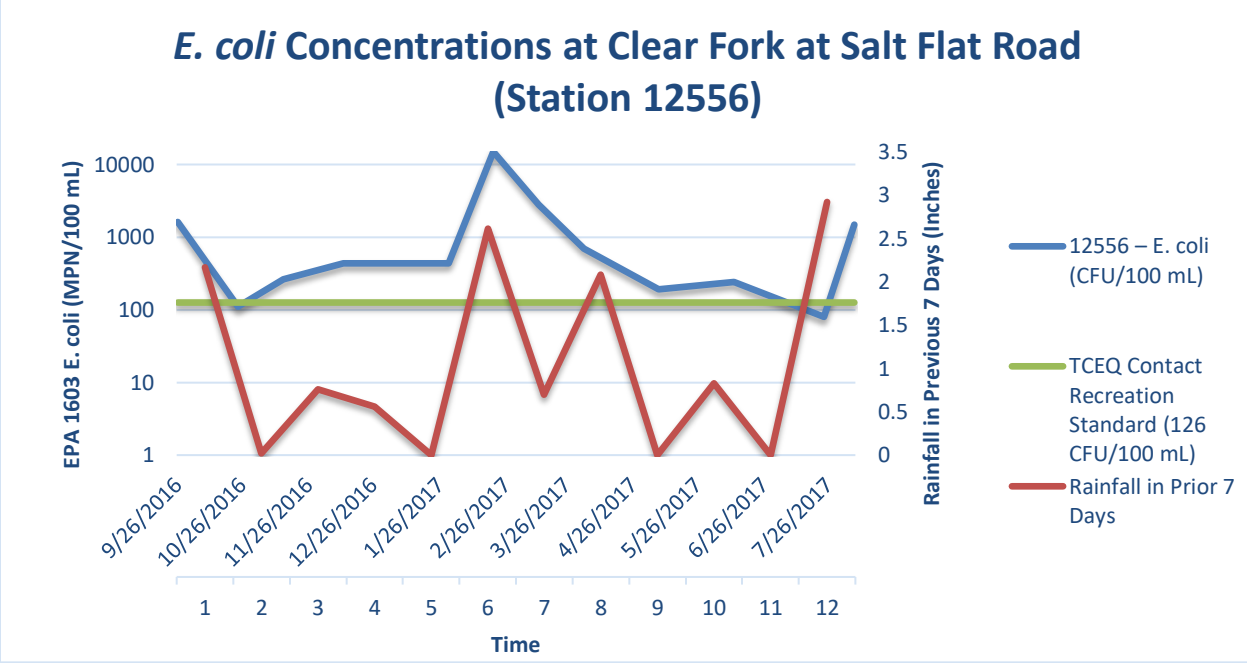


Figure 4. Monthly *E. coli* concentrations with rainfall totals at Plum Creek at CR 202 (Station 12647).

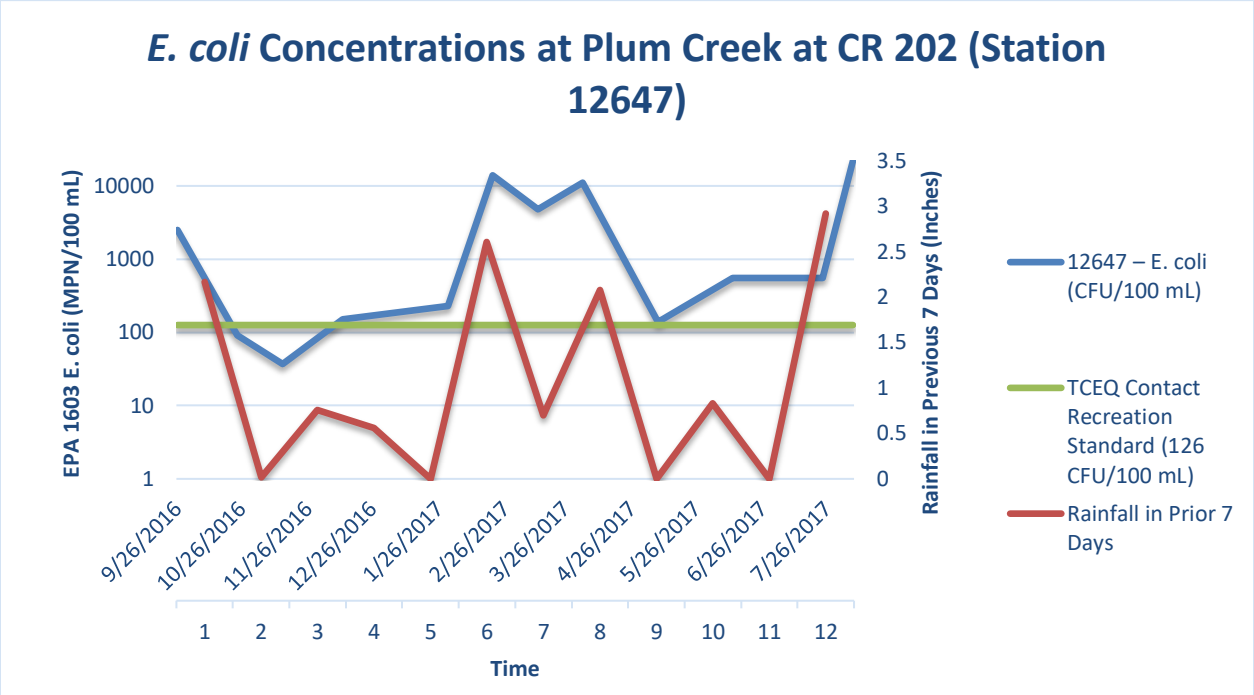


Figure 5. Monthly *E. coli* concentrations with rainfall totals at Plum Creek at Plum Creek Road (Station 17406).

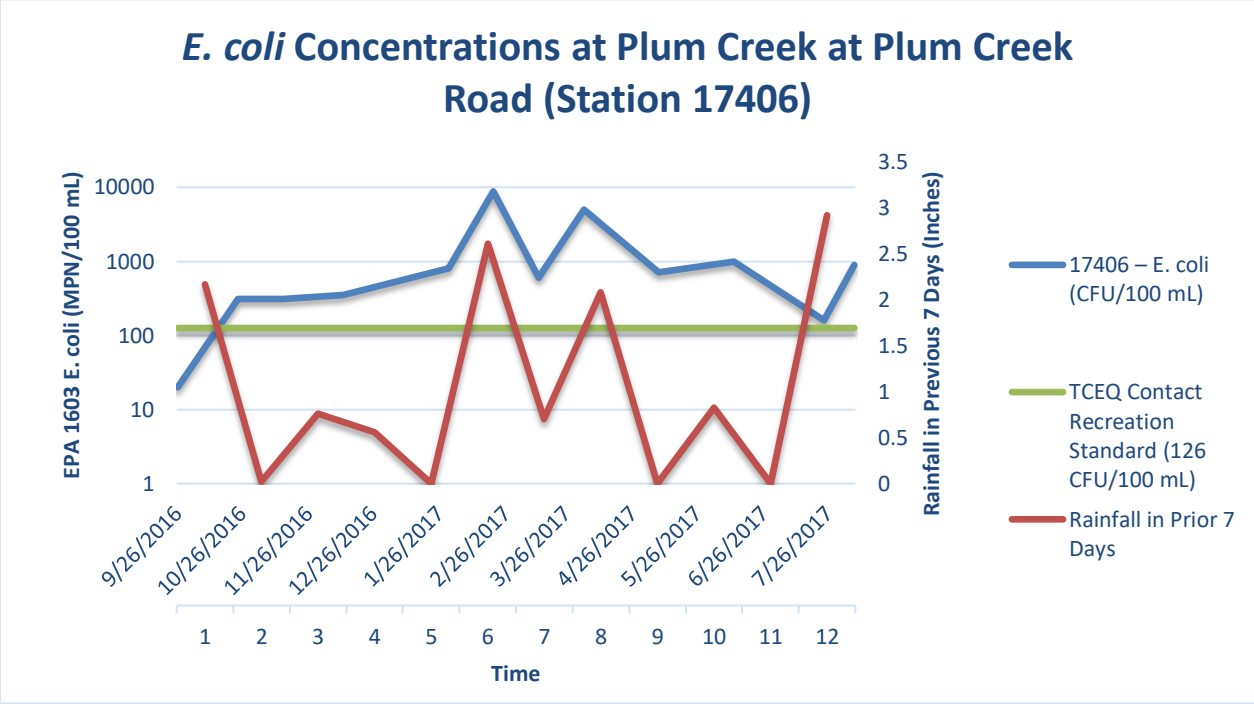
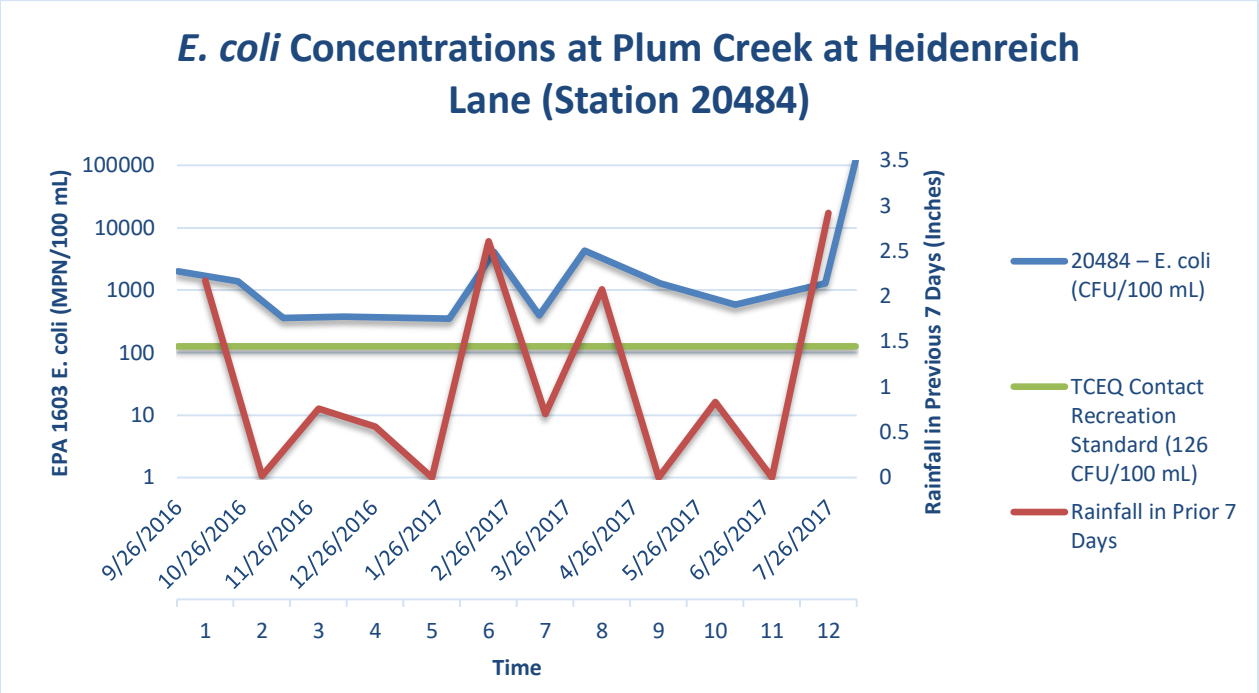


Figure 6. Monthly *E. coli* concentrations with rainfall totals at Plum Creek at Heidenreich Lane (Station 20484).



Discussion

The laboratory testing results of the *E. coli* bacteria at each of the five monitoring stations in the sample study design provided useful insights into the current state of impairment at each station. A t-test comparison of the *E. coli* concentrations from the EPA 1603 *E. coli* method utilized during this project with the traditional IDEXX Colilert 18 Quanti-Tray method has shown that both methods produce substantially similar results for samples in the Plum Creek Watershed. This study has identified the Plum Creek at Heidenreich Lane (20484) station, located in the upper portion of the watershed, as the source of the highest concentrations of *E. coli* in the watershed. The geometric mean for the Plum Creek at Heidenreich Station had nearly twice the concentration of the next downstream station (17406), and the largest single sample grab concentration collected throughout the project. This station had extremely high *E. coli* concentrations during all flow regimes. The monitoring stations located furthest downstream at the Plum Creek at CR 135 (12640) and the Clear Fork tributary (12556) had substantially lower *E. coli* concentrations than the stations upstream throughout the collection period of the project. The Plum Creek at CR202 (12647) station immediately downstream of the City of Lockhart showed the greatest variability during wet weather and dry weather conditions. This station had the highest concentrations during wet weather events and the lowest concentration during dry weather conditions. The variability of these results may indicate that this station is heavily influenced by non-point source runoff pollution and the bacterial source tracking analysis may be particularly useful at this station.

Design Limitations

The monthly temporal collection intervals and one year scope defined by the project may have biased the sampling events towards specific weather conditions. A number of rainfall events occurred during the collection period of the project and the monthly collection requirement may have inhibited the contribution of events targeted for dry weather conditions. A disproportionate number of wet weather influenced sample events were collected during the project and the *E. coli* bacteria that was cultivated for bacterial source tracking analysis may be biased towards sources that are not present in the streams during base flow conditions. The addition of flow, field and conventional monitoring parameters to the work plan would have provided more information to assist with identifying potential sources. An expansion of the project to additional contributing tributaries such as Porter Creek, Town Creek and the West Fork may have provided additional information about the sources of additional bacteria contributions.

Summary

The goal of this project was to collect and enumerate *E. coli* samples from multiple locations in the Plum Creek Watershed and submit the confirmed colonies to the TAMU SAML laboratory for additional bacterial source tracking (BST) analysis. The results of this BST analysis may be used by the Plum Creek Watershed Partnership to direct future implementation efforts and best management practices based upon the sources of bacteria that were found. Although this study was relatively small in scope and the results of the BST analysis are not yet available for interpretation, several conclusions can still be drawn from the data that was collected. The *E. coli* analysis conducted during this project confirms that the bacterial impairment identified by TCEQ still exists throughout the entire length of Plum Creek and this impairment may also extend to the Clear Fork tributary. The extreme variability of the *E. coli* concentrations in the creek during wet and dry conditions indicates that non-point source runoff contributes a large portion of the *E. coli* loading to the Plum Creek watershed and BST analysis should prove to be particularly useful in identifying the origin of these bacteria.

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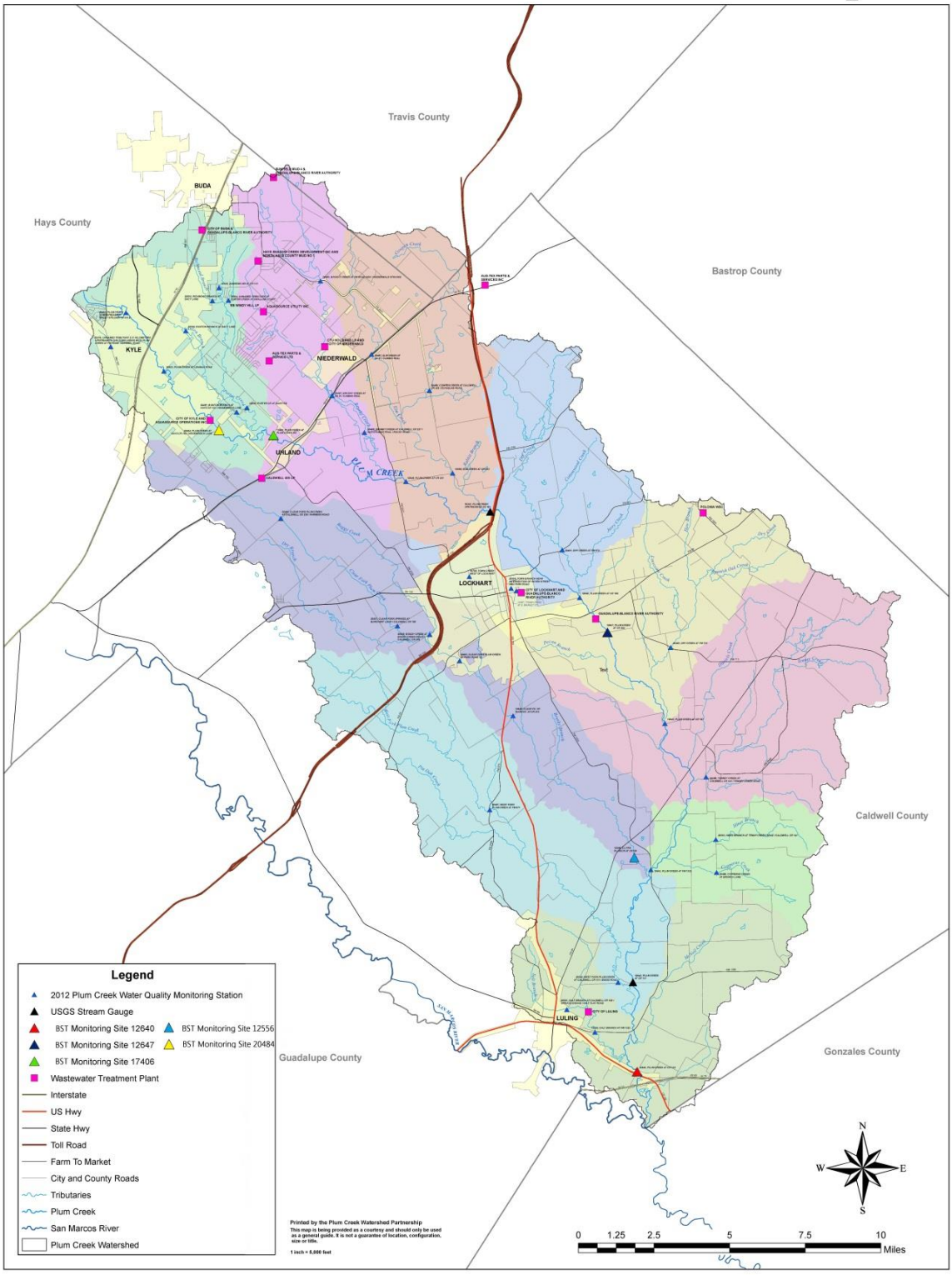
US EPA Methods for Chemical Analysis of Water and Wastewater, Manual #EPA-600/4-79-020., 1983

Appendix A

Maps of Bacterial Source Tracking Monitoring Stations in the Plum Creek Watershed

2016 Plum Creek Watershed Reference Map



Appendix B

TAMU SAML Standard Operating Procedure for Cultivation of *E. coli* from Water Samples and Pre-Processing for Isolation and Bacterial Source Tracking

Soil and Aquatic Microbiology Laboratory
Texas AgriLife Research / Texas A&M University

Standard Operating Procedure SAML-12-105

Cultivation of *E. coli* from Water Samples and Pre-Processing for Isolation and Bacterial Source Tracking

Author: _____
Laboratory Director Terry Gentry

Approved: _____
Laboratory Director Terry Gentry

Revision Record

Revision	Date	Responsible Person	Description of Change
1	November 2012	Terry Gentry	Initial Release

Cultivation of *E. coli* from Water Samples and Pre-Processing for Isolation and Bacterial Source Tracking

1. Follow the EPA Method 1603 Modified mTEC procedure (EPA-821-R-09-007; http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/method_1603.pdf).

2. After 22 +/- 2 hour incubation at 44.5°C, red or magenta colonies are considered 'typical' *E. coli*.

3. Using a black Sharpie or similar marker, mark *E. coli* colonies with a 'dot' on the back of the plate. This helps to ensure that colonies which grew during the incubation period, as opposed to during shipping or storage, are subsequently isolated. If the colonies were counted, please also write the total number of counted colonies on the back of each plate.

4. After incubation and counting, immediately store plates at 4ºC 'media-side up' (i.e., upside down), so condensation does not fall onto the filter during storage.

5. The plates should be shipped as soon as possible (preferably the day after filtration, but no later than three days following filtration) to SAML (address below) via overnight delivery.

6. In preparation for shipment, each plate should be sealed with Parafilm around the edge to protect the cultures from contamination during transit. Dilution series for each sample should subsequently be grouped together and placed in secondary containers such as large Whirl-Pak or zip-top bags.

7. 'Blue-ice' or freezer blocks should be used to keep the plates cool (~4ºC), but not frozen during transport. Do not use dry ice for shipment as this will freeze the media and cultures.

8. Notification of shipment should be sent to SAML (Emily Martin and Heidi Mjelde) via email (emartin@ag.tamu.edu and hmjelde@ag.tamu.edu) no later than the day of overnight shipping. Notification should include the *E. coli* count datasheet (if available), shipment tracking number, and direct contact person for confirmation upon receipt of samples.

9. Ship plates (and COCs) in insulated coolers with sufficient ice packs to maintain ~4°C to:

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