Little Bayou Basin Q Water Quality Improvements

Task 6 –Candidate Restoration Projects and Activities

December 16, 2021

Prepared for:

Engineering and Capital Improvements
City of St. Petersburg
City Project No. 19053-110

Prepared by:

Stantec Consulting Services Inc.
and
Environmental Science Associates

1.0 SCOPE AND BACKGROUND

Stantec and ESA are supporting the City of St. Petersburg (City) with the City's development of a 4e Plan to facilitate the implementation of activities that may provide reasonable assurance to the Florida Department of Environmental Protection (FDEP) that the water quality in Basin Q, including Little Bayou Creek and it's receiving waters in Little Bayou, will be improved and thereby avoid receiving a Total Maximum Daily Load (TMDL) for the basin. The development of the 4e Plan is intended to provide an opportunity for the City to proactively pursue adequate study plans and timeframes necessary to determine the pollutant sources that contribute to the impairments of concern, and to develop the projects and/or programs to address the water quality issues.

This evaluation included the identification of four (4) candidate restoration projects that may contribute appropriate strategies to better identify and subsequently reduce the water quality impairments. These candidate projects were selected based on the results of the pathogen source examination, empirical water quality modelling of existing publicly available data, and communication and coordination with the City, FDEP and local stakeholders including the Tampa Bay Estuary Program.

1.1 INITIAL MOLECULAR SOURCE TRACKING (MST) SAMPLING

Elevated bacteria concentrations in waterbodies may result from a variety of sources including sewage, pets, uncontaminated soils (Fujioka et al. 1999, Solo-Gabrielle et al., 2000) and decomposing vegetation (Ksoll et al. 2007). As a Class III-Freshwater stream, the current bacterial impairment of Little Bayou Creek is for *Escherichia coli* (*E. coli*). In response to coordinated discussions between Stantec/ESA, the City and FDEP, a preliminary bacterial sampling plan was developed and implemented to identify potential bacterial sources to the Little Bayou system (ESA 2021). The sampling plan for this Basin Q analysis incorporated testing for E. coli (and the other traditional bacterial indicators *Enterococci* and Fecal Coliform) as well as quantitative microbial source tracking (MST) to discern if bacterial sources are human in origin. The human-specific indicators evaluated include two techniques, termed "Human Bacteroidetes Identification: Dorei" and "Human Bacteroidetes Identification: EPA method," that look for a human gene biomarker

Surface water samples for bacteriological and MST analysis were collected from Little Bayou Creek on January 25, 2021 and February 10, 2021 at the 7th Street S. (adjacent to the City's current monitoring station) and 4th Street S. monitoring locations (**Figures 1 and 2**). These "dry season" samples were collected during low tidal conditions to maximize the water quality characterization of the freshwater influences. Daily precipitation as reported from the Albert Whitted Weather Station indicated only minor rainfall prior to both sample collection events. Rainfall data from this station show that less than 0.2 inches of rain was recorded on January 16, 2021, more than a week before the initial sampling effort on January 25, 2021. Likewise, less than 0.2 inches of rain was recorded on February 7, 2021, three days prior to the February 10, 2021 sampling effort. (**Figure 3**). Samples were collected consistent with FDEP

Standard Operating Procedures (SOPs), placed on ice, and transported to the receiving laboratories for processing and analysis.

Figure 1. Existing sampling location and initial bacterial sampling sites.

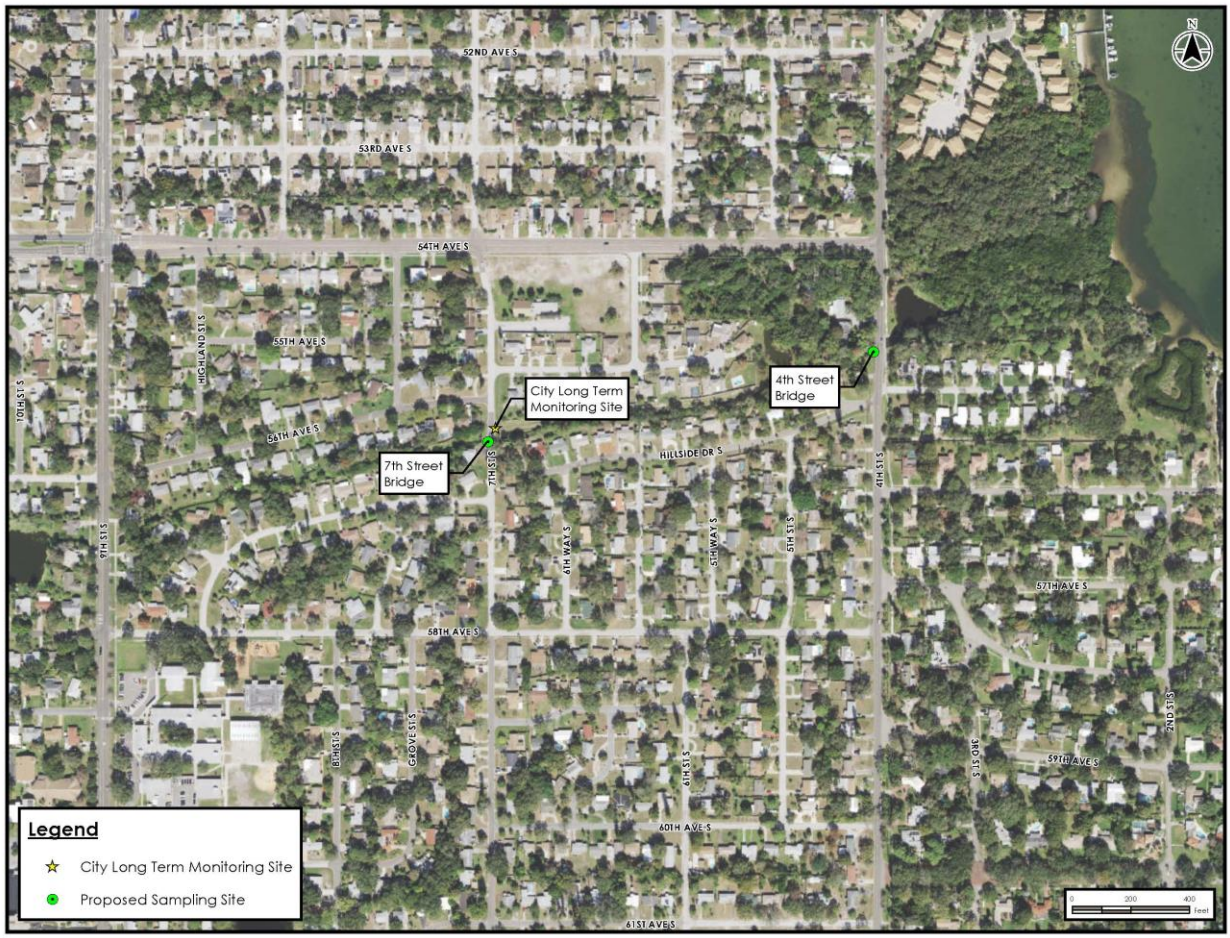
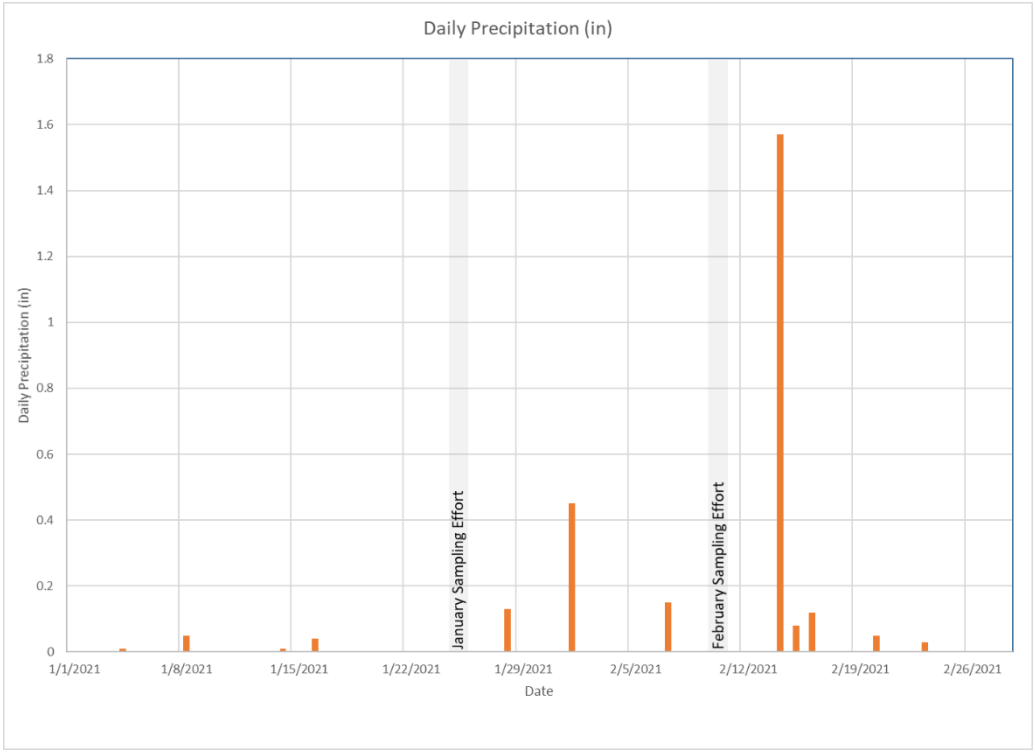


Figure 2. Bacterial Monitoring Stations. A. 7th Street S.. B. 4th Street S. location.

Figure 3. Daily Precipitation in Saint Petersburg, Florida as reported at the Albert Whitted Airport. Sampling events are denoted with a gray bar.



1.1.1 Sampling Implementation and Results

It is important to note, each sampling effort represents conditions on the day of collection. As such, a non-detection result for human-based pathogens does not preclude the existence of human bacterial sources in the water body. Similarly, the documentation of a human-based pathogen source does not preclude other bacterial sources (e.g., wildlife, vegetation, etc.) from being the primary sources of bacteria in the waterbody. Therefore, a best professional judgement weight-of-evidence approach is necessary to ascertain the likelihood for human-based bacterial contamination.

The results of the bacterial analysis identified that traditional bacterial indicator concentrations were greater at the 7th Street S. location compared to the 4th Street S. site, and overall the indicator concentrations were more elevated during the February sampling event compared to the January event. Additionally, E. coli concentrations in the samples analyzed were elevated above the Class III-freshwater criteria (410 cfu/100mL) at both locations during both sampling events (**Table 1**). The FDEP recommends evaluating fecal indicator bacteria (FIB) results by order of magnitude and, based upon the previous monitoring that has occurred in Little Bayou Basin as well as these results, the bacteria concentrations would be classified as "difficult to discern", not requiring immediate action (FDEP 2018).

Table 1. Bacterial Indicator and Human Pathogen (MST) results for Little Bayou.

Analysis	7th Street S.		4th Street S.	
	25-Jan	10-Feb	25-Jan	10-Feb
Bacterial Indicators (cfu/100mL)				
E. coli	520	4,352	529	3,654
Enterococci	1,674	9,804	845	4,352
Fecal Coliform	480	3,500	410	2,800
Human Pathogen (copies/100mL)				
Bacteroidetes dorei (HF183)	581	DNQ	DNQ	1,250
Bacteroidetes EPA Method	ND	ND	ND	ND

DNQ=Detected Not Quantified
ND= Not Detected

The MST results show that human-generated bacteria were detected at both locations during each sampling event based upon the *Bacteroidetes dorei* (HF183) method; however, sufficient material to allow for MST quantification were only available in the January samples collected at the 7th Street S. location (581 copies/100 mL) and the February samples collected at the 4th Street S. site (1,250 copies/100mL). The results from the MST sampling show that the proportion of human-generated bacteria (as quantified by concentrations of *Bacteroidetes dorei*) to the fecal coliform concentrations indicate that a majority of the fecal coliform bacteria at the 7th Street S. site in February could be attributed to a human-generated source, while the *Bacteroidetes dorei* to fecal coliform proportion at the 4th Street S. site in February was much less. These results suggest that there are multiple sources of fecal coliform bacteria within the

Little Bayou Creek which include human-based sources. Based upon the results of these preliminary samples, further investigation into the sources of human-based bacterial loads to the Little Bayou/Basin Q system is recommended.

Appendix A provides the full bacterial indicator and MST results as provided by the laboratories.

2.0 CANDIDATE PROJECTS AND ACTIVITIES

2.1 PROJECT #1: SUPPLEMENTAL WATER QUALITY DATA COLLECTION

2.1.1 Contributing and Receiving Waters

Background and Justification

Little Bayou Creek (WBID 1709D) was previously identified as impaired for dissolved oxygen (DO) and nutrients due to elevated phytoplankton production, as measured by the presence of chlorophyll-*a*. However, it is important to note that during the time of that evaluation, Little Bayou Creek was classified as an estuarine system and therefore, subject to the water quality standards of a Class III Marine waterbody. Since then, FDEP changed the waterbody classification from class III-Marine to Class III-Freshwater, which is the present category for Little Bayou Creek. The receiving waters of Little Bayou (belonging to WBID 1709) remain a Class III Marine waterbody. The water quality standards for pathogens have also changed in recent years, and FDEP's 2018 303(d) list reflects impairment based on water quality targets that have been modified by FDEP. The fecal coliform bacteria standard is no longer used to determine impairment status for Class III marine waters; rather, the standard is now based on the abundance of *Enterococci* bacteria. Prior studies have shown that both fecal coliform bacteria and *Enterococci* bacteria are not restricted to humans as a source, but can come from dogs, cattle, sea birds, and even decomposing vegetation (i.e., Tomasko et al., 2017; Solo-Gabriele et al., 2000; Ksoll et al. 2007).

Existing water quality data within the basin is limited, with current data collected from the single fixed sampling station within Little Bayou Creek located at 7th Street S. This sampling location was originally monitored by FDEP from April 2008 through December 2012, then in June 2013 the City incorporated this site into their ongoing monitoring program (Station 51-01). The most recent data collected from this site was collected in 2019.

Water quality data within the receiving waters of Little Bayou is also limited. As mentioned, Little Bayou is included in WBID 1709, which also contains the waters of Big Bayou. Historical data for WBID 1709 obtained from the most recent FDEP comprehensive water quality database used for impairment determinations (IWR Run 58) are primarily available from the Florida Fish and Wildlife Conservation Commission (FWC) which utilized a randomized, stratified sampling design with sample locations that

varied each time that data were collected. As the primary purpose of these samples was to support fisheries data, the data set for WBID 1709, as included within IWR Run 58, were field parameters such as salinity/specific conductance, DO, pH, and water temperature. There was one station (21FLPDEM50-01) with a more robust dataset including both field parameters as well as nutrients and chlorophyll-*a* data; however, this station was located between Little Bayou and Big Bayou and is therefore not suitable for determining the water quality of the waterbody downstream from Little Bayou Creek itself. Furthermore, the water quality data from this station is limited to the period from January 1991 to November 2002.

Contributing and Receiving Water Quality Sampling Plan

In order to provide sufficient data to evaluate locally relevant dose-response relationships between Little Bayou Creek (and it's contributing watershed) and the conditions within the receiving waters of Little Bayou (and the adjacent waters of the middle segment of Tampa Bay) a detailed sampling plan for impairments of concern (nutrients, DO, and bacteria) could be developed to include:

- Upstream contributing areas
    - Lake Vista Park (creek)
    - Lake Catalina and Lake Coronado (12th St. S./60th Ave. S.)
- Little Bayou Creek
    - 7th Street S. (ongoing)
    - 4th Street S.
- Receiving Waters
    - 4th Street Lagoon
    - Tidal Little Bayou Creek (Cook Park)
    - Little Bayou (multiple sites)

These candidate sampling sites are shown on **Figure 4**.

Figure 4. Candidate locations for proposed contributing and receiving water sampling sites.

Samples should be collected monthly to identify seasonality (wet vs. dry) of impairments. Additionally, the targeted sampling effort may be expanded to include sample collection during rainy periods to capture first flush and rainfall associated concentrations. Sampling methods will follow appropriate FDEP SOPs for collection, handling, and analysis of samples.

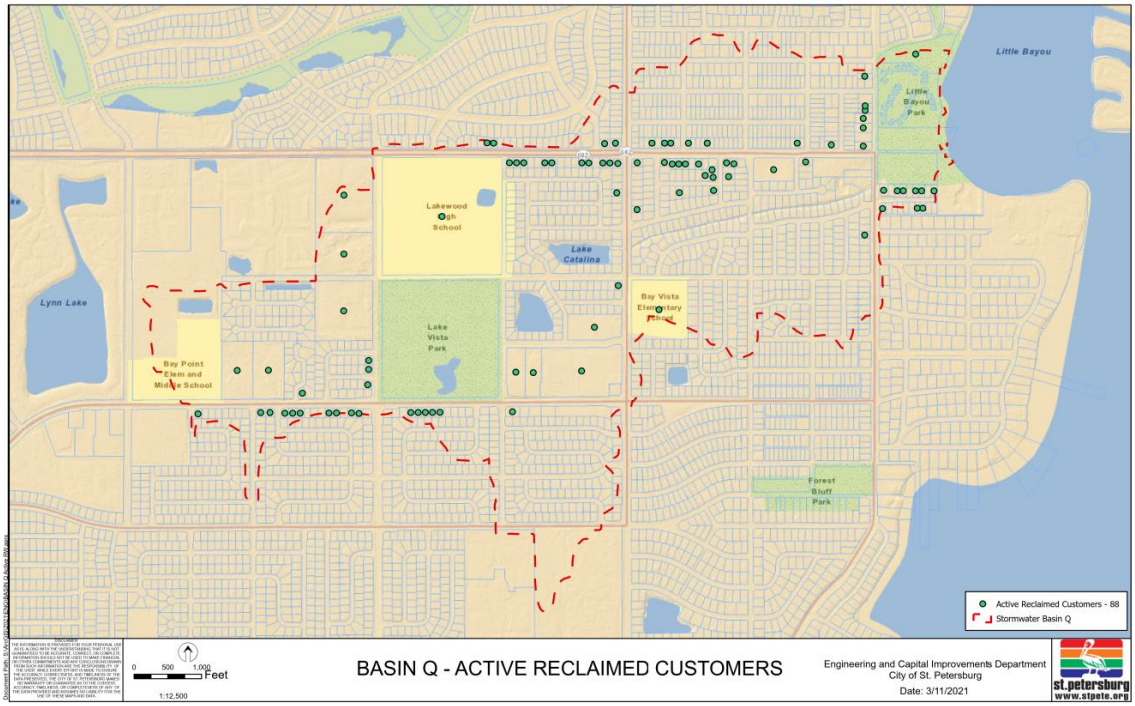
2.1.2 Specific Human/Non-Human Source Tracking

Background and Justification

As mentioned above, the detection of FIB alone does not identify the specific source, as these bacteria may occur from a variety of organisms (human and non-human) and environmental conditions (i.e., decomposing vegetative material). Just as MST sample analysis can be used to target specific non-human hosts, including dogs, birds, decaying seagrass, decaying lawn clippings, etc., there are a variety of human-associated markers that can be identified using additional analysis.

Recently, the City engaged the University of South Florida (USF) for a bacterial tracking study in Frenchman Creek in St. Petersburg. Results from that study provided evidence that the application of some reclaimed water, dependent upon advanced water treatment processes, could obscure the detection of raw sewage within a watershed (USF 2020). In other words, traditional MST methods such as *Bacteroidetes dorei* (HF183) are unable to differentiate between treated and raw sewage as a potential contamination source. In the Frenchman Creek study, additional screening using the human-associated H8 marker in culturable *E. coli* was implemented to identify raw sewage bacterial sources to the waterbody. A review of active connections within Basin Q indicates that the number of connections to the City's reclaimed water system is relatively limited in this area, leading it to be unlikely that this would be a major source of human-based bacterial loading to the watershed (**Figure 5**). However, it was noted from City staff that one of the largest properties—the City-owned Lake Vista Park—recently switched from reclaimed water as its primary irrigation source; but prior to the switch, this park could have been a significant source of human-based bacteria upstream of the fixed sampling location at 7th Street S.

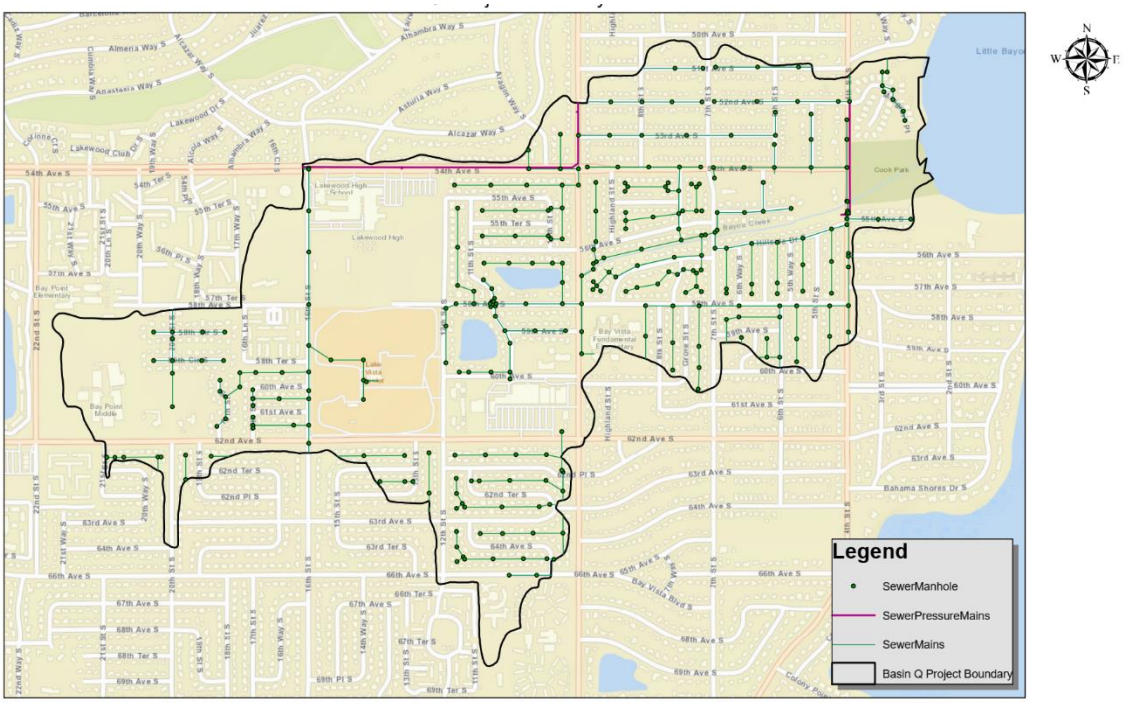
Figure 5. Map of the active reclaimed customers within the Little Bayou watershed



Other common human-based bacterial sources in urban settings can include compromised/aging wastewater infrastructure (e.g., sewer, septic), direct illicit connections, and even homeless encampments adjacent to the waterbody. Project staff from Stantec, ESA, and the City completed a "Walk the WBID" exercise in March 2020 to document any notable observations of potential bacterial sources. During that event, no homeless camps or illicit discharges were identified directly adjacent the creek.

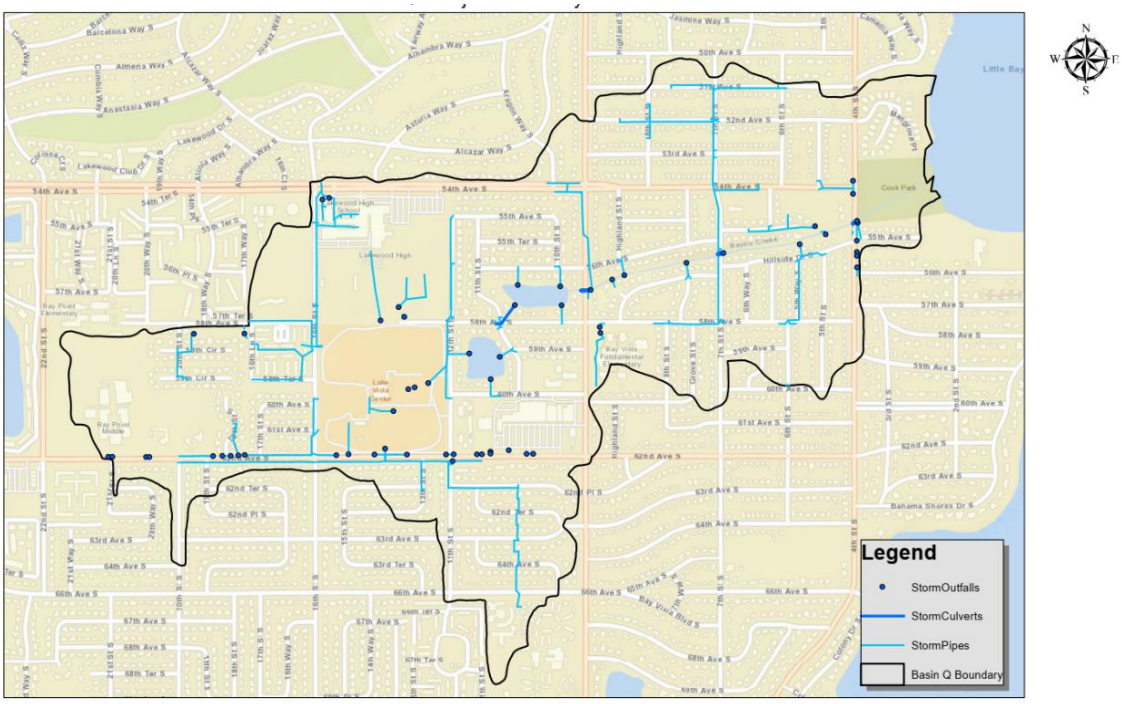
A review of the wastewater infrastructure indicates that the sanitary system is comprised of public sewer lines (**Figure 6**). Typically, bacterial contamination from compromised sewer lines drain to the stormwater system which then discharges to the adjacent surface water. A review of the stormwater infrastructure within the Basin Q watershed identifies multiple locations where the stormwater and sewer system are adjacent to one another (**Figures 6 and 7**). A more comprehensive evaluation of the potential for wastewater effluent to connect with the stormwater distribution system due to aging or compromised infrastructure is recommended.

Figure 6. Map of the sanitary sewer system within the Little Bayou watershed.



It is important to reiterate that human-based bacterial sources are not the sole contributor to pathogens within the watershed. During the prior site visits and MST sampling events, it was observed that many residents and recreational users actively utilize the parks and open spaces/easements in this part of the watershed to walk their dogs in the maintained areas directly adjacent to the creek. This may indicate that pet waste and possibly vegetation clippings could be contributing to the overall bacterial contamination.

Figure 7. Map of the stormwater infrastructure within the Little Bayou watershed.



Additional Microbial Source Tracking Sampling Plan

As described above, the extent of the preliminary MST sampling within Little Bayou Creek was limited to the detection of human vs. non-human indicator bacteria. As both groups were present at each preliminary sampling location, additional sampling could be conducted with specific source-associated markers to quantify the contribution of each constituent to the overall bacterial impairment. A detailed sampling plan could be developed which may include sample analysis for:

- Traditional human-associated MST markers (e.g., HF 183)
- Dog-associated MST markers (e.g., DG37, Dog-Bact, BacCan)
- Decomposing vegetation markers (e.g., 16S rDNA)
- Other as-needed (e.g., bird-associated markers such as GFD)

Additionally, depending on further evaluation of historical and current reclaimed water use, a monitoring program could be established in conjunction with the laboratory at USF to evaluate the potential for bacterial contribution from reclaimed water sources in the basin.

Water Quality Monitoring Implementation

Data collection and monitoring to establish a more robust assessment of baseline conditions for the impairments of concern (i.e., nutrients, DO, and bacteria), as presented in the Contributing and Receiving Water Quality Sampling Plan above, should address the water quality monitoring component required of a 4e Plan. The implementation of this plan may involve establishing a monitoring regime of empirical sample collection and the installation of remote cellular telemetry sensors. Whereas the development and implementation of the Additional Microbial Source Tracking Sampling Plan would be considered above and beyond the baseline condition analysis and should be considered as a proposed project in support the Evidence of Watershed Approach under the 4e Plan. Furthermore, these water quality monitoring programs may be combined with water level/flow monitoring and potential air quality/climate factors to support additional candidate projects as described below.

Monitoring duration for both the baseline constituents of concern as well as the microbial source tracking project should be sufficient to assess seasonal (wet/dry) variation and must be robust enough to support permitting and design elements of subsequent project implementation. Implementation costs will be highly dependent on the number of constituents evaluated, total samples collected, laboratory analysis required, duration of monitoring and technologies utilized to conduct the studies, among other things. The order of magnitude implementation budget may range from approximately $50,000 to $170,000, depending on final scope for the water quality plan.

2.2 PROJECT #2: RESTORING TIDAL CONECTIONS TO LITTLE BAYOU CREEK

2.2.1 Background and Justification

Prior to discharging to Tampa Bay at Little Bayou, Little Bayou Creek enters an approximately 0.5-acre lagoonal area on the east side of 4th Street S. Discussions with City Engineering and Parks Department staff indicate that significant effort is spent to treat nuisance aquatic vegetation in this lagoon each year, including mechanical removal of water lettuce (*Pistia stratiotes*). **Figure 8** shows the lagoon in January 2021, prior to treatment). Based on a review of historical imagery and discussions with City staff, this lagoon drains through Cook Park and connects with the estuary at Little Bayou. However, the presence of water lettuce and other aquatic vegetation like water hyacinth indicates that this lagoon remains primarily freshwater, and tidal connection to Little Bayou has been largely lost.

Figure 8. Photo of Aquatic Vegetation at Little Bayou Creek Lagoon at 4th Street S.

2.2.2 Restoring Tidal Connections and Development of Appropriate Habitat

Based on site visits to the watershed, ecosystem restoration and enhancement projects could be further investigated to reestablish historical tidal connections between Little Bayou and lower Little Bayou Creek. Increasing the reach of tidal waters into the lagoon could increase the salinity, helping to control the growth of nuisance freshwater vegetation.

Development of a study plan could be conducted as a phased approach and may include:

Phase I

- Topobathymetric/LiDAR survey
- Empirical flow monitoring for upstream and tidal portions of Little Bayou Creek
- Initial discussions with regulatory agencies and stakeholder/partner organizations

Phase II

- Computational Fluid Dynamics (CFD) modeling using empirical flow data collected in Phase I
- Conceptual Design
- Detailed pre-application meeting and evaluation of impacts (e.g., identification of potential impacts to mangroves)
- Environmental Resource Permitting (ERP)

Phase III

- Project implementation funding/grant applications
- Project construction bidding
- Construction/project implementation

The completion of conceptual design and permitting efforts would provide the basis for pursuing partnerships and funding opportunities for state and federal grants to implement this project. However, as indicated, initial discussion with partnering agencies and organizations (e.g., SWFWMD, TBEP, etc.) should occur early in the process as part of Phase I.

Tidal Connection Project Implementation

Empirical data collection required to establish baseline conditions within Little Bayou Creek and Little Bayou may be accomplished as described under Candidate Project #1 above. LiDAR data may be available from public sources, whereas a specific purpose topobathymetric survey will likely need to be conducted. A detailed hydrodynamic numerical model can provide a detailed view of parameters such as water levels, velocities, and residence time at a scale and refinement not possible with a field effort, including the ability to test any number of historical and/or hypothetical events as well as a variety of potential solutions or modifications to the system for establishing/improving circulation. Evaluating alternatives, such as storm events, tidal surges, or sea level rise scenarios in this manner is much more time- and cost-effective than implementing them in the real world and waiting in real time to determine their success. Implementation costs for baseline water quality assessments are estimated under Candidate Project #1 above. Additional budget required to support field survey and numeric model development may range from $50,000 to $80,000 depending on model input requirements and total number of model runs or design iterations evaluated.

Detailed design for the tidal portions of Little Bayou Creek, and needed improvements to the 4th Street Lagoon area, should include recommended dimension, pattern and profile, as well as delineated wetland features, erosion and sediment control practices, and construction sequence recommendations. An evaluation of impacts from design iterations will support environmental resource permitting that will be required prior to any construction activities. Conceptual and final design development as well as ERP

permitting efforts may range from $200,000 to $350,000 and should include support for construction sequencing and contractor bidding processes. Additional coordination and site management may be provided during construction implementation phases.

Additional Restoration Implementation Opportunities

The site evaluation, design, permitting, pre-construction, and construction support service elements and associated budget estimates may also be applicable to restoration activities of upstream, non-tidal portions of Little Bayou Creek, depending on the extent of improvements proposed. Evaluation of restoration priorities should be established based on the refinement of impairment source identification and opportunities for project funding should be explored accordingly.

2.3 PROJECT #3: PUBLIC EDUCATION AND TIDAL TRIBUTARY MANAGEMENT TRAINING (IN PARTNERSHIP WITH TBEP)

2.3.1 Background and Justification

Flows into the upstream portions of Little Bayou Creek consist of stormwater inlets and surface flows from residential, commercial, and municipal areas. Lake Vista Park is the largest City-owned property in the basin and includes large areas of maintained turf grass, two baseball/softball fields, a small lake and a surface water that establishes the start of Little Bayou Creek. Along with the park, the City maintains an easement between 7th Street S. and 4th Street S. that together provide approximately 40 acres of property directly adjacent to the creek.

As mentioned previously, in addition to human and animal sources, fecal coliform bacteria have been attributed to decomposing grass clippings and other decaying vegetation (Tomasko et al. 2017) as well as from eroded soils (Fujioka et al. 1999). In addition to causing bacterial contamination, it is known that vegetation clippings and soil runoff can be significant contributors of nitrogen and other nutrients into waterways (Johansson and Greening 1999, Tomasko et al. 2005, Greening and Janicki 2006) making vegetation management in the watershed an important component of water quality maintenance.

2.3.2 Tidal Tributary Management Training

Recent discussions with staff from the Tampa Bay Estuary Program (TBEP), as well as review of the Program's FY2022 Work Plan, indicates an initiative to enhance community knowledge of the economic and environmental services that tidal tributary ecosystems provide (TBEP 2021), and to provide Best Management Practice (BMP) training for enhancing these ecosystems. The objectives of this training, as stated in the Estuary Program's Work Plan (TBEP 2021), are to:

1. Manage urban streams for multiple purposes (i.e., water quality and wildlife habitat, in addition to flood control);

2. Identify native plants and their benefits; and
3. Develop basic consensus-building and conflict resolution techniques for dealing with numerous property owners/stakeholders.

TBEP has partnered with the Pinellas County Sea Grant Extension Agent for the University of Florida/Institute of Food and Agricultural Sciences (IFAS) program to implement the training. Participating local municipal partners may have opportunities to contribute to the curriculum development. Additionally, the TBEP is anticipating that a small-scale pilot restoration project will be implemented to demonstrate concepts discussed in the training and initial discussions with TBEP staff indicate that the Little Bayou Creek system could be a candidate for this pilot effort. Development of the pilot project scope and identification of grant opportunities to support the implementation would be done in partnership with the TBEP.

This BMP training opportunity may be conducted in conjunction with the implementation of a broader stakeholder education and outreach program. Existing targeted campaigns such as the City's Good Neighbor Guides and those from regional partners such as the Be Floridian, Living Next to Nature, Adopt-a-Drain, and Scoop That Poop programs may be promoted to residents and stakeholders within the basin.

3.0 REFERENCES

ESA. 2021. Little Bayou/Basin Q Bacterial Study Basis-Revised. Submitted to the City of St. Petersburg. January 2021.

FDEP. 2018. Restoring Bacteria-Impaired Waters: A toolkit to Help Local Stakeholders Identify and Eliminate Potential Pathogen Problems. Version 3.0. Developed by the Water Quality Restoration Program. Pp. 63.

Fujioka, R. Sian-Denton, C., Borja, M., Castro, J., Morphew, K., Stewart-Tull, D., Dennis, P. and A. Godfree. 1999. Soil: the environmental source of Escherichia coli and Enterococci in Guam's streams. Journal of Applied Microbiology 85: 83S-89S.

Greening, H. and A. Janicki. 2006. Toward Reversal of Eutrophic Conditions in a Subtropical Estuary: Water Quality and Seagrass Response to Nitrogen Loading Reductions in Tampa Bay, Florida, USA. Environmental Management. 38, 163–178.

Johansson, J.O.R. and H.S. Greening. 1999. Seagrass restoration in Tampa Bay: A resource-based approach to estuarine management, p. 279–293. *In* S. A. Bortone (ed.), Seagrasses: Monitoring, Ecology, Physiology, and Management. CRC Press, Boca Raton, Florida.

Ksoll, W., Ishii, S., Sadowsky, M., and R. Hicks. 2007. Presence and sources of Fecal Coliform bacteria in epilithic periphyton communities of Lake Superior. Applied and Environmental Microbiology 73: 3771-3778.

Solo-Gabriele, H., Wolfert, M., Desmarais, T. and C. Palmer. 2000. Sources of Escherichia coli in a coastal subtropical environment. Applied and Environmental Microbiology 66: 230-237.

Tomasko, D.A., C.A. Corbett, H.S. Greening, and G.E. Raulerson. 2005. Spatial and temporal variation in seagrass coverage in southwest Florida: assessing the relative effects of anthropogenic nutrient load reduction and rainfall in four contiguous estuaries. Marine Pollution Bulletin. 50: 797–805

Tomasko, D., Keenan, E., Hudson, J., Propst, C., Logan, M., and J. Vernon. 2017. Finding sources of fecal coliform bacteria in stormwater runoff: The importance of non-fecal origins. Florida Water Resources Journal. July Issue: 34-37.

Tampa Bay Estuary Program. 2021. Final TBEP FY2022 US EPA Work Plan and Budget.

USF. 2020. Microbial Source Tracking in Frenchman Creek, St. Petersburg, FL. Submitted to the City of St. Petersburg. Final Report.

APPENDIX A

MST Laboratory Results

BENCHMARK
EnviroAnalytical Inc.

NELAC Certification #E84167

ANALYTICAL TEST REPORT
THESE RESULTS MEET NELAC STANDARDS

Submission Number : 21011242

Environmental Science Asso.
4200 W. Cypress St. Suite 950
Tampa, FL 33607

Emily Keenan

Project Name : LITTLE BAYOU BACTERIA SAMP. EFF.
Date Received : 01/25/2021
Time Received : 1028

Submission Number: 21011242
Sample Number: 001
Sample Description: LB 4 ST
Sample Date: 01/25/2021
Sample Time: 0745
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	845	#/100 ML	10	10	ENTEROLERT	01/25/2021 11:50	LG
FECAL COLIFORM	410	#/100 ML	10	10	SM9222D	01/25/2021 12:10	LG
E- COLI BY IDEXX QUANTITRAY	529	#/100 ML	10	10	SM9223B	01/25/2021 11:55	LG

Submission Number: 21011242
Sample Number: 002
Sample Description: LB 4 ST- Rep
Sample Date: 01/25/2021
Sample Time: 0745
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	1130	#/100 ML	10	10	ENTEROLERT	01/25/2021 11:50	LG
FECAL COLIFORM	510	#/100 ML	10	10	SM9222D	01/25/2021 12:10	LG
E- COLI BY IDEXX QUANTITRAY	512	#/100 ML	10	10	SM9223B	01/25/2021 11:55	LG

Submission Number: 21011242
Sample Number: 003
Sample Description: LB 7 ST
Sample Date: 01/25/2021
Sample Time: 0815
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	1674	#/100 ML	10	10	ENTEROLERT	01/25/2021 11:50	LG
FECAL COLIFORM	480	#/100 ML	10	10	SM9222D	01/25/2021 12:10	LG
E- COLI BY IDEXX QUANTITRAY	520	#/100 ML	10	10	SM9223B	01/25/2021 11:55	LG

BENCHMARK
EnviroAnalytical Inc.

NELAC Certification #E84167

Submission Number: 21011242
Sample Number: 004
Sample Description: LB 7 ST- Rep

Sample Date: 01/25/2021
Sample Time: 0815
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	3255	#/100 ML	10	10	ENTEROLERT	01/25/2021 11:50	LG
FECAL COLIFORM	490	#/100 ML	10	10	SM9222D	01/25/2021 12:10	LG
E- COLI BY IDEXX QUANTITRAY	576	#/100 ML	10	10	SM9223B	01/25/2021 11:55	LG

Dale D. Dixon / Laboratory Director — 01/27/2021 Date

Tülay Tanrisever - Technical Director/QC Officer

Kara Peterson - QA Officer

DATA QUALIFIERS THAT MAY APPLY:

A = Value reported is an average of two or more determinations.
B = Results based upon colony counts outside the ideal range.
H = Value based on field kit determination. Results may not be accurate.
I = Reported value is between the laboratory MDL and the PQL.
J1 = Estimated value. Surrogate recovery limits exceeded.
J2 = Estimated value. No quality control criteria exists for component.
J3 = Estimated value. Quality control criteria for precision or accuracy not met.
J4 = Estimated value. Sample matrix interference suspected.
J5 = Estimated value. Data questionable due to improper lab or field protocols.
K = Off-scale low. Value is known to be < the value reported.
L = Off-scale high. Value is known to be > the value reported.
N = Presumptive evidence of presence of material.
O = Sampled, but analysis lost or not performed.
Q = Sample held beyond accepted hold time.
T = Value reported is < MDL. Reported for informational purposes only and shall not be used in statistical analysis.
U = Analyte analyzed but not detected at the value indicated.
V = Analyte detected in sample and method blank. Results for this analyte in associated samples may be biased high. Standard, Duplicate and Spike values are within control limits. Reported data are usable.
Y = Analysis performed on an improperly preserved sample. Data may be inaccurate.
Z = Too many colonies were present (TNTC). The numeric value represents the filtration volume.
| = Data deviate from historically established concentration ranges.
? = Data rejected and should not be used. Some or all of QC data were outside criteria, and the presence or absence of the analyte cannot be determined from the data.
* = Not reported due to interference.
Oil & Grease - If client does not send sufficient sample quantity for spike evaluation surface water samples are supplied by the laboratory.

NOTES:

MBAS calculated as LAS; molecular weight = 340.
PQL = 4xMDL.
ND = Not detected at or above the adjusted reporting limit.

COMMENTS:

For questions or comments regarding these results, please contact us at (941) 723-9986.

Results relate only to the samples.

Benchmark EnviroAnalytical,Inc
1711 12th Street East
Palmetto, Fl 34221
941-723-9986
941-723-6061 Fax

Client Information: **Environmental Science Associates**
4200 W. Cypress Street
Tampa, FL 33607
813-207-7200
Emily Keenan 813-207-7211 / Cell 727-433-1200 ekeenan@esassoc.com
Sampler: Jeff Winter Cell: 813-713-4681 jwinter@esassoc.com

Project Name: ~~City of St. Petersburg~~ Little Bayou Bacteria Sampling Effort*
* Project: D2019015.00

Laboratory Submission # 2101142

Sample Name	Sample Type[1] / Sample Matrix[2]	Collection		Container			Preservative[4]	Parameters for Analysis	Laboratory Sample #
		Date	Time	Qty	Capacity	Type[3]			
LB 4 ST	G / SW	25 Jan	0745	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	1
LB 4 ST-rep	G / SW		0745	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	2
LB 7 ST	G / SW		0815	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	3
LB 7 ST-rep	G / SW		0815	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	4

1 "Sample Type" is used to indicate whether the sample was a grab (G) or whether it was a composite (C).
2 "Sample Matrix" is used to indicate whether the sample is being discharged to drinking water (DW), groundwater (GW), surface water (SW), fresh surface water (FSW), saline surface water (SSW), soil, sediment (SDMNT), or sludge (SLDG).
3 "Container Type" is used to indicate whether the container is plastic (P) or glass (G).
4 **Sample must be refrigerated or stored in wet ice after collection. The temperature during storage should be less than or equal to 6°C (42.8°F).**
Under "Preservative," list any preservatives that were added to the sample container.

Instructions:
1. Each bottle has a label identifying sample ID, premeasured preservative contained in the bottle, sample type, client ID, and parameters for analysis.
2. The following information should be added to each bottle label after collection with permanent black ink: date and time of collection, sampler's name or initials, and any field number or ID.
3. All bottles not containing preservative may be rinsed with appropriate sample prior to collection.
4. The client is responsible for documentation of the sampling event. Please note special sampling events on the sample custody form.

* as per EK 1/25/21 pm

Laboratory Sample Acceptability:
pH <2 : ☐ Temperature: 1.1 °C

1	Collected/Relinquished By:	Date 25 Jan	Time 9:30	Received By:	Date ~~1/25/20~~ 1028	Time 1028
2	Relinquished By:	Date	Time	Received By:	Date	Time

BENCHMARK
EnviroAnalytical Inc.

NELAC Certification #E84167

ANALYTICAL TEST REPORT
THESE RESULTS MEET NELAC STANDARDS

Submission Number : 21020521

Environmental Science Asso.
4200 W. Cypress St. Suite 950
Tampa, FL 33607

Emily Keenan

Project Name : LITTLE BAYOU BACTERIA SAMP. EFF.
Date Received : 02/10/2021
Time Received : 0945

Submission Number: 21020521
Sample Number: 001
Sample Description: LB 4ST
Sample Date: 02/10/2021
Sample Time: 0800
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	4352	#/100 ML	10	10	ENTEROLERT	02/10/2021 12:55	LG
FECAL COLIFORM	2800	#/100 ML	100	100	SM9222D	02/10/2021 13:20	LG
E- COLI BY IDEXX QUANTITRAY	3654	#/100 ML	10	10	SM9223B	02/10/2021 12:55	LG

Submission Number: 21020521
Sample Number: 002
Sample Description: LB 7ST
Sample Date: 02/10/2021
Sample Time: 0830
Sample Method: Grab

Parameter	Result	Units	MDL	PQL	Procedure	Analysis Date/Time	Analyst
ENTEROCOCCI	9804	#/100 ML	10	10	ENTEROLERT	02/10/2021 12:55	LG
FECAL COLIFORM	3500	#/100 ML	100	100	SM9222D	02/10/2021 13:20	LG
E- COLI BY IDEXX QUANTITRAY	4352	#/100 ML	10	10	SM9223B	02/10/2021 12:55	LG

Dale D. Dixon / Laboratory Director — 02/15/2021 Date

Tülay Tanrisever - Technical Director/QC Officer

Kara Peterson - QA Officer

DATA QUALIFIERS THAT MAY APPLY:

A = Value reported is an average of two or more determinations.
B = Results based upon colony counts outside the ideal range.
H = Value based on field kit determination. Results may not be accurate.
I = Reported value is between the laboratory MDL and the PQL.
J1 = Estimated value. Surrogate recovery limits exceeded.
J2 = Estimated value. No quality control criteria exists for component.
J3 = Estimated value. Quality control criteria for precision or accuracy not met.
J4 = Estimated value. Sample matrix interference suspected.
J5 = Estimated value. Data questionable due to improper lab or field protocols.
K = Off-scale low. Value is known to be < the value reported.
L = Off-scale high. Value is known to be > the value reported.
N = Presumptive evidence of presence of material.
O = Sampled, but analysis lost or not performed.
Q = Sample held beyond accepted hold time.
T = Value reported is < MDL. Reported for informational purposes only and shall not be used in statistical analysis.
U = Analyte analyzed but not detected at the value indicated.
V = Analyte detected in sample and method blank. Results for this analyte in associated samples may be biased high. Standard, Duplicate and Spike values are within control limits. Reported data are usable.
Y = Analysis performed on an improperly preserved sample. Data may be inaccurate.
Z = Too many colonies were present (TNTC). The numeric value represents the filtration volume.
! = Data deviate from historically established concentration ranges.
? = Data rejected and should not be used. Some or all of QC data were outside criteria, and the presence or absence of the analyte cannot be determined from the data.
* = Not reported due to interference.
Oil & Grease - If client does not send sufficient sample quantity for spike evaluation surface water samples are supplied by the laboratory.

NOTES:

MBAS calculated as LAS; molecular weight = 340.
PQL = 4xMDL.
ND = Not detected at or above the adjusted reporting limit.

COMMENTS:

For questions or comments regarding these results, please contact us at (941) 723-9986.

Results relate only to the samples.

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Client Information: **Environmental Science Associates**
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Tampa, FL 33607
813-207-7200
Emily Keenan 813-207-7211 / Cell 727-433-1200 ekeenan@esassoc.com
Sampler: Jeff Winter Cell: 813-713-4681 jwinter@esassoc.com

Project Name: Little Bayou Bacteria Sampling Effort
Project #: D201901576.00

Laboratory Submission # 2102052 1

Sample Name	Sample Type[1] / Sample Matrix[2]	Collection		Container			Preservative[4]	Parameters for Analysis	Laboratory Sample #
		Date	Time	Qty	Capacity	Type[3]			
LB4ST	G / SW	Feb 10	0800	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	1
LB7ST	G / SW	Feb 10	0830	1	100mL	P	NaThio	Fecal Coliform (MF) E. Coli (SM9223B) Enterococcus	2

1 "Sample Type" is used to indicate whether the sample was a grab (G) or whether it was a composite (C).
2 "Sample Matrix" is used to indicate whether the sample is being discharged to drinking water (DW), groundwater (GW), surface water (SW), fresh surface water (FSW), saline surface water (SSW), soil, sediment (SDMNT), or sludge (SLDG).
3 "Container Type" is used to indicate whether the container is plastic (P) or glass (G).
4 **Sample must be refrigerated or stored in wet ice after collection. The temperature during storage should be less than or equal to 6°C (42.8°F).**
Under "Preservative," list any preservatives that were added to the sample container.

Instructions:
1. Each bottle has a label identifying sample ID, premeasured preservative contained in the bottle, sample type, client ID, and parameters for analysis.
2. The following information should be added to each bottle label after collection with permanent black ink: date and time of collection, sampler's name or initials, and any field number or ID.
3. All bottles not containing preservative may be rinsed with appropriate sample prior to collection.
4. The client is responsible for documentation of the sampling event. Please note special sampling events on the sample custody form.

Laboratory Sample Acceptability:
pH <2 : ☑ Temperature: 1.9°C

1	Collected/Relinquished By:	Date Feb 10	Time 0945	Received By:	Date 2-10-21	Time 945
2	Relinquished By:	Date	Time	Received By:	Date	Time

Chain Of Custody Record

Revision 1.2
Effective Date 8/20/2018

Source Molecular

Source Molecular Corporation
15280 NW 79th CT Suite 107 Miami Lakes, FL 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name	ESA
Contact Name(s)	Emily Keenan
Send Results To	(email) ekeenan@esassoc.com
Phone	727 433 1200
Address	4200 W. Cypress St. Suite 450
City/State/Zip	Tampa, FL, 33607
Billing Info	PO#: ☐ Will call with credit card

Sample ID	Analysis Requested (see pg. 2) Mark boxes with "X": Human Bacteroides - DoCE	Human Bacteroides - EPA					Comments (i.e. special requests, # of containers)	Collection Date	Collection Time
LB4ST mst *	X	X					Quantitative	25 Jan	0745
LB7ST mst *	X	X					Quantitative	25 Jan	~~0830~~ 0815
* reps included									

Completed by Client:
Relinquished By Justin Skarinen
Signature
Date/Time 25 January 1000

To protect confidentiality, confirmation and results will only be sent to email address provided or authorized by contact provided. Signed form indicates agreement with the test limitations on the back of this form and the company's terms of use found here: sourcemolecular.com/about-sourcemolecular/privacy_statement/.

Completed by Source Molecular:
Temperature 8.3°C
Thermometer 001
Cooler Number B5005
Received/Filtered Jenn
Signature Jael Boswell
Date/Time 1/26/21; 10:00am

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ISO 17025 Accredited Testing Laboratory

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: ESA
Date Received/Processed: January 26, 2021
Report Generated: February 10, 2021

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Date Collected	Time Collected	Analysis Requested	Marker Quantified	Result Unit
SM21A26282	LB4ST	1/25/2021	7:45 AM	Human_HF183	DNQ	copies per 100ml
SM21A26283	LB7ST	1/25/2021	8:15 AM	Human_HF183	5.81E+02	copies per 100ml
SM21A26284	LB4ST	1/25/2021	7:45 AM	Human_HumM2(EPA)	ND	copies per 100ml
SM21A26285	LB7ST	1/25/2021	8:15 AM	Human_HumM2(EPA)	ND	copies per 100ml

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 1.4
Effective Date 12/12/19

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: ESA
Date Received/Processed: January 26, 2021
Report Generated: February 10, 2021

SM #	Sample ID	Sample Type	Extraction Date	Analysis Date	Amount Processed	Amount Processed Unit	Analytical Volume (ul)
SM21A26282	LB4ST	Water	2/8/2021	2/9/2021	100	ml	2
SM21A26283	LB7ST	Water	2/8/2021	2/9/2021	100	ml	2
SM21A26284	LB4ST	Water	2/8/2021	2/9/2021	100	ml	2
SM21A26285	LB7ST	Water	2/8/2021	2/9/2021	100	ml	2

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysi s.

Laboratory Comments

Submitter: ESA

Report Generated: February 10, 2021

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the water quality questions to be answered or issues to be resolved.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of Source Molecular Corporation, as well as its agents or representatives, the liability of the company shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to Source Molecular Corp. The company shall not be liable for any damages, either direct or consequential. Source Molecular Corp. provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request. The sample(s) cited in this report may be used for research purposes after an archiving period of 3 months from the date of this report. Research includes, but is not limited to internal validation studies and peer-reviewed research publications. Anonymity of the sample(s), including the exact geographic location will be maintained by assigning an arbitrary internal reference. These anonymous samples will only be grouped by state / province of origin for research purposes. The client must contact Source Molecular in writing within 10 days from the date of this report if he/she does not wish for their submitted sample(s) to be used for any type of future research.

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Chain Of Custody Record

Revision 1.2
Effective Date 8/20/2018

Source Molecular Corporation
15280 NW 79th CT Suite 107 Miami Lakes, FL 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name	Environmental Science Assoc.
Contact Name(s)	Emily Keenan
Send Results To	(email) ekeenan@esassoc.com
Phone	727-433-1200
Address	
City/State/Zip	
Billing Info	PO#: ☐ Will call with credit card

Analysis Requested (see pg. 2)
Mark boxes with "x"
HHRS
Human = DD

Sample ID							Comments (i.e. special requests, # of containers)	Collection Date	Collection Time
LB4ST-mst	x	x						Feb 10	0800
								Feb 10	0830
LB7ST-mst	x	x							

Completed by Client:
Relinquished By: Justin Sarriner
Signature:
Date/Time: Feb 10 1000

To protect confidentiality, confirmation and results will only be sent to email address provided or authorized by contact provided. Signed form indicates agreement with the test limitations on the back of this form and the company's terms of use found here: sourcemolecular.com/about-sourcemolecular/privacy_statement/.

Completed by Source Molecular:
Temperature: 10.4°C
Thermometer: ad
Cooler Number: SF 1500
Received/Filtered: Angelo
Signature:
Date/Time: 2/11/2021 1:23 PM

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: ESA
Date Received/Processed: February 11, 2021
Report Generated: February 22, 2021

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Date Collected	Time Collected	Analysis Requested	Marker Quantified	Result Unit
SM21B11064	LB4ST-MST	2/10/2021	8:00 AM	Human_HF183	1.25E+03	copies per 100ml
SM21B11065	LB7ST-MST	2/10/2021	8:30 AM	Human_HF183	DNQ	copies per 100ml
SM21B11066	LB4ST-MST	2/10/2021	8:00 AM	Human_HumM2(EPA)	ND	copies per 100ml
SM21B11067	LB7ST-MST	2/10/2021	8:30 AM	Human_HumM2(EPA)	ND	copies per 100ml

Reported Results Authorized By: Anda Quintero, Quality Manager

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Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 1.4
Effective Date 12/12/19

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
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ACCREDITED Certificate 4126.01
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Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: ESA
Date Received/Processed: February 11, 2021
Report Generated: February 22, 2021

SM #	Sample ID	Sample Type	Extraction Date	Analysis Date	Amount Processed	Amount Processed Unit	Analytical Volume (ul)
SM21B11064	LB4ST-MST	Water	2/18/2021	2/18/2021	100	ml	2
SM21B11065	LB7ST-MST	Water	2/18/2021	2/18/2021	100	ml	2
SM21B11066	LB4ST-MST	Water	2/18/2021	2/18/2021	100	ml	2
SM21B11067	LB7ST-MST	Water	2/18/2021	2/18/2021	100	ml	2

Reported Results Authorized By: Anda Quintero, Quality Manager

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Laboratory Comments

Submitter: ESA

Report Generated: February 22, 2021

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Fecal Reference Samples

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For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.