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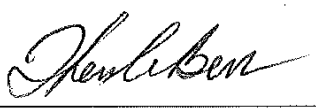
Microbial and Chemical Assessment of Fairview Environmental Park in Montgomery, AL

by

Kyle Lesinger

An Undergraduate Thesis Submitted to
The University Honors Program
Auburn University at Montgomery

In partial fulfillment of the requirements for the degree of
Bachelor of Science in Biology



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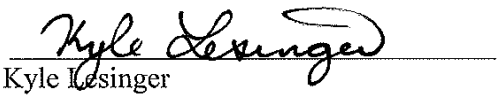

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Honor's Thesis

Advisor – Dr. Ben Okeke

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Microbial and Chemical Assessment of Fairview Environmental Park in Montgomery, AL

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Abstract

Quantification of bacterial pathogens and chemical concentrations are necessary to provide an accurate assessment of a water systems' health. The Fairview Environmental Park was constructed to aesthetically improve the surrounding area and to assist in reducing contamination into Catoma Creek from Genetta Stream (Montgomery Parks and Recreation). Monthly samples were taken from Fairview Environmental Park in Montgomery, AL from the in-flow and out-flow points to determine the water's quality and to determine if the current structure was satisfactory for reducing pathogen load and chemical buildup. After sampling once per month over a 4-month time period, it was determined that pathogen load was not consistently decreased between in-flow and out-flow points and it is recommended that the Fairview Environmental Park should be reconstructed to become more efficacious at reducing pathogen load. Chemical analysis indicated that there were no heavy metals or toxic components in the water and no chemical remediation is necessary with respect to chemicals analyzed.

Introduction

Water is a vital compound that is necessary for human life and its quality can promote a healthier lifestyle. Water serves as a medium that is both non-volatile for most chemicals and it acts as a refuge for numerous bacteria and parasites to survive, grow, and proliferate. Water is also conducive to the breakdown and elimination of

wastes by acting as a solvent, serving to assist in releasing unneeded heat, and acting as a metabolite in photosynthesis and aerobic respiration (Gould 2011 and USGS 2016). Clean water is considered water that is free from harmful pathogens, chemicals, and debris and has a direct correlation with promoting human health.

Bacterial contamination of water has been well documented to produce disease in humans with symptoms including gastroenteritis, diarrhea, vomiting, and abdominal pain (Arnold et al. 2016, Hodge et al. 2016, and Khan et al. 2013). Health issues have also been observed with consumption of chemicals and other heavy metals that may lead to permanent organ damage or death (Cerveny et al. 2016 and Khan et al. 2013). Removal of harmful bacteria and chemicals must remain a priority and more efficacious tests should continue to be researched to properly characterize a water system's health as quickly and efficiently as possible.

Bacterial coliforms and bacterial fecal coliforms are commonly used as water quality indicators and their presence in large numbers is indicative of contaminated water (Galfi et al. 2016, Partyka et al. 2016, and Tong et al. 2016). Testing for coliforms and fecal coliforms has become routine and cost-effective over previous methods when scientists had to run numerous chemical and biologic tests to determine bacterial properties. Bacterial coliforms are Gram negative rod-shaped, non-sporing forming, oxidase negative, aerobic or facultative anaerobes that can usually ferment lactose with the production of gas when incubated at 35^{o}C within 48 hours (Baron and BioLumix). Coliforms include organisms from the genus *Pseudomonas, Klebsiella, Escherichia, Citrobacter,* and *Enterobacter*. Fecal coliforms are bacterial species who come from

animal or human feces and have different growth properties then coliforms but have the same rod-shaped morphology. Fecal coliforms have the ability to ferment lactose between 44.5 ^{o}C-45.5 ^{o}C. These organisms include organisms from genus *Pseudomonas, Escherichia, Citrobacter, Enterobacter, Citrobacter,* and *Klebsiella*. Their presence in drinking water contributes to gastrointestinal complications and disease and may lead to chronic gastrointestinal complications (Hodge et al. 2016 and Seyfried et al. 1985). Although coliforms are ubiquitous in nature and have even known to reside in plants, an increase in fecal coliforms in water indicates that is a potential source of fecal pollution nearby. Quantification of bacterial numbers can be performed through most probable number (MPN) using defined substrate technology. To improve water quality and reduce disease, identification and removal of coliforms and fecal coliforms must continue to be a priority.

Common methods for bacterial identification in water include multi-plex real-time PCR, DNA probes, or biochemical assessment of cultures (Minogue 2013, Santiago 2015, Yipin 2011, and Zimmer-Faust 2016). These technologies have the ability to produce rapid, accurate results, but generally are expensive to own and operate. Performing PCR gives one the ability to generate large amounts of genetic material to analyze the genome for identification. DNA probes are used to hybridize to portions of complementary DNA for identification, but these probes can have errors due to detection limits (Bonvicini et al. 2015 and Kuritza et al. 1986). PCR in conjunction with DNA probes has the ability to produce enough genetical material to hybridize DNA probes to satisfy detection limits. This technology can also identify numerous bacteria

by using different primers and probes and has been shown to detect *Brucella* species in 10 minutes (Sikarwar et al. 2017). Biochemical assessments have the advantage of being inexpensive and identification of particular nutrients or by-products that are made can be identified visually.

HEA was used as the first method of selective and differential selection in identification of unknown microorganisms. This media contains ten times the amount of lactose compared to the other sugars of glucose and sucrose (HEA-QC). Colonies that ferment lactose are generally orange in color and colonies that ferment sucrose or salicin can be white-, blue-, or green-colored. HEA is also specific for most enteric, Gram negative bacteria and the media limits proliferation of Gram positive organisms through the incorporation of bile salts.

The objective of the experiment was to assess the water quality of Fairview Environmental Park through bacterial enumeration, identification, and chemical analyses. I hypothesized that water samples collected from the out-flow will have a decreased number of chemicals and pathogens when compared to the in-flow due to aerobic conditions and biotic foliage in the Park.

Materials and Methods

a) Monitored site

The Fairview Environmental Park, now referred to as the Park, in Montgomery, AL was chosen as the chemical and microbial assessment sampling site. This Park was chosen because of its location to Auburn University at Montgomery and because I have

conducted past water collection at this site. Located at 32.35228°, -86.31911°, the Park was completed in 2015 and constructed to aesthetically improve the surrounding area, to remove contaminated soil from ground sources, and to improve the quality of water that runs from Genetta Stream to the Catoma Creek (see Figure 8) (Montgomery Parks and Recreation). The Catoma Creek flows into the Alabama River which is a recreational site used by many Alabama residents. The Park's construction also included the removal of contaminated soils that had been polluted by excess trash buildup and dumping of materials by a glass company that resided on the current lot. The Park's new construction was also aimed at increasing the filtration of storm water runoff with the use of local plants and trees.

Since 1998, Catoma Creek has been placed on the state's §303(d) river and stream list for pathogens, storm sewers, urban runoff, and pasture grazing (ADEM). Upstream to Catoma Creek is Ramer Creek and downstream of Catoma Creek is the Alabama River. The impaired section had a length of approximately 21.3 miles (TMDL). In 2016, Catoma Creek was not listed as a §303(d) river and stream, but rather had sections that were Category 2B which is interpreted as low priority for remediation based upon funds due to the existence other more polluted waters. In fact, many sections of Catoma Creek are classified as Category 4A waters meaning they the total maximum daily load has been established and are meeting expectations as required by the EPA. The re-categorization of Catoma Creek cannot be attributed to the Park alone, but improving water quality standards can begin in even the smallest watersheds. The Park is considered riparian area because of the interaction between water and land.

Although the Park has a small surface area, it can be considered vital for plant and animal growth and development.

The Park construction project was completed in three separate phases. Phase One was funded through ADEM's Section 319 grant program, a loan from Alabama's EPA-funded Brownfields Revolving Loan Fund, and a HUD Community Development Block Grant (CDBG). The city of Montgomery matched federal dollars for this project. 2D Studio LLC was contracted for creative design and construction of certain portions of the Park including horticulture for remediation of storm water. Phase Two of Park construction focused on green infrastructure additions to the Park. This green infrastructure design added a permeable surface so that runoff is allowed to naturally permeate the ground and to add seating, walking paths and lighting. This phase was funded through EPA's Clean Water State Revolving Loan Fund and HUD CDBG funds. The Third Phase of the Park project has yet to be completed and is awaiting additional funding. The Third Phase proposes to restore ½ mile of culverted stream that is located south of the Park. This restoration would lessen the impact between the modified stream and the concrete ditch between the Park and Catoma Creek. The proposed changes seek to connect the stream with the floodplain located downstream and reduce flood risk and create a more natural ecosystem (Urban Waters Partnership).

b) Field sampling procedures

Over a 4-month period, a total of 4 samples were collected from both in-flow and the out-flow sections of the Park. For simplicity, samplings are labeled according to

sampling day (D1, D2, D3, or D4) and were noted if they were in-flow or out-flow samples. Samples were collected to identify if there was a change in microbial and chemical activity. Out-flow grab samples were conducted first to remove potential for contamination from in-flow, grab-sample disturbances. Temperature measurements were obtained by lowering an Onset HOBO Pendant Logger, created by MicroDAQ, into the water and retrieving Pendant Logger after collection and dissolved oxygen test is complete. D1 temperature recordings were set to catch at 10 seconds, but battery life would soon become an issue. D2, D3, and D4 temperature recordings were captured every 30 seconds to save battery life. PVC Biobailers provided by Solinst were used to retrieve water from an elevated height of approximately 15 feet. Three new 500mL HDPE bottles from VWR were used to collect and store water collected from the out-flow. After collection of water, LaMotte TesTab dissolved oxygen tabs were used to quantify the dissolved oxygen content. A separate tube was filled and stored away for five days at room temperature in the dark to determine BOD content. After water was retrieved, turbidity and odor were observed and recorded. After out-flow samples were retrieved, in-flow water was collected and temperature and dissolved oxygen were measured in an identical fashion to out-flow samples. Outside temperature was gathered via AccuWeather (AccuWeather). All in-flow and out-flow samples were transported to AUM laboratory for further processing.

c) Water quality assessment test selection and procedures

Testing a water's quality can be expensive and time consuming; therefore, testing supplies, procedures, and objectives should be carefully planned prior to field work being performed.

IDEXX Quanti-tray technology has proven efficacious and cost-effective in determining MPN microbial counts in water samples (Bain et al. 2015 and Bram et al. 2011). IDEXX utilizes defined substrate technology to both detect and quantify individual bacterial species. In-flow and out-flow samples were diluted, sealed, and incubated according to the temperature requirements of the test. The sample water, nuclease free water, and Colilert packet was mixed together in a sterile flask. Mixing was accomplished by gently rotating the flask until the sample was homogenous. Samples were then placed in a quanti-tray 2000 containter (96 wells), labelled to ensure accuracy, and transferred to the Quanti-tray Sealer to thermally seal the Quanti-tray 2000 packet. Sample containers mixed with Colilert were incubated at 35^{o}C +/- 0.5^{o}C for 24 hours and evaluated for both color change (for coliforms) and fluorescence under UV light (for *E. coli*). Sample containers mixed with Enterolert were incubated at 41^{o}C +/- 0.5^{o}C for 24 hours and evaluated for fluorescence under UV light (for *Enterococci*). Sample containers mixed with Pseudalert were incubated at 38^{o}C +/- 0.5^{o}C for 24 hours and evaluated for fluorescence under UV light (for *Pseudomonas aeruginosa*).

Coliscan Plus Easygel was used for quantification of *E. coli* organisms and to compare with results from IDEXX Colilert. Samples were first shaken to disturb particulates in the water, then 1mL of in-flow water was placed into the Coliscan Plus Easygel bottle, vortexed, and poured into aseptically designated Coliscan Plus Easygel plate. In another

plate, 2mL of in-flow sample water was added to another Coliscan Plus Easygel bottle, vortexed, and poured into a petri dish. These two steps were repeated again for out-flow samples. Plates were left to sit and solidify at room temperature for 10 minutes then transferred to an incubator at 35^{o}C +/- 0.5^{o}C for 24 hour.

In-flow and out-flow water samples were serially diluted under a biological safety II cabinet to 10^{-1} and 10^{-2} and spread on Hektoen Enteric Agar (HEA) using an Lazy L spreader, and incubated at 35^{o}C +/- 0.5^{o}C for 24 hours. Growth for each plate was observed and individual isolates were chosen for biochemical assessment to determine genus or species name. Isolates were labeled and were inoculated into sterile 5mL Tryptic Soy Broth tubes (TSB). After 24 hours incubation at 35^{o}C +/- 0.5^{o}C, isolates were observed for growth and growth patterns were noted.

TSB subcultures of isolates were then used to inoculate Tryptic Soy agar slant (TSA), Triple Sugar Iron slant agar (TSI), Lysine Iron Agar slant (LIA), Oxidative Fermentation Basal Medium agar deep w/ mineral oil (OF w/), Oxidative Fermentation Basal Medium agar deep w/o mineral oil (OF w/o), Bile Esculin Agar slant (BEA), Potato Dextrose Agar (PDA), Motility Indole Ornithine agar deep (MIO) Simmons Citrate agar slant (CIT), Phenylethyl Alcohol Blood agar plate (PEA), Eosin Methylene Blue agar plate (EMB), Chocolate Agar plate (Chocolate), Brilliant Green agar plate (BGA), and MacConkey agar plate (MAC). All inoculations occurred under a biological safety II cabinet. Aerobic conditions were simulated for TSI, TSA, TSB, BEA, OF w/, OF w/o, MIO, PDA, CIT, PEA, EMB, Chocolate, BGA, and MAC inoculations. Anaerobic conditions were simulated for EMB, MAC, BGA, and PEA plates.

In addition to using liquid media for microbial growth and quantification, dry compact media was utilized for total colony counts for D3 and D4 samples for bacteria and fungi respectively. Hardy Diagnostics has created general purpose, selective, and differential media for microbial quantification. This media is advantageous in terms of storage and interpretation. Plates come joined together and can be stacked to save space or kept together to for easy serial dilution colony count assessment. Dilutions of in-flow and out-flow water include 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. 1mL of diluted in-flow water was pipetted directly into the middle of the dry compact media where diffusion occurred. Out-flow water was also pipetted into 10^{-1} – 10^{-7} dilution. All plates were inverted and placed in incubator for 48 hours at 35^{o}C +/- 0.5^{o}C.

Chemical analyses of water was performed using API Freshwater Master Test Kit to evaluate pH, nitrates (NO^{3-}), nitrites (NO^{2-}), ammonia (NH^{3}/NH^{4+}), general hardness (GH), and carbonate hardness (KH). Tests were performed according to API instruction manual. Copper (Cu) and lead (Pb) were analyzed using the PurTest Lead test kit. Arsenic (As^{3+}) was analyzed using Freshwater Systems Arsenic quick check test kit. Iron (Fe) and Chlorine (Cl) were analyzed using Insta-test strips. 5mL samples of in-flow water was used for each test except the test for arsenic. Water results were visually inspected, compared to reference instruction guide, and recorded. Three water samples were collected from D2 in-flow water and sent to Gulf Coast LabNet for analysis of trichloroethene (TCE). Analysis of TCE included matrix spiked samples and laboratory control samples to ensure accuracy of results.

For viewing bacteria, samples must be fixed to a microscope slide. Isolates were heat fixed onto a microscope slide from TSB subculture and stained with Crystal Violet stain for 1 minute. The slides were washed with deionized water and Gram's Iodine Mordant stain was applied for 1 minute. The slides were washed briefly with a 50% ethyl alcohol/50% acetone Gram decolorizer solution and then rinsed with water. Safranin was then applied to the slide and let sit for 1 minute and then rinsed off with water. The slides were dried using Bibulous paper and microscopic examination occurred using an Olympus CX31 under 100X oil immersion. Color, size, and morphology of bacteria was characterized and used as evidence for bacterial unknown identification.

Calculations

a) IDEXX Quanti-tray (Colilert, Enterolert, Pseudalert)

Analysis and enumeration of microbial numbers has been simplified using the IDEXX Quanti-tray system and MPN chart (see Table 2 and Table 3). Rapid identification of coliforms, *Enterococci*, *E. coli,* and *P. aeruginosa* can be performed and accurately assessed within 24 hours. Results from all four sampling procedures are as follows. For diluted samples, multiplication of IDEXX MPN final number by a dilution factor is needed for MPN calculation. For 1:0 dilution, no dilution factor was needed. For a 1:1 dilution, a dilution factor of 2 was applied. For a 1:2 dilution, a dilution factor of 3 was applied.

For a 1:3 dilution, a dilution factor of 4 was applied. For a 1:4 dilution, a dilution factor of 5 was applied.

b) Coliscan Plus Easygel

Quantification of *E. coli* was determined according to Micrology Laboratory's colony count method per 100mL (Micrology Laboratories). Coliscan Plus Easygel technology provides researchers the ability to distinctly identify *E. coli* based upon both purple color and fluorescence under UV light.

This formula is: a) divide 100 by the number of mL used for sample

b) multiply the count on plate by result obtained

c) disregard light blue, blue-green or white colonies

Analysis of standard deviation could not be conducted since there were different amounts of sample in each bottle for D1, D2, and D3 and only 1 plate was poured for each specific sample volume. For D4 sample analysis, five plates were poured and the average of the five was recorded.

c) Colony forming units/mL

Total colony counts were performed using Hardy Diagnostics Compact Dry media. Calculation of totally colony count was performed by using the formula described in the Environmental Laboratory Manual (Pepper and Gerba 2004). This formula for colony forming units on a plate is:

(# of colonies x inverse dilution) ÷ amount of sample pipetted (mL)
(e.g., $10^{-6} = 10^{6}$)

Quality Control

Quality control (QC) tests were performed to ensure that media was functioning properly. Due to expensive costs of new media, expired media was used for some tests. Media that did not meet QC criteria cannot be considered for this project and cannot be included in results. The only media that did not meet QC requirement was MacConkey agar. All KwikStik QC organisms were purchased from VWR. All plates and tubes were incubated aerobically at 35°C +/- 0.5°C for 24 hours and checked for growth and a visual change in media.

Escherichia coli ATCC 25922 was used as the QC organism for TSI, EMB, and MAC agar. After *E. coli* inoculation on Difco TSI, there was a change in media to an alkaline (yellow)/alkaline (yellow) color. Additionally, signs of gas production were present with no hydrogen sulfide production. This follows proper QC characteristics of the media for *E. coli* (TSI-QC). *E. coli* growth on Levine-EMB was successful and produced a large, blue-black, green metallic sheen (EMB-QC). When *E. coli* was inoculated onto Remel MacConkey agar, only 1 purple colored colony was seen. QC results should have seen no growth inhibition and colonies that were pink to red (MAC-QC). When inoculated in an MIO deep, growth was observed that was both motile and positive for ornithine decarboxylase. With addition of Kovac's reagent, a pink colored was produced showing that the organism could produce indole (MIO-QC).

Streptococcus pyogenes ATCC 19615 was used as the QC organism for BEA and PEA plates. On BEA, no growth was observed and the media did not have any characteristic blackening. This is the expected growth pattern of *S.* pyogenes on BEA (BEA-QC). On PEA, no growth was observed. Growth of *S. pyogenes* on PEA should be observable and not inhibited (PEA-QC). This constitutes a failure for the media, but another inoculation was performed using *Enterococcus faecalis* ATCC 29212.

Enterococcus faecalis ATCC 29212 was used as the QC organism for BEA and PEA. After 24 hours on BEA, positive growth and blackening of the media was observed. This result is positive for hydrogen sulfide production which is a typical for this media (BEA-QC). On PEA, growth was observed and colonies were colorless. This is the expected growth pattern (PEA-QC).

Salmonella enterica subsp. *enterica* serotype *Typhimurium* ATCC 14028 was used as the QC organism for BGA and HEA agar. When grown on BGA agar, clear-colored growth was observed and the media turned bright pink. This is indicative of a lowering of the pH and is indicative of a positive control (BGA-QC). When inoculated on HEA, positive growth occurred and colonies exhibited typical green/blue color with black centers, indicative of hydrogen sulfide production (HEA-QC). When *S. enterica* was inoculated onto LIA, a black precipitate was formed and the butt and slant remained purple. This is indicative of hydrogen sulfide production and lysine decarboxylation which is typical for this organism (LIA-QC).

Klebsiella pneumoniae ATCC 33495 was used to inoculate CIT and EMB. When grown on CIT, a blue color was observed at top of slant which is indicative of sodium citrate use

as carbon source and ammonium dihydrogen phosphate as source of nitrogen. This is a positive QC result for this media (CIT-QC). When *K. pneumoniae* was grown on EMB, growth was observed and colonies were purple colored. This indicates a positive result (EMB-QC).

Pseudomonas aeruginosa ATCC 27853 was used for inoculation of OF w/ mineral oil. Growth was observed and there was no color change in media. This is indicative of no fermentation occurring and is consistent with OF inoculated with *P. aeruginosa* (OF-QC).

A QC check was successful for the following media: HEA, BGA, PEA, TSI, BEA, MIO, LIA, PEA, OF, EMB and CIT. MAC agar failed the QC test.

Results and Discussion

After analyzing samples from both in-flow and out-flow grab samples, there does not appear to be any evidence that the Fairview Environmental Park is consistently increasing water quality with regards to *Enterococci* between the in-flow and out-flow points. Out-flow samples from D1 showed a decrease in *Enterococci* when compared to the in-flow (435.6/100mL and 845.0/100mL respectively for D1) and D4 samples also showed a decrease in *Enterococci* between the in-flow and the out-flow (6016.5/100mL and >12,098/100mL respectively for D4) (see Figure 3 and Table 1). But samples from D2 and D3 grabs show that bacterial numbers are increasing between the in-flow and out-flow. D2 grab samples showed an increase of 1139.6 *Enterococci*/100mL between the in-flow and out-flow and D3 samples showed an increase of 6025.2

Enterococci/100mL between the in-flow and out-flow samples. D4 samples for *Enterococci* were run in duplicates due to availability of IDEXX materials and showed that there was a significant decrease in *Enterococci* concentration between the in-flow and out-flow points. The discrepancies may be attributed to samples not being mixed enough prior to dilution or due to temperature differences between the two sampling sites. Although there was minimal temperature difference between in-flow and out-flow points (x=4 ^{o}F), this temperature difference could affect the number of *Enterococci* present. This error could be fixed by running multiple samples on the same dilution, taking the average, and determining standard deviation between samples for a more accurate assessment.

As temperatures increased, microbial numbers increased in all samples with the exception of D4 outflow for *Enterococci* (46.0/100mL at 78.1 ^{o}F) (see Figure 3). This increase in numbers can be attributed to an increase in metabolic activity and nutrient acquisition. It would be difficult to graph changes in microbial numbers by temperature since all temperatures were different in each grab sample and for different time periods. There is no direct correlation between temperature and microbial growth between the D1, D2, D3, and D4. It was observed that at the highest recorded temperature of 78.1 ^{o}F for D4 out-flow there were less *Enterococci*/100mL than at the lower temperature of 65.1 ^{o}F for D3 out-flow (see Table 1). This is a 13 ^{o}F difference between the two grab samples and recordings indicate that there were 1242 less *Enterococci* when the temperature was raised by 13 ^{o}F. This could be attributed to fauna being more numerous and efficient at higher temperature in reducing pathogen

concentration. The highest recorded concentration of *Enterococci* in out-flow measurements was 7258.8/100mL at 65.1 ^{o}F during D3 grab sample. The highest recorded concentration of *Enterococci* in in-flow measurements was >12098/100mL at 74.1 ^{o}F during D4 grab sample. There does not appear to be a direct correlation between temperature and *Enterococci* concentration as can be seen in Figure 4.

Coliforms were tested for all four grab samples and for in-flow and out-flow using IDEXX Colilert. There was an overall increase in coliforms when compared to temperature (see Figure 5). In each sample collected, the maximum concentration detected was found. This does not allow for a comparison between in-flow and out-flow sites. To alleviate this problem in the future, multiple higher dilutions are needed and these can be run in triplets as well. Without understanding the microbial community interaction, the increase in coliforms cannot be attributed to an increase in temperature alone and it is likely that multiple variables are involved.

E. coli concentrations were analyzed for in-flow and out-flow samples and it was observed that *E. coli* concentrations were increasing in all samples between the in-flow and out-flow (see Figure 6). Two measurements were taken for D4 samples and the average was recorded and incorporated into the graph. The highest recorded concentration for *E. coli* was found in out-flow grab samples from D3 in which the concentration was 4659.3/100mL at a temperature of 65.1 ^{o}F. It is obvious that there is an increase in pathogen load between the in-flow and the out-flow regions of the Park and this may be attributed to excess trash build-up in the Park that is not being cleaned up effectively (see Figure 11 and Figure 12). As can be seen in Figure 13, there does not

appear to be a direct correlation between temperature and *E. coli* concentration. The highest recorded concentration of *E. coli* was 4,659.3/100mL that was observed at a temperature of 65.1 ^{o}F. The highest temperature recorded was 78.1 ^{o}F with an *E. coli* concentration of 2,393.0/100mL. These observations do not show that temperature alone is the driving force behind increasing microbial numbers.

Concentrations of *P. aeruginosa* were observed to increase in D1, D2, and D3 samples between the in-flow and the out-flow samples and D4 grab samples showed a decrease between the in-flow and the out-flow points (see Figure 7). The largest difference between the in-flow and out-flow points was observed during D3 samples in which there was an increase of 97.4/100mL. D4 samples of in-flow and out-flow showed a decrease in *P. aeruginosa* by 12.2/100mL and this may be due to a temperature threshold. The highest observed concentration of *P. aeruginosa* was at temperature of 65.1 ^{o}F in D3 out-flow sample. It is unlikely that temperature is the driving force for growth for this microorganism and that excess debris and improper fauna is the leading causative factor for increased concentration. There does not appear to be any correlation between temperature and *P. aeruginosa* concentration. The highest recorded concentration of *P. aeruginosa* from all samples occurred at a temperature of 65.1 ^{o}F with a concentration of 145/100mL. The highest temperature recorded was 78.1 ^{o}F and only had a concentration of 46.0/100mL. This implies that temperature cannot be the sole variable for microbial numbers of *P. aeruginosa*.

A comparison between Coliscan Easy Plus Gel and IDEXX Colilert was performed to determine if these two technologies could be used interchangeably to accurately

assess *E. coli* concentration in water (see Figure 2). With the exception of D1 and D2 in-flow samples, there were large fluctuations in numbers of reported *E. coli* concentrations within the sample grab sample. This highest difference between the two tests was observed during the D3 out-flow grab sample with a difference of 4540.7 *E. coli* per 100mL. This implies that one of the methods is more accurate in assessing *E. coli* concentrations than the other. In IDEXX's defined substrate technology has proven efficacious in numerous studies and should be viewed as the more accurate approach.

Chemical analyses of in-flow and out-flow water samples showed that composition of water remains fairly constant between in-flow and out-flow sites (see Table 1). Trichloroethylene was undetectable at 0.00050mg/L during the D2 and all tests were negative for lead (Pb), chlorine (Cl), and arsenic (As^{3+}). Nitrates (NO^{3-}) remained at 5ppm for both in- and out-flow samples for each sampling day. There were no nitrites detected in either in- or out-flow samples. Minimal amounts of copper (0-1.3ppm) were detected in D1 samples and no copper (Cu) was detected in any other sample. The detection of copper in D1 sample may have been a misinterpretation error due to the difficulty in reading test strip. Iron (Fe) was identified in both in- and out-flow samples from D1, D2, and D4 samples and was observed to be 0.3ppm. The pH of in flow and out-flow water remained between 7-8 for each sampling and does not appear to have a correlation with temperature or any other factor. The stability of pH is a good sign that the water is stable in terms of normal pH. The process of testing water for pH was not time-consuming nor expensive for NO_3^-, NO_2^-, GH, KH, or pH, but interpretation was tedious. When estimating concentration of a specific element or

compound, one must use a color coded chart that can be misinterpreted easily due to only slight color variations.

It is interesting to note that when compared to the Alabama Department of Environmental Managements results for *E. coli* concentration, there were largely significant reported amounts. Although the same methods were used by ADEM and me for quantification of *E. coli* (IDEXX Colilert), there were large discrepancies in reported concentrations between February 2, 2017 and February 3, 2017. ADEM contracts laboratory work to Environmental Services Laboratory, Inc. and reports findings back to ADEM. It was observed by ADEM that the concentration of *E. coli* in in-flow sample for February 2, 2017 was 86.2/100mL (see Table 4). This is in contrast to my reported findings of 237.4 *E.coli* per 100mL on February 3, 2017 (see Table 1). With out-flow *E. coli* concentrations, ADEM reports concentrations of 397/100mL on February 2, 2017 (see Table 5). This is in contrast to my observed findings of 660.0 *E. coli* per 100mL on February 3, 2017 (see Table 1). The discrepancy may be attributed to a more concentrated sample that I obtained and not the result of a duplicate sample being taken. It is unlikely that there was a significant change in environmental conditions, but since these samples were taken 1 day apart, it is impossible to say due to confounding variables. One variable that could not be accurately assessed was rainfall. When viewing rainfall data, it does not give precipitation rates for specific areas. Rainfall data is on a large scale and includes the entire city of Montgomery. Data indicates that there is also an increase in *E. coli* concentrations from in-flow to out-flow points which

corresponds to the data that I observed. This is an indication that there is indeed increased pathogen loading between the in-flow and out-flow points.

Bacterial total colony counts were performed using Hardy Diagnostics Dry Compact Media. This media is advantageous because it is uniquely suited for colony counting due to its diffusion properties and the gridlines that are built into the plate. Due to cost, only grab samples from in-flow and out-flow samples from D3 were used. The purpose of this portion of the experiment was inspect how this media functioned and to add to existing data for the Park. It was observed that there were lower concentrations of total bacterial numbers between the in-flow and out-flow points when all data was averaged (see Table 6). It was observed that there were approximately 83,000/100mL in the in-flow samples and approximately 80,000/mL in the out-flow samples. It must be noted there at a dilution of 10^{-5} only out-flow samples contained bacteria. This implies that there are actually more bacteria in the out-flow when compared to the in-flow. If more samples had been acquired, a more accurate representation of bacterial numbers could be formed. I would not use this set of data as soleevidence that there are lower numbers of bacterial numbers in the out-flow when compared to the in-flow.

Fungal total colony counts were also performed using Hardy Diagnostics Dry Compact media. This media was specific for fungi and does not cater to any specific type of fungus. As can be seen in Table 7, there were on average more fungi in the out-flow than the in-flow for D4 grab-samples. Sample dilutions were taken in duplicates and the average of the two was reported. It can be seen that for the in-flow, there were

on average 186.67 fungi per 100mL and in the out-flow there were on average 206.83 fungi per 100mL for D4 grab-samples. There were variations in the number of fungi for each dilution; therefore, this media can only be used as an estimate. It was odd to observe that there was only 1 fungus/mL in 10^{-1} dilution and that there was also 1 fungus that was present in the 10^{-3} sample in the D4 in-flow sample. If there was only 1 fungus/mL it would difficult to find any in subsequent dilutions. It also must be noted that some of the media was covered in color and only a few fungi may have been present. This is indicative of spreading (which is normal for hyphae) but this may affect accurate reporting numbers due to competition within the media. More experiments would need to be run to determine the validity of using this media when examining water samples.

If Environmental Services Laboratory, Inc. only performs double experiments on 10% of their samples as indicated by NEMI Quality Control requirements (NEMI), this implies that triplet experiments are never performed. After calling Environmental Services Laboratory, Inc., it was determined that they would not have run a duplicate sample from grab samples by ADEM and the value from one sample would have been reported. This implies that ADEM could be under-reporting or over-reporting values, based on the one sample collected. To alleviate the discrepancy, samples from the same day would need to be tested by another independent laboratory to verify results.

The calculation of biological oxygen demand was calculated using LaMotte TesTabs and required the ascertainment of the dissolved oxygen concentration from the initial sample and the dissolved oxygen after 5 days. This was extremely difficult due to

interpretation issues with the reference color chart given. The reference chart only gave color indications for 0ppm, 4ppm, and 8ppm. 4ppm and 8ppm were difficult to interpret since both were shades of pink/red. Results of dissolved oxygen and biological oxygen demand are visible in Table 1 and do not give an accurate representation of actual dissolved oxygen in the water. After researching calculation methods, it was determined that LaMotte TesTabs are not able to calculate biological oxygen demand effectively. It was observed that the highest biological oxygen demand occurred with D1 out-flow samples with a BOD of ≈6. This implies that there should have been more bacterial numbers in this water sample, but this is not accurate when compared to specific bacteria that were measured. This could mean that there were more bacteria present in the water sample during that time period, but since bacterial enumeration was only performed on a specific number of microorganisms it is difficult to find the true cause for such a high BOD.

There was difficulty in attempting to identify unknown microorganisms due to lack of experience and improper equipment. An attempt was made to identify microorganisms based upon biochemical features using media that is specific for *Enterobactericeae*. The assumption that one can identify water microbes using this information is true only if one has the correct media. Important media that would have been used for identification of *E. coli* include Methyl Red and Voges Proskauer. I did not have this media due to cost and this would have been a useful test. Another difficulty was the assumption that you could simply streak organisms onto a media plate and determine results. Due to the large number of tests that I was running, I made the

mistake of not streaking for colony isolation. Media is created to determine how an individual colony reacts and can give useful information. Since I did not streak plate the majority of the media plates (i.e., EMB, SBA, PEA, MAC), identification could not be ascertained accurately.

Conclusion

In order to accurately determine unknown organism identification, PCR analysis would need to be conducted or shotgun sequencing of community microbiota. Biochemical tests performed were not specific enough to determine the identity of any cultured microorganisms. Clinical microbes are more easily identifiable and there are many resources available to assist in this identification but sources for non-pathogenic environmental organisms are not as numerous. Environmental organisms form complex communities based upon specific nutrient availability, climate, and geographic location. Certain media tests were not completed correctly; therefore, no specific identification was possible.

Overall, data suggests that there appears to be an increase in pathogenic microorganisms between the in-flow and out-flow sections of Fairview Environmental Park. No suggestions are made about possible remediation since the Park, not a recreational water, falls under acceptable standards at current time. There may be multiple factors influencing pathogen proliferation including but not limited to: excess trash and debris located in the Park, improper plant fauna, or a low water flow rate. Future methods can be employed to reduce the microbial numbers entering Catoma

Creek and can include a shotgun analysis of organisms present in the Park, analysis of plant fauna, and a metagenomic analysis of soil microbiota to identify community microbiological interaction. Triplet experiments would be valuable when running environmental samples for accuracy. It is difficult to determine a correlation with limited samples due to confounding variables that may include a high bacterial concentration in an individual sample. This will skew MPN calculations and cause and inaccurate result and individual samples should be avoided.

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Table 1 Comprehensive chemical and microbial analysis of in-flow and out-flow water by sampling date. Data measurements include: temperature, pH, dissolved oxygen, BOD, NO_3^-, NO_2^-,NH_4^+, GH, KH, Cu, Fe, Cl, Pb, *Enterococci*, coliforms, *E. coli*, *P. aeruginosa*, trichloroethylene, and arsenic (As^{3+}).

	D1 (2/3/17)		D2 (3/3/17)		D3 (4/7/17)		D4 (05/10/17)		(IDEXX 2nd sample)			
	In	Out	In	Out	In	Out	In	Out	In	Out		
Temperature	58.3 oF	56.4 oF	61.2 oF	58.1oF	68.1 oF	65.1 oF	74.1 oF	78.1 oF				
pH	8	8	7.6	7.2	7.8	7.6	7.4	7.4				
Dissolved Oxygen (ppm)	4	4	4	4	4	4	4	4				
BOD (5-day) (ppm)							0	0				
NO3- (ppm)	5	5	5	5	5	5	5	5				
NO2- (ppm)	0	0	0	0	0	0	0	0				
NH4+ (ppm)	2	0.25	0.25	0.25	0	0.25	0.25	0.5				
GH (general hardness)	3	3	4	4	7	4	4	4				
KH (carbonate hardness)	8	7	6	6	6	7	8	10				
Cu (ppm)	0-1.3	0-1.3	0	0	0	0	0	0				
Fe (ppm)	0.3	0.3	0.3	0.3	0	0	0.3	0.3				
Cl (ppm)	0	0	0	0	0	0	0	0				
Pb (ppm)	negative	negative	negative	negative	negative	negative	negative	negative				
IDEXX Enterococci/100mL	845.0	435.6	688.2	1827.8	1503.6	7258.8	>12,098	6016.5	12098.0	3433.5		
IDEXX Coliforms/100mL	4839.2	4839.2	7257.0	7257.0	7257.0	7257.0	12098.0	12098.0	>12,098	>12,098	*E. coli* (Coliscan 1mL)	
IDEXX E. coli (IDEXX)/100mL	237.4	660.0	667.2	1032.3	1738.2	4659.3	1724.0	2393.0	1155.0	3433.5	In	Out
IDEXX P. aeruginosa/100mL	10.2	28.6	24.2	49.0	47.6	145.0	54.2	46.0			700.0	5100.0
E. coli (Micrology) 0.5mL/ 100mL	-	-	-	-	4200.0	9200.0	-	-	-	-	In	Out
E. coli (Micrology) 1mL/ 100mL	400.0	200.0	500.0	3400.0	1900.0	9000.0	600.0	4500.0	500.0	3900.0	700.0	3900.0
E. coli (Micrology) 1.5mL/ 100mL	-	-	-	-	3666.7	6799.0	-	-	-	-	In	Out
E. coli (Micrology) 2mL/ 100mL	150.0	300.0	750.0	3750.0	6050.0	5050.0	-	-	-	-	1800.0	4400.0
Tricholorethylene	-	-	Undetect-able a	-	-	-	-	-	-	-		
Arsenic (mg/L)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

Table 1 IDEXX Quanti-Tray calculation table for 96-well trays. Small wells 0-24.

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

09-63235-01

Table 2 IDEXX Quanti-Tray calculation table for 96-well trays. Small wells 25-48.

Environmental Services Laboratory

6000 Richard E. Hanan Drive Montgomery, Alabama 36108 Phone 206-1701

1st Quarter Results

Lab ID# 30220

Report Date: 03/03/17

Sample ID:	Sample Location:	Analysis Date	Analyte Name	Result	MRL	Unit	Analysis Method
Collection Date: 02/02/17							
AM04351	Genetta Stream at Catoma Creek						
		2/17/17	Oil & Grease	< 5	5	mg/L	EPA 1664B
		2/2/17	Nitrate	<MRL	1.00	mg/L	EPA 300.0
		2/2/17	Nitrite	<MRL	0.100	mg/L	EPA 300.0
		2/3/17	E. Coli	192	N/A	#/100 mL	20 SM 9223B-QT
		2/2/17	Turbidity	14.9	0.10	NTU	20 SM 2130 B
		2/2/17	Total Suspended Solids	24	1	mg/L	20 SM 2540 D
		2/7/17	Biochemical Oxygen Demand	2	1	mg/L	20 SM 5210 B
		2/9/17	Total Kjeldahl Nitrogen	<MRL	0.50	mg/L	EPA 351.2
		2/9/17	Total Phosphorus	<MRL	0.5	mg/L	EPA 365.3
Collection Date: 02/02/17							
AM04352	Genetta Stream Inflow						
		2/17/17	Oil & Grease	< 5	5	mg/L	EPA 1664B
		2/2/17	Nitrate	1.1	1.00	mg/L	EPA 300.0
		2/2/17	Nitrite	<MRL	0.100	mg/L	EPA 300.0
		2/3/17	E. Coli	86.2	N/A	#/100 mL	20 SM 9223B-QT
		2/2/17	Turbidity	3.15	0.10	NTU	20 SM 2130 B
		2/2/17	Total Suspended Solids	1	1	mg/L	20 SM 2540 D
		2/7/17	Biochemical Oxygen Demand	2	1	mg/L	20 SM 5210 B
		2/9/17	Total Kjeldahl Nitrogen	<MRL	0.50	mg/L	EPA 351.2
		2/9/17	Total Phosphorus	<MRL	0.5	mg/L	EPA 365.3

Table 4 Environmental Service Laboratory Testing results for Fairview Environmental Park (Genetta Stream) in-flow for February 2, 2017.

Environmental Services Laboratory

6000 Richard E. Hanan Drive Montgomery, Alabama 36108 Phone 206-1701

1st Quarter Results

Lab ID# 30220

Report Date: 03/03/17

Sample ID:	Sample Location:	Analysis Date	Analyte Name	Result	MRL	Unit	Analysis Method
Collection Date: 02/02/17							
AM04353	Genetta Stream Outflow						
		2/17/17	Oil & Grease	< 5	5	mg/L	EPA 1664B
		2/2/17	Nitrate	1.0	1.00	mg/L	EPA 300.0
		2/2/17	Nitrite	<MRL	0.100	mg/L	EPA 300.0
		2/3/17	E. Coli	397	N/A	#/100 mL	20 SM 9223B-QT
		2/2/17	Turbidity	30.0	0.10	NTU	20 SM 2130 B
		2/2/17	Total Suspended Solids	59	1	mg/L	20 SM 2540 D
		2/7/17	Biochemical Oxygen Demand	7	1	mg/L	20 SM 5210 B
		2/9/17	Total Kjeldahl Nitrogen	3.52	0.50	mg/L	EPA 351.2
		2/9/17	Total Phosphorus	1.11	0.5	mg/L	EPA 365.3

CP - Coliform Present

CA- Coliform Absent

MRL - Minimum Reporting Limit

All samples are analyzed by standard USEPA protocols. All results are validated against laboratory control standards. If you have any questions regarding these analyses or procedures, please contact:

Environmental Services Laboratory

Table 5 Environmental Service Laboratory Testing results for Fairview Environmental Park (Genetta Stream) out-flow for February 2, 2017.

Hardy Diagnostics Dry Compact Total Bacterial Colony Count D3		
In-flow	Out-flow	Dilution
TMTC	TMTC	10^{-2}
106000/mL	50000/mL	10^{-3}
60000/mL	90000/mL	10^{-4}
	100000/mL	10^{-5}
AVG = 83000	**AVG = 80000**	
TMTC = too many to count		

Table 6 Calculation of total bacterial colonies using Hardy Diagnostic Dry Compact Media for D4 grab samples. Calculation of averages of in-flow and out-flow fungi/mL were based upon average of two samples.

Hardy Diagnostics Dry Compact Total Fungal Colony Count D4				
In-flow (1)	In-flow (2)	Out-flow (1)	Out-flow (2)	Dilution
10/mL	10/mL	250/mL	160/mL	10^{-1}
100/mL	0/mL	200/mL	0/mL	10^{-2}
1000/mL	0/mL	1000/mL	0/mL	10^{-3}
AVG = 186.67		**AVG = 234.17**		

Table 7 Calculation of total fungal colonies using Hardy Diagnostic Dry Compact Media for D4 grab samples. Calculation of averages of in-flow and out-flow fungi/mL were based upon average of two samples.

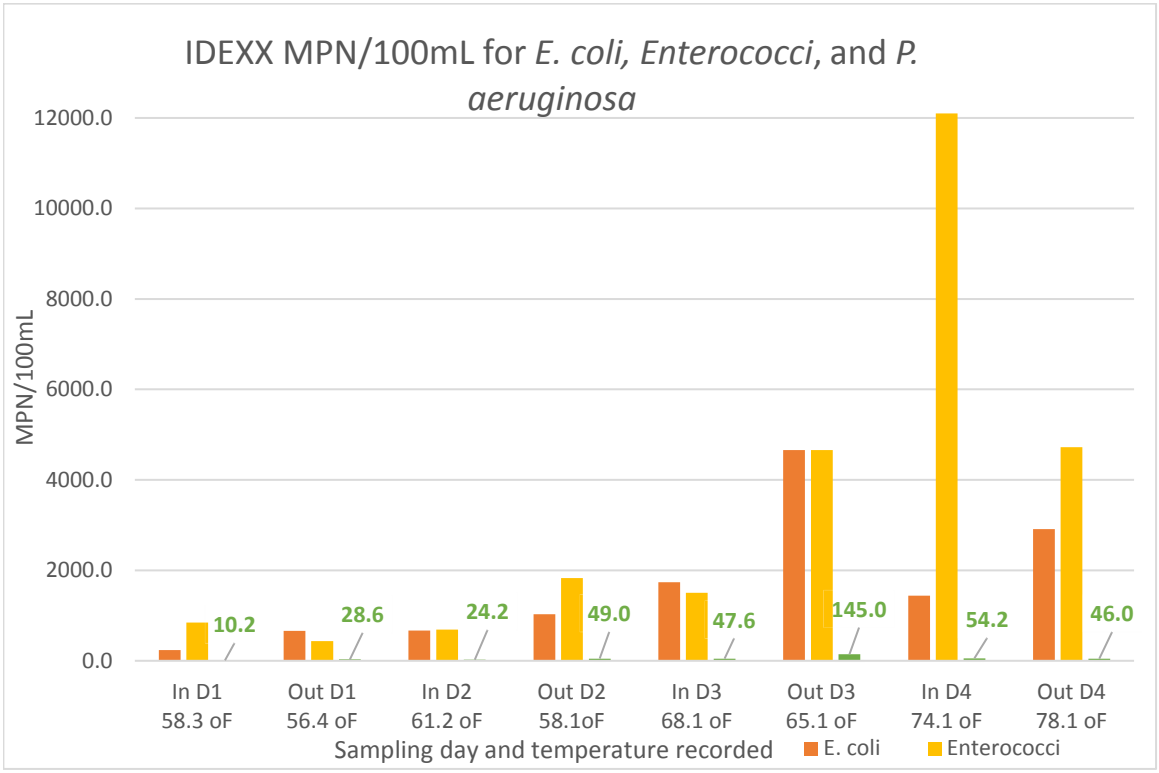

Figure 1 Comparison of MPN calculations of *E. coli, Enterococci*, and *P. aeruginosa* from in-flow and out-flow samples in Fairview Environmental park from D1, D2, D3, and D4 grab samples.

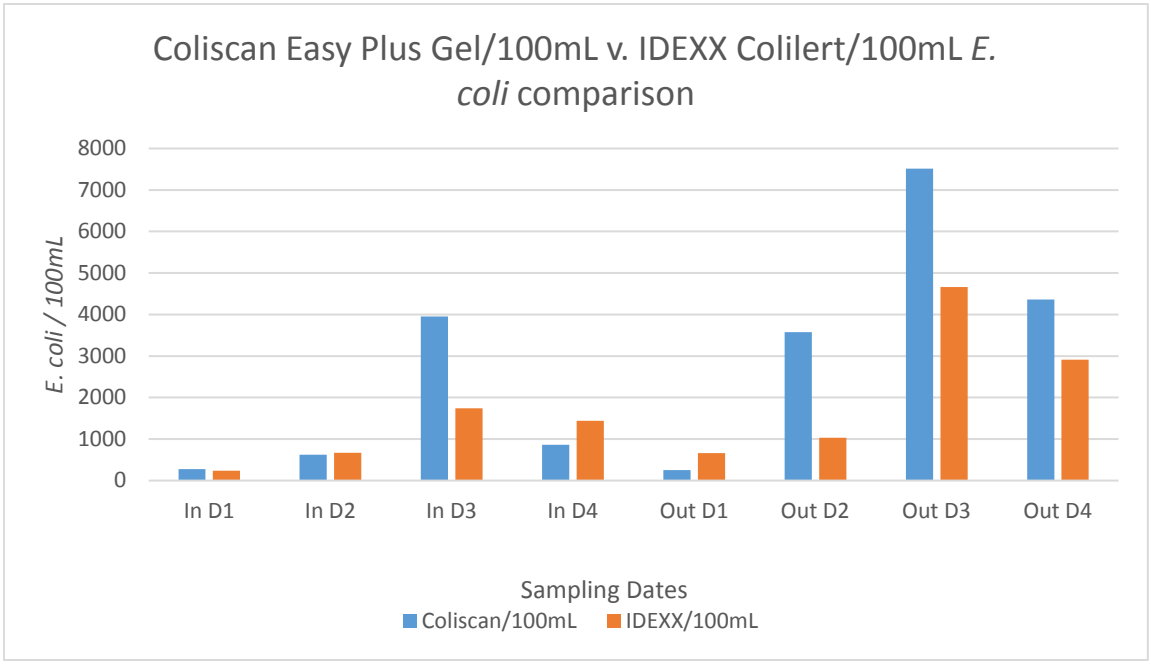

Figure 2 Comparison between Coliscan Easy Plus Gel and IDEXX Colilert for detection of *E. coli* in in-flow and out-flow samples from Fairview Environmental Park.

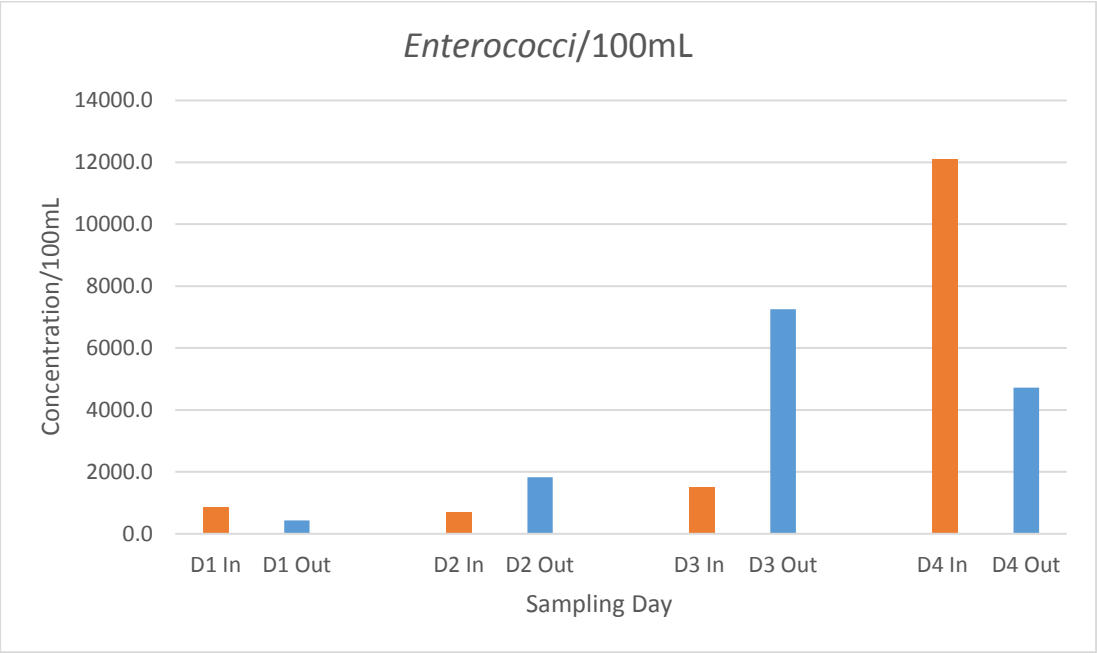

Figure 3 Comparison of in-flow and out-flow *Enterococci* MPN calculations using IDEXX Enterolert.

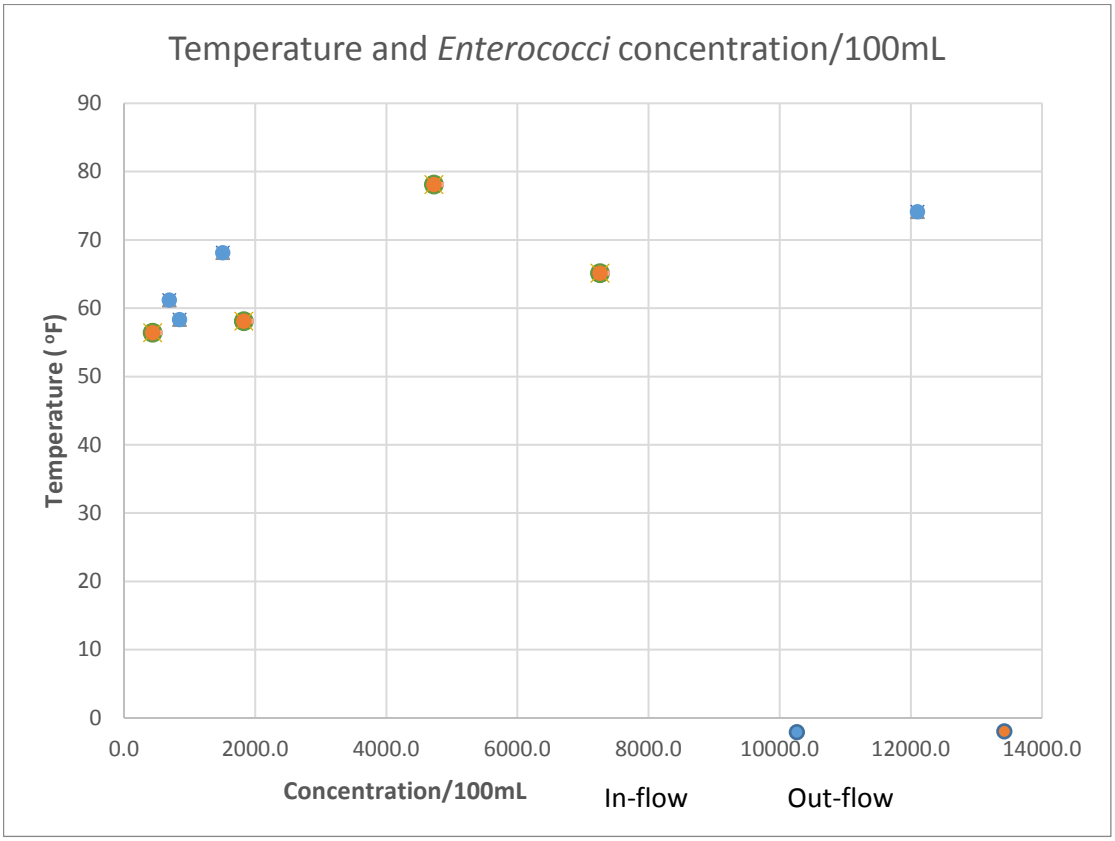

Figure 4 Comparison of *Enterococci* concentration to temperature for all D1, D2, D3, and D4 samples for in-flow and out-flow.

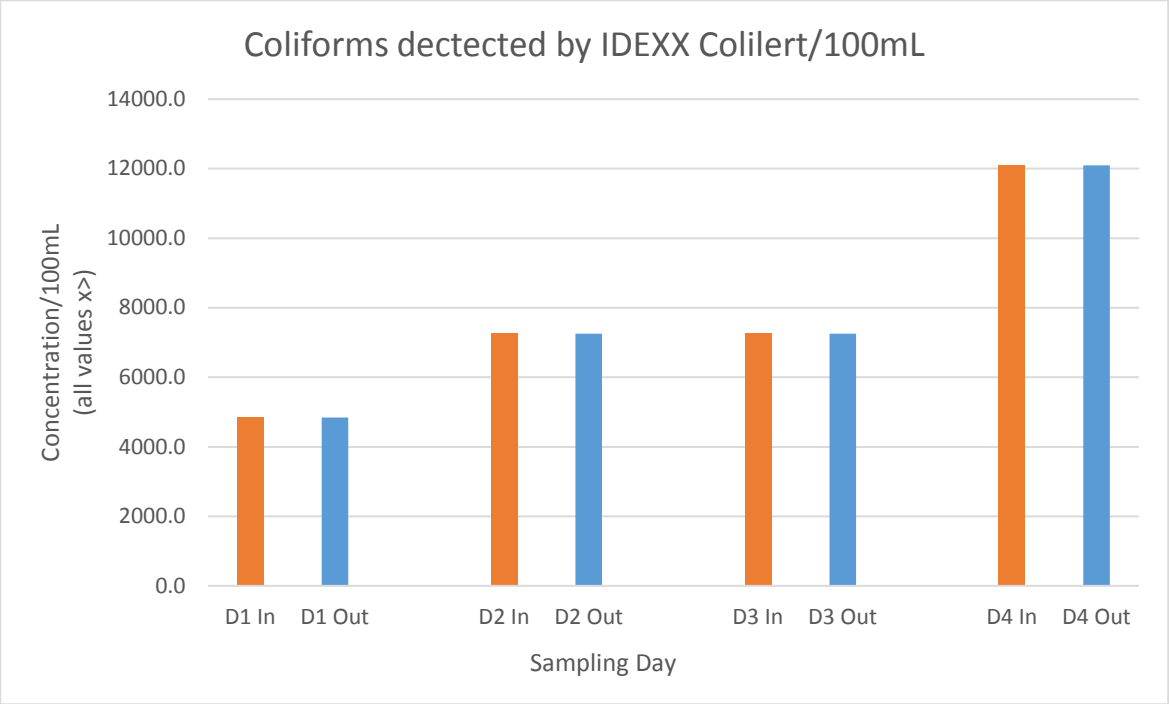

Figure 5 Coliform concentration for in-flow and out-flow samples using IDEXX Colilert. All concentration values the maximum for particular dilution.

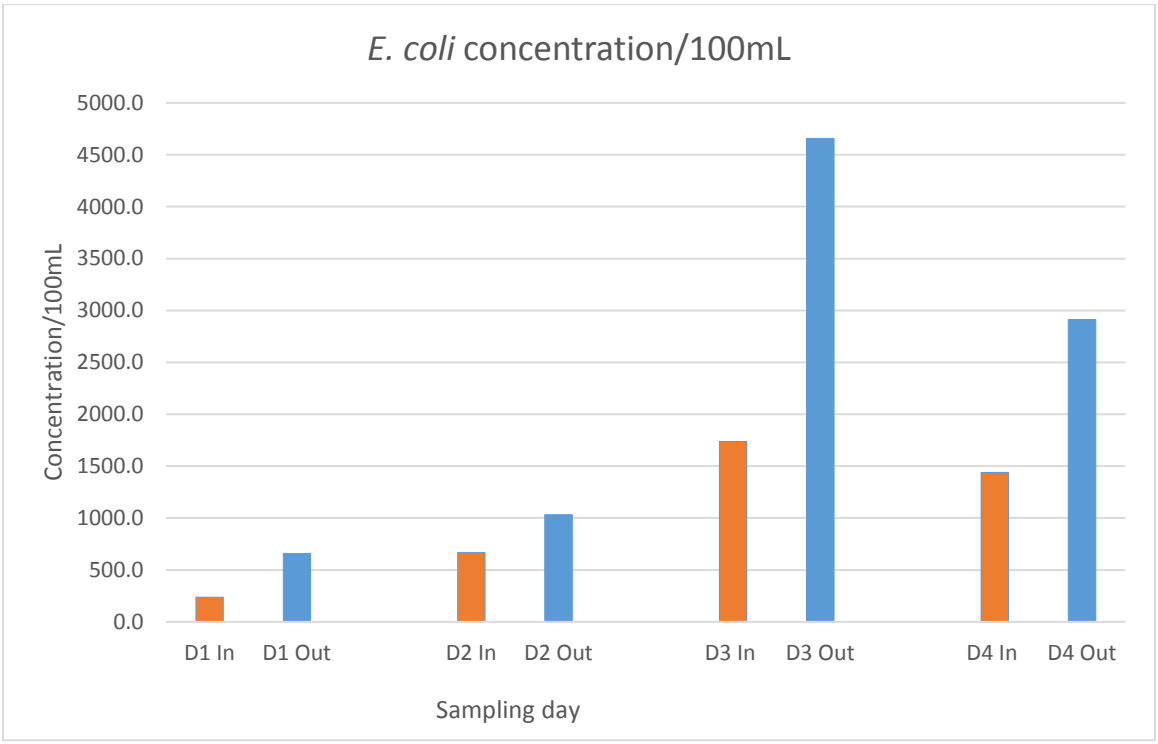

Figure 6 *E. coli* concentration for in-flow and out-flow samples using IDEXX Colilert.

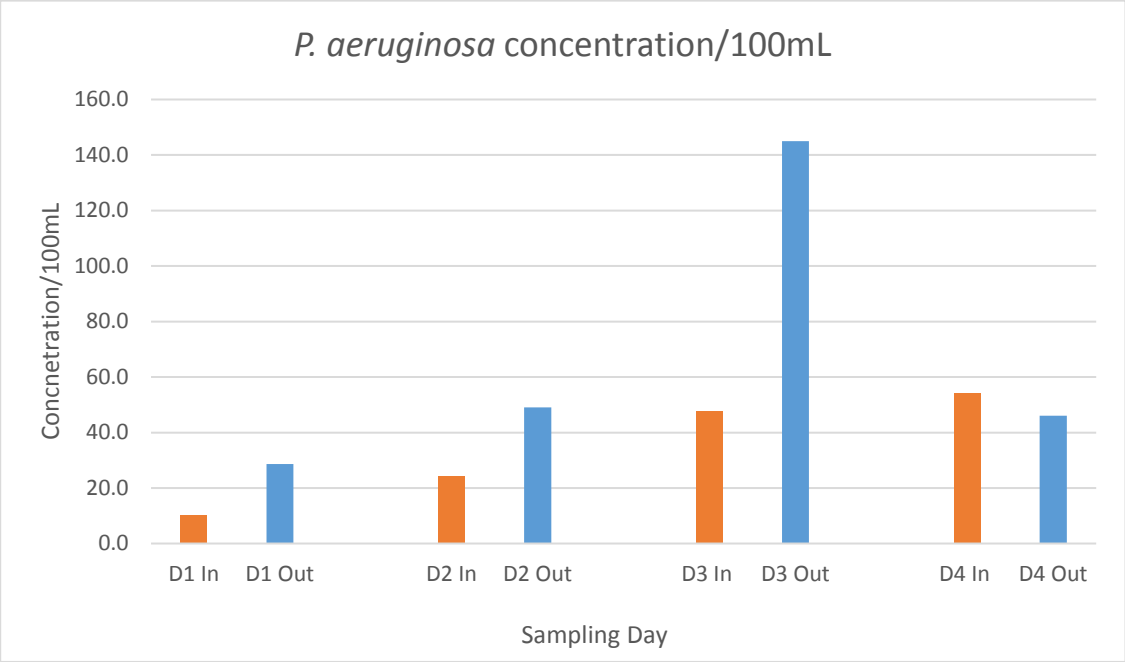

Figure 7 Comparison of *P. aeruginosa* concentrations between in-flow and out-flow grab samples.

Figure 8 Fairview Environmental Park. Photo taken March 3, 2017.

Figure 9 Fairview Environmental Park in-flow. Photo taken March 3, 2017.

Figure 10 Fairview Environmental Park out-flow. Photo taken March 3, 2017.

Figure 11 Out-flow trash debris located in Fairview Environmental Park.
Photo taken February 2, 2017.

Figure 12 In-flow trash debris located in Fairview Environmental Park.
Photo taken February 2, 2017.

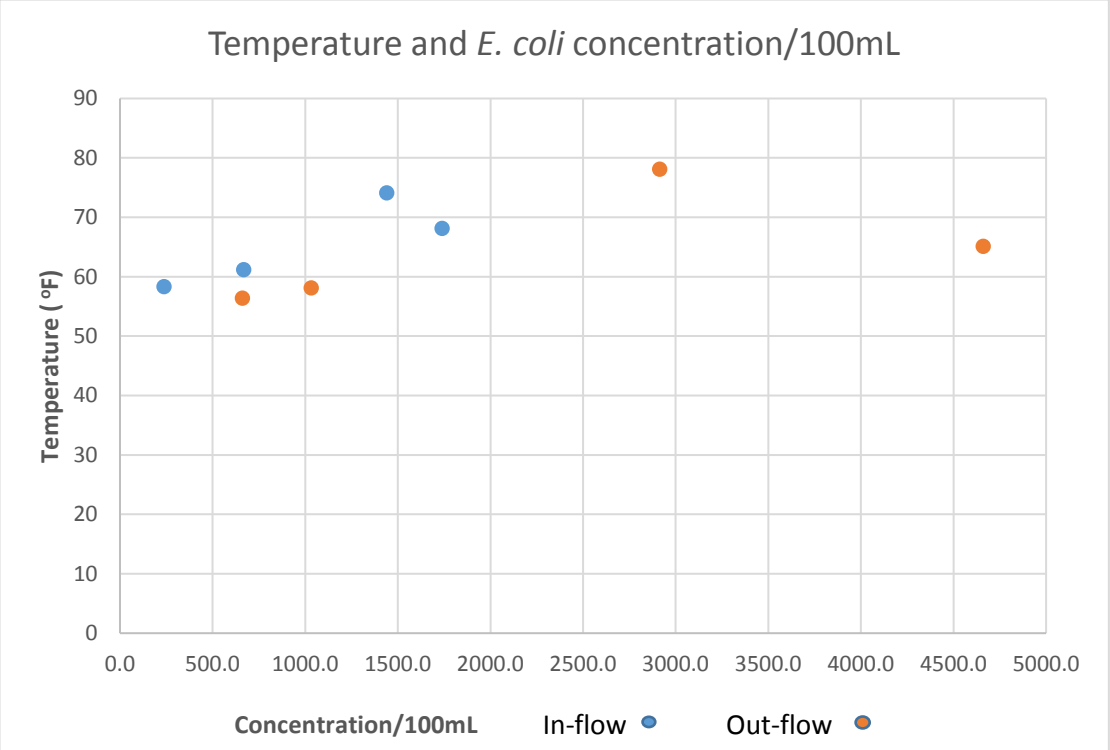

Figure 13 Comparison of *E. coli* concentration to temperature for all D1, D2, D3, and D4 samples for in-flow and out-flow.

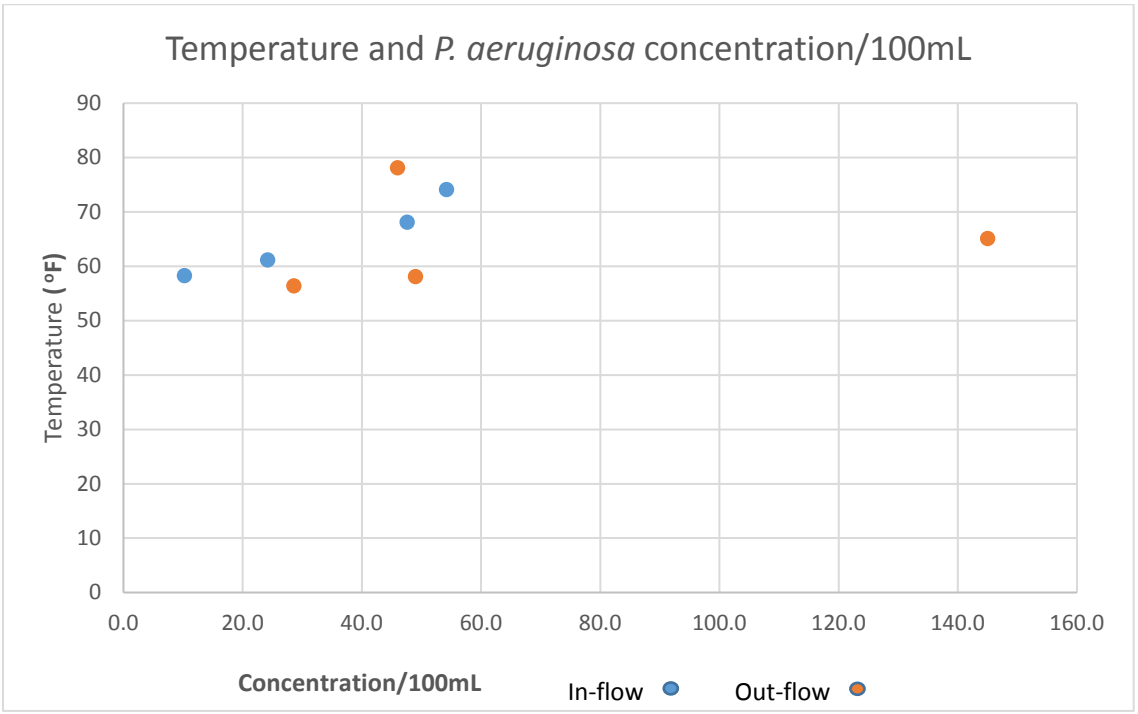

Figure 14 Comparison of *E. coli* concentration to temperature for all D1, D2, D3, and D4 samples for in-flow and out-flow.