Lake Wildwood 2019 Microbial Monitoring Program and Ongoing Response to 2017 *E. coli* O157:H7 Outbreak

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ABSTRACT

This report discusses results from 2019, the second year of an expanded monitoring program that was designed to respond to an *E. coli* O157:H7 outbreak at Lake Wildwood in July 2017. An extensive report was prepared following the first year after the outbreak providing detailed background information (Yanko et al., 2019). This report is a continuation of the 2018 work previously described. Primary goals of the second year were to provide additional documentation supporting the conclusions presented last year and conducting focused experiments to better understand possible causes for the outbreak and provide support for potential management options.

Lake Wildwood Association received a United States Fish & Wildlife Service depredation permit in 2018 to cull 75 geese, in part due to the evidence linking geese to the 2017 outbreak. The permit was received too late in the year to take advantage of the molting season to remove a meaningful number the birds. The majority of authorized birds were taken in June 2019 resulting in an estimated 80% reduction in the number of resident geese. Comparing the 2019 data to 2018 showed indicator *E. coli* average concentrations and exceedances of the EPA recreational limits were lower at all beach shorelines, beach waist-deep sampling locations, and in beach sand after the culling. The results documented a lake-wide reduction in *E. coli* concentrations due to the lower number of geese present in 2019. When high *E. coli* counts did occur in shoreline water, they again correlated to higher amounts of goose feces observed on the respective beaches, confirming the same patterns observed in 2018.

A question examined in both 2018 and 2019 was if *E. coli* grew in warm moist beach sand during the summer. The 2018 results were inconclusive but appeared to suggest the variable sand concentrations were due more to the relative amount of goose fecal contamination than actual growth. During 2019 with fewer geese present, more beaches showed declining or no trends in sand *E. coli* densities during the summer. A very high concentration of *E. coli* detected after sand was purchased for a small private beach showed rapid exponential die off at a shoreline location normally not visited by geese. This unexpected event indicated there was not a tendency for *E. coli* to colonize the sand in the absence of fecal microorganisms and organic material inputs from the geese.

Microbial source tracking analyses indicated ruminant animals were the primary source of contamination to Meadow Park Creek. The specific ruminants were not identified but appeared to not be cattle. The results suggest it may be appropriate to survey the local Deer population for *E. coli* O157:H7 since they may be a source of indicator *E. coli* stream contamination.

Discrepancies were found between two commercial products used for confirming EcO157 Reveal Test positive samples creating uncertainty about the frequency of STEC detected. Outside assistance will be necessary to clarify the inconsistencies.

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CHAPTER 1: INTRODUCTION

1.1 Background

This report summarizes results from the second year of a lake microbial monitoring program developed to investigate and respond to the 2017 recreational water *E. coli* O157:H7 (EcO157) outbreak at Lake Wildwood (LWW). As such, this report should be viewed as a continuation of the microbial monitoring program initiated in 2018. The results of monitoring in 2018 are reported in Yanko et al., 2019. That report contained an extensive review of the background, history, and literature pertinent to the outbreak which will not be repeated in this document. The key findings from the 2018 LWW microbial monitoring program were as follows:

- The study confirmed non-point source localized contamination on park sand beaches caused frequent high *E. coli* levels exceeding the EPA recreational limits in ankle deep water. Goose feces along beach shorelines were determined to be the source.

- No significant *E. coli* contamination was detected in other areas of the lake, except at the sand beaches in the parks, where geese tended to roost.

- The density of *E. coli* in beach sand and probability of exceeding the EPA recreational limits was shown to correlate with the amount of goose feces on beaches.

- EcO157 was detected in two of three creeks that flow into Lake Wildwood. The creek at Meadow Park exhibited chronic contamination. Results suggest creek inflows did not contribute to shoreline contamination at park beaches.

- EcO157 was only detected in shoreline water and beach sand in the latter third of the monitoring program. EcO157 was detected in goose feces during the same time period.

- Given the nature of potential EcO157 sources, the cause of the Lake Wildwood outbreak will probably never be known with certainty. Results suggest the most feasible scenarios include geese, via one or more potential mechanisms, as part of the equation.

- Lake Wildwood will continue to experience high *E. coli* levels at the beach shorelines until goose fecal contamination is mitigated. Recreational activities in other areas of the lake do not appear to be impacted.

A number of changes to the original monitoring program were recommended in the 2018 monitoring report to further substantiate these findings and improve the testing program efficiency. Those changes were instituted as outlined below. In addition to the basic monitoring program, some special experiments and sub-evaluations were also conducted, and will be included with this report. The primary changes instituted in 2019 included:

- A reduction in shoreline monitoring from three times per week (Monday, Wednesday, Friday) to two times weekly on Monday and Thursday.
- A change in the frequency of sand sample collection, with weekly samples collected from three locations (Meadow, Hideaway West, Control), and the remaining beaches (Commodore, Hideaway East, Vista, Explorer) sampled one time per month.
- The addition of new sampling sites at the Commodore Pavilion area swim zone, a near shore waist deep sample location; and mid-lake sample locations in Meadow Bay (in the area of the water ski course) and Hideaway Bay.
- The addition of a private landowner beach sample location as a "control" site, to evaluate *E. coli* levels in shoreline water and beach sand in a similar environment to public park beaches, but without the presence of geese.
- An increase in the sample frequency at Meadow Park Bridge to two times per week and testing of all Meadow Park Bridge water samples for EcO157.
- The use of multi-point sampling at all park beaches and analysis of composite water samples to reduce the number of samples for analysis while providing a conservative public health assessment.
- The collection and analysis of goose feces for EcO157 throughout the monitoring period, and goose fecal swabs from geese taken under the U. S. Fish & Wildlife Depredation Permit.
- Collection and processing of water samples for EcO157 and microbial source tracking analysis in Meadow Creek and Wildwood Creek.
- An evaluation of source irrigation water in Newtown Canal to investigate potential sources of contamination in the Meadow Creek watershed.
- A closure of the beach at Meadow Park for the duration of the recreational season, and installation of goose exclusion fencing at the Meadow Park beach to prevent geese from accessing the beach shoreline.

1.2 Project Objectives

The primary goals of the 2019 microbial monitoring program were to (1) collect data to inform management decisions and ensure protection of lake users (2) provide additional validation for the conclusions presented in the 2018 summary report, and (3) refine the monitoring program and address some specific questions. The project continues to be conducted as a collaboration between Lake Wildwood Association (LWA) under the direction of volunteer PI/Project Manager William Yanko, Sierra Streams Institute, and Cel-Analytical. With this report the responsibility for report preparation is being transferred to SSI under the direction of the PI. Funding was provided by LWA. No external funding was provided for this project.

CHAPTER 2: METHODS

2.1 Sample Locations

The map in Figure 1 shows the location of the designated sampling points for the 2019 monitoring program. There are five sample locations on creeks that flow into Lake Wildwood, including sample points on Meadow Creek, Deer Creek, and Wildwood Creek. Meadow Creek was sampled at two locations. One sample location was at the bridge over the creek that provides access to Meadow Park, with the second sample location just downstream in the slough. There is a flow measurement weir at the Meadow Park bridge that creates a small dam, with the sample point located just upstream of the weir. Downstream of the weir, Meadow Creek widens into a slough that was dredged to provide boat access to some homes located at a point before the creek enters the lake. The volume of water per unit lateral distance increases significantly in the area between the two sample points on Meadow Creek, as the creek transitions into the lake. Deer Creek flows into Lake Wildwood at Lake Wildwood Drive. Deer Creek is the largest tributary that flows into the lake, and provides the majority of flow into the lake year round. Wildwood Creek flows through ponds on the Lake Wildwood golf course before it enters the lake, with the creek sampled upstream and downstream of the golf course.

There are five public parks that provide beach access in Lake Wildwood, including Commodore Park, Meadow Park, Hideaway Park, Vista Park, and Explorer Park. The beaches within each park were sampled during the 2019 monitoring program, with sample locations at the sand/water interface (sand samples), beach shorelines (water samples; ~6 inch depth) and at waist deep depth (water samples; ~3 feet). Commodore Park is the largest park with the largest beach, with the park including a building complex and the marina. There is a pavilion and major lawn area located south of the marina, with the Commodore Park beach located north of the marina. In some respects, the Commodore complex can be viewed as two separate parks, each with its own environment. Meadow Park has a designated beach adjacent to and west of Meadow Creek Slough, with a large lawn located north of the beach. Meadow Park facilities include a boat launch, pickleball and bocce ball courts. Hideaway Park has two separate beaches, designated as Hideaway East and Hideaway West, with sample locations at both beaches. Vista Park Beach has a small concrete curb installed at the shoreline of the beach to prevent sand erosion from boat wakes, with a lawn located south of the beach. Explorer Park is the only public park that does not have a large lawn area, with a natural forest environment located adjacent to and south of the beach.

In addition to the creek and public park beach sample locations, lake and near shore environments were sampled. Three sample locations targeted the open-water areas of the lake, with surface water collected Mid-Lake, at the Waterski course, and within Hideaway Bay. The Mid-Lake location was sampled in the 2018 monitoring program, while the Waterski course and Hideaway Bay locations were added as part of the 2019 monitoring program. Near-shore sample locations without a designated beach area included the Chaparral Greenbelt and

Commodore Park Pavilion, with samples collected at waist deep depth at these locations. The Chaparral Greenbelt is an undeveloped lake front open parcel that is not irrigated and the waterfront is natural, i.e. no sloping sand beach. Commodore Park Pavilion is located within Commodore Park, to the south of the marina, and is a separate sample location from Commodore Park Beach. At Commodore Park Pavilion, a pair of concrete stairs lead to the water, with samples collected at the end of both stairways.

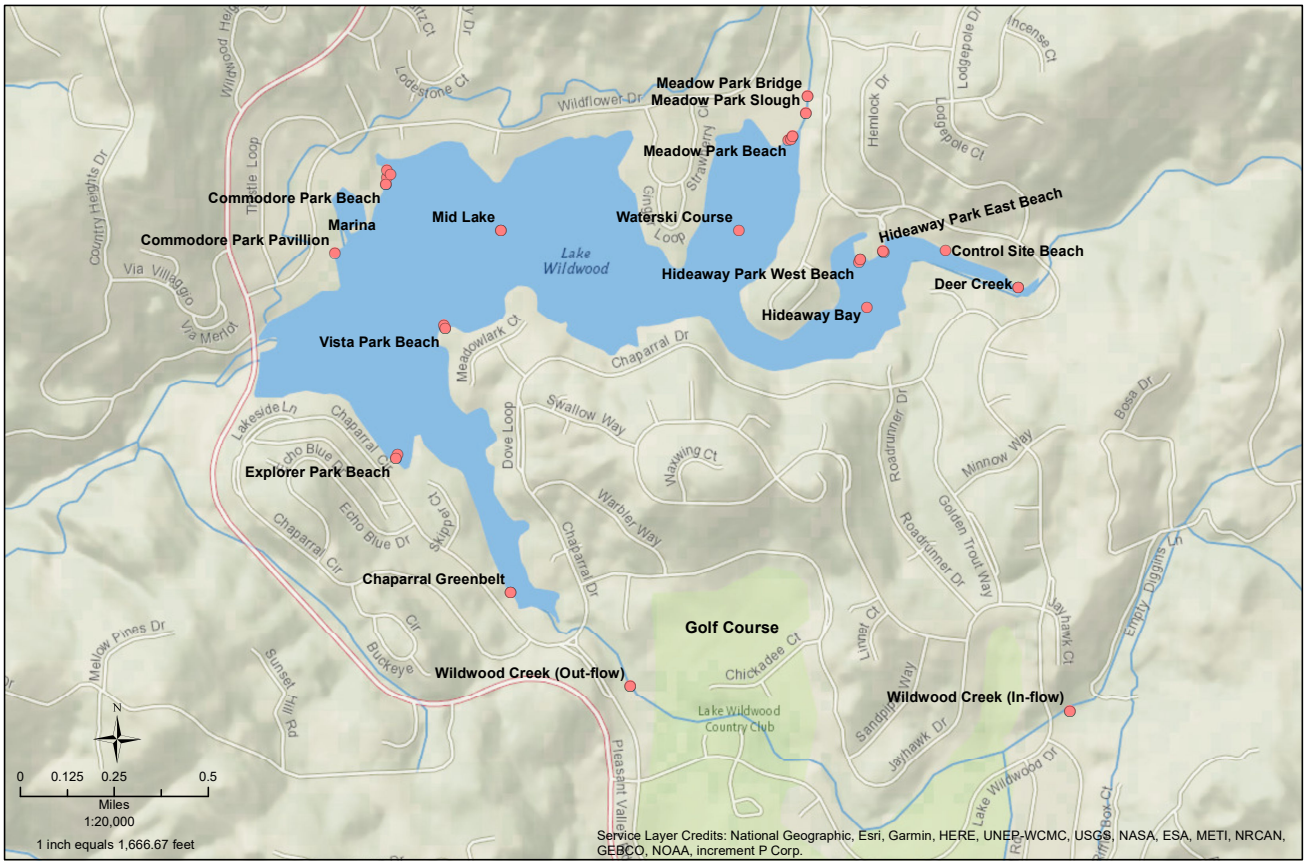

Figure 1: Sample location map showing the location of the regular 2019 monitoring sites.

One private lake front property was sampled on the north shore of the lake, east of Hideaway Park and west of Deer Creek. The private landowner sample location includes a well-maintained sand beach that is similar in nature to the beaches at public parks within Lake Wildwood. This sample location was selected, based on the historic lack of goose activity at the beach, to serve as a control site during the 2019 monitoring program. Beach shoreline water samples and sand samples were collected at the private landowner control site.

2.2 Sample Collection and Analysis

Sierra Streams Institute (SSI) personnel completed sample collection, with the support of LWA Public Works staff. Public Works staff collected the Mid-Lake, Waterski course, and Hideaway Bay samples by boat, and provided the samples to SSI. Sample collection took place on Monday and Thursday morning each week between May 2 and October 7, 2019.

Sample collection followed standard methods established in the Yuba-Bear Watershed Council's Quality Assurance Project Plan, and the State Water Resources Control Board (SWRCB) Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance Program Plan (Yuba Watershed Council, 2013; SWRCB, 2017). In addition to collecting samples, at each sample location the number of geese present at the time of sampling was recorded. The density of fecal matter on each beach was evaluated and rated on a subjective scale from 0 – 3, with a score of 0 corresponding to no visible feces on the beach and a score of 3 indicating very heavy levels of fecal matter on the beach. Fecal density was estimated for three areas at each beach, immediately adjacent to the water at the sand/water interface, within 3 ft of the water line, and for the entire beach. These estimates were referred to as the fecal index.

2.2.1 Water Samples - Fecal Indicator *E. coli*

Water samples were collected from public park beaches at the shoreline and waist deep depth, in creeks, near shore environments, and mid-lake locations. At each sample location, water samples were collected using 100 mL Whirl-Pak sample bags. Three samples were collected at Commodore Park and Hideaway East beach shorelines, while two samples were collected at Meadow, Hideaway West, Vista, and Explorer Park beach shorelines. For each beach shoreline, samples were composited in a 500 mL Whirl-Pak at the SSI laboratory prior to sample analysis. For composite samples, 100 mL of the composited sample was used for analysis.

SSI staff scientists performed sample analyses at the SSI laboratory. Water samples were analyzed for Total Coliform and *E. coli* using the Idexx Quantitray 2000 method, per the manufacturer's instructions. Field sampling protocols and laboratory quality assurance practices were followed as recommended in the California Water Resources Board Surface Water Ambient Monitoring Program Quality Assurance Plan (SWRCB, 2017).

2.2.2 Sand Samples – Fecal Indicator *E. coli*

At each public park beach and the private landowner beach, a composite sample of sand was collected at the water line using 500 mL Whirl-Pak sample bags. The composite was prepared by aseptically collecting equal volume aliquots of the wet sand and mixing them together. Five sample points equally spaced along Commodore Beach were collected to prepare the composite for that location. Three equally spaced points were sampled at Meadow Park, Hideaway Park, Vista Park, Explorer Park, and the control beaches to make the composites for those locations.

For fecal indicator analysis of sand samples, the analytical method was an adaptation of the procedure described by Boehm et al., (2009). The composite sand samples were mixed well at the lab before analysis. For indicator *E. coli,* 15 g of the sand composite was suspended in 150 mL of a sterile washing solution consisting of non-disinfected well water containing 0.3% Tween 80. The sand/washing solution mixture was firmly shaken by hand for two minutes and allowed to settle for 30 seconds before decanting the supernatant solution for analysis. The samples were analyzed at a 1:10 dilution following the Idexx Quantitray 2000 method, per the manufacturer's instructions, to determine the Total Coliform and *E. coli* in MPN/g for each sample. When a result of ">" was obtained for *E. coli*, subsequent dilutions were performed to obtain a quantitative value for *E. coli* in sand. Sterile non-disinfected well water was used to complete the dilutions.

2.2.3 Goose Fecal Samples/Fecal Swabs

To evaluate the presence of EcO157 in the goose population at Lake Wildwood, we collected goose fecal samples and took fecal swabs from geese. Goose fecal sample collection took place at public parks, within the lawn areas where geese were present, during regular monitoring events. Fecal samples were collected off the ground surface, from individual geese, after a goose was observed defecating. Efforts were undertaken to ensure fecal samples were collected from individual geese without re-sampling the same goose on each sample date. Samples were collected using a 100 mL Whirl-Pak sample bag using a metal scoop that was disinfected in ethanol between collecting each sample. Goose feces collection took place three times during the 2019 monitoring program, in May, August and September 2019.

Goose fecal swabs were collected in November 2019 from a small number of geese that were taken under Lake Wildwood's depredation permit. Cloacal swabs were collected from fresh geese by inserting a swab into the vent of the bird and vigorously swabbing the mucosal wall. Two swabs were collected from each goose, one for indicator *E. coli* analysis and the second for EcO157 analysis. After sample collection, each swab was verified for the presence of fecal material and placed in screw cap culture tubes of prepared enrichment media (Idexx test medium for coliforms and Reveal 20 hr. medium for EcO157) for transport and subsequent indicator *E. coli* and EcO157 analysis at the SSI lab. Indicator *E. coli* was confirmed using the Idexx presence/absence format.

2.2.4 *E. coli* O157:H7

Sand, goose feces, fecal swabs, and selected water samples were analyzed for *E. coli* O157:H7 using the Neogen Reveal lateral flow immunoassay in a presence/absence format. All composite sand samples, goose fecal samples, and fecal swabs were analyzed for EcO157. Due to laboratory capacity and funding limitations, only water samples from Meadow Park Bridge and those water samples that exceeded the EPA recreational limits for *E. coli* (320 MPN/100 mL) were tested for EcO157. Samples were enriched using Reveal 20 Hour Medium, a proprietary selective enrichment medium produced by Neogen. Water samples were enriched

by adding 3.68 g Reveal 20 hr medium to a 100 mL sample and mixing to dissolve the dehydrated medium in the water sample. For sand samples, 25g of sand was added to a prepared enrichment medium, consisting of 3.68 g Reveal 20 hr medium dissolved in 100 mL of sterile non-disinfected well water, and swirled to mix. For goose fecal samples, the amount of feces collected from each goose was added to a prepared enrichment medium, as described above, and swirled to mix. For goose fecal swabs, the fecal swab was placed in a test tube containing 10 mL of prepared enrichment medium, as described above, and swirled to mix.

Samples were incubated for 20 hours at 42°C. After incubation, the samples were tested using the Reveal test kit per manufacturer's instructions. Positive Reveal tests were scored using a 1 to 4 scale, with 1 indicating a barely perceptible positive reaction and 4 indicating a positive result equal to, or more intense, than the internal positive control. The 2 and 3 scores were subjective increments between 1 and 4. Positive Reveal test samples were submitted for confirmation by PCR.

2.2.5 Microbial Source Tracking

Water samples were collected in late May 2019 from creeks in Lake Wildwood for Microbial Source Tracking analysis. SSI completed sample collection with support from the project P.I. Three locations were sampled, including Meadow Park Creek at the bridge, Meadow Park Creek Upstream (US) at a point close to where the creek enters LWW, and Wildwood Creek close to where it enters LWW. Samples were collected in 500 mL Whirl-Pak bags and transported to the Lake Wildwood lab in a cooler.

Water samples were processed at the Lake Wildwood small lab room by the sampling personnel, following protocols provided by SCCWRP. A rinse water sample blank was included with the samples. The only significant deviation from the SCCWRP written protocol was the use of autoclaved Crystal Geyser bottled drinking water for the rinse water sample. Water samples were filtered through either 0.4 µm polycarbonate or 0.45 µm mixed cellulose ester membranes. Three trial runs to work out any methodology issues were conducted using the Millipore HA filters that are preloaded in the disposable filtration units. It was found that approximately 500 mL of sample could fairly easily be filtered with the HA filters. The most significant problem encountered was that when using the Polycarbonate (PC) filters, they plugged up rapidly. The PC filter plugged noticeably with about 50 mL of sample. When attempting to filter 100 mL of sample water, it took too much time, and it was apparent it would not be possible to complete the filtrations in the day using the PC filters. The remainder of the sample filtrations were completed using the HA membranes. For Meadow Park Creek at the Bridge, which was the routine sampling point that was frequently positive for O157, there were two PC filters with 100 mL sample each and two HA filters with 400 mL each. For the other samples 300 mL was filtered on replicate HA filters, except the rinse water control, which was done with the PC filter. Membrane filters were placed in Zymo tubes after the filtration process was completed for each sample. The Zymo tubes containing the filters were frozen at -20 C after the filtrations were completed. The sample tubes were shipped frozen to SCCWRP for Microbial Source Tracking analyses.

Microbial Source Tracking laboratory processing and analysis was completed at SCCWRP. Filters were received at SCCWRP frozen and held at -80°C. DNA was extracted using a Zymo Research Quick DNA fecal/soil kit with an additional processing control. Samples were then tested using digital droplet PCR for the following assays: HF183 and Lachno 3 (human fecal markers), Rum2Bac (ruminant animal marker), CowM3 (cattle specific marker), GFD (general bird marker, and 4 assays for pathogenic E. coli: rfbE, STX-1, STX-2, and Z3276.

2.2.6 Source Irrigation Water in Newtown Canal

In October 2019, Lake Wildwood collaborated with NID and SSI to obtain samples from two locations on Newtown Canal to investigate potential sources of *E. coli* contamination in the Meadow Creek watershed. Newtown Canal is the source of the raw water supply to Lake Wildwood water treatment plant, and the canal also supplies the irrigation water to the ranch properties located in the Meadow Creek watershed area. SSI collected samples in Newtown Canal before and after the end of irrigation season, between October 3 – 31, 2019. On the same day, samples were collected from Meadow Creek at the Meadow Park bridge, for comparison against the Newtown Canal results. Historical raw water *E. coli* data was also obtained from NID for Newtown Canal for evaluation purposes. Details regarding the source irrigation water sampling effort in Newtown Canal, including the background, sampling methodology, results, and recommendations were reported previously and are provided in Appendix A.

2.3 PCR Confirmation of Reveal Test *E. coli* O157 Positive Samples

Samples that were positive for *E. coli* O157:H7 using the Reveal immunoassay test were frozen and subsequently submitted for confirmation testing by PCR at Cel-Analytical Laboratory in San Francisco. The frozen samples were packed in insulated shipping containers with Freeze Paks and shipped to Cel-Analytical via overnight shipping for analysis.

2.3.1 DNA Extraction

DNA from enriched water samples was extracted using the QIAamp® DNA Blood Mini Kit -Qiagen (GmbH-Hilden, Germany). Sand and sediment enriched samples were extracted using the DNeasy® PowerSoil® Kit (Qiagen) following the manufacturer's protocol. DNA was eluted in 200 uL resuspension volume.

2.3.2 Real Time PCR Amplification (Qualitative/Quantitative)

1) Mericon Escherichia coli O157 Screen Plus (QIAGEN) uses multiplex Real-Time PCR technology for qualitative detection of the *E. coli* serotype O157 and *E. coli* virulence genes *intimin* (*eae*) and Shiga toxin-like proteins (*stx1* and *stx2*) in select food and environmental samples. The assay is run on QIAGEN Rotor-Gene Q real time PCR instrument able to detect at least 4 channels to screen for potential positives. The test is presumptive and presence of *eae* and *stx1*/*stx2* suggests that further investigation into the presence of *E. coli* O-

serotype O157, or the non-O157 O-serotypes (O26, O45, O103, O111, O121, and O145) is warranted.

Briefly, the optimized reaction mixture contained 10 ul of DNA and 10 ul of the kit Proprietary Multiplex PCR Master Mix in a 20 ul PCR mixture. Amplification includes an initial PCR activation step 5 min 95°C to activate HotStarTaq Plus DNA Polymerase followed by 3-step cycling Data collection at 60°C for green channel (*stx1/stx2)*, crimson channel (*eae*), orange channel (O157) and yellow channel (Internal control-IC); denaturation 15 s at 95°C, annealing 15 s at 60°C and extension 10 s at 72°C for a total of 40 amplification cycles. Amplification at or above CT threshold of 38 is considered positive, with internal control present at Cycling Threshold values (C_T) of 22-30 indicating no inhibition in the sample.

2) GeneSig O157:H7 test kit v2.0 (Primer Design, UK) is a quantitative kit targeting the Z3276 gene and analyzed with the Rotor-Gene 3000 light cycler (Corbett Research, Australia). Optimized qPCR reaction mixtures contained 10 ul of the master mix (Roche Diagnostic LightCycler® 480 Probes Master), 1 ul of the E.coli_O157_v2.0 primer/probe mix, 4 ul of PCR water and 5 ul of template DNA in a 20 ul PCR mixture. The thermocycler amplification protocol consisted of 95°C for 2 min, followed by 45 cycles of denaturation (95°C for 10 s) and annealing/extension (60°C for 60 s). Fluorescence signals were measured once per cycle at the end of the extension step on the FAM channel. The qPCR reactions were performed in duplicate and controls included a no template control and target standards (1×10^1 -to 1×10^6 target copies) supplied in the kit. Copy number was quantified with reference to the standards. The number of gene copies in each test sample was calculated as indicated below:

gene copies = [(copies/reaction)*200 uL/5ul]/(volume of enrichment sample)

where 200 uL was the resuspended volume after DNA extraction and 5 uL volume used /PCR reaction.

2.3.3 qPCR Inhibition Determination

Mericon Escherichia coli O157 Screen Plus kit has an internal control IC that amplifies in the yellow channel of the multiplex qPCR. Cycling Threshold values (C_T) values not in the range of 22-30 will indicate inhibition.

To assess Inhibition of qPCR assay using the gensig kit, each purified DNA samples was spiked with 0.2 µg/mL salmon sperm DNA (ss-DNA). The qPCR inhibition assay followed the cycling conditions used in the test assay (described above) and was performed using the following primers and probe.

Salmon DNA primer and probe set (EPA Method B):
Forward primer: 5'-GGTTTCCGCAGCTGGG-3’
Reverse primer (Sketa 22) : 5'-CCGAGCCGTCCTGGTC-3’
TaqMan® probe: 5'-FAM-AGTCGCAGGCGGCCACCGT-TAMRA-3’

The Cycling Threshold values (C_T) for salmon sperm DNA measured in the test samples was compared to the reference value of salmon sperm DNA using the $\Delta\Delta C_T$ -comparative cycle threshold calculation method. Deviation from expected C_T values ranging from 2-3 C_T values suggest presence of inhibitors and require sample dilution prior to subsequent analysis.

CHAPTER 3: RESULTS

3.1 Sample Results

Sample collection took place at the regular designated sample locations between May 2 and October 7, 2019. Samples were collected at six public park beach shorelines in ankle deep water and at waist deep depth within the delineated swim zone, in three creeks that flow into the lake, at a lakefront homeowner control site, at three locations in the middle of the lake, and at two near shore locations with no sand beach. Sand samples were also collected from each public park beaches and the lakefront homeowner beach for analysis. We collected a total of 604 water samples and 82 sand samples for analysis, excluding duplicate samples and field blanks. The results of water sample collection and analysis for each location category are summarized in Table 1.

Table 1 summarizes the LWW *E. coli* monitoring data grouped by location category. The table provides a snapshot of the overall microbial trends associated with the lake and creeks in 2019. Sites in the Lakefront Homes, Mid Lake and Near Shore location categories are not associated with a sandy beach at a public park. These sample points, in addition to the Waist Deep sample locations, consistently exhibited low indicator *E. coli* levels throughout the monitoring season. No samples at the Waist Deep, Lakefront Homes or Mid Lake locations exceeded EPA recreational standards for indicator bacteria, with only one sample from a Near Shore location exceeding the EPA recreational standards. In comparison, samples from beach shoreline and creek sample locations were in exceedance of both EPA single sample and 30-day recreational standards. There was considerable variability within the location categories, which will be explored in detail in the following sections of the report.

Table 1: *E. coli* results summary grouped by Location Category.

		E. Coli MPN/100ml			No. (%) Exceeding	% Geo Means Exceeding
Location Category	**n**	**Geo Mean**	**Median**	**Range**	**EPA Rec Limit[a]**	**30-Day EPA Limit[b]**
Beaches- Shoreline	240	16.6	13.3	<1 to >2419.6	20 (8.3)	8.6
Beaches- Waist Deep	120	4.8	4.1	1 to 93.3	0	0
Creeks	128	91.7	134.4	1 to >2419.6	45 (35.2)	52.7
Lakefront Homes[c]	16	7.8	6.3	1 to 228.2	0	0
Mid Lake	60	2.2	1.0	<1 to 44.1	0	0
Near Shore- No Beach	40	5.6	5.2	<1 to 2419.6	1 (2.5)	0

(a) 2012 Freshwater *E. coli* limit 320 MPN/100ml
(b) 2012 Freshwater *E. coli* 30-day geometric mean limit 100 MPN/100ml
(c) Private landowner beach shoreline, "control site"

The EPA STV value was viewed as a single sample limit for the data presented in the Tables and Figures within this report. The data sets used to compute the reported means in

Table 1 and other summaries in this report contained some censored data, i.e. values of <1 or >2,419.6 MPN/100 mL. The number of indeterminate values overall was fairly small. Detection limit values were used for computing the means. Given the range of the data, using 1 or some other estimated lessor value for the <1 values would have no significant effect on the means. Use of the censored data at the upper detection limit would not likely have affected the relative ranking of those locations with a high level of microbial contamination versus those that were low, or the frequency of exceeding the EPA recreational criteria. It is of note though, that the true means and medians are somewhat higher than reported in the tables.

3.2 Creeks – Indicator *E. coli*

The water flowing into Lake Wildwood during the summer months, through Deer Creek and Wildwood Creek, is predominantly water purchased from Nevada Irrigation District for irrigation of the golf course and to offset evaporative loss in the lake. Additional NID water from Deer Creek also flows through the lake to provide irrigation water to downstream users. The flow at Meadow Park Creek is most likely excess irrigation water or irrigation drainage from the small ranches located outside of the LWW community. These natural streams are part of a watershed-wide managed irrigation system operated by Nevada Irrigation District during the period from April 15 through October 15.

The results of indicator *E. coli* analysis for individual creek sampling locations are provided in Table 2. A total of 128 creek samples were collected during the 2019 monitoring period. Samples were collected one time per week, with the exception of Meadow Park Bridge, which was collected twice per week.

Table 2: *E. coli* in Creeks that flow into Lake Wildwood.

		E. coli MPN/100ml			No. (%) Exceeding	% Geo Means Exceeding
Creek	**n**	**Geo Mean**	**Median**	**Range**	**EPA Rec Limit**	**30-Day EPA Limit**
Meadow Slough	20	14.3	13.6	3.1 to 70	0 (0)	0
Meadow Park Bridge	46	555.2	547.5	131.4 to >2419.6	37 (80.4)	100.0
Deer Creek	20	8.3	6.9	1 to 112.6	0 (0)	0
Wildwood Creek- In	21	129.4	139.6	9.6 to 686.7	5 (23.8)	73.3
Wildwood Creek- Out	21	72.2	48.7	10.9 to >2419.6	3 (14.3)	20.0

Historically, Deer Creek rarely exceeded the EPA recreational criteria for *E. coli* during the recreational season. During the 2019 monitoring season, no Deer Creek samples exceeded the EPA recreational standards. Based on historical data, bacteria levels in Wildwood Creek are more variable than in Deer Creek, with higher levels of *E. coli* typically observed upstream of the golf course compared to the sample location downstream. This reduction in *E. coli* has been attributed to sedimentation, increased protozoan predation, and increased UV exposure in the golf course ponds. In 2019, five samples from the Wildwood Creek upstream sample location and three samples from the downstream location exceeded the recreational standard for single samples. In addition, the Wildwood Creek upstream location exceeded the 30-day geometric mean standard 73.3% of the time, with 20% of the downstream site samples above the 30-day

geometric mean standard. This represents an increase in exceedances of the EPA recreational standards relative to the 2018 monitoring season, when no samples on Wildwood Creek exceeded the recreational limits.

Meadow Creek at Meadow Park Bridge consistently showed microbial contamination during the 2019 monitoring season, consistent with historical data. At Meadow Park Bridge the creek water exceeded the single sample recreational criteria 80.4% of the time. Meadow Park Bridge also exceeded the 30-day geometric mean standard 100% of the time during the sample season. This indicated Meadow Creek was consistently out of compliance with both the EPA single sample and 30-day geometric mean standards at the Meadow Park Bridge sample location. In comparison there were no exceedances at the downstream sample location in Meadow Slough. Meadow Creek typically has very low flows during the summer months, with dilution as the water flows over the weir and into the slough, which helps mitigate contamination in the creek and potential water quality impacts to the lake.

3.3 Lake – Indicator *E. coli*

Monitoring was completed at locations in the lake including (1) along public beach shorelines in ankle deep water and at waist deep depth in the swim zones, (2) a lakefront homeowner control site, (3) three locations in the middle of the lake, and (4) two near shore locations with no beach. Sand samples were also collected from each public park beach and the lakefront homeowner beach for analysis. Two primary parameters were monitored, including indicator *E. coli* and *E. coli* O157:H7. The results for the lakefront homeowner site, mid-lake, and near shore sample locations were included in Table 1 and discussed below, while the results for beach shoreline, waist deep, and sand samples are summarized below in Tables 3, 4 and 5.

3.3.1 Beach Shoreline Samples

Shoreline samples were collected twice a week at Explorer Park, Vista Park, Hideaway Park East, Hideaway Park West, Meadow Park, and Commodore Park beach shorelines. At each sample location a total of forty samples were collected at ankle depth during the sampling season, with differences between the beaches shown in Table 3.

Each public park beach shoreline location exhibited at least one exceedance of the EPA single sample recreational standard, with the Hideaway West beach shoreline over the limit 25% of the time, and Commodore Park above the standard in 12.5% of samples. Explorer Park, Vista Park, and Meadow Park beach shorelines exceeded the single sample standard one time (2.5% of samples), with two exceedances (5% of samples) at Hideaway East. All four of these sites were in compliance with the 30-day geometric mean standard. Two sample locations, Hideaway West and Commodore Park beach shorelines, exceeded the 30-day geometric mean standard 27.3% and 24.2% of the time respectively. Hideaway West consistently exhibited the highest *E. coli* values in comparison to the other beach shoreline sample locations, with the

highest geometric mean and median *E. coli* concentrations, and the greatest number of exceedances of the EPA single sample and 30-day geometric mean recreational standards. The private lakefront homeowner beach shoreline sample location exhibited a mean of 7.8 and median of 6.3 MPN/100 mL (n=16). The highest observed *E. coli* concentration at this site was 228.2, with no exceedances of the EPA recreational criteria at the lakefront homeowner location.

Table 3: *E. coli* at Beach Shorelines.

		E. coli MPN/100ml			No. (%) Exceeding	% Geo Means Exceeding
Beach Shoreline	**n**	**Geo Mean**	**median**	**Range**	**EPA Rec Limit**	**30-Day EPA Limit**
Explorer Park Shore	40	10.7	9.2	<1 to 920.8	1 (2.5)	0
Vista Park Shore	40	5.0	3.6	<1 to 1732.9	1 (2.5)	0
Hideaway East Beach Shore	40	11.2	9.2	1 to 648.8	2 (5)	0
Hideaway West Beach Shore	40	85.1	61.9	7.5 to 2419.6	10 (25)	27.3
Meadow Park Shore	40	15.1	12.0	1 to 410.6	1 (2.5)	0
Commodore Park Shore	40	27.6	19.0	2 to >2419.6	5 (12.5)	24.2

3.3.2 Waist Deep Samples

Results for waist deep samples collected within the designated swim zone at public park beaches are summarized in Table 4. At each sample location, a total of twenty samples were collected during the sampling season, with samples collected once a week. There were no exceedances of the EPA single sample or 30-day geometric mean standards for the waist deep sample locations. Concentrations trended the same as the ankle deep results, with the highest geometric mean, median, and maximum *E. coli* concentration observed at the Hideaway West sample location. Results for all sites were well below the EPA single sample recreational standard, with maximum values below 100 MPN/100 mL at each sample location. The highest geometric mean and median concentrations were 10.9 MPN/100 mL and 12.2 MPN/100 mL respectively, suggesting low levels of microbial contamination at the waist deep sites, with each site in compliance with EPA recreational standards.

Table 4: *E. coli* at Beach Swim-zone Waist Deep.

		E. coli MPN/100ml			No. (%) Exceeding	% Geo Means Exceeding
Beach Waist Deep Zone	**n**	**Geo Mean**	**Median**	**Range**	**EPA Rec Limit**	**30-Day EPA Limit**
Explorer Park Beach	20	2.9	2.0	<1 to 55.6	0 (0)	0
Vista Park Beach	20	2.0	1.5	<1 to 12.2	0 (0)	0
Hideaway- East Beach	20	5.1	4.7	1 to 35.4	0 (0)	0
Hideaway- West Beach	20	10.9	12.2	2 to 93.3	0 (0)	0
Meadow Park Beach	20	7.7	7.4	<1 to 41.4	0 (0)	0
Commodore Beach	20	5.1	4.1	<1 to 73.3	0 (0)	0

The data presented in Figure 2 highlights the difference between shoreline and waist deep sample locations. The mean and median *E. coli* concentrations, as well as the % of samples exceeding the EPA single sample recreational standard of 320 MPN/100 mL, are plotted in Figure 2. The data shows that *E. coli* concentrations dropped significantly from the

ankle deep water to the waist deep water at each public park beach, and compliance with the EPA recreational criteria notably improved. As reported in 2018, this pattern continues to indicate a source of contamination predominantly affecting the beach shorelines at ankle depth, but not the waist deep sample locations.

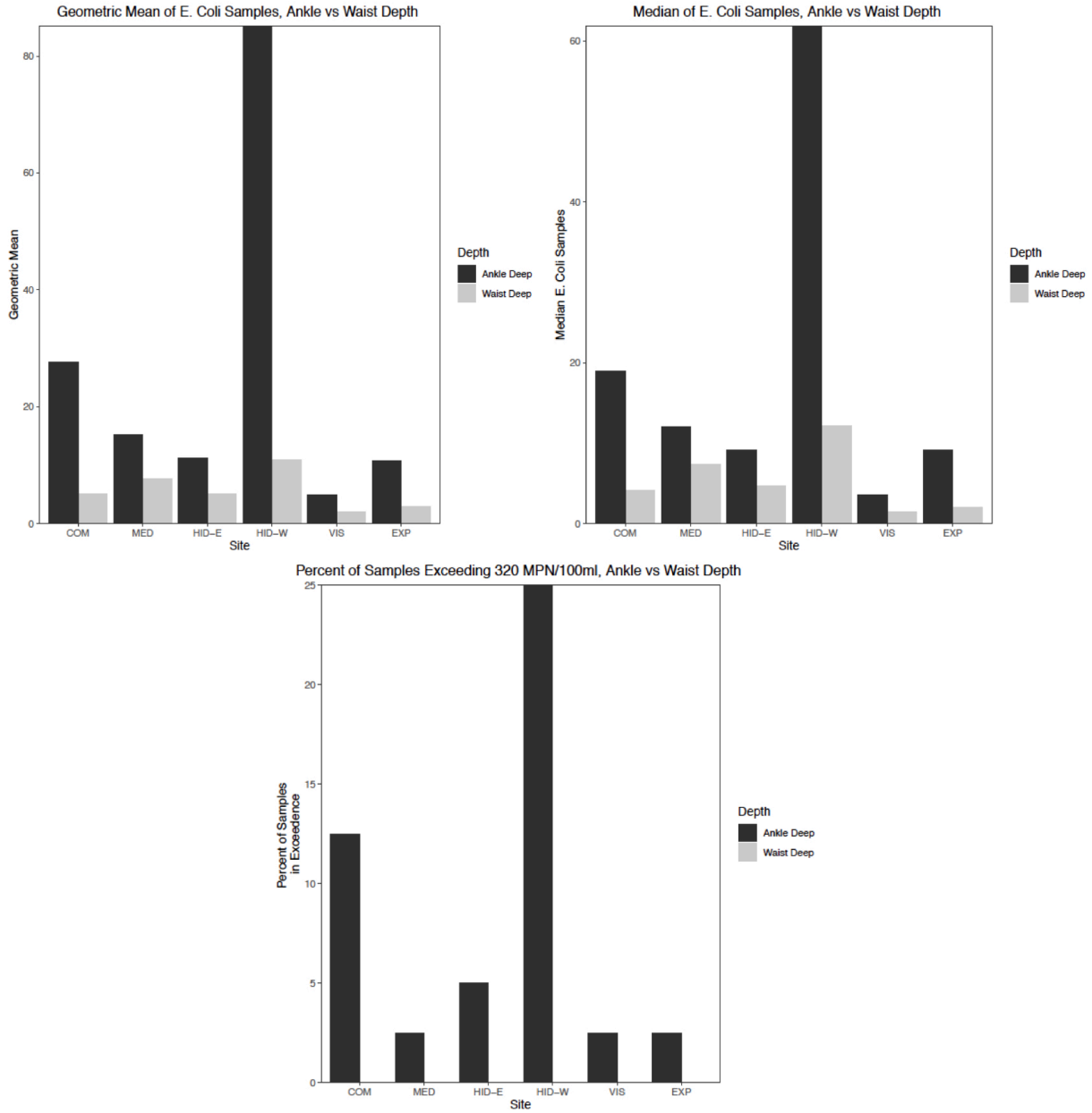

Figure 2: Ankle Deep vs Waist Deep Water: *E. coli* Geometric Mean, Median and % >320 MPN/100 mL

3.3.3 Near Shore and Mid Lake Samples

The Chaparral Greenbelt and Commodore Park Pavilion sample locations (Table 4) were near-shore sample locations with no beach. Although close to the shoreline, the sampling points were waist deep. These sites showed considerable differences to the ankle deep beach shoreline locations, with results more in line with the waist deep depth sample locations. At

near shore sample locations, the mean and median *E. coli* concentrations (n=40) were 5.6 and 5.2 MPN/100 mL respectively. One value exceeded the EPA single sample recreational standard at the near shore locations, while no sites were out of compliance with the 30-day geometric mean standard.

Samples were collected from three locations in deeper water areas of Lake Wildwood, where boating and watercraft recreation occurs. One of these locations designated mid-lake was sampled in 2018, while two additional locations, the waterski course and Hideaway mid-bay, were added in 2019. Combining these sample locations, the mean and median (n=60) were 2.2 and 1.0 MPN/100 mL respectively. The true mean and median was lower, due to numerous <1 values in the data sets. No samples exceeded recreational criteria at those three locations, with 44.1 MPN/100 mL the highest *E. coli* concentration observed at these sites.

3.3.4 Beach Sand Samples

Composite sand samples were collected at the public park beach shorelines and lakefront homeowner beach at the sand-water interface, in the zone that was not underwater but remained wet all the time. A total of 82 sand samples were collected for analysis during the monitoring period. Three beaches, including Hideaway West, Meadow Park, and the lakefront homeowner (Control), were sampled on Thursday of each week. Hideaway West and Meadow Park were sampled every week due to high indicator *E. coli* levels in sand during the 2018 monitoring program. In 2019 a fence was installed at Meadow Park near the waterline, in an effort to manage goose presence on the beach near the shoreline, and mitigate fecal contamination. The fence prevented geese from accessing the beach shoreline directly from the water, or the water directly from the beach shoreline. The private lakefront homeowner control site was sampled weekly, to provide a location for sand sample collection where geese were not present, and there historically was no observable goose fecal contamination. The beaches at Explorer, Vista, Hideaway East, and Commodore Parks were sampled on Thursday once a month, with the exception of the end of June and early July, when samples were collected before and after the July 4th holiday period. The results of beach sand sampling are presented in Tables 5/6 and Figure 3.

Table 5: *E. coli* in Beach Shoreline Sand Site Summary.

		E. Coli MPN/g		
Site	**n**	**Geo Mean**	**median**	**Range**
1S: Explorer Comp Sand	7	28.33	19.7	2 to 648.8
2S: Vista Comp Sand	7	45.84	52.9	7.4 to 184.2
3AS: Hideaway East Beach Comp Sand	7	89.64	105.4	4.1 to 3654
3BS: Hideaway West Beach Comp Sand	19	248.87	387.3	2 to 72700
4S: Meadow Comp Sand	19	21.52	16	1 to 1986.3
5S: Commodore Comp Sand	7	14.37	23.1	1 to 70.3
Control Site Sand	16	16.12	5.2	1 to 24810

The highest indicator *E. coli* level in sand was observed at Hideaway West, followed by the Control site and Hideaway East. Hideaway West had the highest mean and median *E. coli*

concentration in sand, followed by Hideaway East. The results show that there was considerable variability in the sand data, with all sites, excluding Commodore Park, exhibiting variation ranging from <10 to >500 MPN/g.

Table 6: Indicator *E. coli* in Composite Beach Shoreline Sand over the 2019 sampling season.

	E. coli MPN/g						
Sample Date	**Explorer**	**Vista**	**Hideaway East**	**Hideaway West**	**Meadow**	**Commodore**	**Control**
5/2/2019	201.4	35.5	6.3	8.5	13.4	5.2	
5/9/2019	648.8	52.9	105.4	57.3	435.2	6.3	
5/30/2019	167.4	108.1	2419.6	457.0	275.5	49.6	
6/6/2019				488.4	1986.3		47.3
6/13/2019				387.3	24.3		24,810
6/20/2019				72,700	2.0		365.4
6/27/2019	8.5	24.1	122.3	1014.0	5.2	1.0	5.2
7/11/2019	2.0	7.4	15.8	3255	9.7	70.3	1.0
7/18/2019				1664.0	727.0		5.2
7/25/2019				959.0	1.0		2.0
8/1/2019				63.0	5.2		2.0
8/8/2019	2.0	184.2	4.1	2.0	16.0	23.1	1.0
8/15/2019				108.0	1.0		2.0
8/22/2019				2755	474.0		16.1
8/29/2019				63.0	21.6		2.0
9/5/2019	19.7	63.8	3654.0	74.0	3.1	48.0	727.0
9/12/2019				213.0	16.8		49.5
9/19/2019				556	83.9		193.5
10/3/2019				<10	<1		<1

The beach sand sample data distributions are shown in Figure 3 in both arithmetic form (top images) and as $\log_{10}$ normalized data (bottom image). The data clearly shows that Hideaway Park beaches had the highest *E. coli* concentrations in sand. As often is observed with microbiological data, many outliers occurred at the upper end of the distributions. This may reflect the relative amount of fecal material present in any given sample rather than growth of a naturalized *E. coli* population in the sand. Normalizing the data distribution to a $\log_{10}$ basis significantly reduced the number of outliers (bottom image).

Based on the results of the beach shoreline water sample and sand sample analysis, it appears there was a relationship between the concentration of E. coli in beach sand and water. That was very apparent at some beaches, including Explorer Park, Vista Park, Meadow Park, and the Control site. These sites exhibited lower sand concentrations that were coincident with low shoreline water concentrations, had a low rate of exceeding the recreational limit, and no instances of exceeding the 30-day geometric mean limit. The relationship between *E. coli* concentration in beach sand and shoreline water was also apparent at Hideaway West, where the site exhibited higher sand concentrations that were coincident with high shoreline water levels, and had a high rate of exceeding the single sample and 30-day geometric mean recreational limit. In addition, the Hideaway West waist deep sample location had the highest

geometric mean, median, and maximum *E. coli* concentration of all the waist deep sample locations. This relationship was less apparent at Commodore Park beach, where low *E. coli* concentrations were observed in beach sand, but water sample values exceeded the single sample and 30-day geometric mean recreational criteria. This relationship was explored further, for all of the beach shoreline water and sand sample locations (Figure 4).

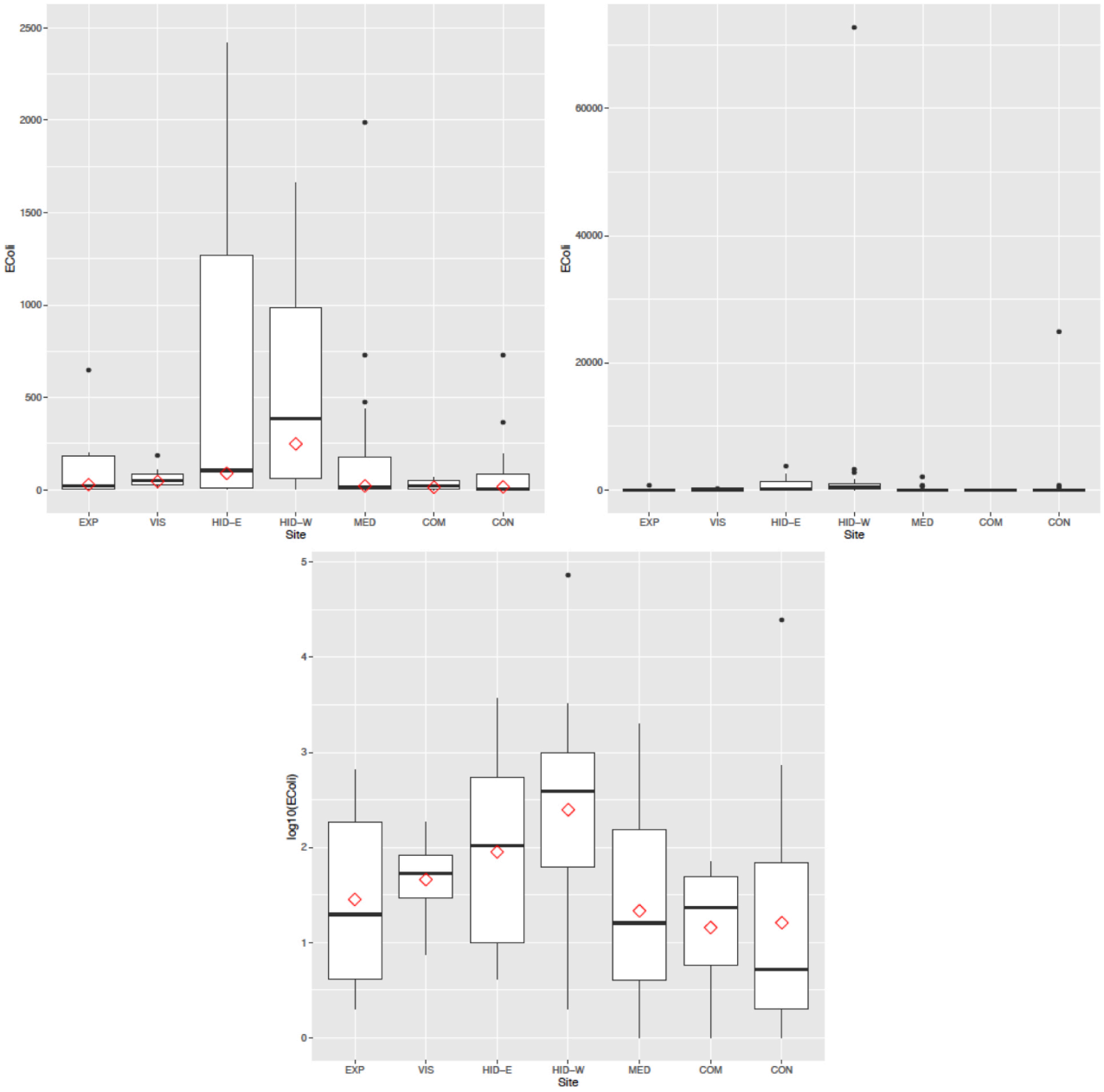

Figure 3: Beach sand sample distribution in arithmetic (top) and as log10 normalized data (bottom).

The relationship between beach sand and shoreline water for the entire data set is shown in Figure 4. Due to the scatter, the R^2 value is low but the trend is apparent and indicates

E. coli concentrations in the beach sand have an effect on the shoreline water concentrations. The relationship in Figure 4 is consistent with other reported studies about the relationship between beach sand and water bacterial densities (Whitman and Nevers, 2003).

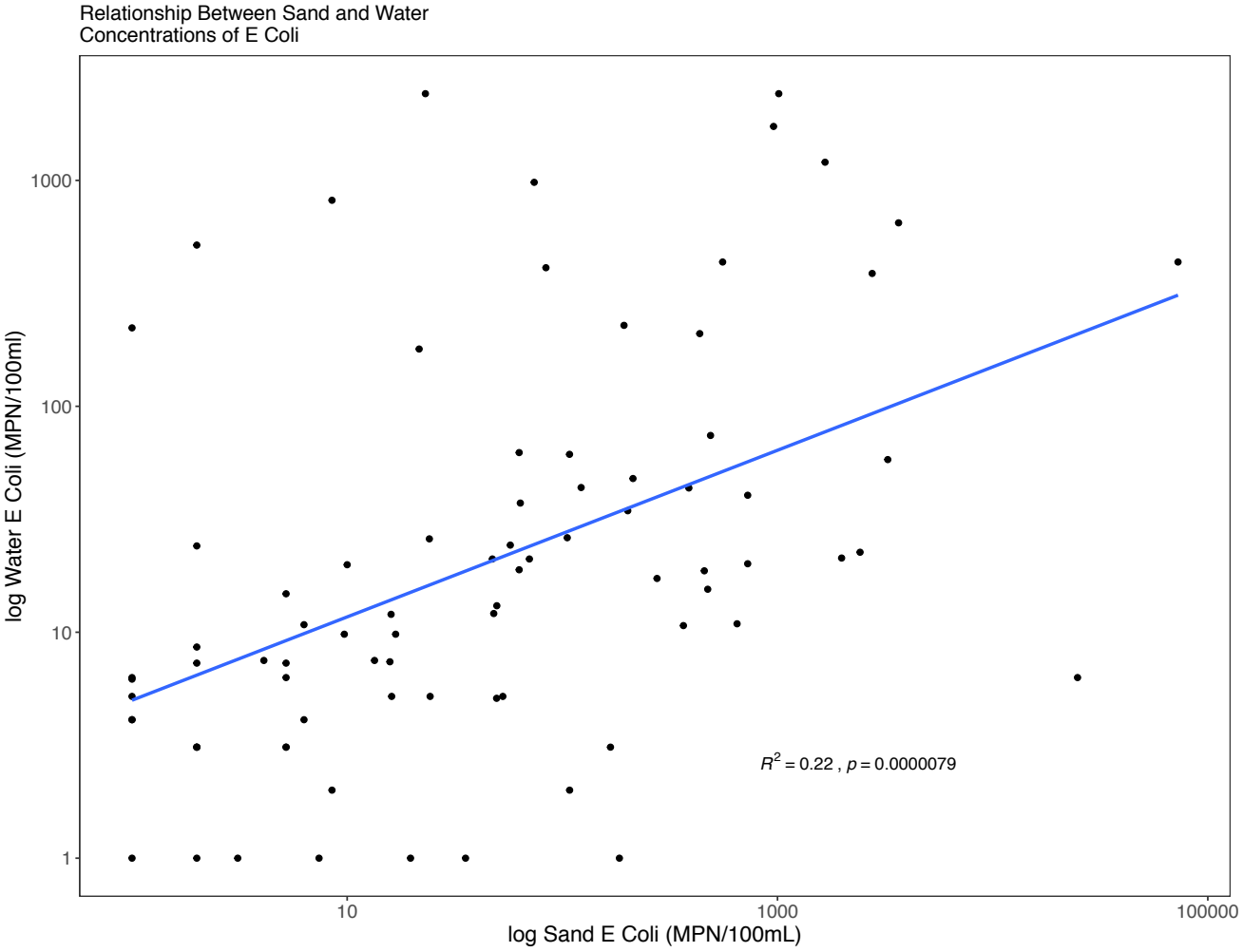

Figure 4: Relationship between sand and water *E. coli* concentrations.

As was the case in 2018, one project goal was to assess if *E. coli* growth occurred in the moist shoreline sand during warm summer weather. It was hypothesized that if indicator *E. coli* were capable of growing in sand, EcO157 could also increase in numbers, and thus increase the potential risk for an outbreak. The data in Figure 5 shows *E. coli* density trendlines over time at each of the beaches. Four of the seven beaches showed negative trend lines, while three beaches showed positive trend lines, with the strongest trend observed at Explorer Park. The results in Figure 5 demonstrate *E. coli* did not exhibit considerable growth in beach sand and highlights the variability of *E. coli* levels in sand over the monitoring period.

3.4 Effect of Goose Feces on Water and Sand *E. coli* Concentrations

For the 2018 monitoring program the amount of goose fecal material on the beach shoreline was recorded each morning as part of the field observations noted when sampling. The same information was gathered during the 2019 monitoring program. The observations were a subjective index, i.e. 0 = no visible feces, 1 = light fecal contamination, 2 = moderate fecal contamination, and 3 = heavy fecal contamination. The observations were used to calculate average fecal index values for the waterline, within 3 ft of the waterline, and the entire beach area. The relationship between the average fecal index at the shoreline and the percent of samples that exceeded the EPA single sample recreational criteria (320 MPN/100 mL) is shown in Figure 6. The data shows that the probability of exceeding the recreational limits increased when more fecal material was observed on the shoreline. There was a relatively strong relationship between average fecal density at the waterline and the % of beach shoreline water samples that exceeded the EPA recreational criteria.

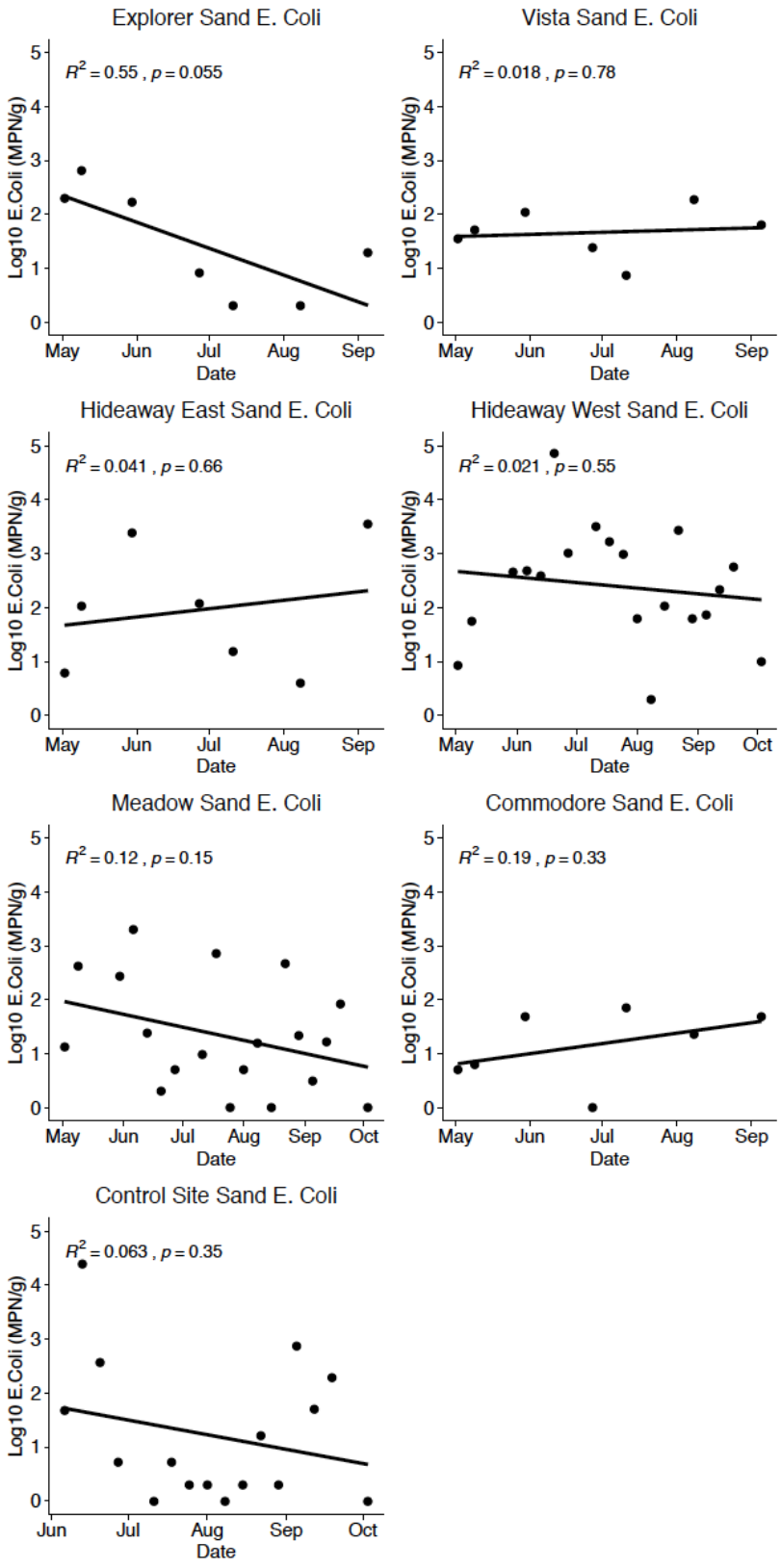

Figure 5: Shoreline composite sand *E. coli* concentrations over time.

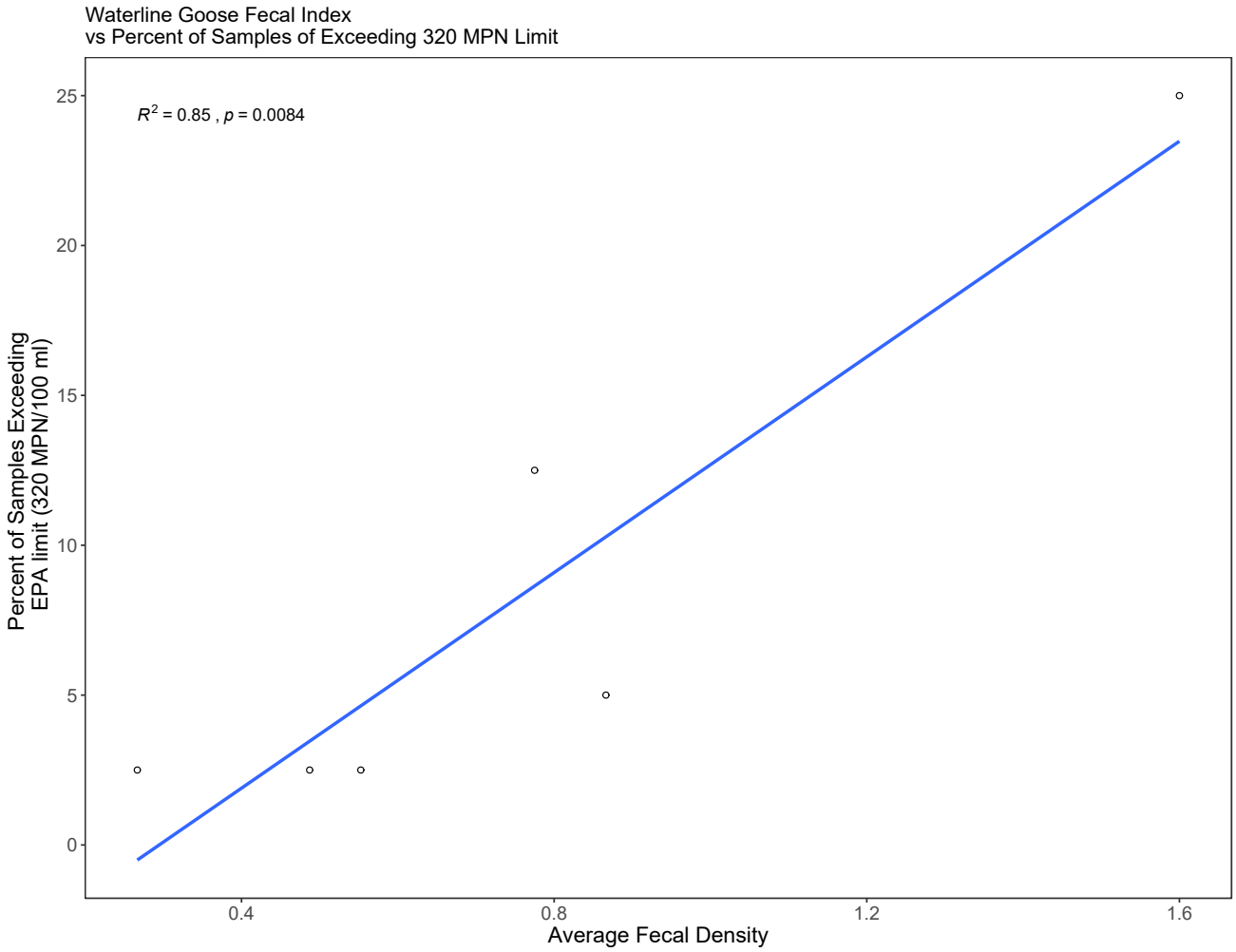

Figure 6: Waterline goose fecal index vs probability of exceeding EPA recreational criteria (>320 MPN/100 mL)

The relationship between the waterline goose fecal index and the mean *E. coli* concentration in sand was also evaluated (Figure 7). The beach sand samples were collected from within the zone where the waterline goose fecal index was noted. The data in Figure 7 shows that higher densities of goose feces on the beach shoreline were associated with higher average *E. coli* concentrations in the wet sand.

3.5 *E. coli* O157:H7 in Water and Sand

The following section is included as a placeholder. Reveal test confirmations were not completed in time to meet the deadline for this draft report for reasons that will be described in the following section and the Discussion. The data summaries below are based on unconfirmed Reveal Test data. This section will be updated when confirmation testing is completed. The numbers in the tables in this section will change when the confirmation data is

available. A subset of samples was analyzed for *E.* coli O157:H7 during the monitoring period. This included all samples from Meadow Park Creek, all sand samples, goose fecal samples, and water samples that exceeded the EPA recreational criteria of >320 MPN/100 mL. *E. coli* O157:H7 was detected in all matrices analyzed during this study, including water, sand, and goose fecal material. Table 7 lists the basic sample categories and shows the total number of samples analyzed for fecal indicator *E. coli*, the number tested for EcO157, and percentage that were positive for EcO157.

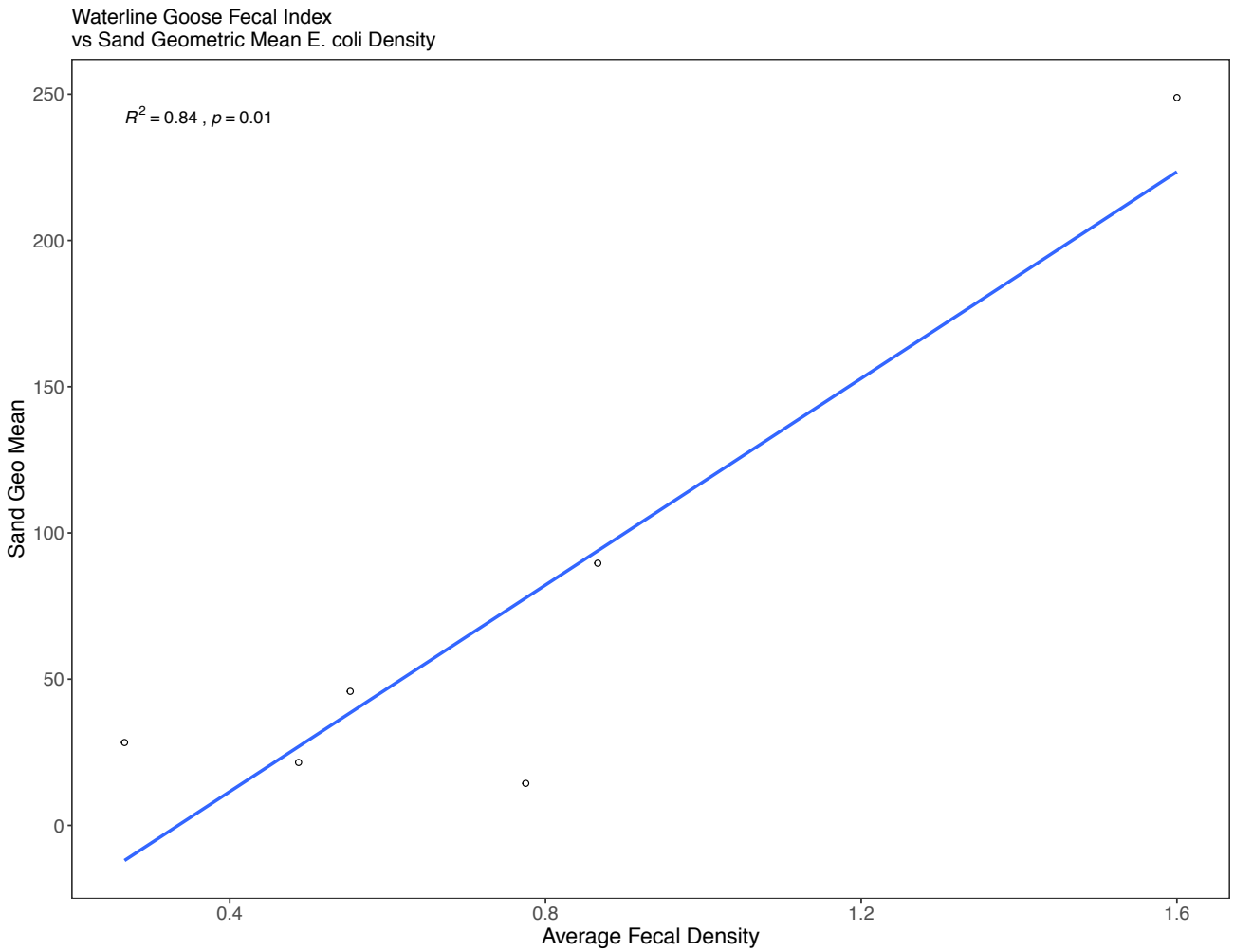

Figure 7: Waterline goose fecal index vs Sand geometric mean *E. coli* concentration in MPN/g.

There were also some miscellaneous samples analyzed for EcO157 during the project, discussed in section 3.8, which are not included here due to the small number of samples.

For the monitoring period, approximately 17% of the water samples and all beach sand samples were tested for EcO157. The percent positive values in Table 7 for the water samples represent the percentage of samples tested for EcO157, not the percentage of total samples analyzed for indicator *E. coli*. For sand samples, 8.5% of analyzed samples tested Positive per

the Reveal method for EcO157. At the creek locations, 23% of analyzed samples tested Positive per the Reveal method for EcO157. For lake samples, 74% of the tested samples were Positive for EcO157 per the Reveal method. It is possible the higher percentage of Positive samples at the lake water locations was due to only samples with an indicator *E. coli* result of >320 MPN/100 mL being analyzed for EcO157 at these locations. In comparison, all of the sand samples and creek samples from Meadow Park Bridge were analyzed for EcO157, regardless of indicator *E. coli* concentration.

The occurrence of EcO157 was not uniformly distributed throughout the lake. Table 7 focuses on sample matrix, but the specific locations where the EcO157 positive samples occurred can provide more insight into the spatial distribution of EcO157. The specific sites and frequency where EcO157 was detected is presented in Table 8. It is important to note that at some sample locations, such as Deer Creek, no samples were tested for EcO157. This was because EcO157 tests were only run on water samples that exceeded the EPA recreational limit (320 MPN/100 mL), with the exception of the creek samples from Meadow Park Bridge and beach shoreline samples at Hideaway West from July through October.

Table 7. *E. coli* O157:H7 Detection Frequency showing the total samples analyzed for indicator *E. coli*, the number tested for EcO157, and the % of samples that tested Positive for EcO157 per Reveal method.

Type	Total Samples Analyzed	No. Tested for O157	No. O157 Positive	Percent Positive
Beach Sand	82	82	7	8.54
Creek Water	128	52	12	23.08
Lake Water	476	54	40	74.07

(a) Based on Reveal test positive samples.

Table 8: Location and frequency of O157:H7 Positive samples, per Reveal method.

Type	Site	No. Positive
Creek Water	6: Meadow Park Bridge	12
Beach Sand	3BS: Hideaway West Comp Sand	5
	4S: Meadow Comp Sand	1
	Control Site Sand	1
Lake Water	2: Vista Park Shore	1
	3A: Hideaway East Shore	3
	3B: Hideaway West Shore	21
	4: Meadow Park Shore	2
	5: Commodore Park Shore	13

In Meadow Park Creek at the bridge, 37 of 46 samples (80.4%) exceeded the EPA single sample recreational criteria, while the 30-day geometric mean standard was exceeded 100% of the time. At Meadow Park Bridge, EcO157 was detected in 26% (12/46 samples) of the tested samples, per the Reveal method. In spite of the high indicator *E. coli* levels and EcO157 detections in Meadow Park Creek at the bridge, none of the samples in the creek at Meadow Park Slough exceeded the recreational limits, and there was only one exceedance of the EPA criteria at Meadow Park Beach shoreline. EcO157 was detected at each public park beach shoreline, excluding Explorer Park, per the Reveal method. Hideaway West (21) and Commodore Park (13) had the most EcO157 detections, followed by Hideaway East (3),

Meadow Park (2), and Vista Park (1). At Hideaway West beach shoreline, the EPA single sample criteria was exceeded 25% of the time with the standard exceeded 12.5% of the time at Commodore Park, the sites with the two most frequent detections of EcO157. In beach sand, EcO157 was detected in 8.5% of samples (7/82 samples) at two public park beaches and the control site beach. The majority of positive sand samples occurred at Hideaway West Park Beach (5 detections), followed by Meadow Park and the Control site (1 each). The *E. coli* O157:H7 detections in beach sand at Hideaway West appear consistent with the frequent detection in the beach shoreline water at that location.

The temporal occurrence of EcO157 is presented in Table 9. The seasonal sampling program was approximately six months between May 2 and November 7 in Meadow Park Creek, and five months between May 2 and October 10 at the other sample locations. The first observation of EcO157 was made on May 13, 2019 in Meadow Park Creek at the bridge. Eco157 was detected in beach shoreline water on June 24, with observations in beach sand on July 11. The final observation of EcO157 in water and sand was on October 3, in beach shoreline water. The pattern of EcO157 detections observed in 2019 was similar to 2018, with detections of EcO157 in Meadow Park Creek approximately one month prior to observations in beach water and sand. There were no changes in the analytical methodology for EcO157 during the sampling period that might have contributed to a change in recovery efficiency.

The temporal occurrence of EcO157 in sand has important implications for management. The fact that EcO157 was not detected in sand during the first two months of the monitoring program suggests that beach sand was not an important reservoir of EcO157, carried over from the previous season. The data patterns observed during this monitoring project suggest the beach sand will not act as a significant recurrent source of EcO157, if sources of fecal contamination are mitigated.

Table 9: Temporal occurrence of EcO157 in Meadow Park Creek, Beach Sand and Water.

Parameter	First Observation	Final Observation	Total Days Interim
LWW Seasonal Sampling	2-May	7-Nov	189
Occurrence in Meadow Park Creek	13-May	9-Sep	119
Occurrence in Beach Sand	11-Jul	19-Sep	70
Occurrence in Beach Water	24-Jun	3-Oct	101

3.6 Reliability of Reveal Test for Detecting *E. coli* O157:H7 in Water and Sand

The 2018 LWW monitoring program (Yanko et al. 2019) utilized a simple lateral flow immunoassay, the Neogen Reveal test, as a screening tool to detect *E. coli* O157:H7. The enrichment broth from positive Reveal test samples was then submitted to a commercial laboratory (Cel-Analytical) for confirmation by PCR. The Z3276 gene was targeted using a kit produced by Genesig (Li et al.,2017, Li et al., 2012). The confirmation rate overall for the Reveal positive samples was about 80 percent. The same approach was planned for the 2019 testing program. When the initial sample set from the 2019 testing was ready to confirm, it was learned that the Genesig test kit was backordered and it was unclear when it would be

available. For that reason, the laboratory switched to using a mericon EcO157 test kit from Qiagen. The Qiagen product was a multiplex PCR test that would detect EcO157:H7 and the virulence genes stx1/stx2 and *eae*, thus potentially providing additional information about the pathogenicity of the strains detected.

Twenty-one Reveal test positive samples were initially analyzed with the Qiagen multiplex assay. All were negative for EcO157:H7; however, one was positive for stx, and 12 were positive for *eae*. These results were inconsistent with the confirmation data obtained during 2018 using the Genesig product. The Qiagen product literature did not indicate what gene was targeted as diagnostic for EcO157:H7, so the manufacturer was consulted. The company representative indicated that was proprietary information, but did confirm that Z3276 was not the target. With no information available about the gene targeted by the Qiagen product for EcO157, it was not possible to examine potential reasons for the apparent discrepancy. As this problem was being deliberated, the Genesig test kit became available and it was decided to retest the samples using that product. Table 10 shows the results for all samples originally tested with the Qiagen product and also includes the corresponding results for those later retested with the Genesig test. All samples listed had been considered positive with the original Reveal Test immunoassay.

Table 10: Confirmation of O157:H7 Positive Reveal Test Samples by Qiagen Multiplex and Genesig PCR Analyses

Sample		Reveal Test	Qiagen Multiplex			Genesig
Date	Location	Intensity	EcO157:H7	Stx1/Stx2	Eae	Z3276
7/22/19	Commodore Shoreline	2	negative	negative	negative	NT
7/29/19	Commodore Shoreline	2	negative	negative	negative	NT
5/13/19	Meadow Park Bridge	1	negative	negative	negative	**Positive**
5/20/19	Meadow Park Bridge	4	negative	**Positive**	**Positive**	**Positive**
6/3/19	Meadow Park Bridge	1	negative	negative	negative	**Positive**
7/1/19	Meadow Park Bridge	1	negative	negative	**Positive**	**Positive**
7/29/19	Meadow Park Bridge	1	negative	negative	**Positive**	**Positive**
6/20/19	Hideaway-W Shoreline	2	negative	negative	**Positive**	**Positive**
7/1/19	Hideaway-W Shoreline	2	negative	negative	negative	NT
7/11/19	Hideaway-W Shoreline	1	negative	negative	**Positive**	**Positive**
7/25/19	Hideaway-W Shoreline	2	negative	negative	**Positive**	**Positive**
7/29/19	Hideaway-W Shoreline	2	negative	negative	negative	NT
7/11/19	Hideaway-W Sand	1	negative	negative	**Positive**	NT
7/25/19	Hideaway-W Sand	1	negative	negative	**Positive**	NT
5/23/19	Goose Poop 1	4	negative	negative	negative	**Positive**
5/23/19	Goose Poop 2	2	negative	negative	**Positive**	**Positive**
5/23/19	Goose Poop 3	3	negative	negative	**Positive**	negative
5/23/19	Goose Poop 4	3	negative	negative	negative	negative
5/23/19	Goose Poop 5	3	negative	negative	negative	negative
8/5/19	Goose Poop 1	4	negative	negative	**Positive**	**Positive**
8/5/19	Goose Poop 2	4	negative	negative	**Positive**	negative
	Positive Control	—	**Positive**	**Positive**	**Positive**	**Positive**
	Negative Control	—	negative	negative	negative	negative

Fifteen of the twenty-one samples originally tested with the Qiagen test were retested with the Genesig kit. Eleven of the fifteen were positive for EcO157:H7, more in line with the confirmation rate observed in 2018. It is interesting to note that most of the samples that confirmed with the Genesig test were also positive for the Eae gene with Qiagen test. In that respect, the Qiagen test appeared to be consistent with the Genesig result. The 5/20/19 Meadow Park Bridge sample is particularly notable in that it was positive for O157:H7 with the Genesig test and positive for both Eae and stx with the Qiagen test, while being negative for O157 in the Qiagen multiplex analysis. One possible explanation for these results that has been considered is that atypical environmental strains of EcO157 are being detected.

Thirty Reveal Test positive samples were tested with the Genesig assay. Table 11 lists those results, *i.e.* the 15 that were retests of the original Qiagen analyses plus 15 others that had not been previously tested. The reveal test confirmation rate for this data set is 73%, slightly lower than observed in 2018. Four of the samples that confirmed had indicator *E. coli* results that were below the EPA recreational criteria.

Due to the discrepancies between the Qiagen and Genesig PCR results for EcO157:H7, analysis of positive Reveal Test samples was significantly delayed. A total of seventy-two samples, representing all matrices sampled during the project, were positive for EcO157 during the monitoring program per the Reveal method. This included 52 water samples, 7 beach sand samples, and 13 goose fecal samples. Forty-two positive Reveal Test samples have not been submitted for confirmation as of the writing of this report. No additional analysis of the EcO157 data will be reported at this time. This section of this interim report will be updated when those analyses are completed.

Table 11: Positive Neogen Reveal Test *E. coli* O157:H7 Confirmation Results by Genesig PCR for Z3276, Samples Grouped by Location

Date 2019	Sample Location	Sample Type	Indicator *E. coli* Concentration MPN/100 mL	Reveal Test Intensity	Reveal Confirmed by PCR	qPCR (gene copies /aliquot)
5/13	Meadow Park Creek @ Weir	Water	160.7	1	Yes	$1.1x10^5$
5/20	Meadow Park Creek @ Weir	Water	307.6	4	Yes	$6.6x10^4$
5/23	Meadow Park Creek @ Weir	Water	131.4	4	Yes	$1.1x10^3$
6/3	Meadow Park Creek @ Weir	Water	866.4	1	Yes	$1.5x10^3$
7/1	Meadow Park Creek @ Weir	Water	727.0	1	Yes	$2.2x10^3$
7/29	Meadow Park Creek @ Weir	Water	1553.1	1	Yes	$1.0x10^5$
8/26	Meadow Park Creek @ Weir	Water	235.9	2	Yes	$3.2x10^3$
9/5	Meadow Park Creek @ Weir	Water	517.2	2	Yes	$1.8x10^3$
6/20	Hideaway Beach-W Shoreline	Water	435.2	2	Yes	$2.0 x10^4$
7/11	Hideaway Beach-W Shoreline	Water	58.1	1	Yes	$3.2 x10^6$
7/25	Hideaway Beach-W Shoreline	Water	1732.9	2	Yes	$3.7 x10^5$
8/5	Meadow Park Shoreline	Water	3.1	1[(a)]	Yes[(a)]	$2.3x10^4$
8/8	Meadow Park Shoreline	Water	12.0	2	Yes	$6.4x10^4$
9/2	Commodore Shoreline	Water	98.4	3	**No**	<100
9/5	Commodore Shoreline	Water	12.1	3	**No**	<100
9/9	Commodore Shoreline	Water	12.2	3	Yes	$1.2x10^6$
9/12	Commodore Shoreline	Water	14.8	3	**No**	<100
5/23	Goose Droppings-1	Feces	NA[(b)]	4	Yes	$1.9x10^2$
5/23	Goose Droppings-2	Feces	NA	2	Yes	$2.0x10^4$
5/23	Goose Droppings-3	Feces	NA	3	**No**	<100
5/23	Goose Droppings-4	Feces	NA	3	**No**	<100
5/23	Goose Droppings-5	Feces	NA	3	**No**	<100
8/5	Goose Droppings-1	Feces	NA	4	Yes	87
8/5	Goose Droppings-2	Feces	NA	4	**No**	<100
9/5	Goose Droppings-1	Feces	NA	2	Yes	$8.2 x10^2$
11/14	Goose Cloacal Swabs-1	Feces	Pos[(c)]	2	Yes	$5.4 x10^4$
11/14	Goose Cloacal Swabs-2	Feces	Pos	2	Yes	$7.1x10^3$
11/14	Goose Cloacal Swabs-4	Feces	Pos	2	Yes	$3.5x10^6$
11/21	Goose Cloacal Swabs-1	Feces	Pos	1	**No**	<100
11/21	Goose Cloacal Swabs-2	Feces	Pos	1	Yes	$2.3x10^2$

(a) Positive O157 is for 1-liter sample volume concentrated on membrane filter

(b) NA = not analyzed

(c) Cloacal swabs tested for indicator *E. coli* by Idexx P/A format

Three of those were the standard 100 mL Reveal test analyses used for this project; one was a 1-L sample that was concentrated by membrane filtration.

3.7 Relationship between Indicator *E. coli* and *E. coli* O157:H7

The following section is included as a place holder. Reveal test confirmations were not completed in time to meet the deadline for this interim report for reasons discussed in the previous section. The data summaries below are based on unconfirmed Reveal Test data. This section will be updated when confirmation testing is completed. The numbers in the tables in this section will change when the confirmation data is available.

As part of this project, we looked at the association between indicator *E. coli* and the occurrence of *E. coli* O157:H7, in both water and sand. For water samples, excluding two sites, only those samples with results >320 MPN/100 mL were analyzed for EcO157. This limits the ability to complete a classic correlation analysis with the entire water sample dataset, as the indicator *E. coli* data is censored, with only those water samples that exceeded the recreational limit being tested for EcO157. However, at two sites, water samples were tested for EcO157 independent of indicator *E. coli* value. Every creek sample from Meadow Park Bridge was analyzed for EcO157 by the Reveal method, regardless of indicator *E. coli* level. Starting in July 2019 at the Hideaway West beach shoreline, after multiple high indicator *E. coli* and EcO157 observations, every sample was analyzed for EcO157 by the Reveal method. This provided a small dataset for evaluating the relationship between indicator *E. coli* and presence of *E. coli* O157:H7. For beach sand, every sample that was collected during the monitoring period was tested for both indicator *E. coli* and *E. coli* O157:H7. This provided a beach sand dataset for evaluating the relationship between indicator *E. coli* and EcO157.

The data in Table 12 shows the distribution of positive EcO157 samples, in comparison to indicator *E. coli* concentration range, for water samples. A total of 52 positive samples were evaluated in Table 12, with indicator *E. coli* range divided into six groups based on concentration, and the resulting number of positive EcO157 samples presented for each group range. The greatest number of positive EcO157 samples was observed at *E. coli* concentrations between 10-99 MPN/100 mL, well below the EPA recreational criteria, with 20 positive samples per the Reveal method. There were thirteen positive samples between 321-1000 MPN/100 mL, followed by nine positive samples between 100-320 MPN/100 mL, five positive samples at indicator *E. coli* values of <10 MPN/100 mL, four positives between 1001-2419.6 MPN/100 mL, and one positive sample above the quantitative detection limit of the Idexx test method (>2,419.6 MPN/100 mL). For the water samples that were EcO157 positive per the Reveal method, 65% of the samples had indicator *E. coli* values that were below the EPA single sample recreational criteria (320 MPN/100 mL).

Table 12: Distribution of Positive EcO157 Samples Relative to Indicator *E. coli* Concentration Range in Water.

Indicator E. coli Concentration Range MPN/100mL	Number of Positive EcO157 Samples
<10	5
10-99	20
100-320	9
321-1000	13
1001-2419	4
>2419	1

The data in Table 13 shows the distribution of positive EcO157 samples, in comparison to indicator *E. coli* concentration range, for beach sand samples. A total of seven positive samples were evaluated in Table 13, with indicator *E. coli* range divided into three groups based on concentration, and the resulting number of positive EcO157 samples presented for each group range. The greatest number of positive EcO157 samples was observed at *E. coli* concentrations between 1,000-10,000 MPN/g, with three positive samples per the Reveal method. There were two positive samples between 99-1,000 MPN/g, and two positive samples

between 10,000-100,000 MPN/g. Due to the small dataset (n=7) of positive samples, and lack of apparent trends in the data, it is difficult to make any definitive conclusions regarding the relationship between indicator *E. coli* concentrations in sand and positive EcO157 observations.

Table 13: Distribution of Positive EcO157 Samples Relative to Indicator *E. coli* Concentration Range in Sand.

Indicator *E. coli* Concentration Range MPN/g	Number of Positive EcO157 Samples
99-1000	2
1000-10000	3
10000-100000	2

As noted at the beginning of this section, the reported observations are based on raw Reveal Test data, not confirmed data. These results will likely change when confirmation testing is completed.

3.8 Miscellaneous Samples

This section discusses results of work that was separate from the basic LWW work plan. Background information was provided as appropriate in the Methods and Results Chapters, to provide context.

3.81 E. coli O157:H7 in Geese

A small number goose fecal samples were tested for EcO157:H7 toward the end of the 2018 monitoring program. Fecal samples were not included in the initial 2018 sampling plan due to concerns about the potential for Reveal Test matrix issues associated with testing feces. All five fecal samples tested in 2018 were confirmed positive. Based on those results, additional goose fecal sampling and analysis was conducted in 2019. These samples included both feces collected from the ground and cloacal swabs collected from culled birds. Results are summarized in Table 14.

Quantitative analyses were not practical with fecal swabs. All goose fecal swabs were positive for indicator *E. coli* with the Idexx presence/absence test. Fecal samples collected from the ground were not tested for indicator *E. coli*. As noted in the Methods Section, the fecal samples collected from the ground were observed to come from different birds. Although the data-base is small, these results suggest that half to two-thirds of the resident geese may be carrying EcO157:H7.

Table 14: Goose Fecal Analyses during the 2019 monitoring program.

Date 2019	Sample Location	Indicator *E. coli* P/A[(a)]	Reveal Test Intensity	Reveal Confirmed by PCR[(b)]	qPCR[(b)] (gene copies /aliquot)
5/23	Goose Droppings-1 Meadow Park	NA[(c)]	4	Yes	$1.9x10^2$
5/23	Goose Droppings-2 Meadow Park	NA	2	Yes	$2.0x10^4$
5/23	Goose Droppings-3 Meadow Park	NA	3	**No**	<100
5/23	Goose Droppings-4 Hideaway-E Shore	NA	3	**No**	<100
5/23	Goose Droppings-5 Hideaway-E Shore	NA	3	**No**	<100
8/5	Goose Droppings-1 Hideaway-W	NA	4	Yes	87
8/5	Goose Droppings-2 Hideaway-W	NA	4	**No**	<100
9/5	Goose Droppings-1 Hideaway Pavilion	NA	2	Yes	$8.2 x10^2$
11/14	Cloacal Swabs-Bird 1	Pos	2	Yes	$5.4 x10^4$
11/14	Cloacal Swabs-Bird 2	Pos	2	Yes	$7.1x10^3$
11/14	Cloacal Swabs-Bird 3	Pos	0	NA	NA
11/14	Cloacal Swabs-Bird 4	Pos	2	Yes	$3.5x10^6$
11/21	Cloacal Swabs-Bird 1	Pos	1	**No**	<100
11/21	Cloacal Swabs-Bird 2	Pos	1	Yes	$2.3x10^2$

(a) Goose cloacal swabs tested for indicator *E. coli* by Idexx Presence/Absence format

(b) Confirmation by Genesig O157:H7 PCR test

(c) NA = Not analyzed

3.82 Meadow Park Creek Source Tracking

The following was a collaborative effort conducted through a consulting contract Nevada County Health Department had with Southern California Coastal Water Research Project (SCCWRP). Background information is briefly reviewed first.

On August 13, 2017, approximately one month after the LWW EcO157:H7 outbreak, 50-L beach water samples were concentrated by Nevada County Environmental Health personnel and shipped to the Centers for Disease Control laboratory in Atlanta GA for analysis. The samples were tested for the presence of EcO157:H7 and for microbial source tracking (MST) markers to help assess the probable source of the beach contamination. The genetic MST markers tested were human, deer and geese. Only goose markers were detected. This was the first evidence that geese may have played a role in the outbreak. Additional evidence was subsequently accumulated establishing the role of geese during the 2018 LWW monitoring and investigation conducted by Lake Wildwood Association (Yanko et al. 2019). Geese were documented to be the predominant source of shoreline contamination at the beaches. Additional evidence was developed that the geese were carrying EcO157, but it was not possible to determine where or how the geese became infected. One theory was that geese may pick up O157:H7 when foraging at nearby cattle pastures. Another possibility was that geese pick up STEC from water in the lake. In either event, a few infected resident geese could then continue to infect others in the flock by defecating on the park lawns where they regularly graze.

It was also reported in the 2018 report that Meadow Park Creek was chronically contaminated and the microbial contamination frequently included EcO157:H7 (Yanko et al., 2019). Based on the initial reports of creek contamination with STEC, Nevada County

Environmental Health (NCEH) coordinated with Centers for Disease Control (CDC) to analyze samples from Meadow Park Creek for Microbial Source Tracking (MST) markers to help assess the source of the contamination. Five samples including both water and sediment were collected by NCEH and shipped to CDC. These were tested for EcO157 and MST markers. Locations sampled were Meadow Park Beach shoreline, Meadow Park Creek at the Bridge, and Meadow Park Creek approximately 0.5 mile upstream (sediment only). CDC reported that E. coli O157:H7 was detected in water and sediment in the creek at the bridge location, and in the sediment at the upstream location. Isolates from those two locations had matching Pulse-Field Gel Electrophoresis (PFGE) patterns, and whole genome sequencing (WGS) demonstrated that the isolates were clonal, with 0-1 single nucleotide polymorphisms (SNPs) indicating a common source. *E. coli* O157:H7 was not detected in either water or sand at the beach location.

In the creek at Meadow Park Bridge, Human and Ruminant MST markers were detected in the water; Goose, Deer and Cow markers were not detected. The corresponding creek sediment sample was negative for all of the animal markers and inconclusive for the human marker. The upstream sediment sample was negative for all markers, although it had been positive for O157:H7. At the Meadow Park Beach location, the water was positive for goose markers, but negative for all other markers, and sand was negative for all markers (cow not tested in the sand). The CDC report did not provide the qPCR data and did not provide any discussion or conclusions about the MST results. The full CDC report was included in the 2018 report, Appendix H (Yanko et. al. 2019). Without the qPCR results, it is difficult to assess the relative significance of the reported ruminant and human marker results.

In general, the CDC MST data appeared to be generally inconclusive. It is possible there could be some human contamination from septic systems outside of the LWW property or the sewer lines within LWW. That area of the sewage system was not examined after the outbreak because it was not located close to Commodore Beach where the outbreak occurred. Finding Ruminant markers, but not Deer, is surprising because the LWW Deer population is large and the Meadow Park stream flows for about a mile through a greenbelt area. There could be some ruminant animals such as goats or sheep in the properties outside of LWW producing the Ruminant signal. It also seemed unusual that the markers were detected in the creek water but not in the streambed sediments. The longterm persistence of specific enteric pathogens compared to selected fecal MST markers in sediments is not well established. For the beach shoreline water sample, only goose markers were detected. That was consistent with the MST analyses conducted soon after the outbreak and the subsequent conclusions that geese were the primary source of contamination at the beaches.

Based on the earlier analyses discussed above, another effort was made to determine the source of contamination in Meadow Park Creek during 2019. Samples were collected and concentrated on-site by LWW project personnel. The sample filters were shipped to SCCWRP and analyzed for MST markers under the direction of Dr. John Griffith. Three samples were collected on May 27, 2019 and concentrated on site as described in the **Methods** Section of this report. The timing of the sampling was related to a series of spring rainstorms. Figure 8 shows a flow hydrograph for Deer Creek downstream from Lake Wildwood for a period of 18 days that

guided the Meadow Park Creek sampling. USGS maintains a flow measuring station downstream from LWW that reports real-time data. Similar flow data for the smaller Meadow Park Creek or Wildwood Creek that were sampled for MST were not available. The flows from these smaller watersheds would be substantially smaller than Deer Creek, but timing of the storm flows should be reasonably similar.

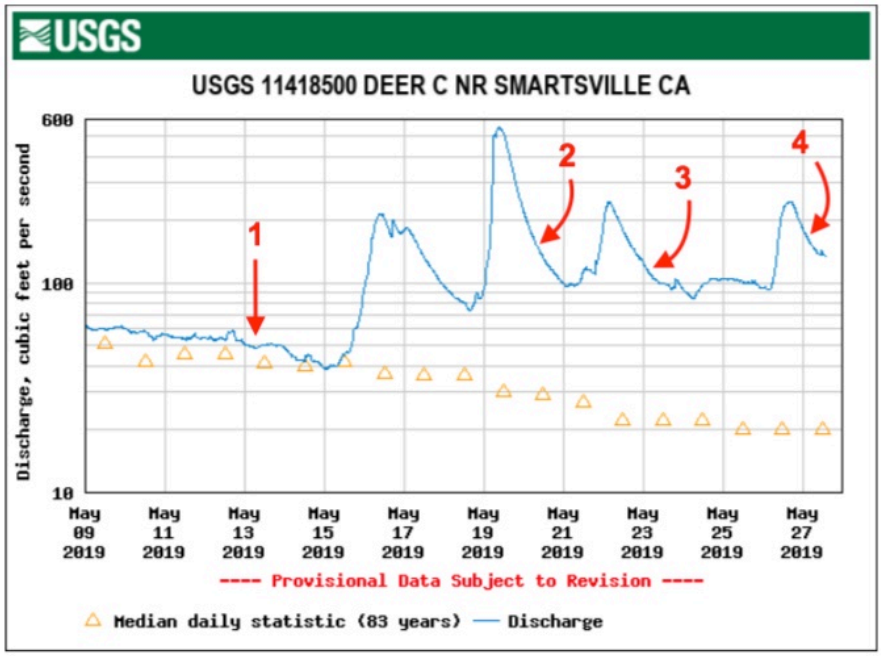

Figure 8: Flow hydrograph for Deer Creek downstream from Lake Wildwood in May 2019.

Point #1 on Figure 8 is when the first EcO157 positive sample was collected. There had not been significant rainfall since about April 19-20 and flows were in a declining baseline condition, just slightly above the historic average (shown by the gold triangles). The first O157 positive was very weak, producing a barely perceptible signal on the Reveal test strip. A few days after that, a series of unseasonable spring storms started and flows rose quickly with each storm. Sample #2 was collected during the declining leg of the hydrograph the morning after the second storm, i.e. it was still in the increased flow period. That sample showed a very strong positive for O157 with the Reveal test. Sample #3 was the same scenario; the sample was collected the morning following a storm while still in the elevated flow stage, again producing a very strong positive for O157. That was the rationale for trying to capture the last of the forecast storms for source tracking analyses.

Samples were collected from Meadow Park Creek at the Bridge near where it enters the lake and about a mile upstream close to where the creek enters the LWW development. A third sample was collected from Wildwood Creek on the other side of the lake. That sample was also collected close to where the creek enters the LWW community. The MST markers utilized in this sampling are listed in Table 15.

Table 15. Genetic Markers Used for Microbial Source Tracking

ID Code	Source Tracking Genetic Marker
HUM-1	Human – HF183 Bacteroides human specific marker
RUM	Rum2Bac – Bacteroides common to range of ruminant animals
COW	Cow – CowM3 Bacteroides–like bacteria cow specific marker
HUM-2	Lachno3 – Human specific Lachnospiraceae spp.
BIRD	Bird – Bird GFD Helicobacter – broad range avian marker
rfbE	rfbE – E. coli gene in E. coli O157 - Does not distinguish non H7 strains
STX-1	Stx1 – Shiga toxin 1 gene
STX-2	Stx2 – Shiga toxin 2 gene
Z3276	Z3276 gene – unique E. coli O157:H7 gene considered diagnostic

Results of the Source Tracking analyses are shown in Table 16 and Figure 9. Note the Y-axis of Figure 9 is a $\log_{10}$ scale. The most significant source of contamination to Meadow Park Creek was a ruminant animal, and the ruminant signal increased significantly between the upstream and downstream sample points. The cow marker was generally low at all sample locations and did not change between the Meadow Park Creek upstream and downstream locations. Other ruminant animals that could impact the stream would include sheep, goats, and deer. Deer would be the only ruminant that could explain the increase observed in Meadow Park Creek as it flowed through Lake Wildwood. The Human-1 marker (HF183) also increased in Meadow Creek, but that may be an artifact. The Human-1 marker tracked proportionally to the Ruminant marker. Other research has shown some cross reactivity may occur between Deer and the Human-1 marker (Griffith et al., 2013). For that reason, a second human marker (Lachno3) was tested. The Human-2 marker was low and did not increase as the water flowed downstream. Although trace human contamination was detected, the levels were below that considered to represent human health concerns (Personal Communication, John Griffith, SCCWRP).

Birds appeared to be the second primary source of contamination following Ruminant contamination at the time these samples were collected. Geese do not directly go into the areas that were sampled. Wild turkeys would be the most probable source within LWW, although chickens, turkeys and game birds on the ranch properties outside of LWW could also potentially contribute.

Table 16: Microbial Source Tracking Sample Information & Results for Samples Collected May 27, 2019.

Sample Location & Replicate No.		Volume Filtered	Filter Type[b]	Source Marker ID Code[a]: Copies/100mL except BRD = Probe/100 mL									% Recovery Extraction Control[c]
				HUM-1	RUM	COW	HUM-2	BIRD	rfbE EcO157	STX-1	STX-2	Z3276 EcO157	
Meadow Creek[d] Upstream	1	300	HA	88	1370	44	40	201	6	0	0	13	39
	2	300	HA	92	416	23	18	126	0	0	0	0	78
	3	300	HA	0	640	0	157	130	0	8	0	5	61
Meadow Creek at Bridge	1	100	PC	853	3626	0	75	298	0	0	0	0	22
	2	100	PC	661	2883	0	55	28	0	0	0	0	58
	3	400	HA	1529	8907	85	37	374	0	6	0	0	42
	4	400	HA	1477	5962	41	0	207	0	0	5	0	45
Wildwood Creek	1	300	HA	0	186	70	46	69	0	0	1	3	83
	2	300	HA	0	72	0	71	107	0	0	0	0	47
Rinse H_20[e]	-	300	PC	0	0	0	0	0	0	0	0	0	108
NTC[f]	-	-	-	0	0	0	0	0	0	0	0	0	-
+ Control	-	-	-	4925	38191	3594	18681	7977	196528	757671	89826	223233	-

(a) See Table 15, p. 39
(b) HA – 47 mm Millipore cellulose acetate; PC – 47 mm Millipore polycarbonate
(c) NP – Halophile
(d) Sample point ~100 ft downstream from LWW fence
(e) Autoclaved Crystal Geyser bottled drinking water
(f) No template control

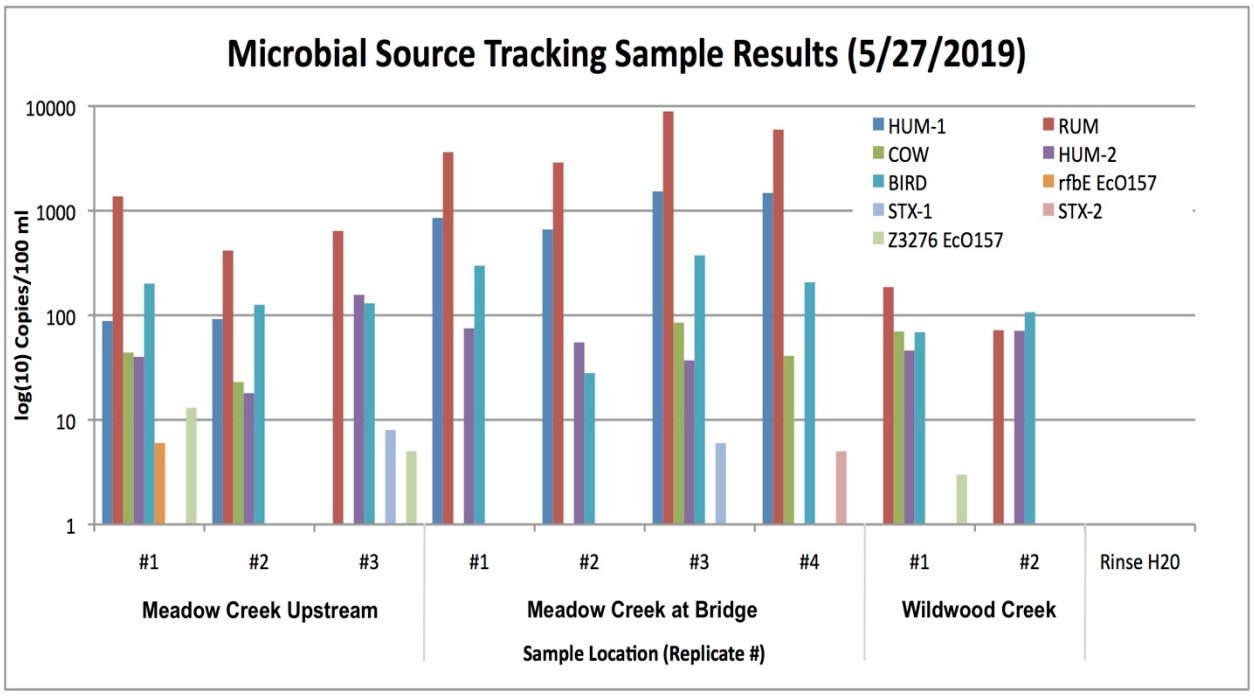

Figure 9: Microbial Source Tracking results for Meadow and Wildwood Creek in May 2019.

Very little evidence of any significant *E. coli* O157:H7 presence was detected in these samples. While minimal, there was some consistency in that all samples that showed trace levels of the Z3276 gene were also positive for one of the other EcO157:H7 virulence genes. Stx toxin genes were detected at trace levels in two samples independent of any O157 markers.

3.83 Newtown Canal (Appendix)

At the end of 2019 monitoring program, a separate focused sub-study was conducted to compare Meadow Park Creek *E. coli* concentrations to Newtown Canal, the irrigation source

water provided to the small Meadow Creek watershed area. That work was reported separately and is included in Appendix A.

3.9 Sample Results – 2018 vs 2019 Comparison

Two seasons of microbial monitoring were completed during 2018 and 2019. Data was collected at many of the same sample locations in both years, including public park beaches in ankle and waist deep water, beach sand, creeks that flow into Lake Wildwood in near shore environments with no sand beach, and the middle of the lake. This allows for comparison of sample results and changes over time at sites which can be evaluated in the context of management actions.

3.9.1 Creeks

Samples were collected in 2018 and 2019 from five locations in three creeks that flow into Lake Wildwood (Figure 10). Sample locations included Meadow Creek at Meadow Park Bridge (MED-B) and Meadow Slough (MED-S), Deer Creek (DC), and in Wildwood Creek at sites located upstream (WW-I) and downstream (WW-O) of the golf course. Indicator *E. coli* concentrations decreased slightly in Meadow Creek at Meadow Park Bridge from 2018 to 2019, with a similar proportion of samples exceeding the EPA recreational criteria each year. Concentrations also decreased in Meadow Slough from 2018 to 2019, with no exceedances of the EPA recreational standards in 2019. In Deer Creek, *E. coli* concentrations were similar in 2018 and 2019, with low levels of indicator *E. coli* and no exceedances of the EPA recreational criteria in either year. Observations in Wildwood Creek suggest *E. coli* levels increased in 2019 relative to 2018, at both the upstream and downstream sample locations. No samples from Wildwood Creek exceeded the EPA recreational standards in 2018, while in 2019 both sites exceeded the EPA single sample and geometric mean criteria. The results suggest a similar level of chronic microbial contamination in 2018 and 2019 for Meadow Creek at Meadow Park Bridge, with regular exceedance of the EPA environmental criteria. In addition, the Wildwood Creek upstream and downstream sample locations exceeded the EPA single sample standard one-quarter and one-fifth of the time in 2019, which was a considerable increase in comparison to the results from 2018.

3.9.2 Beach Shoreline Samples

Samples were collected in 2018 and 2019 from six public park beach shorelines in ankle deep water. Sample locations included Commodore Park (COM), Meadow Park (MED), Hideaway Park East (HID-E) and West (HID-W), Vista Park (VIS), and Explorer Park (EXP). At each site, the mean and median *E. coli* concentrations decreased in 2019, and a smaller percentage of samples exceeded the EPA single sample recreational standard (Figure 11). With the exception of Commodore Park, compliance with the EPA 30-day geometric mean standard improved considerably from 2018, with only Hideaway West and Commodore Park exceeding the geometric mean standard in 2019.

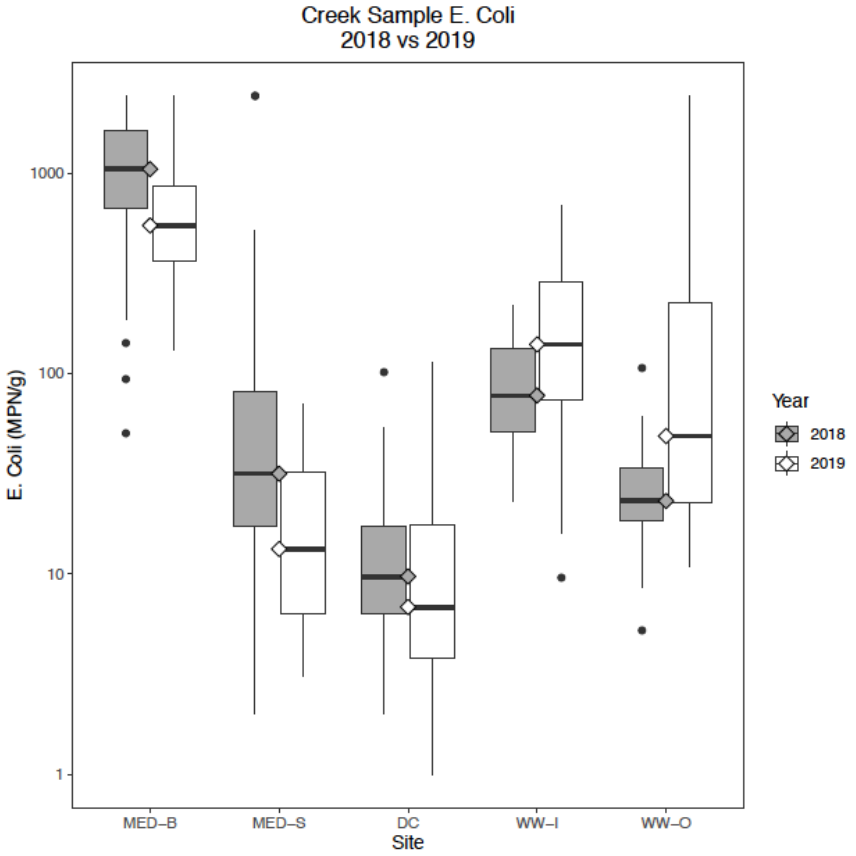

Figure 10: Comparison of indicator *E. coli* in creeks from 2018 and 2019.

Hideaway West exhibited the highest *E. coli* levels and greatest number of exceedances of the EPA recreational criteria in both 2018 and 2019. Indicator *E. coli* concentrations dropped considerably at Meadow Park in 2019, where a goose exclusion fence was installed during the recreational season, with fewer exceedances of the recreational criteria. Overall, the results show a considerable decrease in *E. coli* concentrations at each beach shoreline ankle deep sample location, and improved compliance with EPA recreational standards in 2019.

3.9.3 Waist Deep Samples

The same beaches were also sampled within the designated swim zone at waist deep depth in 2018 and 2019. Sample locations are designated the same as above. Hideaway West, Meadow Park, and Hideaway East exhibited the highest mean and median *E. coli* levels in 2019, as was observed in 2018 (Figure 12). At each site, the mean and median *E. coli* concentrations decreased from 2018 to 2019, with the most pronounced decreases observed at the Hideaway Park and Meadow Park sample locations. No waist deep samples exceeded the EPA single sample recreational standard in 2019, compared with multiple exceedances at three sample locations in 2018. Overall, the results show a considerable decrease in *E. coli* concentrations at each waist deep sample location, with results in compliance with EPA recreational standards in 2019.

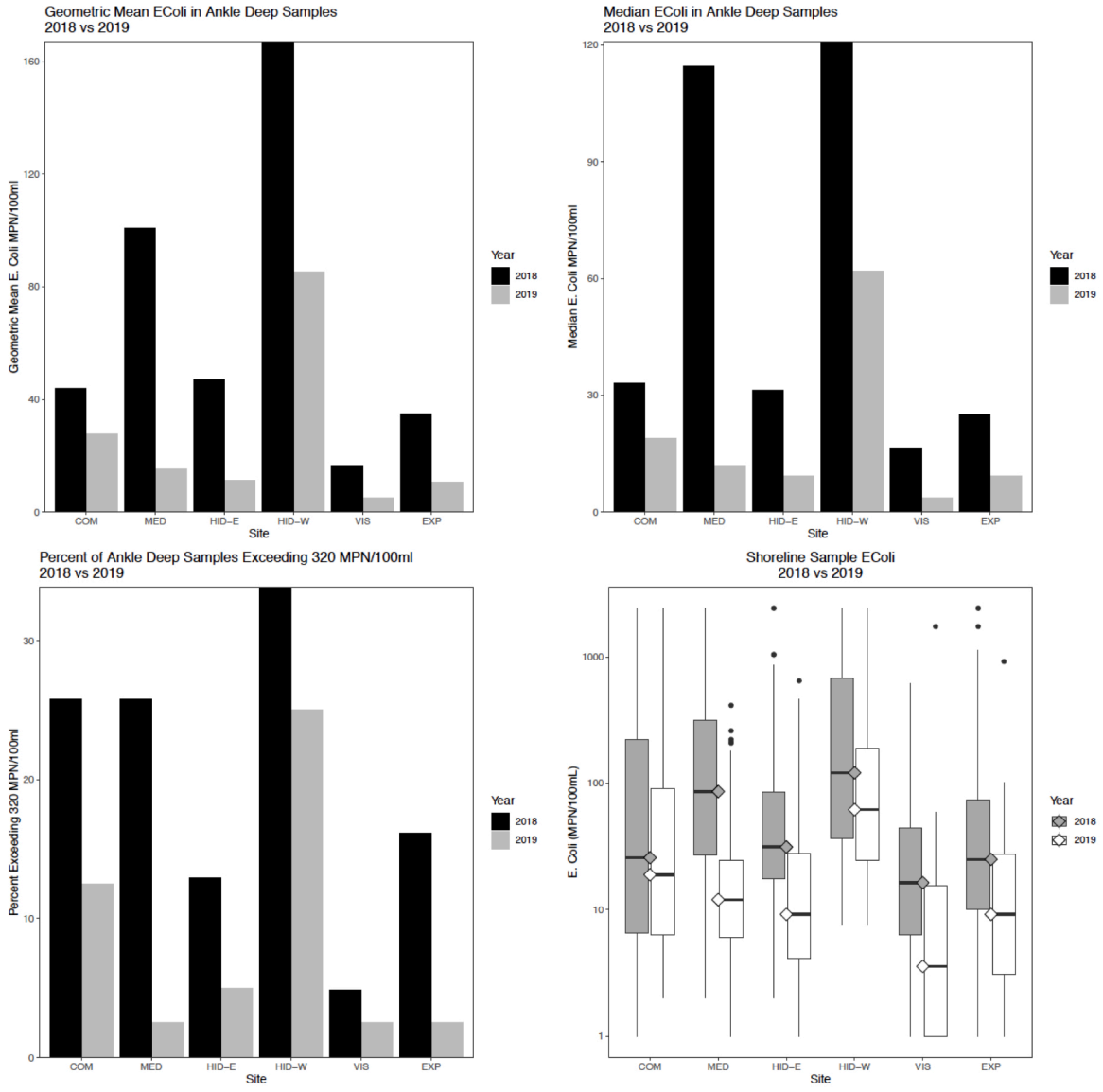

Figure 11: Comparison of beach shoreline sample results from 2018 and 2019.

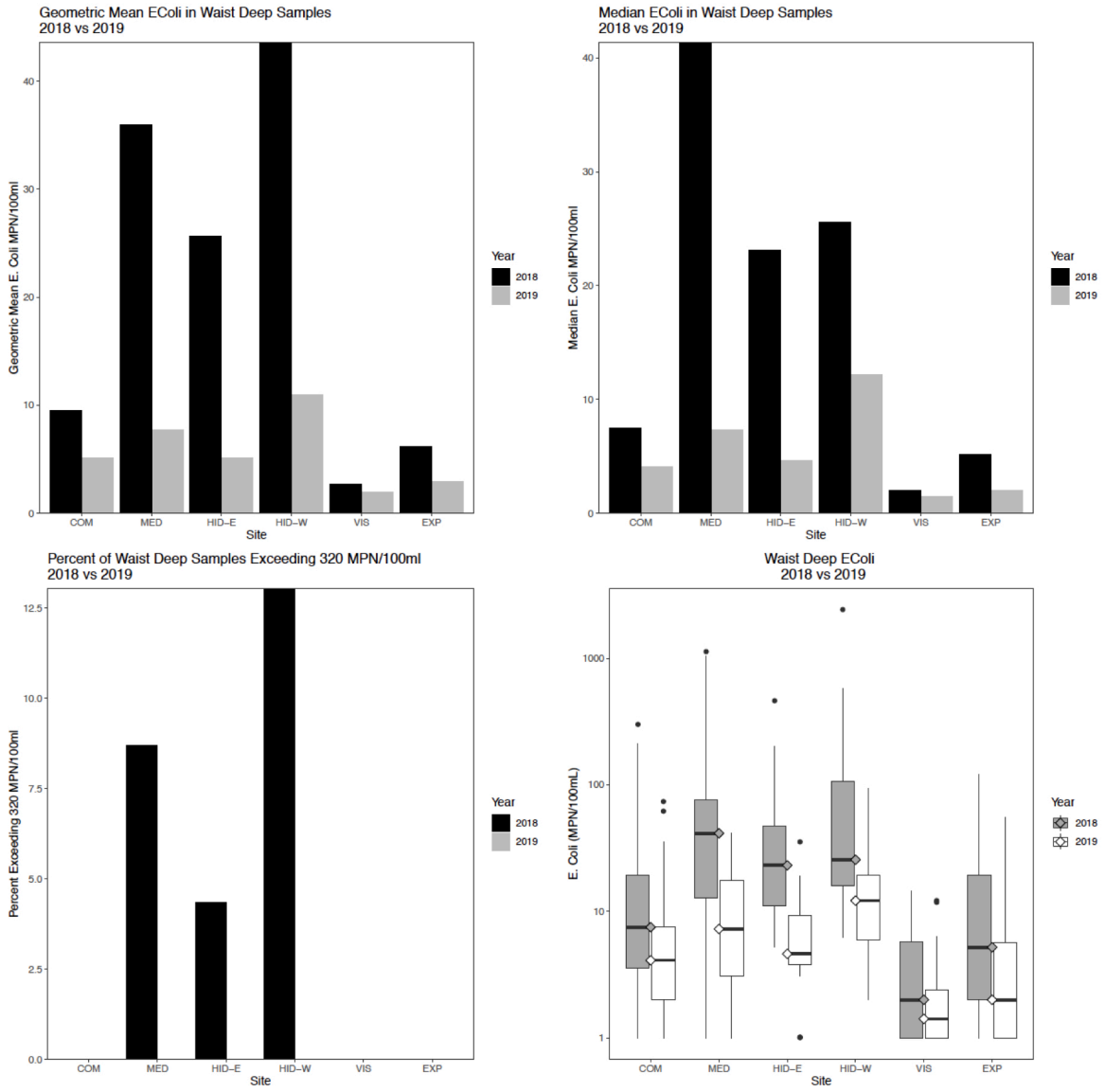

Figure 12: Comparison of swim zone waist deep sample results from 2018 and 2019.

3.9.4 Near Shore and Mid Lake Samples

Samples were collected in 2018 and 2019 from one near shore location with no beach and one location in the middle of the lake, referred to as the Chaparral Drive Greenbelt and Mid Lake sample sites. Low *E. coli* concentrations were observed at both sample locations, with similar results documented in 2018 and 2019 (Figure 13). The results from 2018 and 2019 indicate *E. coli* levels were consistently low at these two sample locations, with no exceedances of the EPA criteria.

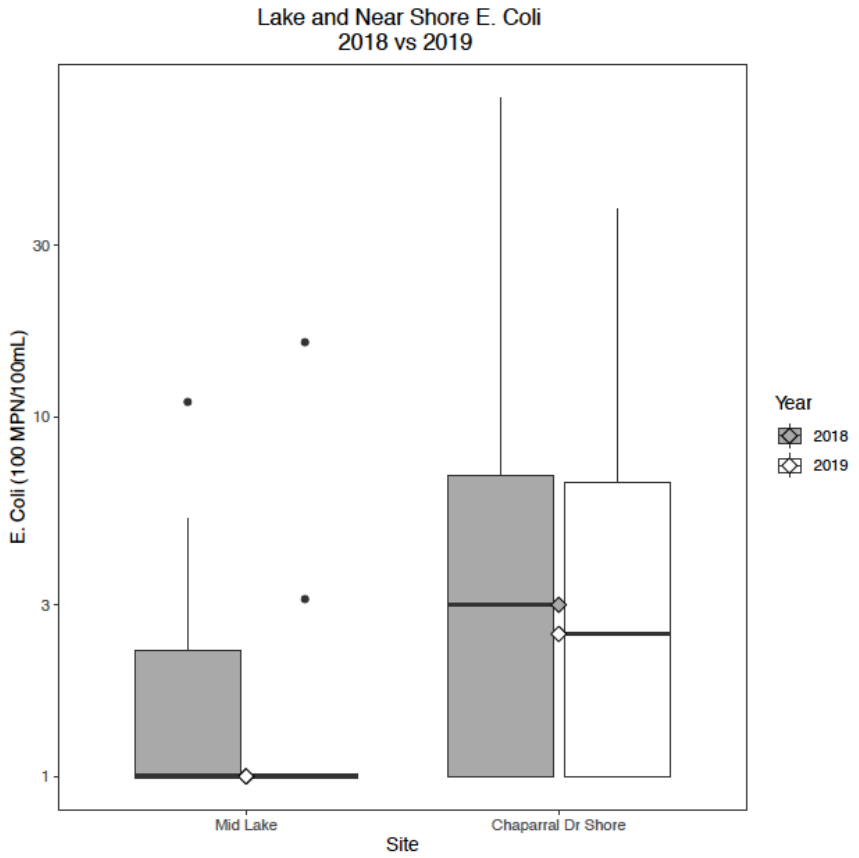

Figure 13: Comparison of mid-lake and near-shore (Chaparral Drive) sample results from 2018 and 2019.

3.9.5 Beach Sand Samples

Composite sand samples were also collected from the six public park beach shorelines at the sand-water interface in 2018 and 2019. At each site, the mean, median and maximum *E. coli* concentrations decreased in 2019 (Figure 14). The highest observed values in 2018 were at Hideaway East (2,419,600 MPN/g) and Hideaway West (195,600 MPN/g), with the maximum value observed at Hideaway West (72,700 MPN/g) in 2019. This marks a considerable decease in the peak *E. coli* concentrations observed in beach sand from 2018 to 2019. Evaluation of the average waterline fecal index densities in 2018 and 2019 further confirms a decrease in the amount of goose fecal material present on the beach shorelines and in beach sand. The highest average waterline fecal index density in 2018 was 2.1 at Meadow Park, followed by 2.0 at Hideaway West, versus a maximum average density of 1.6 observed at Hideaway West in 2019. This represents a decrease in the amount of visible feces observed on the park beaches in 2019 compared with 2018. Overall, the results show that there were lower *E. coli* concentrations in the sand at each beach shoreline sample location in 2019 compared to 2018, consistent with the lower fecal index values.

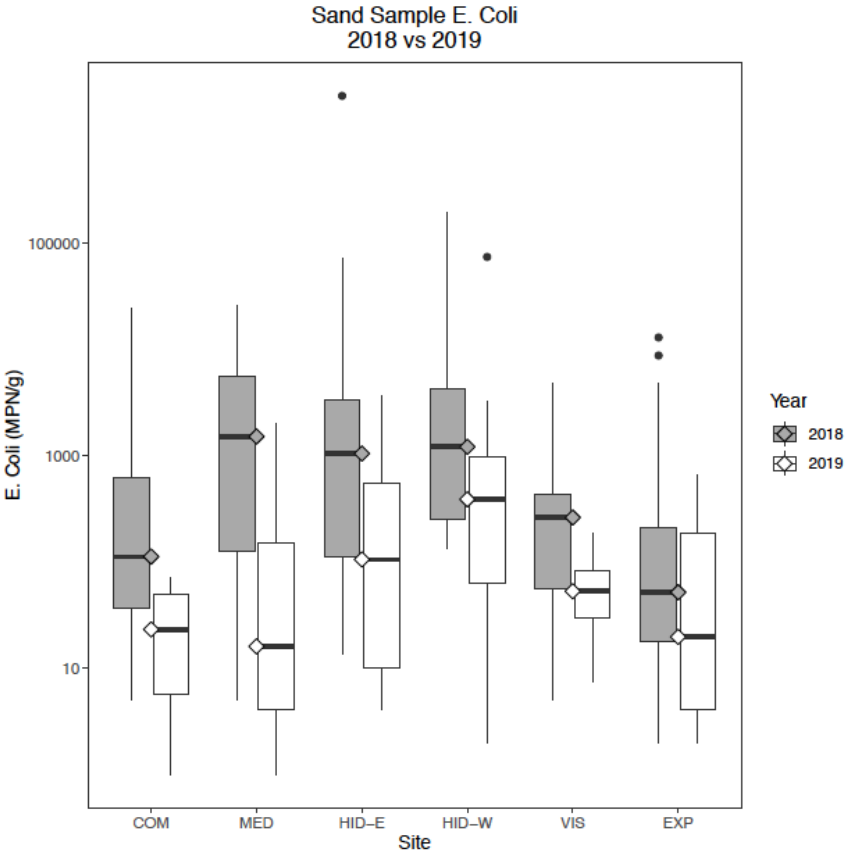

Figure 14: Beach sand composite sample results from 2018 and 2019.

CHAPTER 4: DISCUSSION AND RECOMMENDATIONS

4.1 Discussion

In addition to the analytical results described in this report, some changes were made to the community website data reporting program instituted in 2018 (Yanko et al., 2019). A map of Lake Wildwood showing all of the lake sampling points was included as the opening page for the lake report. The map indicated a simple color code for each location showing the test results for the most recent sampling. Red indicated the last result was over the EPA recreation limit. Yellow indicated the caution range, 100 to 320 *E. coli* MPN/100 mL, continuing to observe Lake Wildwood's conservative caution level. Green indicated the most recent result was less than 100 *E. coli* MPN/100 mL When repeated exceedances occurred in shoreline samples, LWA took a conservative approach and voluntarily closed the beach. As in 2018, no new cases of STEC were reported during 2019. Overall, management approaches being employed at LWW, most notably reducing the resident goose population, appear to be improving water quality and reducing recreational risk.

4.11 Effects of Goose Management on *E. coli* Results

The results from the 2019 monitoring program clearly demonstrate the effects of lake management activities and the goose depredation effort on reducing indicator *E. coli* levels at public park beaches. In June 2019, as part of a combination of approaches to managing the goose population, Lake Wildwood Association began the process of culling geese during the summer molt. Prior to culling, the goose population was estimated to be in the range of 110 – 125 individuals, based on goose count data from Lake Wildwood goose patrol volunteers. After goose depredation, data indicates the goose population in Lake Wildwood decreased significantly, to between 15 – 25 resident geese. As of this report, there is no evidence to suggest significant repopulation of Lake Wildwood by a new resident goose population after the 2019 goose depredation effort.

During the 2019 monitoring program, as a result of the culling program, significantly fewer geese were observed during sample collection in comparison to 2018. At the public park beach shoreline and waist deep sample locations, indicator *E. coli* concentrations decreased from 2018 to 2019, with fewer exceedances of the EPA single sample and geometric mean recreational criteria. Indicator *E. coli* concentrations in beach sand also decreased, as did the average waterline fecal density (amount of visible feces on public park beaches). These observations and results are consistent with a decrease in the overall goose population and sources of fecal contamination within Lake Wildwood from 2018 to 2019.

Geese were routinely observed at Meadow Park on the lawn and beach during sample events in 2018, with associated *E. coli* contamination, presence of EcO157, and exceedances of EPA recreational criteria a concern at the shoreline and waist deep sample locations. To address these concerns and mitigate potential risk to recreational users, Meadow Park beach was closed during the middle of summer in 2018, and a temporary fence was installed to

exclude geese from the shoreline area. The fence prevented the geese from roosting at the waterline, with indicator *E. coli* concentrations in both sand and water declining after fence installation (Yanko et al., 2019). While effective at keeping geese off the beach, the fence also interfered with human use of the beach. A similar approach was explored in 2019 at Meadow Park for the entire recreational season and monitoring period to substantiate the limited 2018 data.

Indicator *E. coli* concentrations dropped considerably in 2019 at Meadow Park in response to a reduced goose population on the lake and installation of the fence at the beach waterline. The fence provided a visual and physical barrier that prevented geese from easily accessing the beach shoreline and roosting at the waterline. The fence at Meadow Park resulted in reduced goose presence on the beach, less goose feces at the sand-water interface, lower indicator *E. coli* concentrations in water and sand, and fewer exceedances of the EPA recreational criteria. The results demonstrate that the management approach employed at Meadow Park beach in 2019 was effective at limiting goose presence and defecation on the beach and reducing *E. coli* contamination from goose feces. Similar, but more user friendly methods could be explored at Meadow Park and other parks where geese are routinely present and observations of indicator *E. coli* and EcO157 are of concern, such as Hideaway Park or Commodore, in an effort to address goose fecal contamination and risk to lake users in these locations.

4.12 Growth of *E. coli* in Sand

The 2018 monitoring program investigated the potential for *E. coli* to grow in warm, moist beach sand at public park beaches. While some sites exhibited positive trends, the results were generally inconclusive, and suggested that the variability in sand *E. coli* concentrations was related more to fecal contamination from geese rather than microbial growth (Yanko et al., 2019). Potential for indicator *E. coli* to grow in sand was investigated at the park beaches again in 2019, with the addition of a lakefront homeowner private beach site where no geese were historically present. *E. coli* levels at four of the seven beach sample locations showed negative trends in 2019, while the other three sites showed weak positive or essentially flat trends, indicating little or no growth of *E. coli* in the beach sand. With fewer geese present in Lake Wildwood relative to 2018, a greater number of beaches showed declining or no trends in *E. coli* levels. The results demonstrated that significant growth of *E. coli* in sand was not documented during the 2019 sampling period, and that *E. coli* levels in beach sand tended to be controlled and limited by input of fecal material from sources including geese.

Growth of *E. coli* was also evaluated in the context of an unexpected event at the Control Site beach during the monitoring season. After the first week of sample collection in early June 2019, the landowners of the control site imported new sand for their small private beach. The following week, high *E. coli* levels (24,810 MPN/g) were detected in the beach sand at the control site, with no visual observations of fecal material present on the beach to explain the results. This suggests that the imported sand was potentially contaminated with *E. coli*. Subsequent observations at the control site demonstrated a rapid decrease in concentrations within two weeks, likely attributed to a rapid exponential die off of *E. coli* (Figure 15). The

results of this impromptu experiment, in combination with the seasonal trends at the beach sites, indicated that there is not a tendency for *E. coli* to grow in the beach sand in the absence of organic and fecal material inputs from geese.

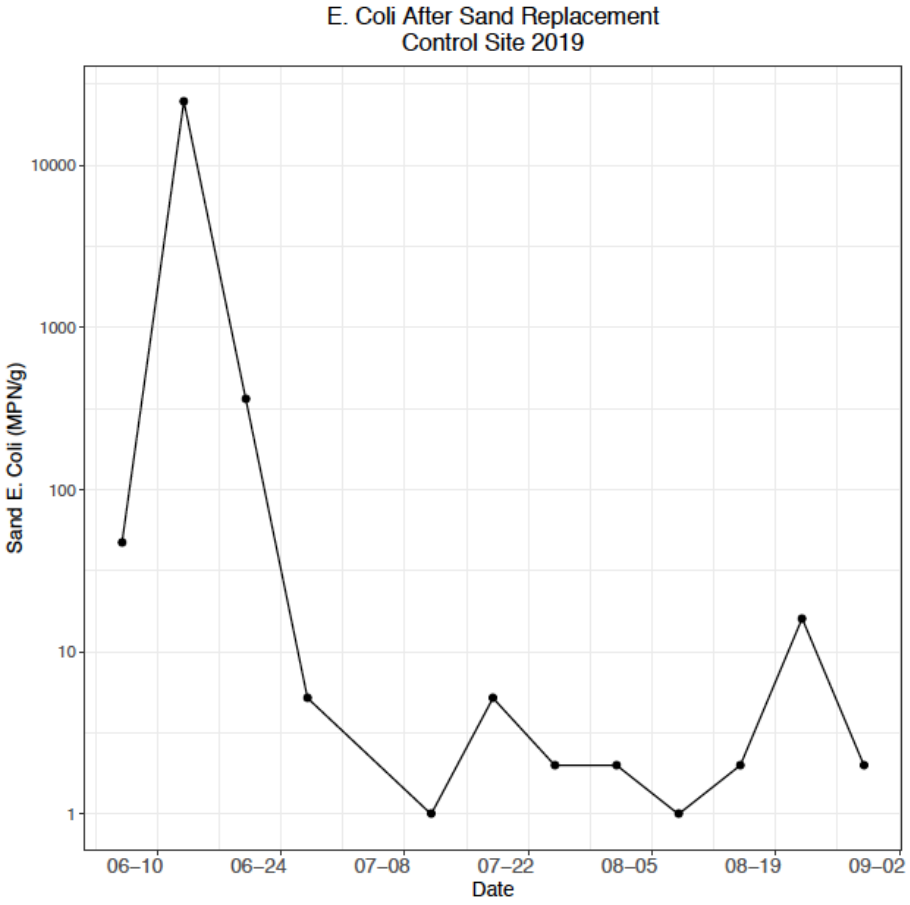

Figure 15: Indicator *E. coli* in beach sand at the control site, before and after importing contaminated beach sand. The graph is log scale on the y-axis. Note the rapid, exponential die off of *E. coli* after 6/13/2019.

4.13 PCR Confirmation Issues with *E. coli* O157:H7

The apparent discrepancy between the Genesig and Qiagen mericon PCR assays used for confirming Reveal Test EcO157:H7 positive samples presented an unexpected challenge for interpreting project data. The reliability of the Z3276 gene targeted by the Genesig assay for identifying EcO157:H7 appeared to be well documented in the scientific literature (Li et al., 2012; Li et al., 2017). Qiagen considered the details of their test to be proprietary information, so it was not possible to review supportive literature documenting reliability of the specific genetic target used for identifying EcO157:H7. One multi-laboratory study did report that the Qiagen mericon assay was equivalent to a standard reference method for detecting STEC including O157 in selected food products (Bird et al, 2018). Given the reasonably robust published documentation available supporting the Genesig PCR test, it was decided to accept the positive Genesig PCR results as evidence that EcO157 was present given that there was also a concurrent positive antibody test.

Nevertheless, questions remain. The Qiagen multiplex assay also tested for the stx toxin genes. All but one of the samples were stx negative suggesting that all but one of the corresponding samples judged positive for O157 by the Genesig assay were non-toxigenic strains. At this time the results remain difficult to reconcile. One possible explanation that has been discussed is that there may be a larger population of atypical O157 strains present in the environments sampled during this project. Clearly more work is needed to address these questions, but that is beyond the local laboratory capabilities available for this project, and funding to pursue this issue is not currently available. This remains an important question.

Forty-two Reveal test samples from 2019 still remain to be tested to confirm the positive immunoassay results. It was decided to not delay an interim release of this report until that testing is completed. It is planned to complete the remaining samples as funding permits; this section of the report will be updated at that time.

It was noted that some samples had indicator *E. coli* concentrations well below the EPA Recreational Limits but were confirmed to contain EcO157:H7. This has also been observed in other environmental studies employing PCR based detection methods (Duris et al., 2009; Partyka et al., 2018). Two points need to be considered here. (1) The EPA recreational criteria were not based on detecting pathogens in the water. The criteria were related to the probability of disease occurring. Obviously, the presence of pathogens is significant, but numerous other variables are involved in the development of water borne infections. (2) The virulence of the EcO157 strains detected by the methods employed in this project is not known. As noted above, there is a possibility that many of the O157 positive samples may be atypical non-toxigenic strains representing minimal risk, but that remains an unknown.

4.14 *E. coli* O157:H7 in Geese

More goose fecal samples, including cloacal swabs were tested in 2019. When LWA received a renewal of the original U. S. Fish & Wildlife depredation permit, the new permit included an unexpected requirement to test cloacal swabs collected from culled birds for both generic *E. coli* and STEC. The first data resulting from that requirement was reported here. At this time, it is being estimated the EcO157 carriage rate in the LWW resident geese ranges from half to two-thirds of the birds. That is significant, but it is also subject to the caveats discussed above concerning pathogenicity. It not currently known with certainty what actual level of risk is associated with the geese. It was documented by the California State Health Department that one goose fecal sample contained the identical strain of *E. coli* O157:H7 that caused the illnesses during the outbreak. Given the potential severity of STEC infections, LWA cannot gamble with children's lives because pathogenicity information associated with the test methods used is not as robust as one would wish. Conservative assessments of recreational safety will need to continue based on the presence and concentration of indicator *E. coli* in the shoreline waters. That, in turn, is directly related to the number of geese present.

Recently the results and findings from the LWW studies were discussed with a research microbiologist from The Western Center for Food Safety at U.C. Davis. There is interest in the data being developed in the LWW project due to its potential relationship to goose

contamination of food crops. Although not firm yet, there is a reasonable possibility some assistance may become available to help address some of the questions posed by the LWW data.

4.15 Meadow Park Creek

Meadow Park Creek (aka Wildflower Creek) continues to be a concern due to the chronic contamination in that stream. The fence experiment on Meadow Park Beach provided additional evidence that the creek represented a minimal amount of beach contamination compared to the geese. Nevertheless, it cannot be ruled out that the creek may be a source of contamination for the geese, and the geese in turn amplify the numbers of EcO157 and vector STEC to other locations. It remains an appropriate goal to mitigate that source of contamination.

E. coli analyses of Newtown Canal water and a review of NID historic data indicated that the irrigation water delivered to the Meadow Park Creek watershed area is not the source of the creek contamination. Microbial Source Tracking analyses conducted by Southern California Coastal Water Research Project under contract to Nevada County Health Department suggested that ruminant animals were the predominant source of the contamination detected following a rainstorm. That source increased as the stream flowed through LWW to the lake. While the ruminant marker was significantly higher than any other markers, cow specific marker was relatively low. Other ruminant animals besides cattle potentially outside of LWW include goats and sheep and deer. The only ruminant that would affect that creek inside LWW would be deer.

Federal and State regulatory approaches consider source control to be the primary approach to controlling microbial contamination when sources can be identified. It has been definitively demonstrated that geese are responsible for causing the shoreline contamination at the park beaches, and aggressive action has been instituted by LWA to control that source. But there is no evidence implicating geese with the contamination in Meadow Park Creek. Ruminant animals outside of the LWW community may be contributing to that microbial load. LWA has no jurisdiction to investigate that source. Three meetings with the County Health agencies and County Agricultural Commission have taken place to discuss strategies for assessing potential sources of contamination to Meadow Park Creek, but those have not resulted in any substantive outcome. At some future point in time, LWA may have to consider requesting State Water Quality Control Board intervention to address this issue.

Given the large deer population in the area, randomized testing of deer feces should also be considered to determine if they carry detectable levels of EcO157. Deer have been implicated in other STEC outbreaks (Laidler et al., 2013; Probert et al., 2017). A small number of deer fecal samples tested for EcO157 by the State Health Department immediately following the outbreak were negative. A more extensive survey would seem appropriate now in light of the Microbial Source Tracking results for Meadow Creek to assess if deer may be a contributing factor to the presence of EcO157 in the creek.

4.2 Recommendations

It was noted in the 2018 Monitoring Report (Yanko et al, 2019) that the coauthors of the report were instrumental in the conduct of the project, but they were not tasked with the responsibility for recommending specific actions to the Lake Wildwood Association in response to the *E. coli* O157:H7 outbreak. Responsibility for report preparation was being transitioned to Sierra Streams Institute with this report, however, the following recommendations continue to be solely those of the P.I., William Yanko, and reflect his personal experience and professional judgment.

1. The 2019 monitoring data further emphasized the importance of controlling and minimizing the resident goose population at Lake Wildwood to reasonably assure safe swimming conditions. Removal of the entire resident goose population now will provide an opportunity to assess if the occurrence of EcO157 in the resident goose population is an anomaly, or if new geese that may attempt to colonize LWW in the future are also carrying STEC.

2. Depredation is the most expedient, and only feasible way to remove the STEC infected birds. At the same time, it is incumbent on the community to develop a longer term non-lethal strategy for discouraging the development of another large resident goose population. Keeping geese off the park beaches will be an ongoing obligation to maintain safe swimming conditions. Discussions should continue regarding institutionalizing this function as a long term Association responsibility.

3. Beach & lake testing should continue at the same frequency as last year to further validate the benefits of reducing and potentially eliminating the current resident goose population.

4. Two changes are recommended to the sampling locations for 2020:
    - Eliminate the mid lake location at the Waterski Course.
    - Add a sampling location in Wildwood Creek Bay in the inlet area where the creek enters the lake. The purpose would be to determine if Wildwood Creek has a measurable impact on the water in that inlet area (See Figure 16).

4. Continue monthly sampling of beach sand for indicator *E. coli* to further substantiate the effects of reducing the goose population.

5. Complete testing of Reveal positive EcO157 samples from 2019 monitoring program in order to complete data analyses.

6. Stop testing beach water and sand for EcO157 in 2020. It is unlikely that additional useful information will be developed at this time.

7. Focus continuing EcO157 analyses on fecal samples from geese. Additional effort can then be devoted to clarifying exactly what the current testing protocol is detecting. It is anticipated

this will be a relatively small number of samples, depending on the success of the scheduled June culling.

8. Initiate a randomized survey of deer and turkey feces for EcO157 to assess possible creek contamination sources.

9. Continue to pursue the opportunity for possible collaboration with Western Center for Food Safety at U.C. Davis.

10. Continue emphasizing community education about the importance of observing published Lake test data and visually examining beach shorelines for presence of goose fecal material before permitting children to play at the shoreline.

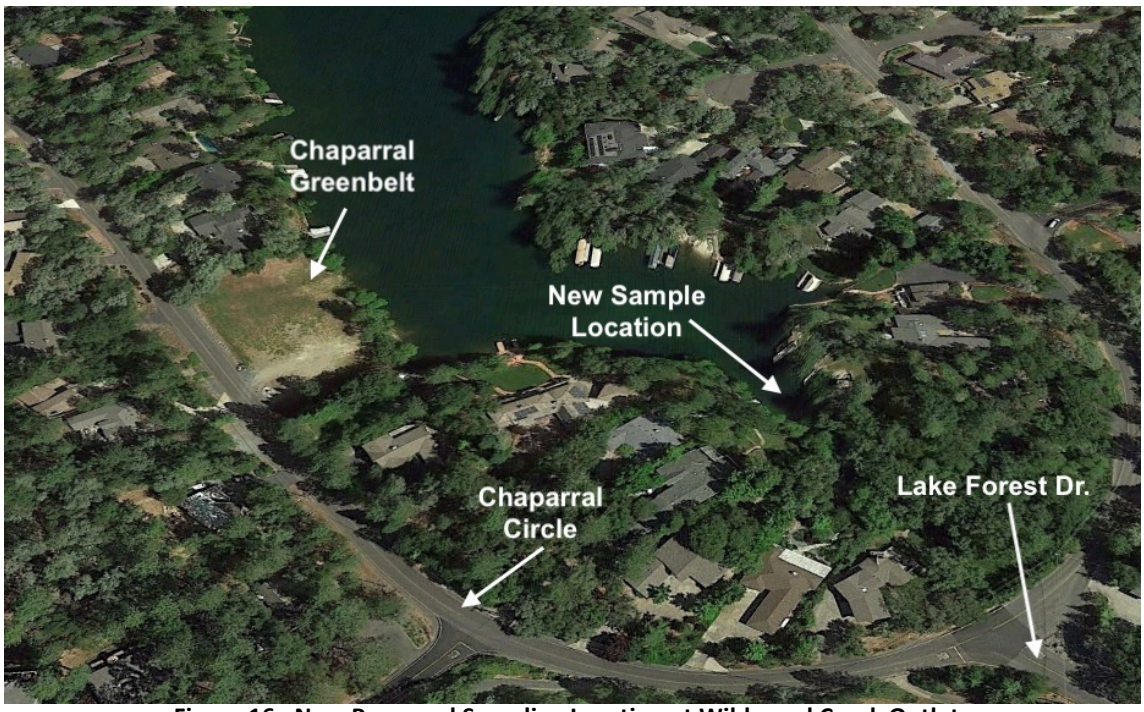

Figure 16. New Proposed Sampling Location at Wildwood Creek Outlet.

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Appendix A

E. coli Contamination in Meadow Park Creek (aka Wildflower Creek) Occurring in the Area Between Bitney Springs Road and Lake Wildwood

William A. Yanko
Lake Wildwood Association
December 10, 2019

Introduction:

On September 4, 2019 there was a meeting of representatives of the Nevada County Agriculture Commission, Nevada County Public Health, and Nevada County Environmental Health. Also attending was Sue Hoek, Nevada County Supervisor, Dan Macon, U. C. Davis Extension, and this writer representing Lake Wildwood Association (LWA). The purpose was to review cattle ranching practices in Nevada County and discuss any potential linkages to the 2017 Lake Wildwood (LWW) *E. coli* outbreak. An introductory presentation by Dan Macon reviewed ranching practices in the county and discussion followed. The discussion eventually focused on the area just north of LWW where the small creek called Meadow Park Creek (aka Wildflower Creek) originates. This creek is chronically contaminated with high concentrations of indicator *E. coli* and nutrients (nitrogen and phosphorus) when it reaches Lake Wildwood. *E. coli* O157:H7 was frequently detected in Meadow Park Creek during the 2019 LWW Monitoring program (Yanko, *et al*, 2019) and in one sample sent to CDC for analysis. For this reason, LWA considers mitigation of the contamination in this creek a priority item.

Two "deliverables" resulted from this meeting. (1) It was requested that LWA continue some *E. coli* monitoring of Meadow Park Creek for a period of time after the end of the NID irrigation season on October 15. Yanko agreed to that. (2) A representative of Nevada County would attempt to obtain a monitoring point on the creek somewhere between Bitney Springs Road and Lake Wildwood to start the process of isolating the source of the contamination.

Separate from the above meeting, LWA representatives were provided a tour of NID facilities associated with LWW water supply on September 23, 2019. This included both potable supply and irrigation water provided to the golf course and lake. The tour included the Lake Wildwood water treatment plant, which is located in the watershed area for Meadow Park Creek. The tour provided an opportunity to learn more about that specific location. Newtown Canal is the source of the raw water supply to the Lake Wildwood water treatment plant. Newtown Canal also supplies the irrigation water to the ranch properties located in the Meadow Park Creek watershed area. We found there were two locations where Newtown canal crossed Bitney

Springs Road, providing access points for sample collection that did not necessitate entering private properties. We also learned that NID tests the raw water supply for *E. coli* and had a database going back many years. Based on this information, we requested NID authorization to include irrigation source water samples with our extra testing of Meadow Park Creek where it enters the lake. We also requested and received a copy of the historical Newtown Canal raw water *E. coli* data.

This report contains the test results for Meadow Park Creek for the month of October 2019, before and after the end of the NID irrigation season. Newtown canal was sampled on the same days for comparison to the creek water. The report also includes a summary comparison of the NID Newtown canal historical data and the LWW Meadow Park Creek data for the past 13 years.

Methods:

Figure A1 shows the location of the sampling points and the area the creek flows through before entering the lake at Meadow Park. Newtown canal was sampled at the two

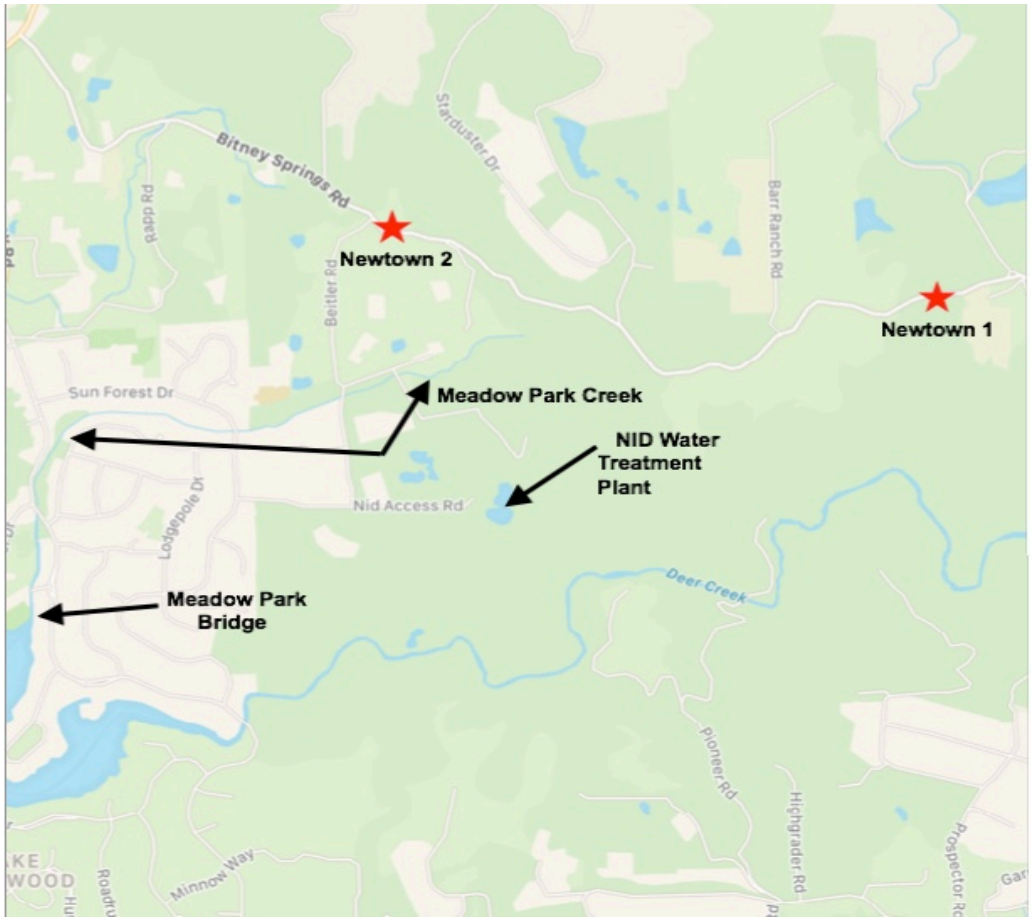

Figure A1. Meadow Park Bridge and Newtown Canal Sampling Locations

points indicated by the red stars. The creek was sampled at Meadow Park Bridge before entering the lake. The creek sample location is behind a flow measuring weir located ~100 feet upstream from where the creek flows into the lake. The NID source water samples were collected by NID personnel at a point located between the two Newtown canal sample locations utilized by LWW. LWW samples were collected and analyzed by Sierra Streams Institute and NID samples were analyzed by their laboratory. Both labs use the Idexx Quantitray method for *E. coli*, per standard testing protocols. Prior to 2013, LWW samples were analyzed by LWA staff using the m-Coli Blue membrane filtration method. Samples (100 mL) listed in Table 1 were tested for *E. coli* O157:H7 using the Neogen Reveal assay as described in Yanko et al, 2019.

Results and Discussion:

Table A1 summarizes the results for the October sampling before and after the end (shaded) of the irrigation season. There was no significant difference between the two Newtown Canal sample points. The Newtown #2 sample location was dry on the last two sampling days. This is most likely due to NID cutting off the irrigation water flow. The Newtown Canal #1 location was located before the diversion to the Lake Wildwood water treatment plant. During this sampling period, the *E. coli* concentrations in the creek increased by more than an order of magnitude compared to the raw irrigation water both before and after irrigation ended. Every creek sample exceeded the USEPA *E. coli* recreational limits. No corresponding canal irrigation water samples exceeded the recreational limits. All samples listed in Table A1 were also screened for E. coli O157:H7 using the Neogen Reveal test. All samples (100 mL) were negative.

Table A1. Newtown Canal and Meadow Park Creek (aka Wildflower Creek) Samples for E. coli Before and After October 15, 2019 End of NID Irrigation Season

	E. coli MPN/100 mL		
Date	Newtown 1	Newtown 2	Meadow Park Bridge
10/3/19	48.8	42.8	461.1
10/7/19	73.3	39.9	866.4
10/13/19	29.5	28.5	866.4
10/17/19	90.9	90.8	920.8
10/21/19	57.8	Dry	517.2
10/31/19	53.8	Dry	580.3

Tables A2 and A3 summarize the NID historical raw water sampling for the Lake Wildwood water treatment plant and the LWW historical data for Meadow Park Creek at Meadow Park Bridge from 2007 through 2019. The LWW sampling program is seasonal, roughly corresponding to the NID irrigation season. The NID data set was edited to include only those samples collected during the April 15 through October 15 time period each year so that data sets were comparable representing the same time period. Given the similarity of the Newtown sample points 1 and 2 summarized in Table A1, it is reasonable to assume the NID raw water data was representative of the irrigation water distributed in that area.

The LWW data (Table A3) had been tabulated previously using the 1986 EPA Recreational criteria as the compliance limit. For convenience, the NID canal data were evaluated using the 1986 limits for comparison to the LWW data. The overall *E. coli* mean for the Newtown source water to the drinking water plant during the reported time period was 35.9 MPN/100 mL. For the same 13 year period, the *E. coli* mean in the creek at Meadow Park Bridge was 559.8 MPN/100 mL, reflecting the same greater than 10 fold increase observed in the Table A1 data.

Table A2. NID Newtown Canal *E. coli* Monitoring Summary 2006 – 2019
***E. coli* data reported as MPN per 100 mL[a]**

Year	Date Started[b] M/D	Date Ended[b] M/D	No. of Samples Tested	*E. coli* Geometric Mean	*E. coli* Range	% Exceeding 235 Single Sample Limit[c]	% Exceeding 126 30-day Geometric Mean[d]
2007	4/26	10/15	12	26.7	4.1 – 81.3	0	—
2008	4/22	10/8	13	16.3	3.0 – 83.3	0	—
2009	4/17	10/7	12	26.2	5.2 – 103.6	0	—
2010	4/22	10/13	12	28.0	2.0 – 135.4	0	—
2011	4/22	10/8	12	42.9	9.6 – 156.5	0	—
2012	4/19	10/2	12	17.6	3.0 – 53.8	0	—
2013	4/27	10/3	12	52.5	19.9 – 146.7	0	—
2014	4/15	10/13	13	28.8	1.0 – 387.3	7.7	—
2015	4/20	9/30	11	22.1	4.1 – 178.2	0	—
2016	4/16	9/26	14	38.6	4.1 – 307.6	7.1	—
2017	5/27	10/1	14	107.4	24.3 – 1,046.2	35.7	—
2018	5/15	10/10	11	38.4	6.3 – 435.2	9.1	—
2019	4/25	8/14	9	20.6	5.2 – 166.4	0	—

(a) Samples analyzed by NID laboratory using the Idexx Quantitray method.
(b) Selected NID samples collected between April 15 to October 15 to correspond with LWW seasonal monitoring program.
(c) Compliance reference to 1986 USEPA recreational water criteria: Single sample not to exceed 235/100 mL and 30 day geometric mean not to exceed 126/100mL
(d) Not enough samples per month to compute 30 day geometric mean.

For most of the years listed in Table A2 there were no canal samples that exceeded the single sample limit. Only two raw water samples were collected each month, so there were not enough data to compute the 30 day geometric mean. The 2014, 2016 and 2018 data reflect a single sample each year that exceeded the recreational limit. In 2017 there were 6 samples that exceeded the recreational limit. If one used the 2012 EPA criteria, the 2016 exceedance would drop out, and the 2017 results would change to 4 samples over limit rather than 6. It should be noted that the 2017 cluster of high *E. coli* values in the canal occurred in the same year as the LWW outbreak. However, closer examination shows that all of the high values occurred in August through early September, well after the Lake Wildwood outbreak. It

appears highly unlikely there is any relationship between the small cluster of high indicator *E. coli* results in 2017 and the Lake Wildwood outbreak.

Using the 2012 EPA criteria to evaluate the Meadow Park Creek data (Table A3) would result in slightly lower numbers in the single sample limit column and higher values in the 30-day geometric mean column. The basic conclusion that Meadow Park Creek is chronically contaminated with *E. coli* would not change. The results summarized here would fall within the State Water Resources Board definition of an impaired water body. The data further demonstrate that the creek contamination is not caused by the irrigation water delivered to the properties in that small watershed.

Table A3. Meadow Park Creek (aka Wildflower Creek)
***E. coli* Monitoring Summary 2006 – 2017**
***E. coli* data reported as CFU or MPN per 100 mL[a]**

Year	Date Started[b] M/D	Date Ended[b] M/D	No. of Samples Tested	*E. coli* Geometric Mean	*E. coli* Range	% Exceeding 235 Single Sample Limit[c][d]	% Exceeding 126 30-day Geometric Mean[c]
2007	4/05	10/04	26	133.0	10-1,100	42.3	53.8
2008	5/08	10/02	22	578.8	10-5,760	81.8	72.7
2009	5/21	9/24	18	579.4	5-2,830	83.3	84.2
2010	5/13	9/23	19	580.1	50-1,920	84.2	84.2
2011	5/12	9/29	21	184.3	5-1,550	52.4	61.9
2012	5/10	10/4	21	740.9	5-202,000	71.4	76.2
2013	5/09	10/23	25	1,178.2	104.3-2,419.6	92.0	88.0
2014	5/07	10/08	23	461.4	26.9-2,419.6	65.2	69.6
2015	5/20	10/14	22	151.5	1-1,413.6	63.6	45.4
2016	5/11	9/28	21	519.4	34.5-2,419.6	76.2	85.7
2017	5/24	8/16	13	883.2	410.3->2,419.6	100.0	69.2
2018	4/30	9/24	23	732.0	50.4 – 2419.6	78.3	77.3
2019	5/2	11/17	46	559.8	131.4->2419.6	87.0	95.5

(a) Samples prior to 2013 tested by membrane filter m-coli blue method. Samples starting 2013 tested by Idexx Quantitray method
(b) Monitoring is seasonal, starting in the spring and ending in fall
(c) Compliance reference to 1986 USEPA recreational water criteria:
Single sample not to exceed 235/100 mL and 30 day geometric mean not to exceed 126/100 mL
(d) Creeks flowing into lake are not designated for recreation and are not retested when single sample limit exceeded.

In July 2018 CDC conducted a microbial source tracking analysis of a sample from the creek at Meadow Park Bridge. That sample was reported positive for human and ruminant DNA markers. It was negative for goose, deer and cow markers. Another sample the same day at Meadow Park Beach was positive only for goose DNA. The other markers were negative. Ruminants commonly observed in the watershed, excluding deer and cattle, could include goats

and sheep. Human sources could include septic tank contamination from properties outside of Lake Wildwood or sewer leakage from within the community near that creek. The LWW sewage system in that area was not examined following the outbreak due to the distance from Commodore Park. A more focused effort is clearly warranted and necessary to address the *E. coli* contamination in this creek.

References:

Yanko, W.A. Wood, J., Dearborn, Y. 2019. "Lake Wildwood 2018 Microbial Monitoring Program and Response to 2017 *E.coli* O157:H7 Outbreak." Lake Wildwood Association, 11255 Cottontail Way, Penn Valley, CA 95946.