



McKay Creek Bacterial Pollution Control Plan

WIBIDs 1633 and 1633B

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Attachment B: Pre and Post Rainfall Event Monitoring with Human and Canine Biomarker Analysis for McKay Creek in Pinellas County, Florida

Introduction

In 1998, the Florida Department of Environmental Protection initially listed McKay Creek on the 303(d) list as being impaired for fecal coliform bacteria based on best available data. Subsequently McKay Creek was split into tidal and non-tidal portions, (1633 and 1633B waterbody identification or WBID, respectively; see *Figure 1*).

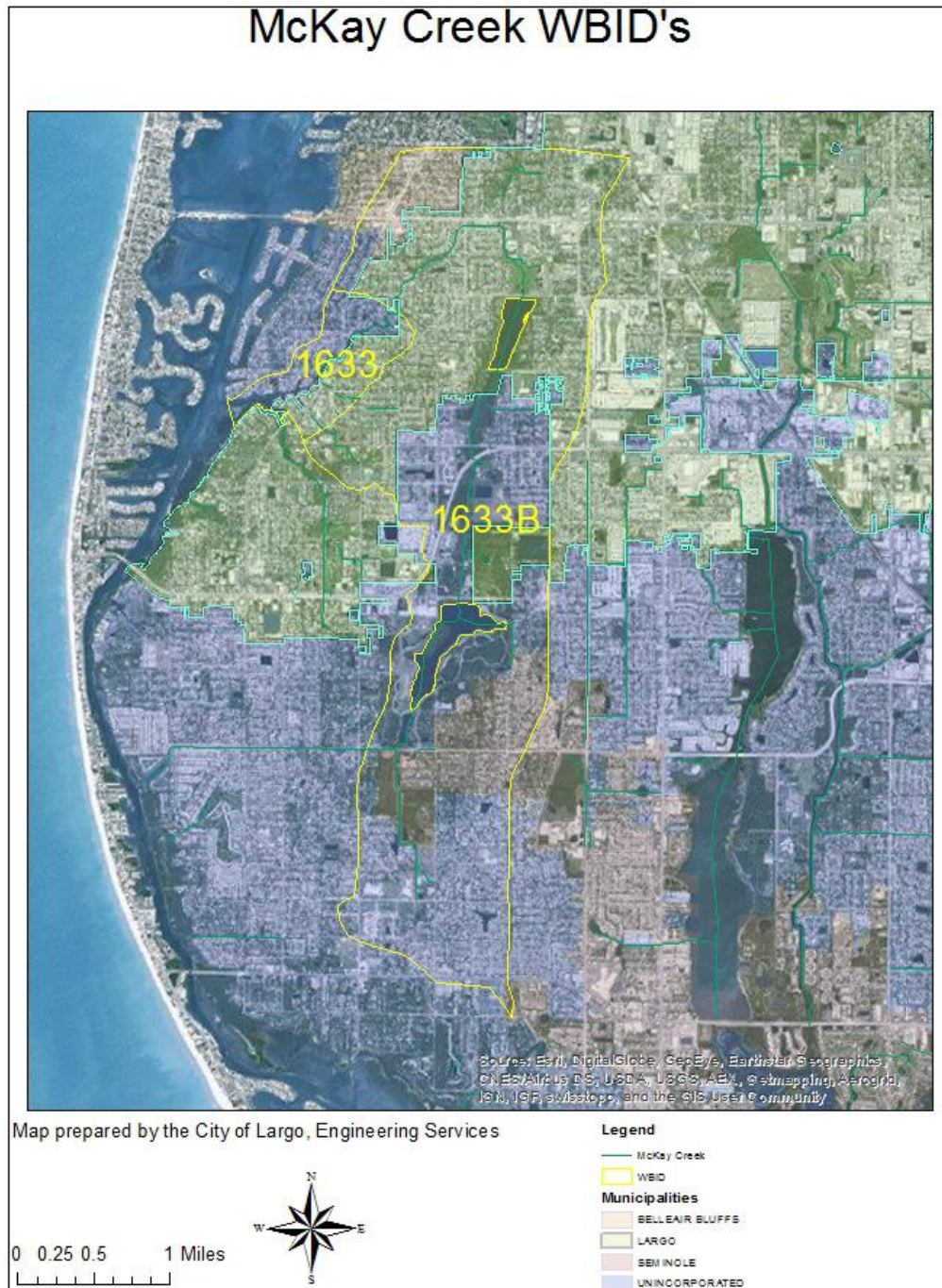


Figure 1: McKay Creek WBIDS 1633 (tidal) and 1633B (non-tidal)

In 2009, the system was included on the Verified List of impaired waters for the Springs Coast Basin. During Cycle 2 assessment, the non-tidal portion of McKay Creek was again determined to be impaired for fecal coliforms. The assessment of water quality data for the tidal section of McKay found that the tidal section of McKay no longer met the criteria as impaired. However, it was determined that water quality was not sufficient to elicit de-listing. The TMDL (Total Maximum Daily Load) was written in 2012 and applies to the tidal and non-tidal sections of McKay Creek (Rojas, 2012).

According to the TMDL, the waste load allocation (WLA) for McKay Creek non-tidal (WBID 1633B) requires a 91% reduction in fecal coliform. Based on available Cycle 2 data for McKay Creek tidal (WBID 1633), no reduction is required. According to DEP, “It is anticipated that if the percent reduction for the freshwater segment of the system is met, the entire McKay Creek system should be restored to meet its applicable water quality criterion for fecal coliform.” Based on this FDEP assessment, this Bacterial Pollution Control Plan (BPCP) has been primarily developed by the City of Largo to address efforts to reduce fecal coliform loadings into the non-tidal portion of McKay Creek, assuming that such efforts will presumably result in a downstream reduction in the tidal zone of the creek.

Data Summary

City of Largo Studies

In August and September of 2014 the City of Largo implemented the *McKay Creek Sampling Plan* to better determine fecal coliform sources in McKay Creek utilizing Microbial Source Tracking (MST) (City of Largo 2014; *Attachment A*). Findings were included in the City of Largo report *Fecal Coliform Investigation: Pre and Post Rainfall Event Monitoring with Human and Canine Biomarker Analysis for McKay Creek in Pinellas County, Florida* (*Attachment B*).

For this study, nine sampling site locations were chosen based on their ability to pinpoint a geographic source. Drainage enters McKay Creek at two main outfalls, both of which were included as sampling sites, in addition to upstream and downstream locations. A map of sampling sites is included as *Figure 2*.

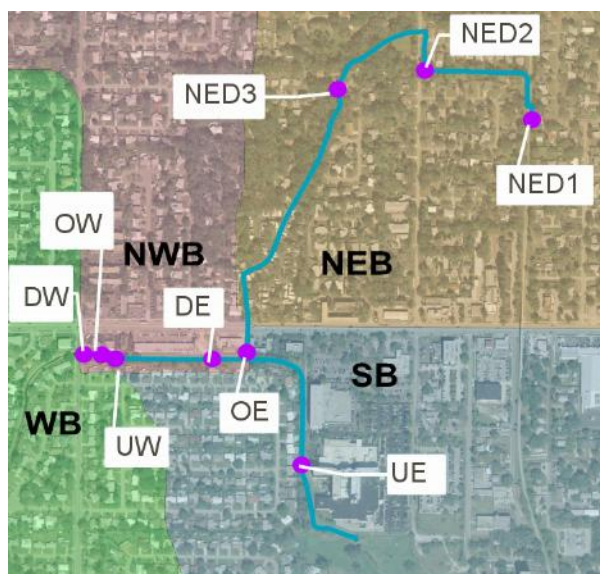


Figure 2: Location of sampling sites (purple) with Site IDs (boxes) along the McKay Creek and its northeastern tributary (blue)

Sampling occurred semiweekly over a 30-day period in August and September of 2014. Fecal levels at each site tended to rise immediately following rainfall events and subsequently recede after approximately two days. A summary of sampling data is included in *Table 1* and *Figure 3*. Overall fecal levels on dry-condition days were significantly lower than levels observed on wet-condition days. Thus it can be assumed with confidence that precipitation has an amplifying effect on local fecal levels. This suggests that non-point sources such as pet wastes likely play a major role in fecal contamination of McKay Creek when they are mobilized into stormwater. This may be exacerbated by stream morphology, as the creek banks at all sites were steep and would facilitate the influx of terrestrial matter during times of increased precipitation.

Site ID	Dry Fecal Levels (cfu/100mL)	75% Confidence Interval (CI)	Wet Fecal Levels (cfu/100mL)	75% Confidence Interval (CI)	Percent Increase (%)
NED 1	1400	463	283800	9.13E+05	+20171
NED 2	8200	1738	23900	6044	+191
NED 3	4900	657	13000	4251	+165
UE	4900	2630	12700	3048	+159
OE	2500	617	15100	3566	+504
DE	2900	720	10800	1726	+272
UW	3100	751	18100	10756	+484
OW	31700	82842	298800	6.66E+05	+843
DW	3500	1127	68600	80960	+1860

Table 1: Fecal levels (colony forming units/ 100mL) found at each site during both wet and dry conditions, as well as the 75% CI and percent increase in fecal levels from dry to wet conditions.

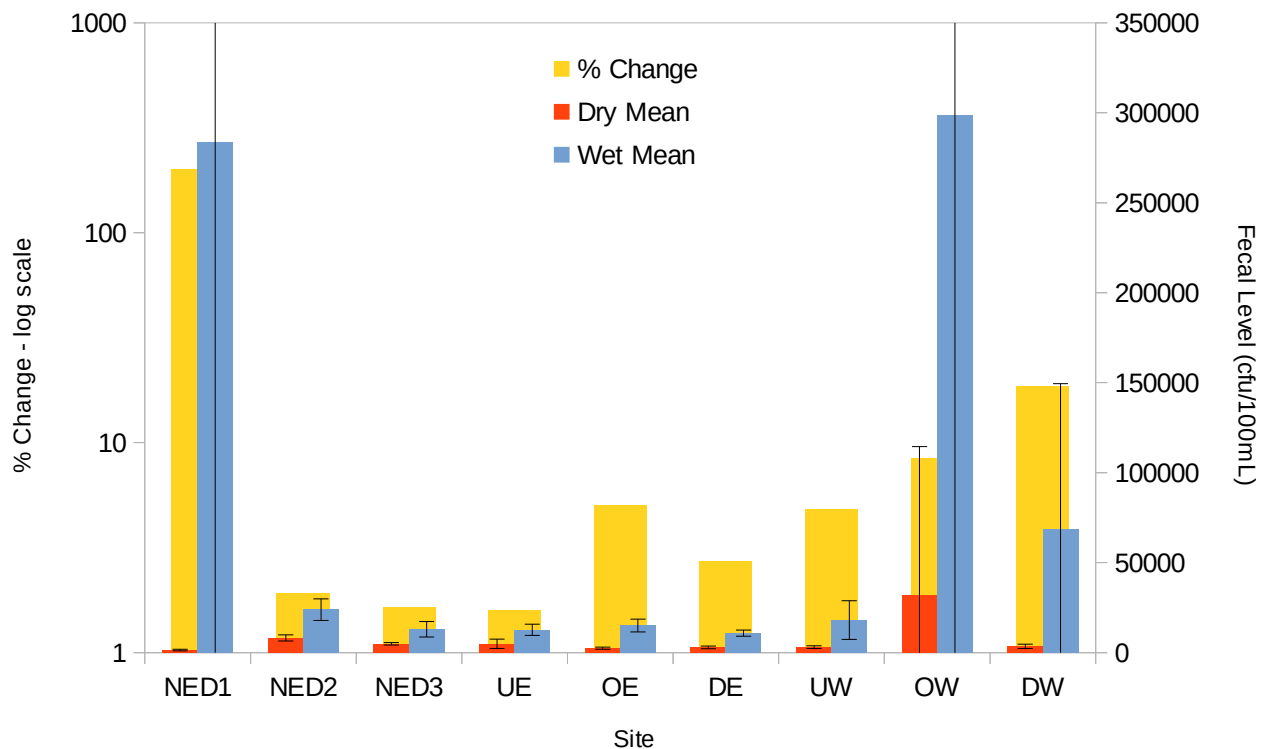


Figure 3: Comparison of the average fecal levels (cfu/100mL) in dry (red) and wet (blue) conditions. 75% CI are shown as error bars (NED1 and OW were the most variable, so had CI that far exceeded values shown on graph—913,000 and 666,000, respectively). The change from dry to wet is represented by the percent change (yellow) on a log scale.

Septic system seepage or sanitary sewer inflow and infiltration are also a known contributing factor to fecal contamination. As part of the study, two sites were tested for human and canine biomarkers. A summary of sampling results is included in *Table 2* and *Figure 4*. One of the sampling sites (OW), located in the same area of McKay Creek that a Florida Department of Environmental Protection sampling site, tested positive for the canine biomarker and negative for the human biomarker during wet event sampling. This suggests that anthropogenic sources are not a significant contributing factor at this location. In fact, the research suggests that, due to a high background level of non-anthropogenic fecal contamination during dry events, wildlife sources may be the source of elevated levels in this area. This was supported by field observations of waterfowl and turtles in the vicinity of the sampling site, both known contributors to fecal levels (Harwood 1999). In terms of the BPCP Plan, this suggests the City should consider targeting anthropogenic source minimization efforts in other portions of McKay Creek.

Site	Fecal Level	Human 1	Human 2	Canine
Pre-Rain OW	1700	ND	ND	ND
Pre-Rain NED 2	7450	<LOQ	142	403
Post-Rain OW	270000	ND	ND	366
Post-Rain NED 2	25000	225	404	6470

Table 2. Fecal (cfu/100mL) and Biomarker (# copies/100mL) levels on Pre- and Post-Rain Event days (9/02 and 9/03, respectively). A non-detect (ND) result meant that the biomarker was not present or was not present at a high enough level to detect. If the biomarker was present, but not at a high enough level to quantify, it was reported as below the limit of quantification (<LOQ).

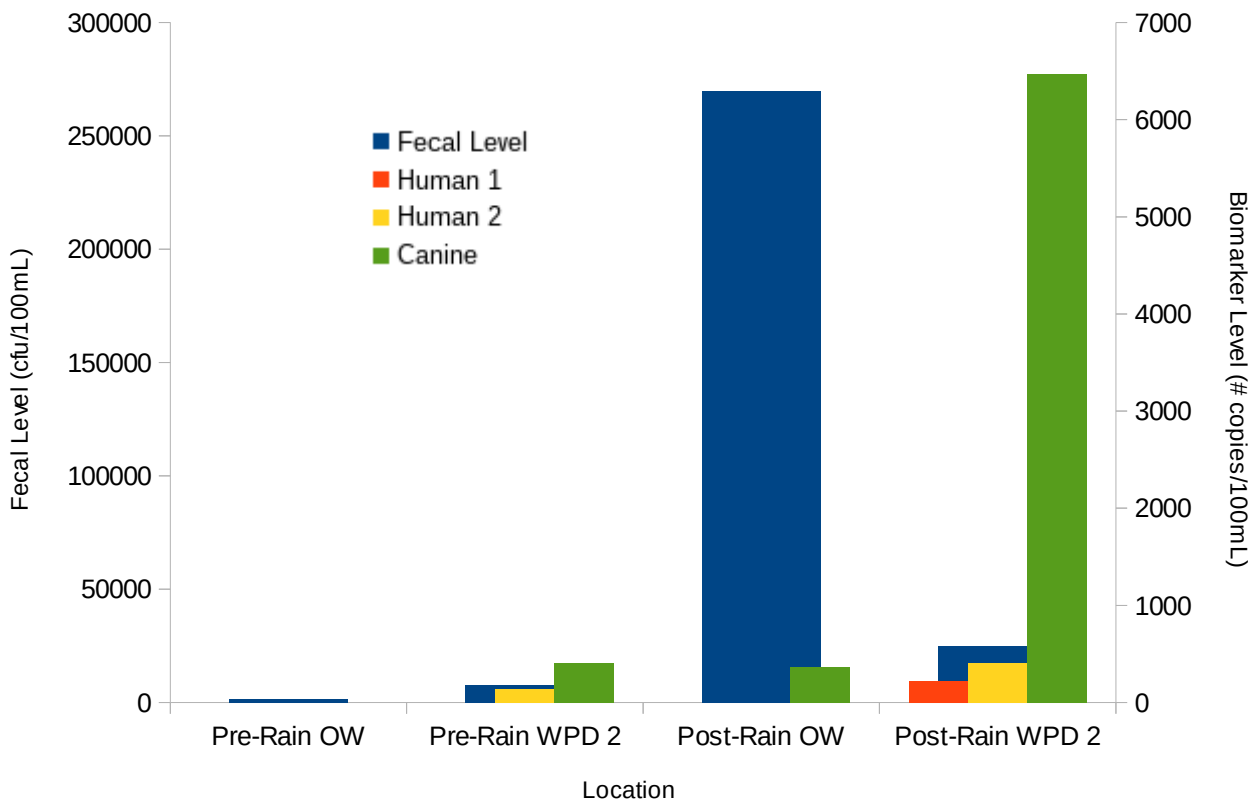


Figure 4: Pre-and Post-Rain Event (9/02 and 9/03, respectively) Fecal and Biomarker (Human 1, Human 2, and Canine) levels at sites OW and NED 2. Trace amounts of Human Biomarker 1 were present on 9/02 at NED 2, however not at high enough levels to be able to quantify.

The second site (NED 2), located within a northeastern tributary named Alcove Creek, tested positive for both canine and human biomarkers under wet and dry conditions. Based on comparison with upstream sampling results, which exhibited much lower levels of contamination during dry conditions, it was determined that anthropogenic fecals were likely introduced in close proximity to this sampling site. This site also experienced a dramatic increase in canine fecal contamination correlated with significant rain events. The Pinellas Trail, a recreational resource frequented by pet owners, is located adjacent to the creek just upstream of the sampling site. Failure to remove pet excreta from the Trail is a likely contributor to the canine biomarkers found downstream. The City plans to examine this area more closely to pinpoint potential sources.

Future Sampling Efforts

Although critical insights were gained from the 2014 fecal coliform investigation, only two sites were sampled for canine and human biomarkers due to prohibitive laboratory costs associated with MST sampling. Additional funding has been located, however, and two interns have been hired to assist with additional sampling efforts during the summer of 2016. MST testing is planned for multiple sites throughout the creek. Specifically, canine sampling will be conducted at the site adjacent to the Pinellas Trail, where a 20,171% percent increase in fecal loading was observed following rain events (1400 cfu during dry sampling and 283,800 in wet sampling). Should the results be positive, the City plans on providing this data to Pinellas County as further justification for the installation of pet waste stations in this area.

The OW site, an outfall for a county-owned stormwater conveyance, tested the highest of all sites in both dry and wet conditions (31,700 cfu during dry sampling and 298,800 cfu during wet sampling). However, the site tested negative for human biomarker during MST analysis, with some canine DNA being detected. The low levels of canine markers appears to indicate that another source is present. The City plans to conduct testing for avian markers to identify another potential source. Unfortunately, if wildlife is identified as the source, there is little the City can do to reduce levels at this site. Should the MST results be inconclusive, additional sampling is planned within stormwater infrastructure upstream of OW in hopes of identifying other potential sources of contamination.

The 2014 sampling event also revealed three sites that had high fecal levels even during dry conditions. It is thought that *E. coli* is a more accurate representation of anthropogenic sources than fecal coliforms. In addition, it is the adopted Class III surface water quality standard. Based on FDEP recommendations, the City plans to take both fecal and *e coli* samples upstream of these sites to identify potential sources. Depending on results, MST analysis may be conducted in these areas.

FDEP recommended sampling for enterococci at at least one site in the tidal portion of the creek. The City will carefully identify the optimal location for sampling and will likely also include fecal sampling to compare results with historic data.

In addition to reporting absolute counts in future sampling efforts, FDEP recommended that the City use surface water quality standard for determining impairment. Each sample will be assessed individually to determine whether it is over the standard (> 400 counts/ 100 ML for fecal, > 410 counts/ ML for *E. coli*), and 130 counts/ 100 ML for enterococci) and if 10% or more of the samples exceed, impairment will be confirmed.

Based on FDEP recommendations, the City is also considering conducting sampling for acetaminophen and sucralose to determine potential anthropogenic source influence.

Non-Anthropogenic Source Reduction

Pet Waste

As previously stated, MST was utilized to identify potential presence of indicator bacteria and the results at two of the sites tested positive for canine sources. (City of Largo, 2014). This suggests that minimization of pet waste influence should be a key component to the McKay Creek BPCP.

Pet Waste Ordinance

While the City of Largo does not have an official city-wide pet waste ordinance, there are two portions of the Code of Ordinances that directly or indirectly apply to proper disposal of animal waste:

Sec 5-34. Removal of dog excreta from certain public and private property

It shall be unlawful for any dog owner or person in custody of a dog to fail to remove deposits of dog excreta made by a dog in that person's charge when the deposit of dog excreta is known or should be known to the dog owner or person in custody of the dog on any public property including, but not limited to, municipal parks and public rights-of-way; or on private property not owned or occupied as a residence by the dog owner or person in custody of the dog. If such depositing of excreta occurs, the dog owner or person in custody of the dog shall immediately cause its removal for disposal at the premises of the dog owner or person in custody of such dog.

Sec 17-17. Domestic animals

(c) It shall be unlawful for any person owning or having custody of any dog on city property upon occurrence to fail to remove immediately the dog's excrement therefrom.

There are additionally two portions of the Code of Ordinances that prohibit discharge of pollutants to stormwater:

Sec. 23-215. Illicit discharges

(a) General prohibitions. Except as set forth under subsection (c) of this section or in accordance with a valid NPDES permit, any discharge to the separate storm sewer system that is not composed entirely of stormwater is prohibited.

Sec. 23-216. Spills and dumping

(a) General prohibitions. Except as set forth under subsection (c) of this section or in accordance with a valid NPDES permit, any discharge to the separate storm sewer system that is not composed entirely of stormwater is prohibited.

Efforts to enhance public education relating directly to pet waste impacts on water quality will be undertaken, and if improvements in water quality aren't observed, an official pet waste ordinance

will be further explored. Existing pet waste ordinances in comparable jurisdictions will be examined to determine their effectiveness in preventing pet waste from entering surface waters. Considerations may include assessment of the penalty structure, acceptance by the public, and ability to enforce (Loch 2014). Nonetheless, public and political acceptance of a pet waste ordinance will likely be higher after educational efforts are implemented.

Citizen Outreach

Phase I of the pet waste citizen outreach program will include the identification of high-risk areas in the McKay Creek watershed, such as pet-friendly apartment complexes, parks, pet businesses, and portions of Pinellas Trail.

Phase II will involve targeted outreach to identified high risk areas. The City worked with its marketing department to produce over three thousand dollars worth of pet waste-focused outreach materials that concentrate on behavioral change messages. These materials are anticipated to be available for distribution by June 2016, and will include educational door hangers, rack cards, and promotional items such as pet waste bag dispensers, leashes, frisbees, and tennis balls emblazoned with a “Love Largo: Scoop Poop” slogan. The visually engaging door hangers and rack cards, as seen below, focus on educating the public about bacterial pollution:



Figure 5: Educational Rack Card/ Doorhanger to be distributed to homes, veterinary offices, and pet groomers within the McKay Creek watershed.

When a citizen uses a Largo-issued leash, tennis ball, or frisbee, the slogan is a constant reminder to clean up after their pet. City outreach staff have demonstrated that promotional items

compliment educational materials by initiating the conversation with eventgoers and other target audiences. City staff anticipate distributing the materials to:

- Homeowners
- Local pet adoption organizations such as Pinellas County Animal Services and the SPCA
- Pet-friendly apartments
- Local special event and festival participants at events such as Pawfest and Family Extravaganza
- Local veterinarians
- Local pet groomers

Initial priority will be given to canvassing neighborhoods, veterinary offices, and pet groomers located within the McKay watershed. However, the City will also be taking a wider outreach approach, such as distributing materials to local adoption organizations, in recognition that these efforts will benefit both the McKay watershed and future TMDL implementation in other areas (Allen's Creek and Cross-Bayou).

Based on initial feedback at local events, the "Scoop Poop" promotional items have been a huge success in distributing effective messaging to the public.

Pet Waste Stations

Based on the wet event sampling results, there may be a large contributing source of pet waste along the Fred Marquis Pinellas Trail. In addition to planned outreach efforts to better inform citizens of appropriate disposal options, the City will investigate whether it is feasible to install and maintain pet waste stations along the Pinellas Trail, which is currently maintained by Pinellas County Parks & Conservation Resources. The City will obtain quotes from vendors and examine the budget to determine whether funds can be allocated to this project. Coordination with County staff will be required to determine whether resources are available to maintain the stations.

Other County and City-owned parks are located within the McKay Creek watershed. To determine where stations are needed, the City will identify target parks and public recreation spaces and visit them to conduct an inventory of existing pet waste stations, if any, and/or trash receptacles. Areas where high volumes of pet feces are observed will be prioritized. In addition, City Engineering staff will coordinate with City and County Parks staff to determine whether resources are available to maintain the stations (remove waste and stock bags).

Insomuch as existing trash receptacles are located in close proximity to target areas, signage may be a more reasonable option due to decreased costs associated with design, purchasing, installation and maintenance of pet waste stations. Should the signage program prove successful, it may be expanded to privately owned high-risk areas identified during Phase I of the outreach plan.

Other Non-Anthropogenic Sources

There is likely a background level of fecal coliform input from urban wildlife sources, including coyotes, opossums and raccoons. Coyotes could be a contributing component of the positive canine MST results. There is anecdotal evidence of coyotes within the watershed; locations of

citizen sightings as reported to Pinellas County are referenced in *Figure 5*. Currently there is little that can be done to address the fecal coliform inputs contributed by wildlife.

Outside of the City of Largo's municipal limits, there are hobby horse farms located within the McKay Creek Watershed. Current code requires all excreta to be removed within 24 hours. Should future annexation efforts include those parcels, targeted outreach to the new residents will be undertaken to ensure the hobby farms are taking all precautions to eliminate impacts to water quality. Future MST efforts may include testing for equine DNA.

Future MST sampling efforts should also include avian assays to better determine possible contributions from the natural bird populations, particularly those that congregate upstream in Taylor Lake Park. The park is also home to many alligators that can also be contributors to fecal coliform levels downstream.



Figure 6: Public coyote sightings reported to Pinellas County from 2011- July 2015

Fecal coliform bacteria do not actually have to come from feces, whether human or animal (Roll and Fujioka 1997). In the Wagner Creek TMDL report, FDEP indicated that “Potential sources can include non-human fecal material, decay of vegetation, and naturally occurring soil bacteria.” There is evidence to suggest that grass clippings are a greater source of fecal coliforms than even pet feces. Therefore the City will conduct targeted outreach to encourage lawn care best management practices such as avoiding blowing grass clippings into the street, sweeping any clippings on the street or sidewalk back on the lawn, and never using a hose to wash them down the storm drain.

Anthropogenic Source Reduction

Upgrades to Sanitary Sewer

Microbial Source Tracking efforts also detected human sources of fecal indicator bacteria at one sampling site in both wet and dry conditions, and additional anthropogenic sources are suspected. In 2015, the City of Largo commenced on upgrading portions of the sanitary sewer system throughout the City to decrease the occurrence of overflows during rain events. The 38.1 million dollar Wet Weather Project encompasses 14 miles of new forcemain and seven new lift stations, three of which are located within the McKay Creek watershed. This is particularly important, as lift station failure in the watershed has resulted in periodic sanitary sewer overflows (SSOs) into the creek. The project is anticipated to be completed in June 2017. Figure 6 illustrates these projects.

As part of the City's preventative maintenance plan, 20% of sanitary sewer conveyances are inspected each year using closed-circuit television (CCTV) cameras, with historic problem areas inspected more frequently. Line condition is assessed and repairs made as needed to prevent seepage and infiltration that may contribute fecals to the McKay Creek watershed. In recent years, the City has dramatically expanded its efforts to seal, line, and replace sanitary sewer pipe. For example, in 2013, only 1,053 linear feet (lf) was sealed, lined, or replaced. In 2014, this jumped to 14,476 lf. Furthermore, the City's proactive inspection program has been expanded to the extent that 248 inflow/infiltration incidents were uncovered and resolved in 2014, as opposed to 191 in 2013. The City hopes to continue to expand preventative maintenance and proactive inspection efforts, which may ultimately contribute to reduced fecal levels in the McKay Creek watershed.

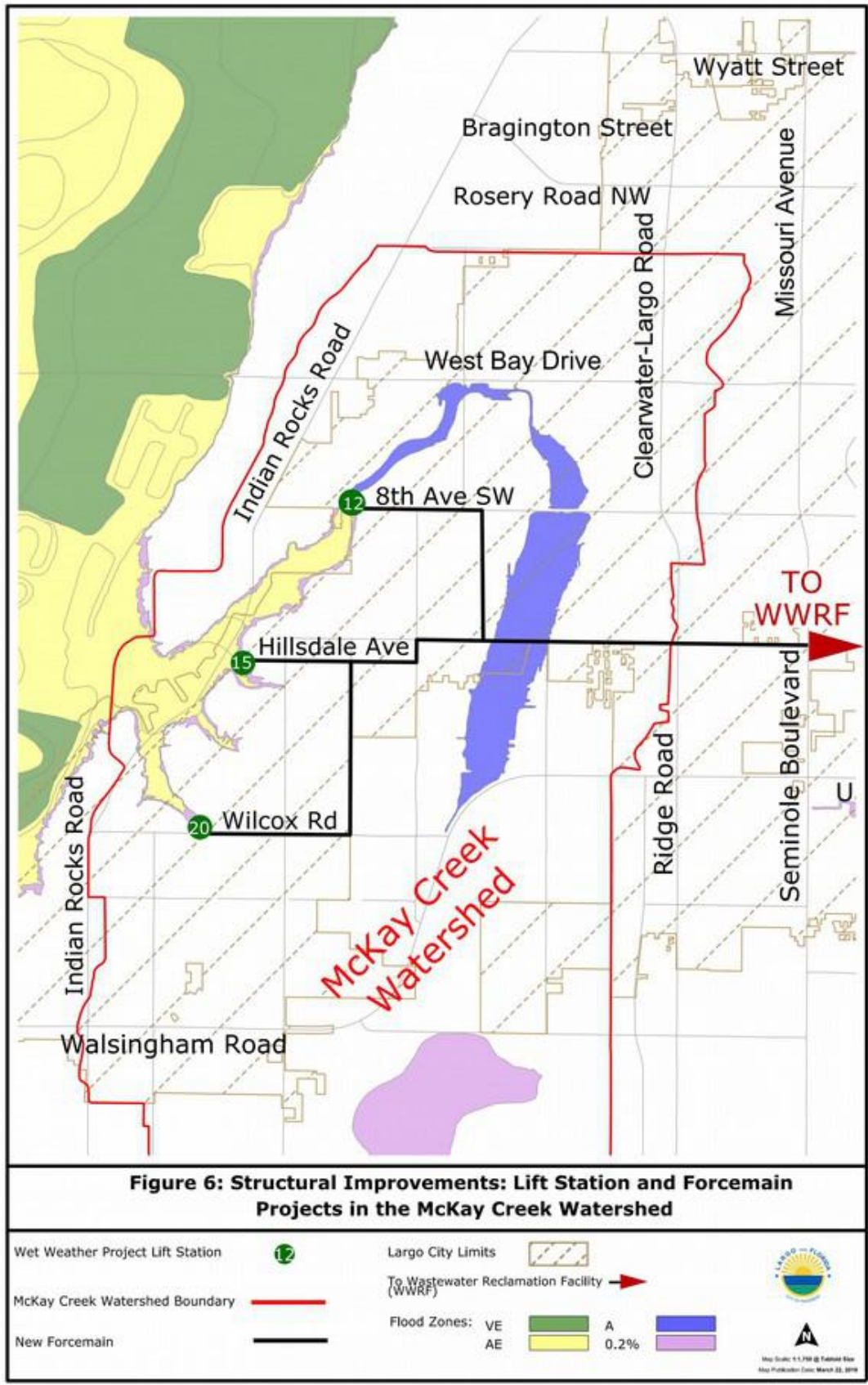


Figure 7:

Stormwater Asset Management Plan

In addition to the Wet Weather Project, the City has hired a consultant to develop a citywide comprehensive Stormwater Asset Management Plan. Begun in September 2015, the Plan will provide the City with a framework for achieving sustainable stormwater management, operations, maintenance, and capital improvement programs. A primary goal is to develop a stormwater program that meets regulatory requirements for water quality. Phase I of the initiative includes development of a stormwater asset inventory (hard and natural assets), condition assessment, and state of the system report. This will be done initially through the creation of a comprehensive map that includes all stormwater infrastructure such as canals, culverts, ditches, ponds, pipes, inlets, and manholes, to name a few, as well as their condition. Phase I is anticipated to be completed by July 2016. Phase II of the initiative includes analysis of management resources and systems (soft assets), level of service evaluation, and stakeholder and public outreach, and is estimated to be complete by June 2017.

The comprehensive inventory will be instrumental to anthropogenic source reduction for multiple reasons. In order to comply with TMDL requirements, the City must first and foremost fully understand its storm sewer system and all its intricacies. Once the system is mapped, existing sanitary sewer maps may be overlain onto the stormwater inventory map in order to identify potential areas of concern, such as areas where the systems are located in close proximity and seepage may occur. The map will also identify sanitary lines with potential to discharge to McKay Creek so that their condition may be monitored closely and repairs/upgrades made in order to prevent potential discharge. Furthermore, the map will assist in identifying optimal locations for structural stormwater best management practices, which will be discussed later in this document.

Fats, Oils, and Greases Program

When they accumulate in sanitary sewer lines, fats, oils, and greases (FOG) and non-dispersibles can create sanitary sewer blockages and SSOs. In order to prevent these blockages, the City issues permits for applicable commercial users that discharge to the sanitary sewer. These facilities, which typically have grease traps, grease interceptors, lint traps, or oil/water separators, are inspected by the City's Environmental Control Division at least annually. The inspectors verify that the systems are functioning correctly and appropriately maintained. When necessary, corrective actions are taken in response to inspection findings.

Environmental Control also identifies hotspots for FOG blockages and conducts targeted outreach activities in those areas. A line cleaning inspection is conducted before and after the events to gauge outreach effectiveness. Moving forward, Engineering has partnered with Environmental Services to discover four blockage hotspots located within the McKay Creek watershed and prioritize them for outreach and line cleaning. The outreach program will continue to be expanded as new hotspots are discovered within the watershed.

Beyond targeted efforts in the watershed, Environmental Services conducts outreach at community events and neighborhood presentations. Over 6,700 promotional items and brochures have been handed out, and FOG materials have been distributed at events with attendees totaling over 32,800 participants. These efforts will be continued into the foreseeable future.

Privately Owned Collection and Transmission Systems

403 privately owned collection and transmission systems and 10% of privately owned manholes are inspected annually by Environmental Control as a preventative strategy to minimize system failure. Operators are also required to conduct monthly inspections of their systems, and these records are reviewed during annual inspections. These efforts minimize the potential for illicit discharges from privately owned systems into McKay Creek.

In addition, the City launches rigorous investigations when leaks are suspected. There are still septic tanks throughout the McKay Creek watershed, including some within Largo's jurisdiction. The City connects properties previously utilizing septic tanks to sanitary sewer as service connections become available.

Pipe Inspections

Engineering Services has also partnered with Environmental Control to conduct sanitary sewer line inspections at locations where lines cross McKay Creek. Any structural issues with pipes shall be promptly repaired.

General Initiatives

Watershed Awareness

Efforts will be made to enhance the public's awareness of McKay Creek and the watershed, including installation of signs that will display the waterbody name on City roads that cross McKay Creek or a tributary. The City's stormwater website is currently being updated and new outreach initiatives are being developed to better inform the public regarding general good practices, provide additional avenues of public information, and streamline reporting of water quality issues. In addition, Engineering staff is coordinating with the City's Multimedia Coordinator to pursue alternative avenues of reaching the public, including social media, video content, and other digital engagement strategies.

Environmental Control also has multiple outreach programs geared toward preventing SSOs in the watershed. The "Toilet is Not a Trash Can" campaign includes promotional giveaways, a program-specific webpage, and a truck wrap displaying marketing slogans. Environmental Control will continue to pursue alternative avenues for public engagement on these topics.

Litter

While litter is not a direct source of fecal coliform bacteria, litter containing food matter frequently ends up in stormwater ponds or ditches, becoming an attractive nuisance for wild and domestic animals. By decreasing litter in our stormwater conveyance systems, the chances of animals defecating in the systems may also decrease. The Engineering Department is taking under consideration a neighborhood clean-up initiative, where the residents will embark on clean-up activities within their respective neighborhoods. The benefits of such a program will not only include less litter in the stormwater system, but will also serve to enhance residents' sense of stewardship.

Sedimentation

High levels of turbidity in the water column will decrease the likelihood of sunlight being able to deactivate indicator bacteria, including fecal coliform and *Escherichia coli*. Sources of sedimentation will be investigated during rain events to eliminate land-based sediment sources and identify erosional areas of concern along the banks of McKay Creek and tributaries. In addition to blocking sunlight in the water column, excess sedimentation provides substrate for fecal indicator bacteria to survive, potentially multiply, and become re-suspended in the water column under appropriate conditions (Rojas, 2012).

The City has made efforts to stabilize portions of McKay Creek and reduce sedimentation. The McKay Creek Stormwater Retrofit Project, begun in 2012 and completed in 2014, involved reshaping tributary slopes, installing sediment traps and planting vegetation to enhance water quality within the watershed.

City staff currently conduct inspections of all active construction sites within city boundaries to verify that sedimentation and erosion control best management practices are adequate. Special consideration will be given to projects located within the McKay Creek watershed, where increased inspection frequencies will be implemented for high-risk areas, particularly during the rainy season.

Structural Stormwater Best Management Practices

Catch Basin Inserts

Currently, the City has a number of catch basin inserts installed in the McKay Creek watershed that are designed to absorb hydrocarbons in stormwater prior to discharging to the creek. Other proprietary products are capable of inactivating fecal indicator bacteria in addition to absorbing hydrocarbons. The City will investigate whether the original funding source will allow switching to an alternative brand of catch basin insert, and whether the other product will work in existing infrastructure. Furthermore, the City will prioritize catch basins for alternative insert installation based on proximity to likely sources or sampling sites that have historically had high bacteria counts.

Ditches

In-line options utilized in other areas of the country as part of BMP treatment trains have resulted in reductions of downstream fecal indicator bacteria measurements. The City hopes to deploy such alternatives in appropriate areas throughout the watershed as budget and opportunities for ditch rehabilitation arise and are determined to be feasible.

Retrofits

Throughout many of the residential neighborhoods there is a wide right-of-way along the roadways. These right-of-ways may present opportunities for bioretention retrofits or other green infrastructure opportunities to divert stormwater from the existing grey infrastructure and focus efforts on stormwater volume reduction and to source control in order to address the fecal coliform and pathogenic bacteria levels found in McKay Creek. Considerations that will determine site appropriateness will include existing utilities, neighborhood demographics (rentals

or owner-occupied) and soil type, with particular attention being paid to the capital improvement pavement schedule to minimize neighborhood inconvenience during construction.

Current Projects

One retrofit project that is currently under design is the Trotter Road Reconstruction project, which is located within the McKay Creek Watershed. Over 50 acres have been identified to drain to this area. Water quality improvements are a primary focus of this project, and bioswales are planned for both sides of the roadway. Importantly, studies have demonstrated that bioswales can reduce bacterial pollution in waterbodies. Mechanisms for bacterial removal include retention of particles to which microbes adsorb and mortality due to grazing by protozoans harbored by the soil (Barley et al, 2014). The City hopes the installation of the bioswales will contribute to a downstream reduction in fecal contamination.

Conclusion: The Path Forward

In summary, the City has undertaken the following initiatives in support of its Bacterial Pollution Control Plan:

- In order to further delineate hotspots of fecal contamination, and as resources permit, Engineering plans to conduct additional wet and dry weather sampling during the summer of 2016, and additional sampling sites may be added as needed.
- Efforts to enhance public education relating to pet waste impacts on water quality will be undertaken, including distributing doorhangers to residents within the watershed, participating in local festivals, and working with local veterinarians and pet adoption organizations. Brochures and promotional items will be distributed.
- An official pet waste ordinance will be further explored.
- The City will investigate whether it is feasible to install and maintain pet waste stations along Pinellas Trail.
- The Wet Weather Project, which replaces three lift stations and associated forcemain located within watershed boundaries, is anticipated to be complete by June 2017. The efforts are intended to prevent SSOs, subsequently reducing fecal levels in McKay Creek.
- Phase I of the Stormwater Asset Management Plan, which involves a complete inventory/map and condition assessment of the City of Largo's stormwater system, is estimated to be complete by July 2016. Phase II of the Plan, which includes an analysis of management resources and systems, is anticipated to be completed by June 2017. These initiatives are crucial to the implementation of structural and nonstructural best management practices in the McKay Creek watershed.
- Environmental Control outreach programs such as the FOG and “Toilet is Not a Trash Can” campaigns will continue to be expanded as new problem areas are discovered. Engineering has partnered with Environmental Services to target and prioritize four hotspots located within the McKay Creek watershed.
- Privately owned collection and transmission systems will continue to be inspected annually.

- The City will continue to connect properties previously utilizing septic tanks to sanitary sewer as service connections become available.
- The City's stormwater website will continue to be expanded with citizen outreach materials. In addition, Engineering staff will be coordinating with the City's Multimedia Coordinator to pursue alternative avenues of reaching the public, including Facebook, video content, and other digital engagement strategies.
- Increased focus will be placed on construction projects located within the McKay Creek watershed, where increased inspection frequencies will be implemented for high-risk areas.
- Feasibility analysis will be conducted for structural stormwater BMPs such as catch basin inserts, ditches, and retrofits.
- The Trotter Road Reconstruction project will include bioswales that the City hopes will play a role in reducing downstream fecal contamination.

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Attachment A
McKay Creek Sampling Plan



McKay Creek Sampling Plan

**Environmental Services Department
City of Largo**



July 2014



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I. Purpose and Background

The purpose of this sampling plan is to satisfy requirements set by Pinellas County's Municipal Separate Storm Sewer System (MS4) permit (permit #FLS000005). The goal of this sampling plan is to locate the geographic and biological source of the fecal contamination in the freshwater segment of McKay Creek, which is maintained by the City of Largo as co-permittee with Pinellas County's MS4 permit. To accomplish this goal, existing site sampling and monitoring data will be analyzed to pinpoint a general source location, which will lead to the development of specific sampling locations for this plan. From these locations, key sites will be designated and samples will be collected from them and tested to determine whether the biological source is human, canine, or other.

McKay Creek is located in west central Pinellas County. The creek is found in the Narrows watershed and is a part of the Springs Coast basin (1). The creek is divided into two sections; the tidal portion (WBID 1633) and the freshwater portion (WBID 1633B). Both sections are classified as impaired by the standards of the United States Environmental Protection Agency (EPA) and Florida Department of Environmental Protection (DEP). The freshwater portion of McKay Creek is impaired for levels of fecal coliform and dissolved oxygen (1).

A well-documented correlation exists between fecal coliform in surface waters, population, and land use; particularly the prevalence of impervious surfaces such as roofs and paved roads and driveways (3). Largo's land use is approximately 87% "urban, residential, and built-up" and 10% natural area (forest, water, and wetlands) (2).

Standard measures are currently being implemented to reduce contamination from the MS4, such as managing and preventing overflow. A new course of action will be determined (or best management practices or BMPs will be developed and implemented) upon completion of the sampling plan. The results may spur further investigation of geographic source if the contamination is proven to be from a human source. If the contamination is from a canine source, Pinellas County and the City of Largo may need to initiate an educative program to encourage residents to pick up their dog's waste, as it can significantly impact surface waters through runoff. The success of the educational program may be measured with continued monitoring of fecal coliform levels from the same high fecal content sites from the sampling plan.

The monitoring will be conducted by the City of Largo's Environmental Control department who will be working in conjunction with the city's Engineering department.

II. Objectives and Project Description

The proposed monitoring will determine the physical location(s) and the biological source of the contamination. Suspected biological sources that will be investigated are human (from septic tanks and sanitary sewer overflows), pet waste, or from another source altogether. The geographic source is suspected to be a specific neighborhood or neighborhoods or recreation area (such as the Pinellas Trail).

There is reason to believe that storm events that cause a surge in runoff drainage to the MS4 system significantly contribute to the fecal coliform colony count in the creek samples. Sampling data from a three day period at sampling Site 3 (conducted by City of Largo, see Figure 1) show an abnormally high count of coliform colonies (40,000 cfu/100mL) on September 25th, 2013. The subsequent two days see a drop to 1,200 cfu/100mL and 147 cfu/100mL, respectively. The same pattern can be seen with slightly lower levels of cfu (colony forming units) at Site 5 downstream of Site 3 (see Figure 1). Upon reviewing USGS water level data for McKay Creek (Station number 02309110) for that three day period, it seems as if a rainfall event occurs in the area as



the water level (measured in feet) spikes on the first of the three sample dates (see Figure 2). Due to a lack of data, a relationship between rainfall events and elevated fecal coliform levels cannot be conclusively verified.

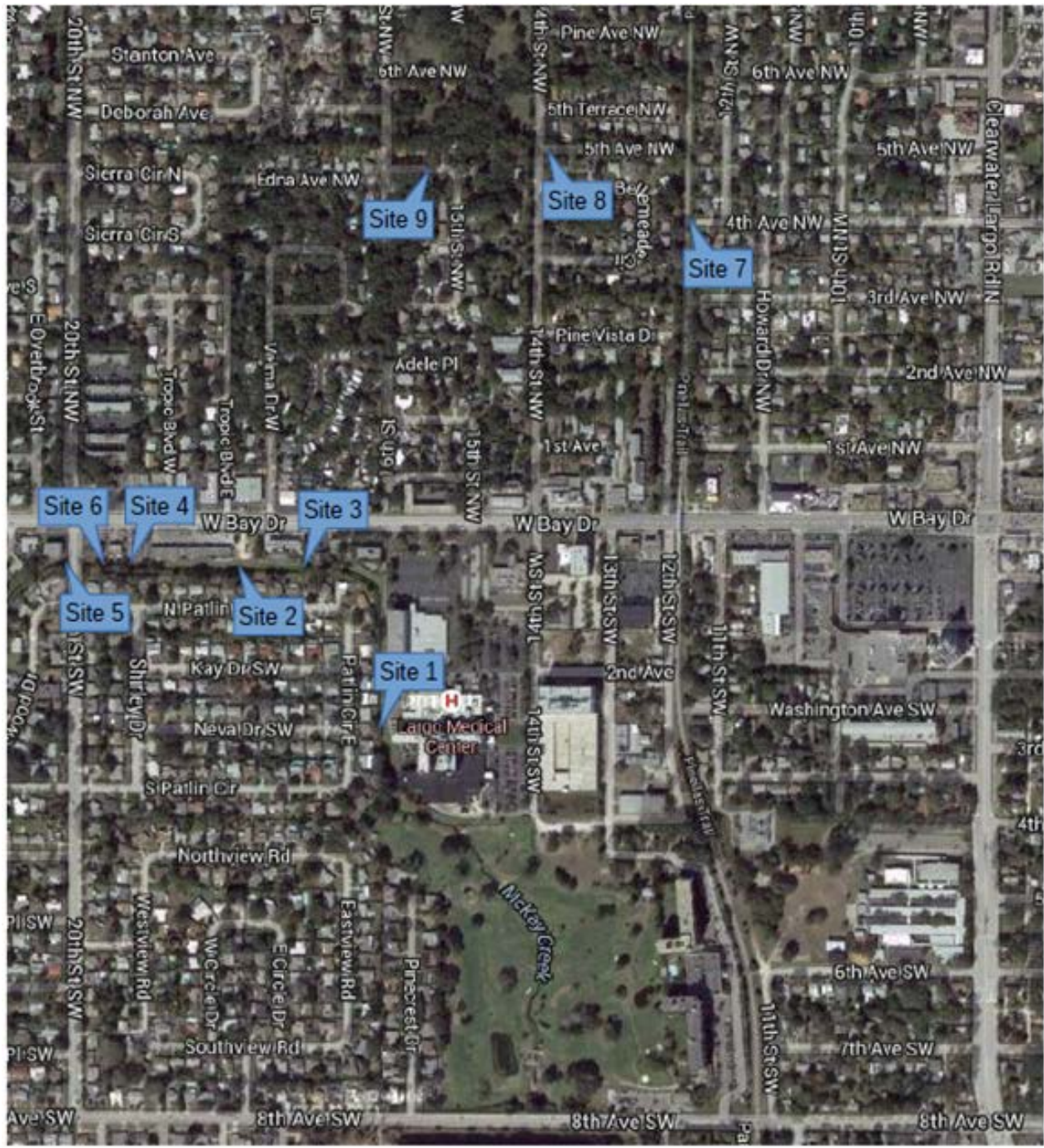


Figure 1. McKay Creek Fecal Sampling Sites

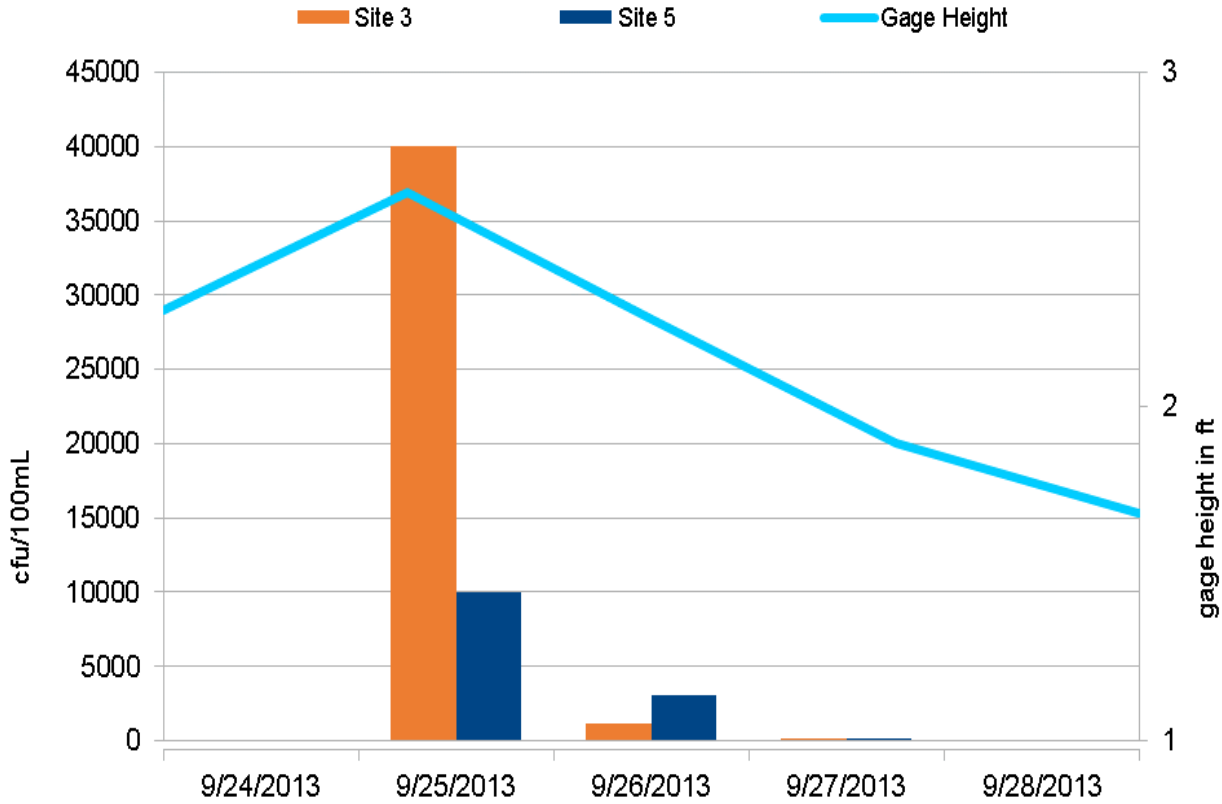


Figure 2. Fecal Coliform and Gage Height Relationship September 24-28, 2013 Water Level Data (Source: USGS and City of Largo)

*Note: Fecal coliform data was only collected September 25-27. Only gage height is displayed for September 24-28.

III. Sampling Sites

Upon analyzing McKay Creek station data from the past ten years (pulled from the DEPs STORET database), there is a single station whose average fecal coliform levels far surpass those of the other stations. Pinellas County Station 27-09 (found in between Site 5 and Site 6) is near the east side of the bridge on 20th Street NW just south of West Bay Drive (see Figure 1). This station is downstream of two stormwater structures through which several neighborhoods drain to the creek.

Data suggest that high Fecal Coliform at Pinellas County Station 27-09 is a result of the northern drainage area that flows through the east stormwater outfall which enters McKay at Site 3. In February 2014, the City of Largo investigated a citizen concern for a foul odor near 5th Avenue NW in Largo. After observing obvious impairment at the site, samples were collected and analyzed, which returned results showing high levels of Fecal Coliform at Sites 8 and 9. Samples taken upstream, a Site 7, showed Fecal Coliform levels inside the normal range. Samples taken downstream at Site 3 typically showed high levels, but lower than levels in samples taken at Sites 8 and 9.

The proposed sample stations can be found in Table 1, along with the sample analysis that will be conducted at each station.



Table 1. Sample Sites

Sample Site	Type	Location Description	Latitude	Longitude
1	Fecal Coliform	Upstream of east stormwater structure	27.914328	-82.804788
2	Fecal Coliform	Downstream of east stormwater structure	27.916187	-82.806463
3	Fecal Coliform	Outfall of east stormwater structure	27.916308	-82.805823
4	Fecal Coliform	Upstream of west stormwater structure	27.916194	-82.808267
5	Fecal Coliform	Downstream of west stormwater structure	27.916283	-82.809023
6	Fecal Coliform	Outfall of west stormwater structure	27.916283	-82.808522
7	Fecal Coliform	4 th Ave and Pinellas Trail	27.920482	-82.800546
8	Fecal Coliform & Biomarker	5 th Ave NW and 14 th St	27.921342	-82.802526
9	Fecal Coliform & Biomarker	Edna Ave	27.920985	-82.804150

To prove or disprove that the contamination stems from the area that drains through the east stormwater structure on McKay Creek, upstream, downstream, and outfall samples must be taken from both east and west structures (Sites 1-6).

To rule out the possibility that the contamination comes from further upstream of Alcove Creek, a sample must be taken upstream (Site 7). Sample Sites 8 and 9 will determine the distribution of fecal coliform levels as the stream flows towards the stormwater outfall at Site 3.

All sample sites are accessible by foot but some sites have denser vegetation. For outfall grab samples and to obtain the most representative samples from the creek banks, a sampling pole should be used. For Alcove Creek, a dipper should be used as the creek banks are very steep and unsafe to traverse. All sample sites are accessible to those who would conduct the sampling.

Due to exceptionally and consistently high levels of fecal coliform, Sites 8 and 9 have been designated as key sites. Samples from key sites will be subject to further analysis, as will be explained further in the next section.

IV. Analytes and Field Measurement

Although the creek is verified as impaired for dissolved oxygen and fecal coliform, this sampling plan is for the purposes of identifying the source of the fecal contamination. Thus, the only parameter in consideration is fecal coliform. All samples will be analyzed to determine the number of colony forming units per 100 milliliters (cfu/100mL).

Samples from key sites will also be analyzed for human and canine genetic biomarkers by the Source Molecular Corporation laboratory in Miami, FL. Microbial source tracking determines the species of the host responsible for fecal contamination through detection of species-specific strains of bacteria in the submitted samples.



High fecal coliform levels have been found throughout McKay Creek and tributaries, however laboratory analysis for genetic biomarkers is costly. For this reason, only two key sites that have exhibited the highest concentrations within the suspected source area have been selected for genetic analysis.

Equipment needed in the field includes:

- Sample bottles (500 mL for key site samples and 125 mL for all other sample sites)
- Bottle labels
- Waterproof pen (if labels or sample bottles aren't pre-prepared or pre-labeled)
- Sampling stick
- Dipper
- Intermediate sampling containers
- Cooler
- Wet ice
- Deionized water
- Appropriate forms (chain of custody, etc.)

V. Other Field Documentation

Records and notes for each sample shall include:

- Sample ID
- Date
- Time sample was taken
- Name of sampler
- Weather conditions
- Flow conditions
- Abnormal or concerning environmental conditions
- Any equipment issues

VI. Schedule and Target Conditions

There will be a total of ten sampling events over a period of thirty days (See Table 2.). Fecal coliform samples will be obtained as a grab sample from all sample sites consecutively during the same calendar day for each sampling event. Samples for key Sites 8 and 9 will be obtained for biomarker analysis during an observed rainfall event.

Sampling is scheduled to begin late July to early August. All reasonable effort will be made to perform sampling for at least one rainfall event. For this sampling plan a rainfall events will be defined as a distance weighted average of 24-hour rainfall greater than 0.5 inches. The centroid for the study area for this sampling plan shall be approximated as Site 9, (See Table 1 for coordinates), when calculating the distance weighted average. Samples will be taken within 48 hours before the anticipated rainfall, during the rainfall, and within 24 hours after the rainfall event. Reasonable effort will be made to obtain biomarker samples from Sites 8 and 9 as part of the sampling performed 24 hours after a rainfall event.



Table 2. Tentative Sample Schedule

Periods	Locations	Samples
Week 1 thru 4 (Mondays, Wednesdays)	Sites 1 thru 9, Fecal Coliform Only	36 per week – 9 Fecal Coliform, 9 Duplicates. Total of 144 samples over 30 day period
Rainfall Events	Sites 1 thru 9, Fecal Coliform and Biomarker	9 Fecal Coliform, 9 Duplicates, (plus 2 Biomarker). Total of 18 (+2) samples per event.
Post Rainfall Events	Sites 1 thru 9, Fecal Coliform Only	9 Fecal Coliform, 9 Duplicates. Total of 18 samples per event.

VII. Field Protocols

Samples will be collected using grab sample techniques in accordance with the City of Largo's internal operating procedure (IOP) for surface water sampling, unless specified otherwise in this sampling plan (See Appendix 1). The IOP should be taken into the field as a referential guide during the sampling event. Largo's IOP is fully compliant with the Department of Environmental Protection's surface water sampling standard operating procedures.

Grab samples will may be obtained with a sampling pole from the banks of the creek or another appropriate adjacent structure. Samples from key sites will be collected in 500 mL plastic sample containers, as requested by the laboratory that will analyze these samples. Samples from all other sites will be collected in 125 mL plastic sample containers. Proper sampling techniques shall be conducted as outlined in the IOP.

The wastewater reclamation facility biological laboratory must be notified of incoming fecal coliform samples once they are collected and stored in the cooler on wet ice at a temperature of 4° Celsius.

Samples collected from the key sites must be prepared for transportation to the Source Molecular laboratory immediately, as the holding time for fecal coliform samples is 6 hours. The packaging instructions recommended by the laboratory on their company website are as follows (Sending):

1. Wrap leak proof bottles with abundant paper towels and put them individually in zip lock bags.
2. Ice packs should be packed with sample(s). The ice should also go into a zip lock bag.
3. Wrap the zip lock bag with abundant paper towels and insert everything in another zip lock bag.
4. Please make sure that the ice packs do not touch directly the samples (adding additional packing material will prevent this).
5. As an added precaution, please put all your zip lock bags and packing material in two overlapping garbage bags.
6. Wrap tightly the garbage bags and put everything in a sturdy cooler.
7. Complete the chain of custody sheets and include with the samples in double zip lock bags.
8. Ship the samples overnight to the laboratory at the following address:

Source Molecular Corporation
4985 SW 74th Court
Miami, Florida 33155 USA



VIII. Quality Assurance and Control

To ensure the accuracy of the sample results, field duplicates should be taken at each sampling site. Field duplicates will verify the integrity of the data. Chain of custody records must accompany the samples at all times, and forms must be properly completed. Labels on the samples bottles shall include sample site, the date and time the sample was taken, and the sampler's initials.

IX. Data Processing, Quality Assurance, and Analyses

The results from these samples will determine whether further investigation is needed (if the results are inconclusive) or the course of action the City of Largo or Pinellas County must take to mitigate the fecal pollution that is entering McKay Creek. If the sample results cannot be replicated with the field duplicates, the data from that sample will be unusable. If the data results are deemed unusable or inconclusive, the sampling event must be repeated or a new plan must be devised and implemented. If it is determined that the source of the pollution is anthropogenic, an investigation regarding sanitary sewer overflow and septic tank usage may follow. If the source is canine, a program may be initiated to educate the public about the impact of dog waste in runoff on surface waters. The data will be analyzed and a conclusion will be made jointly by the Environmental Services and Engineering Division of the City of Largo.

X. Field Safety

Field safety measures include the use of proper safety and personal protective wear such as Nitrile gloves, appropriate footwear, eye protection, reflective vest, and field communication devices. Maintenance of transportation equipment must be used as necessary when in the field.

XI. Staff Expertise and Training Needs

The sampling required by this plan will be executed by the City of Largo staff from the Environmental Control program. All staff performing sampling will be familiar with internal operating procedures and Florida Department of Environmental Protection's standard operating procedures for surface water sampling. Many Environmental Control staff members already possess the skill, training, and experience to perform the required sampling adequately. It is within their job duties to conduct surface water sampling.

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<http://www.sourcemolecular.com/send-samples.html#packaging>



Appendix 1: Internal Operating Procedure Governing Surface Water Monitoring

Printed copies of this procedure are considered uncontrolled unless the revision number matches the current revision number in the Environmental Control Program released documents database.

Title: Internal Operating Procedure Governing Surface Water Monitoring	Procedure Number: ES/EC/IP-003-SW Revision: 4
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Program: Environmental Control	<i>Draft Procedure,</i>	Review Period: 730 days
Subarea:	<i>March 12, 2014</i>	
Area:		

1.0 Purpose

The goal of this procedure is to provide guidelines for surface water sampling for the purposes of permit compliance, investigation due to citizen concern and possible contamination, and in response to unauthorized discharge (line breaks, etc.) and sanitary sewer overflow.

2.0 Scope

This procedure applies to the sampling of surface water bodies under the jurisdiction of the City of Largo.

3.0 Responsibilities

- 3.1 Environmental Compliance Officers (ECOs) are responsible for:
 - 3.1.1 Scheduling and conducting the sampling event
 - 3.1.2 Scheduling and conducting follow-up inspections, if necessary
 - 3.1.3 Properly documenting inspection findings using approved sampling forms
 - 3.1.4 Providing findings to the Environmental Compliance Specialist
 - 3.1.5 Informing the Environmental Control Supervisor of unusual findings in the field
 - 3.1.6 Issuing Notices of Violation (NOV), as necessary
 - 3.1.7 Communication with Customers
- 3.2 Environmental Compliance Specialist is responsible for:
 - 3.2.1 Scheduling and conducting the inspections
 - 3.2.2 Scheduling and conducting follow-up inspections, if necessary
 - 3.2.3 Entering the findings of inspections into the existing electronic database
 - 3.2.4 Informing Environmental Control Supervisor of unusual findings in the field
 - 3.2.5 Communication with customers
- 3.3 Environmental Control Supervisor (ECS) is responsible for:
 - 3.3.1 Reviewing and modifying procedures
 - 3.3.2 Reviewing field documentation
 - 3.3.3 Determining additional actions, when necessary



- 3.3.4 Creating Compliance/Consent/Administrative Order documents, when necessary
- 3.3.5 Coordination with other City Departments, when necessary
- 3.3.6 Communication with customers
- 3.3.7 Communication with Environmental Manager regarding any follow-up activities
- 3.4 Environmental Manager (EM) is responsible for:
 - 3.4.1 Reviewing, modifying and approving procedures
 - 3.4.2 Reviewing and approving correspondence with customers
 - 3.4.3 Creating Compliance/Consent/Administrative Order documents, when necessary
 - 3.4.4 Coordination with other City Departments when necessary
 - 3.4.5 Issuing penalties and fines when necessary
 - 3.5.6 Approving Compliance/Consent/Administrative Order documents
 - 3.5.7 Communication with customers

4.0 Definitions

- 4.1 Intermediate vessel – Sampling container attached to string or pole used for collecting intermediate grab samples in difficult to reach surface waters.
- 4.2 MOT equipment – Maintenance of traffic equipment (flags, cones, etc)
- 4.3 COC form – Chain of custody form for laboratory samples

5.0 Equipment

- 5.1 Whirl-pak bags or 100 mL sterile container (for fecal coliform samples)
- 5.2 Pre-labeled sample bottles
- 5.3 Intermediate vessel (sampling cup, etc.)
- 5.4 Cooler
- 5.5 Wet ice
- 5.6 Pole
- 5.7 Waterproof pen
- 5.8 COC form
- 5.9 Map (for location, direction of streamflow, etc.)
- 5.10 Appropriate related forms for sampling event (refer to specific sampling event IOPs)
- 5.11 Deionized water

6.0 Procedure

- 6.1 Determine location of sample site. If the site exists in the surface water sampling spreadsheet, review existing site data.
- 6.2 Obtain pre-labeled sample bottles from the laboratory supervisor unless sampling event is unplanned.
- 6.3 Ensure that all equipment necessary (see section 5.0) is loaded into the vehicle.
- 6.4 Arrive at location and park in a safe area. Turn on vehicle light bar and set up any MOT equipment, as needed.
- 6.5 Follow all safety and quality control protocol during the sampling event (use latex gloves, appropriate footwear and eye protection, etc.)



- 6.6 Observe and record the apparent condition of the water body and surrounding environment. Any particular odors or unusual or seemingly recently altered physical conditions in and around the water body should be noted.
- 6.7 General considerations for sampling include:
- 6.7.1 If wading, collect samples upstream of where you are standing.
 - 6.7.2 Avoid disturbing sediments in the immediate sampling area.
 - 6.7.3 Consider the characteristics of the water body in choosing the most representative location for a sample.
 - 6.7.4 Unless required by permit, sampling near structures such as dams or weirs may not provide the most representative sample.
 - 6.7.5 Collect samples from downstream towards upstream.
- 6.8 Determine appropriate method of obtaining the sample, either by direct grab or intermediate grab, based upon depth or accessibility of water body to be analyzed. All surface samples are to be obtained from the top twelve inches of the water column. Follow guidelines stated in DEP-SOP-001/01 FS 2110 Section 1.1. Avoid skimming the surface of the water and avoid disturbing sediments, especially in instances of very shallow water. Follow DEP sampling guidelines for obtaining samples for individual parameters. To determine what parameters to sample for, refer to the IOP guidelines for the specific sampling event (e.g. sanitary sewer overflow, ambient water sampling, etc.).
- 6.8.1 To obtain a direct grab sample, refer to the guidelines found in DEP-SOP-001/01 FS 2110 Section 1.1.1.
 - 6.8.1.1 Remove container cap and rinse sample container with sample water three times if sampling for fecal coliform. For other parameters, do not rinse the sample container.
 - 6.8.1.2 Slowly submerge the container, opening first, into the water.
 - 6.8.1.3 Invert the bottle so the opening is upright and pointing towards the direction of water flow (if applicable). Allow water to slowly fill container.
 - 6.8.1.4 Bring filled container quickly to the surface.
 - 6.8.1.5 If a preservative is needed, pour out a small volume of sample downstream of the sampling location to allow room for preservatives and sample expansion. Add the appropriate amount of preservatives, if necessary, then properly seal and label the container.
 - 6.8.2 To obtain an intermediate grab sample, follow guidelines found in DEP-SOP-001/01 FS 2110 Section 1.1.2.
 - 6.8.2.1 Rinse the intermediate vessel three times with sample water or deionized water. In shallow conditions, rinse with deionized water or rinse with sample water downstream of where final sample will be pulled. Discard rinsate downstream after each rinse.
 - 6.8.2.2 Fill intermediate vessel with sample water, minimizing agitation of sample.
 - 6.8.2.3 Fill sample containers from the intermediate vessel. The intermediate vessel must not touch the sample container.
 - 6.8.2.4 If preservatives are required, add them to the sample container before adding sample from the intermediate container. Once the sample is added



to the sample container from the intermediate container, properly seal and label the container.

6.9 Once samples are obtained, preserve on wet ice and follow instructions for preservation, temperature and maximum holding time as per DEP-SOP-001/01 Table FS 1000-4 for individual parameters.

6.10 Notify laboratory of incoming samples.

6.11 Ensure all appropriate forms are completed (COC, Unauthorized Discharge Report, Sewer Overflow Sample Tracking Report, etc.)

6.12 Update surface water sampling spreadsheet (see section 8.1) and other databases, as necessary.

7.0 Safety Information

7.1 Approved foot, hand, and eye protection field work equipment

7.2 Latex gloves

7.3 Reflective vest

7.4 MOT equipment, as necessary

7.5 Communication devices (radio, cell phones)

8.0 Associated Documents/Procedures

8.1 /home/largo/es/EC/Sampling/Surface_Water_Sampling_Locations.ods

8.2 DEP-SOP-001/01 FS 2100 Surface Water Sampling

8.3 DEP-SOP-001/01 Table FS 1000-4

8.4 City of Largo Sanitary Sewer Overflow Discharge Response Plan

8.5 Chain of Custody form

Attachment B

Pre and Post Rainfall Event Monitoring with Human and Canine Biomarker
Analysis for McKay Creek in Pinellas County, Florida



**Fecal Coliform Investigation: Pre and Post
Rainfall Event Monitoring with Human and
Canine Biomarker Analysis for McKay Creek in
Pinellas County, Florida**

**Environmental Services Department
City of Largo**



November 2014



BACKGROUND

Purpose

Per requirements set by Pinellas County's Municipal Separate Storm Sewer System (MS4) permit (#FLS000005), the City of Largo (City), as co-permittee, is responsible for the maintenance of portions of McKay Creek and upstream tributaries. The City is therefore charged with addressing the issue of fecal contamination in the creek's freshwater segment within municipal boundaries. This project aimed to take steps in locating both the geographic and biological sources of the contamination. Fecal levels from various parts of the impaired creek were analyzed to pinpoint potential source locations. Additionally, biomarker samples were taken at select sites in order to classify the biological source as human, canine, or other.

Sample Area

McKay creek is located in the west-central part of Pinellas County and is part of the Narrows Watershed (*Figure 1*). It consists of two parts: a tidal and a freshwater portion, both of which are considered impaired by EPA and DEP standards (*City of Largo. 2014*), but only the freshwater portion was sampled.

The sample area spanned three sub-basins (*Figure 2*) that drained into the creek at various outfalls. The three sub-basins were:

- 1) the Southern Sub-Basin (SB), whose drainage entered the creek most upstream;
- 2) the Northeastern Sub-Basin (NEB), whose drainage entered the creek at the sample area's eastern outfall; and
- 3) the Northwestern Sub-Basin, whose drainage entered the creek downstream of the other two at the western outfall.

Sample locations

McKay creek runs through an urban area steep banks that backed up into both commercial and residential areas. During the sampling period, a USGS station (#02309110) located downstream of the sample site recorded water level changes around 6 inches with rainfall (USGS 2014); that value was used to reflect the value in the sample area upstream.

The residential area that contributed to the NEB drainage was also sampled. The locations were located upstream of the creek's eastern outfall, near the Pinellas Trail, which sees daily pedestrian and bicycle traffic, including dog walkers.

Preliminary Sampling

Previous sampling was done to determine where sampling should take place and which sites were potential locations for biomarker sampling. The results from that project showed high contamination near an outfall on the eastern end of the creek and even higher contamination at two out of three sites in the NEB neighborhood (McKay Creek Sampling Plan 2014). Based on these results, it was suspected that the NEB sites were substantially contributing to the impairment of the creek at the eastern outfall.

SAMPLING METHODS

Site Locations

Based on previous sampling, contamination in McKay Creek was thought to result from an influx of fecal-rich



drainage water from nearby neighborhoods. Site locations (*Figure 2, Table 1*) were chosen based on their ability to pinpoint a geographic source. The drainage enters McKay Creek at two main outfalls: an eastern outfall (OE) and a western outfall (WO). Sample sites are located at each outfall, as well as both upstream (UE and UW), and downstream (DE and DW) of the outfalls. Additionally, contamination was expected to come from drainage that originated in the sub-basin to the northeast then ran through the eastern outfall, so samples were taken from three locations, starting upstream, in the area (NED1, NED2, and NED3).

Rain Gauges

There was not a rain gauge directly measuring rainfall at McKay Creek. Instead, a linear distance weighted average of the rainfall at the five closest gauges (*Figure 1*) was determined and used to produce the daily rainfall estimate in the sample area.

Sampling Schedule

Regular sampling occurred semiweekly, over a 30-day period. It was usually done on Mondays and Wednesdays (with some exceptions due to holidays). Rain event sampling was done the next day regardless of the previous sampling date.

Originally, biomarker samples were only to be taken on one day during a rain event, at the two sites which showed the highest fecal levels. However, biomarker samples ended up being taken on two days. The first were taken on the 29th day of sampling, which was not a rain event, but collection occurred to be sure sampling fell within the 30-day sampling period. Then, between the 29th and 30th days of sampling, a rain event did occur, so another set of biomarker samples was taken to fulfill the rain event criteria.

Regular Sampling

A sample and a duplicate were taken at each site using a sampling pole with sterile, 100mL bottles attached by zip ties. Before samples were taken at each site, both the pole and zip ties were thoroughly rinsed with DI water. Following each sample a Chain of Custody form was filled out, which included the notation of sampling time and anomalous conditions observed at each site.

Sampling was done in two groups, each from highest to lowest expected concentration. The first group consisted of sites NED1, NED2 and NED3, in that order, while the rest were sampled in the second group where upstream and downstream of an outfall were sampled, followed by the outfall itself. This was done for both the eastern and western outfalls (UE > DE > OE > UW > DW > OW). It was more convenient for us to do the NED group first, then the others second, but may be done in the converse order if preferred.

Sampling was done from suspected low concentrations to high concentrations to mitigate the chances of crossover contamination from one site to the next. However, the transition from NED 3 to UE goes from a presumed high concentration to a low concentration, thus increasing the chance of contamination. To check for crossover, a blank and duplicate were taken between the sites.

Once collected, samples were stored in a cooler with ice until given over to lab for analysis.

Rain Event Sampling

In addition to regularly scheduled sampling, *rain event* sampling was also required to obtain biomarker samples. A *rain event* was originally defined as a cumulative rainfall over 0.50 inches in 24-hours. However, that number was lowered for the analysis due to time constraints. The new threshold for a rain event became 0.40 inches.



Rain event sampling continued as previously described, but with the addition of another sampling day, including two biomarker samples and their duplicates (4 total samples). The biomarker samples were taken at the two sites that consistently had the highest average fecal levels (OW and NED 2). The samples and duplicates were taken in the same manner as normal sampling, but were done using 250mL bottles provided by the lab doing the analysis.

Biomarker samples were stored in the cooler along with the other samples until sampling was done. Once sampling was complete, biomarker samples were immediately packed in a separate cooler and shipped to Source Molecular, the lab performing the microbial source analysis. Packing and shipping were done as specified by the lab (Source Molecular Corporation 2014).

Sample Analysis

Once collected, all regular (non-biomarker) samples were given over to the lab where a fecal coliform analysis was performed using membrane filtration. Samples were tested for the presence of fecal coliform colony forming units (cfu) and, if verified, were quantified (cfu/100mL).

Lab protocol states that if a plate count exceeds 200 colonies the reported result is TNTC (too numerous to count) and the specific number of colony forming units was not reported. When this occurred (8/13/14 OW), the number of colonies for that dilution factor was 200 and the reported value (20,000 cfu/100mL) was calculated based on the dilution factor. The reported value told us that fecal levels were at least that high, however it should be noted that in this instance, fecal levels were likely higher than the reported value.

There were instances where a plate did not verify as positive for fecal coliform. If neither the sample nor the duplicate verified, then the site data on that day was not used for further analysis. If one verified, but the other did not, only the verified sample was reported and used for analysis.

Biomarker samples, once collected, were immediately shipped to Source Molecular Corporation laboratory for microbial source analysis. Source Molecular used Quantitative Polymerase Chain Reaction (qPCR) DNA analysis to test for presence and quantity of specific gene biomarkers associated with human or canine sources. Two indicators were used to test for human presence, *Bacteroides dorei* (Human 1) and an EPA Developed Assay (Human 2), and one to test for the canine-specific bacteria: *Bacteroidetes* (Source Molecular).

DATA ANALYSIS

Usable Data

The raw data gave two values for each site on each day (sample and duplicate). As only one value was desired, the average of the values was taken and the percent error was recorded. In order to eliminate outliers and misleading values, data was classified as either '*usable*' data that was used in all subsequent calculations, or '*unfit*' data that was not used. Data was defined as *usable* when a site's percent error was less than 2.5 times the average of all percent errors. This calculation resulted in about 93% of the data classified as usable, while 7% were unfit because their percent errors were too high.

The value of data point 'x' was considered *usable* IF:

$$\%Error_x \leq 2.5 * \%Error_{average}$$



Effect of Rain on Fecal Levels

To determine the effect rain had on fecal levels, samples were first split into 'wet' and 'dry' groups. The wet groups referred to samples taken close enough to a significant rainfall, so that the rain could have had an influence—for this project that was within three days following a rain event. Dry groups were any sample taken outside of that three day limit; they represented samples taken when rain couldn't have had an effect on fecal levels.

Rain effect analysis was performed using the log-normal distribution of the data; all means and standard deviations (SD) were calculated using the log-normal mean (μ) and log-normal variance (σ):

$$\begin{aligned} \text{Mean} &= e^{\mu + 0.5\sigma^2} \\ \text{Variance} &= e^{2(\mu + \sigma^2)} - e^{2\mu + \sigma^2} \\ \text{SD} &= \sqrt{\text{Variance}} \end{aligned}$$

The rain effect was investigated and a 75% confidence interval was calculated at each site in wet and dry conditions. A paired, one-tailed t-test was performed to test for significant statistical difference between wet and dry conditions. Next, the averages of all wet day samples at each site were compared to the corresponding averages of each site's dry day samples, and finally, the percent changes in fecal levels from dry to wet conditions were calculated for each site.

Rain Event

Only one rain event (September 2nd-September 3rd) was successfully sampled where collection took place immediately before and immediately following significant rainfall—as opposed to a couple day lag in sampling dates.

Fecal samples were defined and grouped as either Pre-Rain (those taken on September 2nd) or Post-Rain (September 3rd). The fecal levels and percent changes before and after rainfall were used for comparison.

Biomarker Analysis

Biomarker analysis tested for the presence of genetic material in fecal samples associated with Human 1, Human 2 and canine biomarkers. These results reported biomarkers as being present, present, but only in trace amounts, or absent. If biomarkers were present, results were also quantified and reported as the measure of gene copies per 100mL (#/100mL); as a non-detectable amount (ND), meaning they could have been present but not enough to be detected by this form of analysis; or as below limit of quantification (<LOQ), meaning biomarker was detected and considered present in the sample, but not at a high enough level to be able to quantify.

RESULTS

Fecal Levels Over Sampling Period

Fecal levels at each site tended to follow a similar pattern where values rose immediately following rainfall, then fell back down after a couple days (*Figure 3*).

There was a large amount of rain that persisted for four days from August 8th through August 11th, which was the sample period's most prolonged rain event. All sites showed a decrease in fecal levels during the dry period



before the rain (except for OW), then, at the end of the rain event (August 11th), fecal levels increased at all sites.

The largest single day rain event of the sampling period occurred on August 15th (1.5 inches) with low-rainfall days on either side of it (both were below 0.2 inches). However, all samples taken three days later, on August 18th, showed a decrease in fecal levels ranging from 21-82%.

August 17th through September 2nd, 2014 was a relatively dry period, the few days that had rain fell below 0.2 inches. During that period, there was an initial decrease from 21-94%, then fluctuating values at most sites for the remainder of the period.

A rain event occurred between the September 2nd and September 3rd, 2014 sampling events, bringing a rainfall of 0.444 inches (*Figure 4*). All sites saw an increase in fecal levels ranging from a 54% (UW) to almost 35,000% increase (NED 1).

Effect of Rain on Fecal Levels (Independent of Day)

Overall fecal levels on dry days were significantly lower than the levels observed on wet days ($p < 0.05$) (*Table 2; Figure 5*).

The highest, dry-condition fecal levels were seen at sites OW and NED 2—31,700 and 82,000 cfu/100mL, respectively. During wet conditions, the fecal levels at OW increased to extremely high levels (298,800 cfu/100mL, an 843% increase), while NED 2 didn't see as significant of a change; it increased to 23,900 cfu/100mL (a 191% increase).

The NED 1 location, on the other hand, had the lowest fecal levels during dry conditions (1400 cfu/100mL), but skyrocketed to 283,800 cfu/100mL in wet conditions, which was by far the greatest increase (+20,171%). The DW (+1,860%) and OW (+843%) locations were also largely affected by precipitation, though not to as great of an extent.

The sites which appeared least rain-affected were UE (+159%), NED 3 (+165%), and NED 2 (+191%), though fecal levels still more than doubled from dry to wet conditions.

Biomarkers

Neither human nor canine biomarkers were detected in OW before rain (*Table 3, Figure 6*). After the rain event, there was still no detection of human 1 or 2 biomarkers, but the canine biomarker was detected (366 copies/mL).

All three biomarkers were present in NED 2 samples, both before and after rain, though the Human 1 biomarker was only present at trace levels before the rain event. Human 1 and 2 biomarkers saw slight increases from pre-rain (<LOQ and 142 copies/100mL, respectively) to post-rain values (225 and 404 copies/100mL). The canine biomarker increase, however, was substantially greater. It increased from 403 copies/100mL (pre-rain) to 6470 copies/100mL (post-rain), which is an increase of about 1500%.

DISCUSSION

Rain Effect



It can be assumed with confidence that precipitation has an amplifying effect on local fecal levels. This was seen at every site, both during a rain event and when all samples taken in wet conditions were compared to those taken in dry conditions. The creek banks at all sites were steep and would facilitate the influx of terrestrial matter during times of increased precipitation, this could include material containing fecal coliform. The effect of rain on fecal levels fell into three categories: 1) Low-Dry, High-Response; 2) High-Dry, Low-Response; or 3) High-Dry, High-Response.

The Low-Dry, High-Response (LDHR) sites consisted of the majority of the sites (NED 1, DE, OE, UW, and DW). At these sites, fecal levels were lower than average during dry conditions, but showed a strong response to rain with a large increase in wet conditions. An inference can then be made that the source of the fecal coliform was washed in with rain from either the surrounding banks or was flooded in from nearby, upstream water sources.

High-Dry, Low-Response (HDLR) sites were characteristic of NED 2, and to a lesser extent, NED 3 and UE. These sites had higher than average fecal levels during dry conditions, but didn't see much of a change with increased precipitation. This means that the primary source of fecal contamination must have been fed into the stream at a relatively constant rate. The increases in overall fecal levels were likely due to secondary source, such as the influx of fecal-contaminated material being washed in from nearby banks or impaired waters upstream.

The only High-Dry, High-Response (HDHR) site found in this project was OW. The fecal levels were consistently much higher than fecal levels at other sites in dry conditions and then, in wet conditions, the levels increased to an even greater extent, showing a noticeable response to precipitation. These characteristics likely indicate two prominent sources of contamination in the area. The first being a steady influx of contamination not dependent on rainfall; the second was likely a large influx from the nearby banks or upstream waters that is directly correlated with increased precipitation.

Sources by Site

The two sites that were tested for biomarkers were NED 2 and OW. For those two sites, direct assumptions about the biological and geographical sources could be made based on the biomarker and fecal results. For the other sites, however, inferences had to be made that were based on the results of the sampled sites, combined with findings from similar studies.

NED 1, NED 2, NED 3

Site NED 2 was located in a residential area in the sub-basin to the northeast of McKay Creek. It tested positive for human biomarkers 1 and 2 in both dry and wet conditions. Though there was a minor increase in samples taken during wet conditions, the fecal levels (both total and human-source) stayed relatively constant. Therefore, it can be presumed there may be a continuous low-level input of human-source fecal contamination feeding into the site. This could be a result of leaks in pipes or septic systems similar, to what a study in Clearwater, FL found (Whitlock 2002); or direct human input could be a cause, as was the case in a UCLA study (Jay 2014). The upstream site (NED 1) was unlikely the source of human contamination. If it were the source, one would expect consistently high fecal levels, independent of rainfall, to continuously feed downstream. That was not the case, instead site NED 1 was an LDHR site that had some of the lowest fecal levels during dry conditions, but had a strong response to rainfall and produced fecal levels way above those of the other sites. Because Site NED 1 was not the source, the human-associated fecals must have been introduced in close proximity to NED 2.



In addition to human-related contamination, site NED 2 experienced a dramatic increase in canine fecal contamination correlated with significant rainfall, so, as increased precipitation appeared to have caused the influx, the high fecal levels were due to input from either nearby banks or upstream. In this case, upstream contamination from NED 1 was the most likely source. After rain, the site saw the most dramatic increase in fecal levels out of all the sites, a pattern which was reflected by the canine biomarker levels at site NED 2. NED 1 was directly adjacent to the Pinellas Trail and saw a lot of pedestrian traffic, much of which were dog walkers. It was likely that walkers neglected to pick up after their dogs, resulting in waste accumulation near the creek's banks. Some of that waste may have seeped into the nearby soil, as was seen in the Whitlock study (2002) so that when it rained, both waste and contaminated soil would have washed into the creek, thus increasing the overall canine-sourced fecal levels in the water and would have flowed downstream to site NED 2. The dominant source of contamination at site NED 1 was assumed to be canine rather than other wildlife because the spike in canine indicators at site NED 2, coincided with the dramatic increase in fecal levels at NED 1.

NED 3 was not an area of concern because it seemed to be a result of the impairment at sites NED 2 and NED 1. The dominant influence was determined to be site NED 2 because its fecal levels were mirrored by site NED 3, though at lower concentrations, while there was much less of a correlation between NED 1 and NED 3.

UW, OW, DW

The second biomarker sample was taken at OW. In dry conditions the overall fecal level was higher than the levels at the other sites, but neither human nor canine sources tested positive in the biomarker analysis. After rain, the fecal levels rose by over 15,000% and a canine source of fecal contamination was found. It is unlikely that the bulk of the fecal level increase is due to canine-sourced contamination for two main reasons: 1) the fecal levels were high even in dry conditions when canine source indicators were absent and 2) the increase in overall fecal levels is disproportional to the increase in canine source indicator levels. More than likely, the high fecal levels in dry conditions were due to local wildlife waste. This was supported by personal observations of waterfowl and turtles in and around the sample area, both known contributors to fecal levels (Harwood 1999 and 2000). Furthermore, in wet conditions when the fecal levels increased substantially (specifically the post-rain event day), large amounts of duckweed were seen in the sample area, indicating an influx from an upstream pond or other stagnant body of water. Waste likely accumulated in the other water source during dry conditions, then was flushed out downstream due to increased precipitation, significantly increasing fecal levels.

The site downstream of OW (DW) saw a large increase in fecal levels during wet conditions, which was most likely also a result of the upstream source flowing through OW. This idea was further supported by the lack of rain response at site UE, which is upstream of OW and DW, so therefore should have been (and was) unaffected by anything flowing into OW from the Northwestern Sub-Basin.

UE, OE, DE

None of the sites at or around the eastern outfall had substantial levels of fecal contamination in dry or wet conditions, or any noteworthy changes to spur further investigation as it is unlikely that any of these sites were major contributors to overall contamination of McKay Creek. It could be noted that UE had characteristics of a HDLR site, similar to NED 2, which had human-source fecal contamination. If future investigations were to show the HDLR sites to be characteristic of human-related contamination, then the UE site should be considered for investigation.



Future Actions

The main sites of concern were OW, NED 1, NED 2 and possibly UE. The OW site was extremely high, but the source appears to be non-anthropogenic and therefore challenging to remedy. Even so, microbial source analysis should be done to verify the source is wild. The two sites in the northeastern sub-basin, NED 1 and NED 2, appear to be impaired because of human-related reasons and so remedial actions are necessary. Testing for pipe or septic system damage should be done in the neighborhood near NED 2, as well as additional, more continuous, water quality testing. If a break is found, steps should be taken to repair it, if not, further source testing will need to take place. Microbial source analysis should be performed at site NED 1 to test for both canine and wild sources. If confirmed canine, steps will have to be taken to reduce canine waste in the area. Site UE is unlikely a source of contamination, but could be considered for further source analysis because the characteristics at the site are similar to the human-impacted site NED 2.

The site characteristics LDHR, HDLR and HDHR may be possible indicators of fecal sources in McKay Creek, which could potentially aid in assessing impaired creeks. However, further testing for a longer, more continuous sampling period would be necessary to test their validity.

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FIGURES

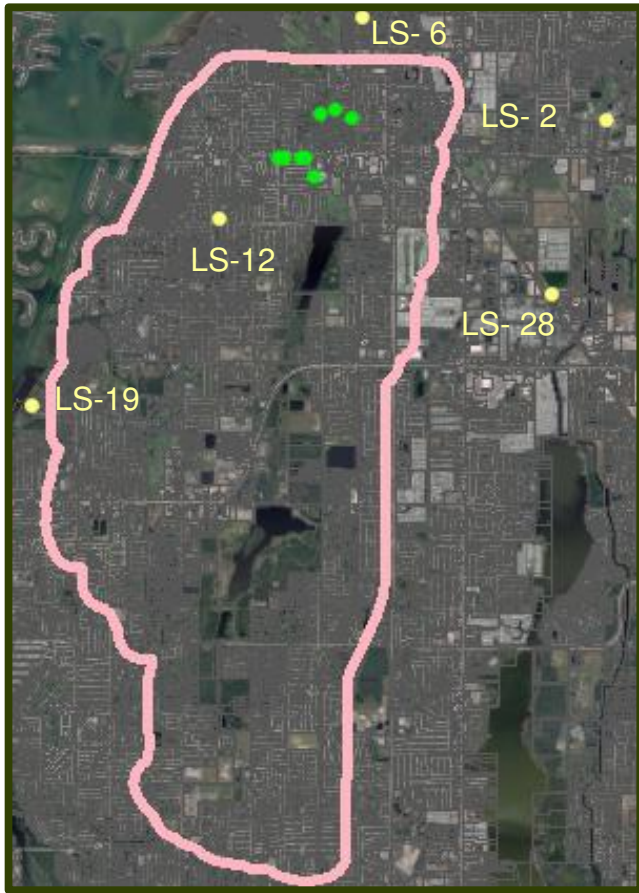


Figure 1 - Map of the Narrows Watershed (Pink). Also given are the locations and IDs of all 5 lift stations that were used as rain gauges (yellow) with respect to sample locations (green).

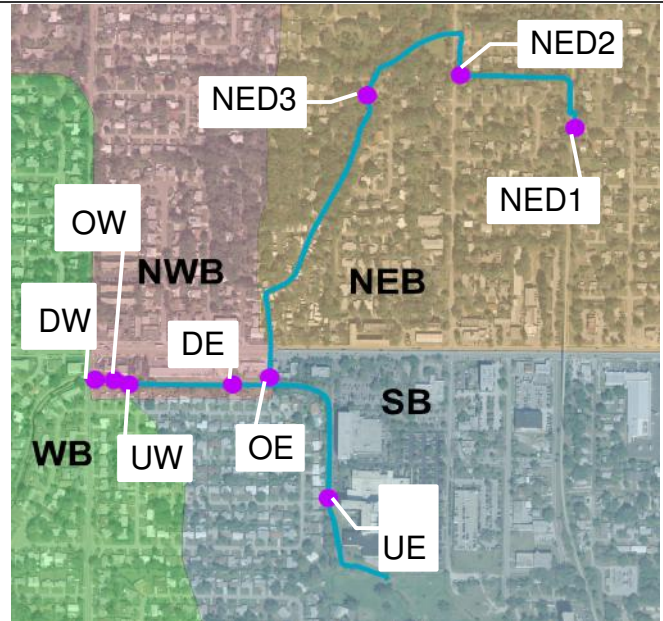


Figure 2 – Location of sampling sites (green) with Site IDs (boxes) along the McKay Creek and its northeastern tributary (blue).

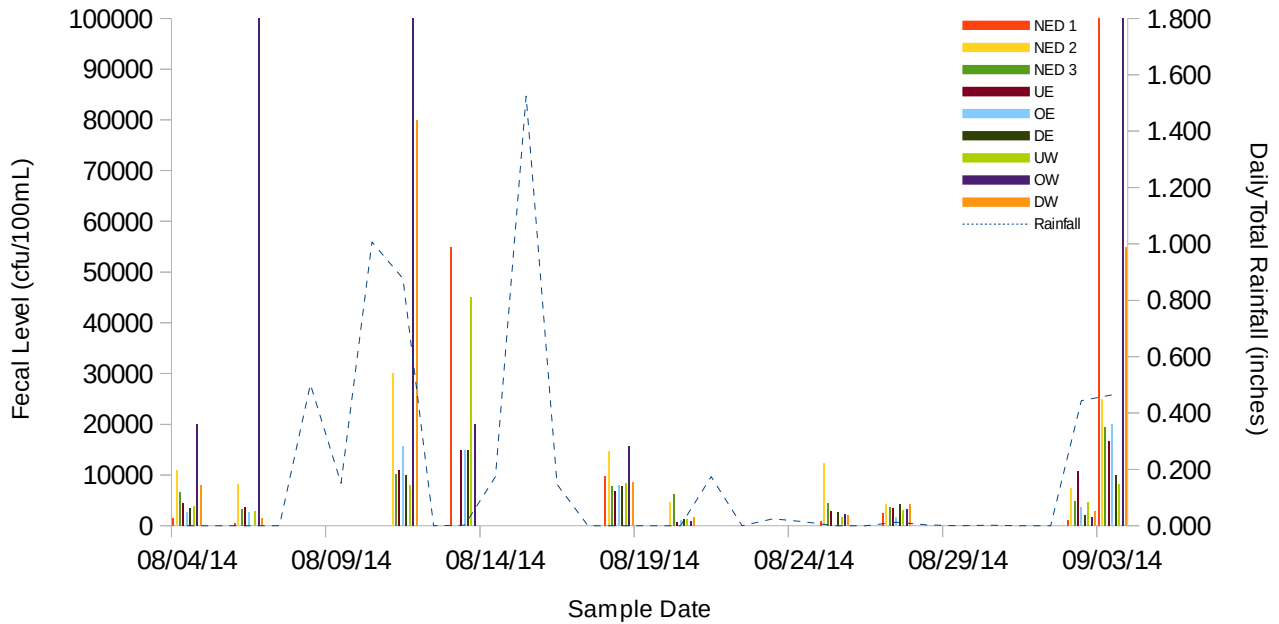


Figure 3 – Daily total rainfall (dashed line) and daily average fecal levels (bars) throughout the sampling period.

Coliform Level During a Rain Event

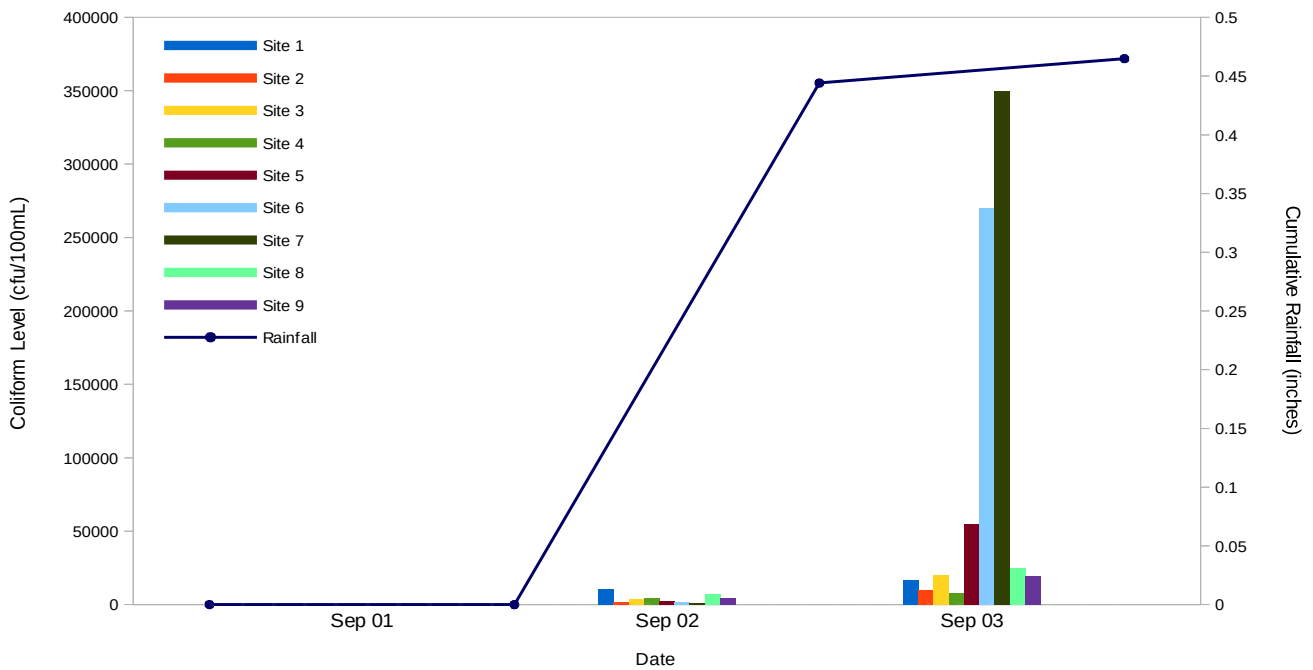


Figure 4 – Fecal levels (bars) before and after a rain event that occurred between sampling on September 2nd and September 3rd and the cumulative rainfall over the monitoring period (solid line).

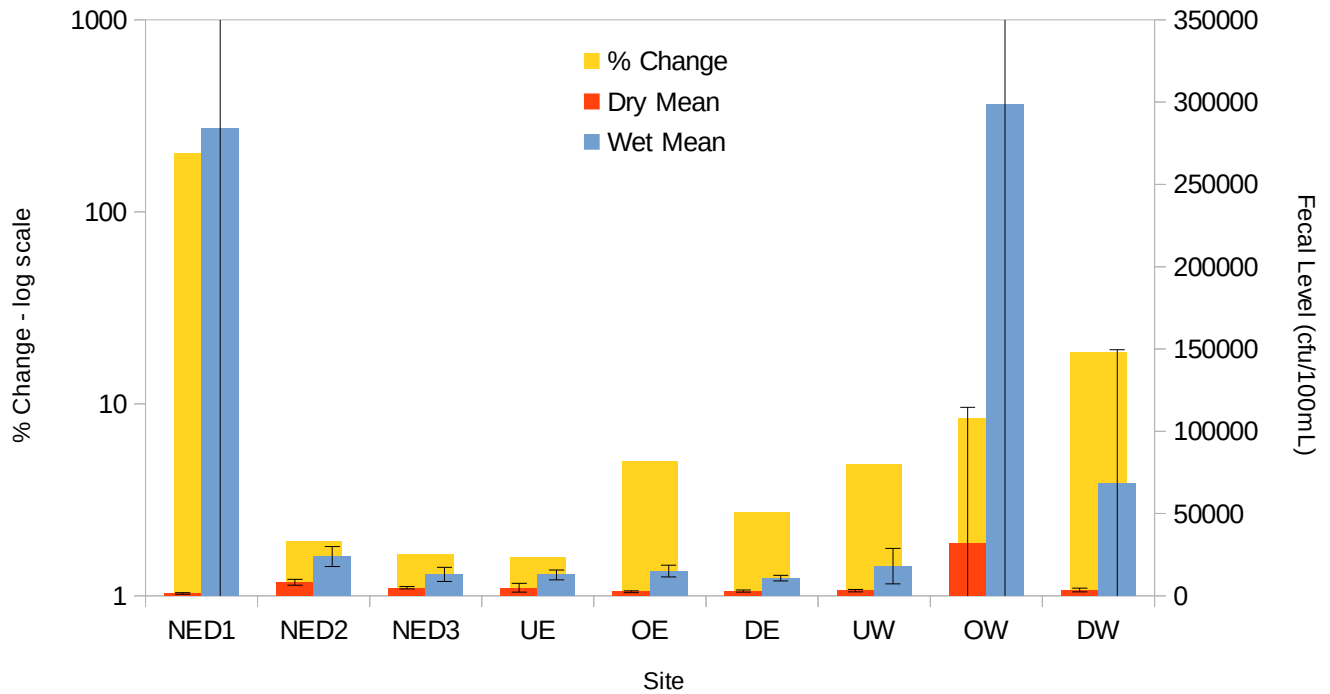


Figure 5 – Comparison of the average fecal levels (cfu/100mL) in dry (red) and wet (blue) conditions. 75% CI are shown as error bars (NED1 and OW were the most variable, so had CI that far exceeded values shown on graph 913,000 and 666,000, respectively). The change from dry to wet is represented by the percent change (yellow) on a log scale.

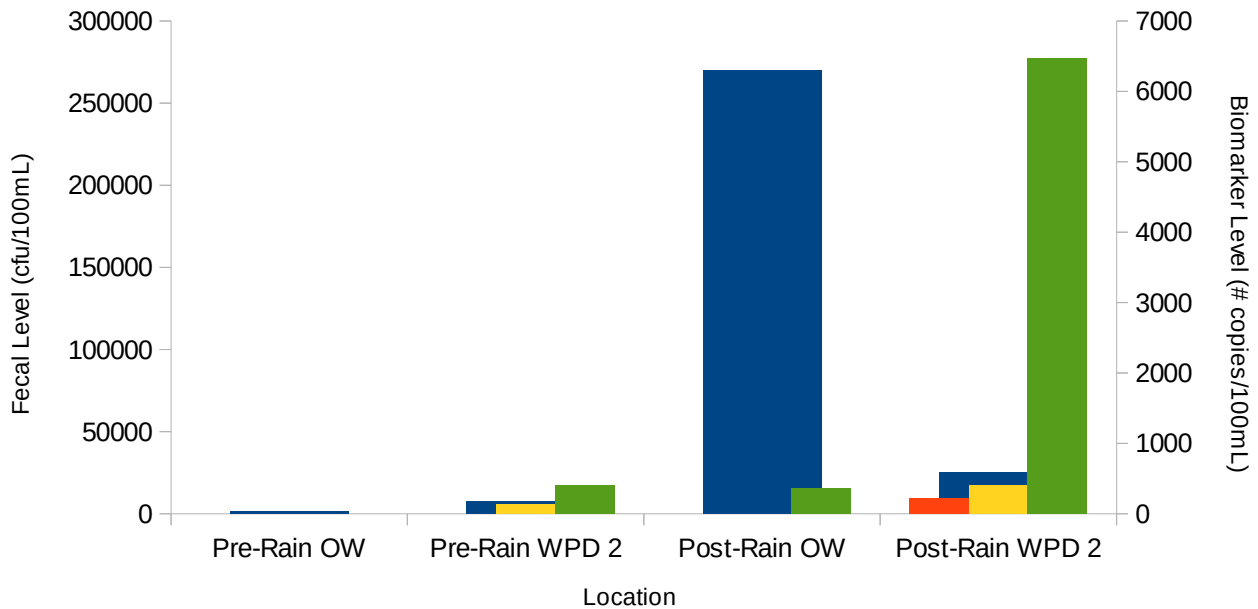


Figure 6 – Pre- and Post-Rain Event (9/02 and 9/03, respectively) Fecal and Biomarker (Human 1, Human 2, and Canine) levels at sites OW and NED 2. Trace amounts of Human Biomarker 1 were present on 9/02 at NED 2, however not at high enough levels to be able to quantify.



TABLES

Site ID	Description	Sub Basin Represented	Sample Type	Latitude	Longitude
UE	Upstream of eastern outfall	South	Fecal	27.914328	-82.804788
DE	Downstream of eastern outfall	Northeast and South	Fecal	27.916187	-82.806463
OE	Outfall of eastern storm water structure	Northeast	Fecal	27.916308	-82.805823
UW	Upstream of western outfall	Northeast and South	Fecal	27.916194	-82.808267
DW	Downstream of western outfall	All (South, Northeast, Northwest)	Fecal	27.916389	-82.808889
OW	Outfall of western storm water structure	Northwest	Fecal and Biomarker	27.916283	-82.809023
NED 1	Northeastern Sub-Drainage Basin, furthest upstream	Northeast	Fecal	27.920482	-82.800546
NED 2	Northeastern Sub-Drainage Basin, mid-stream	Northeast	Fecal and Biomarker	27.921342	-82.802526
NED 3	Northeastern Sub-Drainage Basin, furthest downstream	Northeast	Fecal	27.920985	-82.804150

Table 1 - Table describing sample site attributes. Includes site ID name, physical location description, which sub-basin drains into the area, sampling type (daily fecal sampling or biomarker sampling coinciding with rain event), and the latitude and longitudes (decimal degrees).

Site ID	Dry Fecal Levels (cfu/100mL)	75% Confidence Interval (CI)	Wet Fecal Levels (cfu/100mL)	75% Confidence Interval (CI)	Percent Increase (%)
NED 1	1400	463	283800	9.13E+05	+272
NED 2	8200	1738	23900	6044	+504
NED 3	4900	657	13000	4251	+484
UE	4900	2630	12700	3048	+1860
OE	2500	617	15100	3566	+159
DE	2900	720	10800	1726	+843
UW	3100	751	18100	10756	+20171
OW	31700	82842	298800	6.66E+05	+191
DW	3500	1127	68600	80960	+165

Table 2 - Fecal levels (colony forming units/ 100mL) found at each site during both wet and dry conditions, as well as the 75% CI and percent increase in fecal levels from dry to wet conditions.



Site	Fecal Level	Human 1	Human 2	Canine
Pre-Rain OW	1700	ND	ND	ND
Pre-Rain NED 2	7450	<LOQ	142	403
Post-Rain OW	270000	ND	ND	366
Post-Rain NED 2	25000	225	404	6470

Table 3 - Fecal (cfu/100mL) and Biomarker (# copies/100mL) levels on Pre- and Post-Rain Event days (9/02 and 9/03, respectively). A non-detect (ND) result meant that the biomarker was not present or was not present at a high enough level to detect. If the biomarker was present, but not at a high enough level to quantify, it was reported as below the limit of quantification (<LOQ).