

Microbial Source Tracking at the Santa Ana Watershed

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Acknowledgement

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Executive Summary

The Santa Ana Watershed experiences influxes of bacterial growth throughout the year and frequent water quality monitoring is an important public health objective for this area. The primary objective of this project is to characterize water quality issues among disadvantaged communities located within the Santa Ana River Watershed. The recent substantial developments of homeless encampments along the river raises the concern that this underrepresented population is potentially exposed to poor water quality. In addition, the increased density of encampments has unknown impacts to the water quality of this area. This study aimed to investigate the relationship between areas with high human activity and water quality using microbial source tracking (MST) through qPCR analysis of samples for host-specific indicators of human and animal interaction. Human host-specific marker, HF183, was found at all sampling sites and the results indicate the possibility of a profound association between areas containing high-concentration of human activity and poor water quality. Additional water monitoring parameters such as pH, temperature, and dissolved oxygen (DO) experienced fluctuations that were in-line with weather events and emphasized the importance of monitoring water quality in this area. While human activities were implicated as a potential source of fecal contamination in the Santa Ana River, our current methods are unable to differentiate among the diverse human-related activities occurring in the Santa Ana River such as wastewater effluent discharges, recreational uses, and/or homeless populations. Our findings indicate homeless populations may be disparately exposed to poor water quality and a comprehensive study of the Santa Ana River is needed to understand the contributions of the different human activities to microbial water quality in this region.

Project Objectives

The primary objective of this project was to characterize the water quality of the Santa Ana River Watershed as it relates to disadvantaged communities that utilize this water source, in order to describe potential public health risk. This project will use gene-specific markers of fecal contamination, microbial community profiles, land use patterns, and water quality parameters to identify major sources of pollution within a section of the Santa Ana River located in Riverside, CA. The goal of this project was to create a pollution map of sampling sites along the Santa Ana River in Riverside in order to study the relationship of water quality and areas of human activity. In order to achieve this, the first objective was to find strategic sampling sites through field surveying and utilizing the help of Inland Empire Waterkeeper (IEWK). The second objective was to gather water quality data through regular intervals of sample collection. The third objective was to analyze the samples in the laboratory using filtration, DNA extraction, and qPCR for host-specific gene markers. The final objective of the project was to compile the data to establish research findings through the use of graphs and scientific reasoning. The results will be used to expand the current understanding of water systems that are located in proximity to human activity and contribute to public health officials efforts in maintaining safe water systems in urban areas.

Project Approach

Site Selection

Our sampling locations consist of several points along the Santa Ana River between Van Buren Blvd and W Dunn Ranch Rd. This portion of the Santa Ana River is full of biodiversity and proximal to human impact sources including wastewater effluent discharges, homeless encampments, and seasonal recreational uses. Sites were chosen based on information provided

by IEWK. The map provided below includes locations related to homeless encampments, water treatment sites, IEWK testing sites, and general land use in Riverside, CA. Locations were visited and assessed prior to selection. Site #1 was located downstream of Veolia North America, a water treatment plant. This location was selected as baseline due to its distance from the homeless encampments and recreational sites (not pictured in Figure 1). Site #2 was located at Martha McLean Anza Narrows Park. Through information from IEWK, this site was chosen because there is a presence of both homeless encampments and recreational uses. Site #3 was located under the Van Buren overpass. This section of the river constitutes the greatest cluster of homeless camps. Site #4 was located behind Paradise Knolls Golf Course at the Santa Ana River Regional Park. This location was considered to be both downstream of the encampment hotspot and an area of public recreation.

Field sampling

Locations were sampled approximately once a month between the months of October 2018 and February 2019. All but one sampling event took place in the morning between 9am to 12pm. 8 sterile 1L Nalgene bottles (2 per site) were pre-labeled with site name and date. Waders were used to wade into the middle of the river for sample collection. Water was collected from below the surface without disturbing the sediment. Samples were then stored in a cooler for transfer. GPS coordinates and elevation information were also recorded at the location of sampling.

Data collection

Data were collected and recorded on various parameters using instruments and data sheets provided by IEWK. Parameters included weather conditions (precipitation, cloud presence, and wind conditions), pH, DO, water temperature, air temperature, conductivity, and

water characteristics (water flow and water clarity). Each parameter was collected twice at every site. The data were averaged and plotted in Appendix B. Data on flow and precipitation within the last 72-hr was accessed from USGS. We define a precipitation event as one that resulted in one inch of rain. Wet weather samples were obtained within a 72-hr period after the rain event (Figure 2).

Sample Processing

Water filtration method. EPA standard methods for water quality analysis were used to filter samples. Field samples were placed in a refrigerator at 4 °C for storage until ready for filtration. Work-station was sanitized and all instruments were autoclaved before use. Aseptic techniques were used to assemble the filtration apparatus and throughout sample processing. A 0.45 micron filter was used to filter the 1L volume sample. After samples were processed, the filter was aseptically transferred to sterile a metal tray where it was cut up for placement into 2-mL microcentrifuge tubes for DNA extraction processing. As there were two samples per site, each site required two filters (8 total).

DNA Extraction. DNA extractions were performed using a ZymoBIOMICS DNA Miniprep kit (Zymo Research, Irvine, CA) following the manufacturer's instructions. Briefly, filters were placed in lysis tubes with lysing solution and added to a bead beater to break down product collected on the filter. The solution was then centrifuged and the supernatant was decanted into a filter tube and centrifuged once again. Filter was discarded and buffer was added to the remaining solution. The subsequent steps involved multiple buffer washes and centrifugation to collect the final DNA product. DNA extracts were labeled with project name, sampling date, and sampling location and stored in a freezer at -18 °C. DNA concentration and

purity was measured using a Nanodrop Spectrophotometer (ThermoFisher, Waltham MA) and are reported in Appendix C.

qPCR. Quantitative polymerase chain reaction (qPCR) was utilized due to its advantage in rapid detection of markers and the ability to determine the relative concentrations of targeted gene fragments (Staley, Gordon, Schoen, & Hardwood, 2012, pg.20). DNA was amplified using PCR chemistry (Sybr green), mastermix information, primer concentration and annealing temperature. All qPCR reactions were performed on a StepOnePlus Real-Time PCR System (ThermoFisher, Waltham, MA). Primer information for the different host-specific targets are presented in Table 2. Amplification using qPCR was carried out in a 20- μ L final volume, of which 2- μ L was the DNA sample. Each sample was tested in triplicates, along with a standard curve. MST primer assays for canine, equine, and swine were tested for presence/absence (See Appendix F).

Sequencing. Samples were sent out to Zymo Research (Irvine, CA) for next generation sequencing of the V3-V4 hypervariable region of the 16S rRNA gene. The results will offer a comprehensive microbial community signature for each location. The microbial signature will offer insight as to which bacteria are associated with certain contamination sources (homeless encampments, agricultural uses, and recreational uses among others).

Analytical techniques. Flow data were retrieved from the National Water Information System of the U.S. Geological Survey (USGS) for Site#11066460 (U.S. Geological Survey, 2019). This gage is located between Site 2 and Site 3 at 33°58'07" and 117°26'51". Flow rates were averaged over a 24h period. Chemical water quality parameters were measured using instruments provided by IEWK. Measurements were taken at the site of sample collection. Each parameter was measured twice. Instruments were placed in water for about a minute in order for

it to be acclimated to the water. Physical parameters were based on the data recorder's observation. The data sheet provided by IEWK listed options for precipitation (none, misty, rain, heavy rain), wind (none, breezy, windy, blustery), water flow (stagnant [no flow], trickle [<1 quart/second], moderate [<5 gallon/second], high [>5 gallon/second]), water clarity (clear, cloudy, murky, other), and sky conditions (no clouds, partly cloudy, heavy clouds, overcast).

Project Outcomes

Flow Data

Figure 2 shows the flow data retrieved from the USGS for Site #11066460 (U.S. Geological Survey, 2019). Sampling began during a dry period in the middle of the fall 2018 season on 10/27/2018. The two wet weather sampling dates took place on 11/30/2018 and 12/7/2018, within 72 hours of the rain event. Sampling on 1/11/2019 and 2/1/2019 took place in between rain events. The fall 2018 and winter 2019 season brought various and distributed rain events that allowed us to collect a variety of water samples. During this time last year (Fall 2017 and Winter 2018), there was only one rain event (occurring January 2018) of similar magnitude to the ones observed during our sampling period (Figure 3).

pH, Conductivity, and DO

Observance of pH during sample collections from the end of October to the beginning of February showed a slightly alkaline trend of approximately 8.5, which is shown on Figure 4. One exception is the last sample from Site 4, measuring slightly acidic at 6.25. Figure 5 showed that DO concentration relatively remained steady over time. However, a period of low dissolved oxygen was observed after a wet-weather sampling event occurring on 11/30/2018. Areas of known human activity at Site 2, 3, and 4 resulted in lower levels of dissolved oxygen compared with Site 1. Site 2 ranked the lowest in dissolved oxygen level compared to other sites. The

conductivity at all sites increased in the month of November, coinciding with wet-weather events recorded on Figure 6. Site 2, 3, and 4 had higher levels of conductivity than Site 1. Overall, Site 2 experienced high levels of conductivity compared to Site 1, 3, and 4. Water and air temperature recorded were comparable throughout the sampling time frame with the November and December sampling months experiencing the lowest temperatures drop at approximately 13 °C (Figure 7). Site 2 experienced lower air and water temperatures while Site 3 and 4 experienced higher water and air temperature recordings.

Presence/absence and abundance of host-specific genes

Targeted gene assays were tested to confirm the identity of PCR fragments and to generate appropriate positive controls for use in qPCR assays. Following the manufacturer's instructions, gel electrophoresis method (E-Gel Power Snap, ThermoFisher, Waltham, MA) was utilized to visualize positive gene fragments from PCR reactions on environmental water samples. To separate DNA, the PCR product was run on a 2% agarose gel and the fragment size of each positive sample was approximated using a 50 bp DNA ladder. Bands were visualized under blue light and positives were confirmed based on size (Table 2). With the exception of our October 2018 baseline sampling location, the results indicated that human-associated HF183 gene marker tested positive across all sampling sites (See Appendix D).

Presence/absence testing was conducted for canine, equine, and swine targets (Appendix F). Canine primer assays tested positive for all sites except for various sampling dates at Site 1. Equine primer assays tested positive for all sampling sites in December but mostly negative on other sampling days. The exception was on 11/30/2018 when equine tested positive at Site 3 and on 01/11/2019 at Site 4. The swine primer assays tested negative at all sampling sites.

A positive qPCR result means that the targeted region of the 16S rRNA amplified during the reaction, indicating that the host-specific targeted gene exists within the sample. The results from qPCR shows that human-associated HF183 genes were found at all locations (See Appendix D). During the first sampling date in October, HF183 concentration was found to be higher at Site 2 and Site 4. On subsequent sampling dates, HF183 was detected at all sites. Collectively, HF183 concentrations were consistently elevated at Sites 2, 3, and 4. Site 2 and Site 4 consistently had higher concentrations of HF183 than Site 1 and 3. In addition, concentrations of HF183 was high after wet weather events when compared with the dry sample in October.

Total Bacteria

The results from qPCR analysis showed an elevated total bacteria concentration ($6.65\text{E}+07$ copies per 100mL) on 10/26/2018 at Site 1 when compared to Sites 2, 3, and 4 ($4.86\text{E}+07$, $4.20\text{E}+07$, and $2.82\text{E}+07$, respectively) as seen on Figure 9. However, subsequent sampling dates showed higher total bacteria concentration levels at Sites 2, 3, and 4 when compared to Site 1 (Figure 9). This suggests that human activity related to these specific locations has an impact on the microbial water quality of this river. Total bacteria concentrations were highest at all sites on 11/30/2018 and 12/7/2018. This is also when DO was the lowest. The total bacteria results on these dates suggest there was a high biological oxygen demand (BOD), which can affect water quality. Total bacteria concentrations were also high on 2/1/2019, about two weeks after a rain event. Further research should assess the amount of HF183 that contributed to this total bacteria count.

HF183 Abundance

The human target (HF183) was found at all sites throughout the sampling period, with the exception of Site 1 on 10/26/2019. The absence of HF183 at this site supported the use of Site 1

as the baseline. Even though HF183 can persist after water treatment, the HF183 concentration was the lowest at Site 1 when compared to the other sites across the sampling period (Figure 9). This is in line with the total bacteria results, suggesting that the human activity that occurs downstream of this site is impacting water quality in the Santa Ana River. The concentration on the first sampling date was lower at all sites when compared to the concentration found on 11/30/2018 and 12/7/2018. This is likely due to the rain events that took place on the latter dates, which could be explained by resuspension of microorganisms from the sediment-water interface deposited along the river bed. The HF183 concentrations were similar to that of the 10/26/2018 event at all sites (except Site 4) on 1/11/2019. It had not rained between the 12/7/2018 and 1/11/2019 sampling dates. Site 4 had the highest concentration on 1/11/2019. This may be because this site experienced the most alteration of its riparian zone.

Conclusions

Along the Santa Ana River, a rapidly increasing disenfranchised homeless population raises concerns for the negative impacts polluted water systems could have on human health. According to researchers, wet weather patterns have a high correlation with microbial growth in surface water (Cao et al., 2013). The results from our study corroborate the literature due to elevated total bacteria counts and elevated HF183 concentrations, which is linked to waterborne illnesses and pose a serious risk to public health (Harwood, Staley, Badgley, Borges, & Korajkic, 2014). Testing locations experienced higher wet weather days than expected and are useful for comparison-analysis with dry samples. During the same time last year, the region had not experienced high river flow rates during rain events (Figure 3). There was only one big rain event that took place in January 2017. Figure 5 showed that after the first dry sample, subsequent samples that were affected by wet weather events decreased in DO levels across all sites. This is

most apparent at the 11/30/2018 sample date, which followed the first rain event in the fall season. Low levels of DO may indicate high BOD, often associated with wet weather samples and poses a risk to aquatic animals and potential communities with access to water in this area (Bailey & Admadi, 2014). Temperatures continually dropped during our testing period, which may have curbed some microbial growth, but during the warmer months ahead, the water quality of this area could worsen. Our wet-weather samples also yielded higher levels of HF183 and total bacteria concentrations, which is alarming when factoring in the rise in homeless encampments and consequent activity in this area. This fact becomes more pressing as our results indicate that the water quality of the river may be hazardous to human health.

The results from our study indicate that there is a stronger association between HF183 markers and areas of high recreational activities and homeless populations (Sites 2 - 4) compared to areas of low activity (Site 1). Site 3, a highly impacted area of homeless encampments, consistently showed high levels of the human-specific marker, HF183. Throughout the sampling period, we observed various changes in landscape. When sampling first began, Site 2 contained a large homeless encampment along the river bank. The encampment was eventually removed by the end of the sampling period. HF183 rebounded to elevated levels at this location in February after the removal, suggesting that human activities other than the presence of these encampments may be associated with high HF183 concentrations. After the various rain events, the riparian zone changed drastically, shifting the flow of the river and flora. This was especially true at Site 2 and 4. Site 4 experienced areas of stagnant water, and soil that was once underwater became fully exposed. We had to cross over this newly exposed area to reach the flowing river for sampling on 2/1/2019 (Table 1). The disruption of sediment can contribute to the resuspension of microbial agents from previous pollution events (Grant et al., 2011). At one point, a caution sign

was posted at Site 4 warning residents and recreational users to avoid this area of the river due to poor water quality. However, a disenfranchised population like the homeless that establish encampments along the river would unknowingly be exposed to poor water quality as a result of the draining stormwaters. Concrete data has yet to be documented on how this community may interact with the water source and also how they may utilize this area of the river. However, our data indicate that there is an observable difference, which emphasizes the importance of future research that may employ a higher frequency of sample collections and testimonies of residential impacts.

The research is currently ongoing but the results could potentially deliver tremendous input for local environmental health officials in implementing beneficial plans for all communities at risk. The potential risk of surface water contamination along this area of the Santa Ana River due to human activity brings attention to the importance of maintaining the water quality in this area through diligence in water monitoring and reporting.

Working on this project has really motivated Ms. Cindy Puga to pursue opportunities that allow her to help marginalized populations, such as the homeless. There are several environmental health issues that affect such populations, and these deserve awareness and action. Ms. Puga has always had an interest in microbial water quality, and this internship allowed her to learn more about it. She is interested in not only learning more about water testing, but also learning about regulations relating to water quality.

The opportunity to work on a project that is so important not only for the immediate Santa Ana watershed communities, but for all areas impacted by high population growth and water quality concerns, has been a highly rewarding experience for Ms. Loanne Nguyen. This unique learning experience has helped Ms. Nguyen bridge her background in safety and

environmental engineering. It has also given her insight into microbial source tracking and provided an edge in knowledge that would diversify her professional portfolio for years to come. Ms. Nguyen is passionate about protecting the safety of the public and working with the Santa Ana watershed has been a humbling experience. This work has nurtured her dedication to providing service that would protect vulnerable people of the community. She is immensely appreciative of this opportunity and hopes to continue expanding her knowledge in water research.

Appendix A

Homeless Encampments Santa Ana River

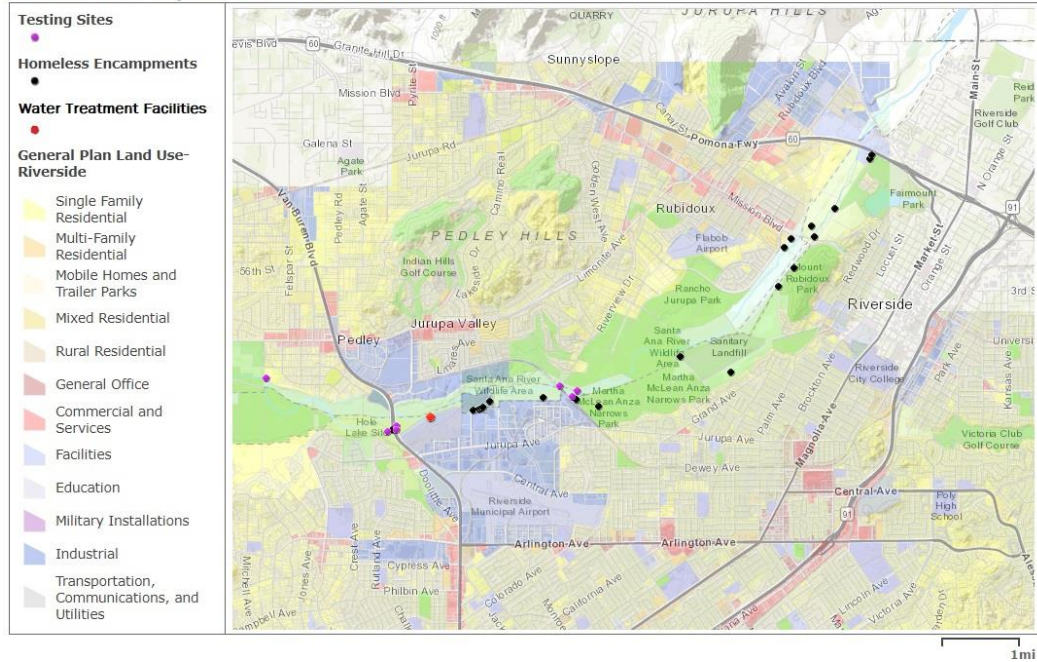


Figure 1. Previous testing sites and homeless encampments maps provided by IEWK.

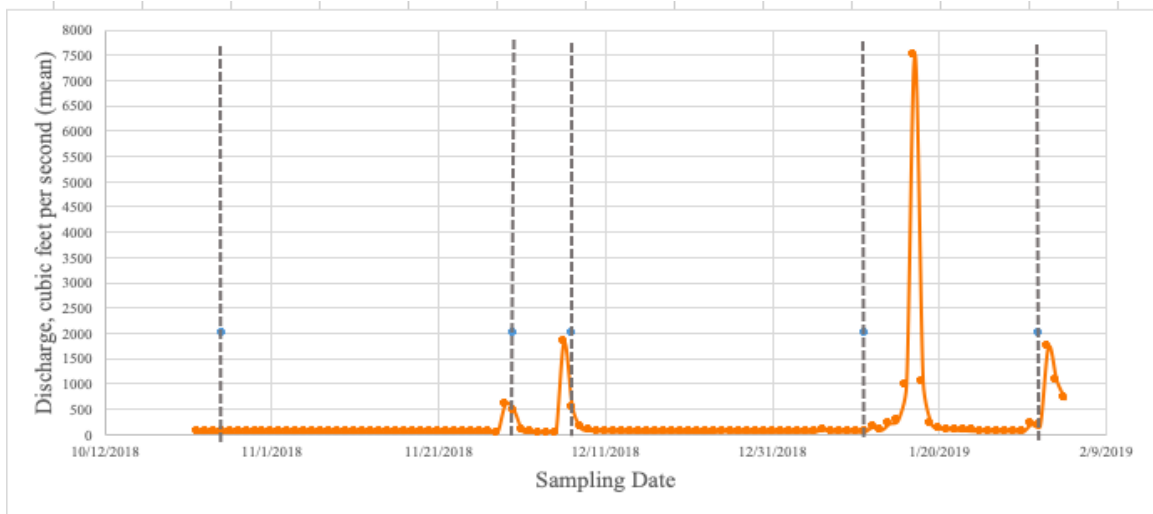
Table 1. Study locations with GPS coordinates.

Site Number	Location	Latitude	Longitude
1	Veolia WTP	34°02.887'	117°21.392'
2	McLean Park	33°58.099'	117°26.104'
3	Van Buren Bridge	33°57.797'	117°27.905'
4	Paradise Knolls GC	33°58.204'	117°29.296'
	Paradise Knolls GC	33°58.181' ³	117°29.272' ³

1. WTP: water treatment plant

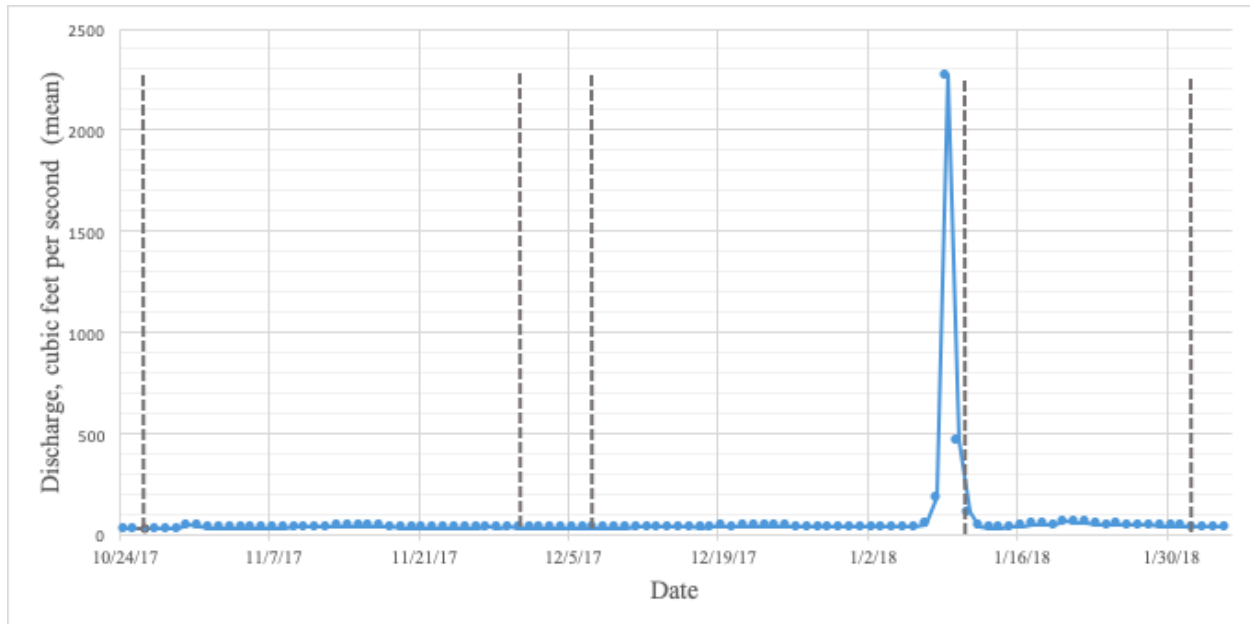
2. GC: golf course

3. Sample was taken at a different location within the Paradise Knolls GC vicinity on 2/1/2019



Note: Dotted lines represent sampling dates.

Figure 2. USGS discharge data from USGS Site 11066460 SANTA ANA R A MWD CROSSING CA for October 2018 to February 2019.



Note: Dotted lines represent sampling dates in 2017 and 2018.

Figure 3. USGS discharge data from USGS Site 11066460 SANTA ANA R A MWD CROSSING CA for October 2017 to February 2018.

Table 2. Target primers utilized in qPCR analysis.**Primers**

Assay Name	Source	Frag; Anneal T	Target/Gene	Primer Sequence (5'-3')	Primer Name	Citation
UniBac	Total Bacteria/Archaea	180bp; 61.5°C 180bp; 61.5°C	16S rRNA	AAACTCAAAGGAA TTGACGG	926F	(De Gregoris et al 2011)
				CTCACRRACGAG CTGAC	1062R	
HoF597	Equine	354bp; 58°C	16S rRNA	CCAGCCGTAAA ATAGTCGG	HoF597F	(Dick et al., 2005)
		354bp; 62°C		CACATG TTCCTC CGCTCGTA	Bac708 R	
Bac3	Bovine	NA; 62°C	16S rRNA	CTAATGGAAAATG GATGGTATCT	Bac3F	(Shanks et al., 2010)
				GCCGCCAGCTCA AATAG	Bac3R	
PF163F/ Bac708R	Swine	NA; 53°C	16s rRNA	GCGGATTAATAC CGTATGA	PF163F	(Dick et al., 2005)
				CAATCGGAGTTC TTCGTG	Bac708R	(Bernhard and Field, 2000)
HF183/BacR28	Human	N/A	16s rRNA	ATCATGAGTTCAC ATGTCCG	HF183	(Green et al., 2014)
				CTTCCTCTCAGAAC CCCTATCC	BacR28	
DG3	Canine	N/A	GTF2I	TTTTCAGCCCCTTG TTTCG	Dg3-fw	(Green et al., 2014)
			GTFI	GACCATGAACACC ATCAAGTGAA	Dg3-rv	

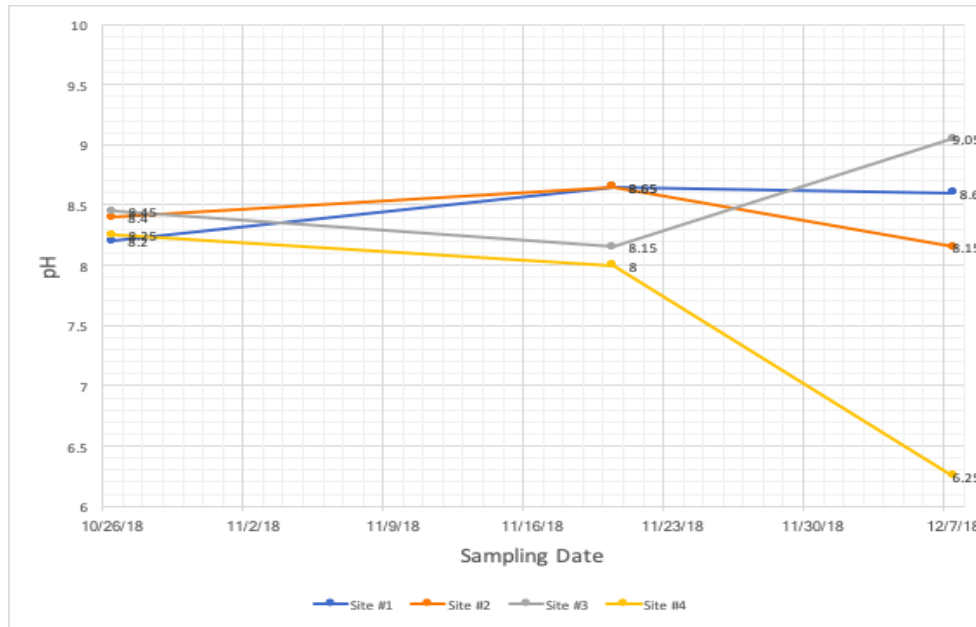
Appendix B

Figure 4. pH Levels at 4 Sampling Locations Along the Santa Ana River.

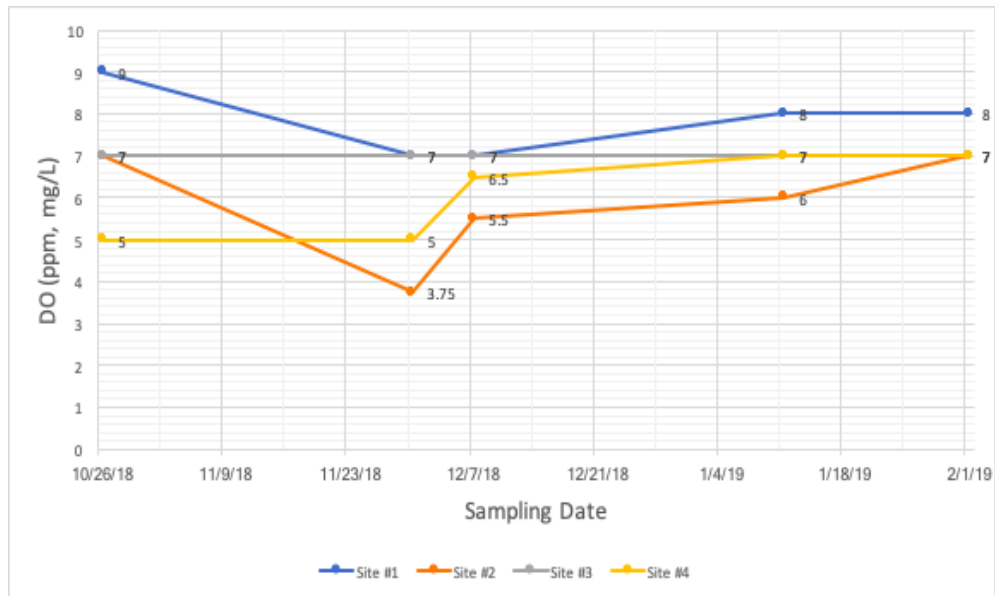


Figure 5. Dissolved Oxygen (DO) at 4 Sampling Locations Along the Santa Ana River.

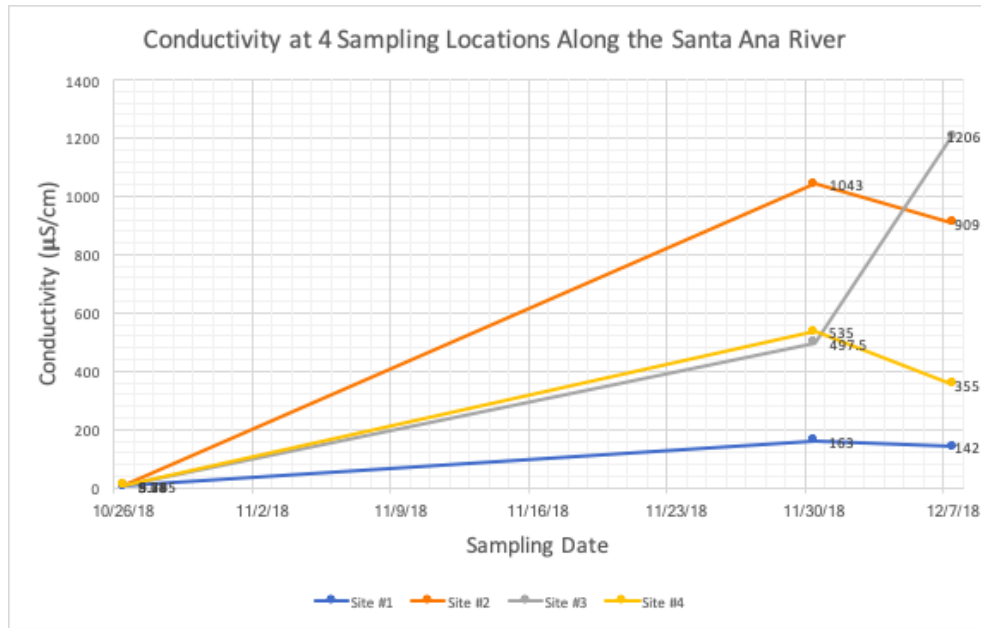


Figure 6. Conductivity at 4 Sampling Locations Along the Santa Ana River.

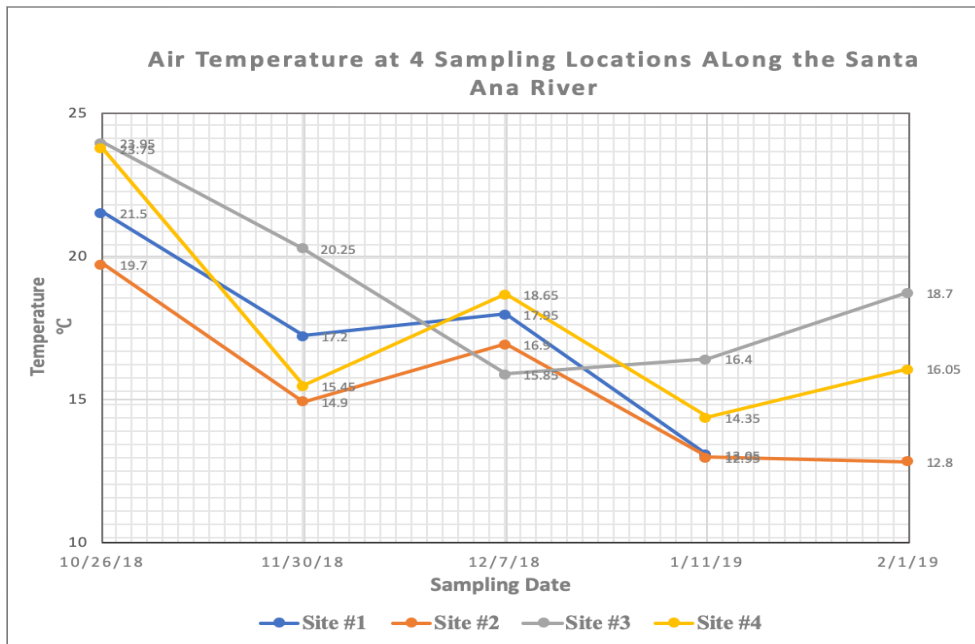
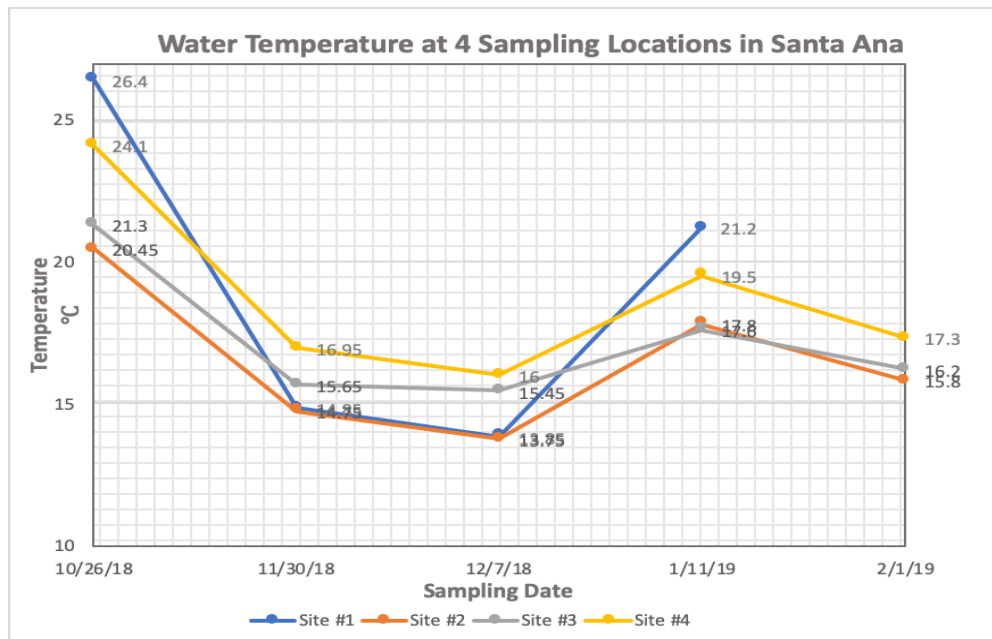


Figure 7. Air Temperature at 4 Sampling Locations Along the Santa Ana River.



Figures 8. Water Temperature at 4 Sampling Locations Along the Santa Ana River

Appendix C

Table 3. DNA Concentration from Nanodrop results in ng/uL.

Nanodrop DNA Concentration (ng/uL)									
Sample	Site #1	S.D	Site #2	S.D2	Site #3	S.D3	Site #4	S.D4	
10/26/18 Sample 1	7.4	2.4	5.3	0.3	12.9	3.5	16.6	10.9	
10/26/18 Sample 2			9	0.3	10.1	8.0	6.0	1.0	
11/30/18 Sample 1	11.6	3.0	15.4	0.8	35.8	0.4	98.6	5.2	
11/30/18 Sample 2	6.4	0.3	19.3	0.2	213.2	72.4	27.5	0.5	
12/7/19 Sample 1	12.7	0.5	24	4.5	26.1	0.5	17.6	2.6	
12/7/19 Sample 2	6.6	0.5	18.5	4.2	27.8	1.0	26.8	0.6	
1/11/19 Sample 1	8.6	0.8	5.3	0.7	7.6	0.5	7.9	0.5	
1/11/19 Sample 2	4.6	0.2	10.5	2.2	2.3	0.6	16.7	6.1	
2/1/19 Sample 1	4.3	0	22.5	3.7	29	0.8	31.4	2.2	
2/1/19 Sample 2	5.1	0.3	34.4	3.6	9.5	0.2	15.8	1.5	

Appendix D

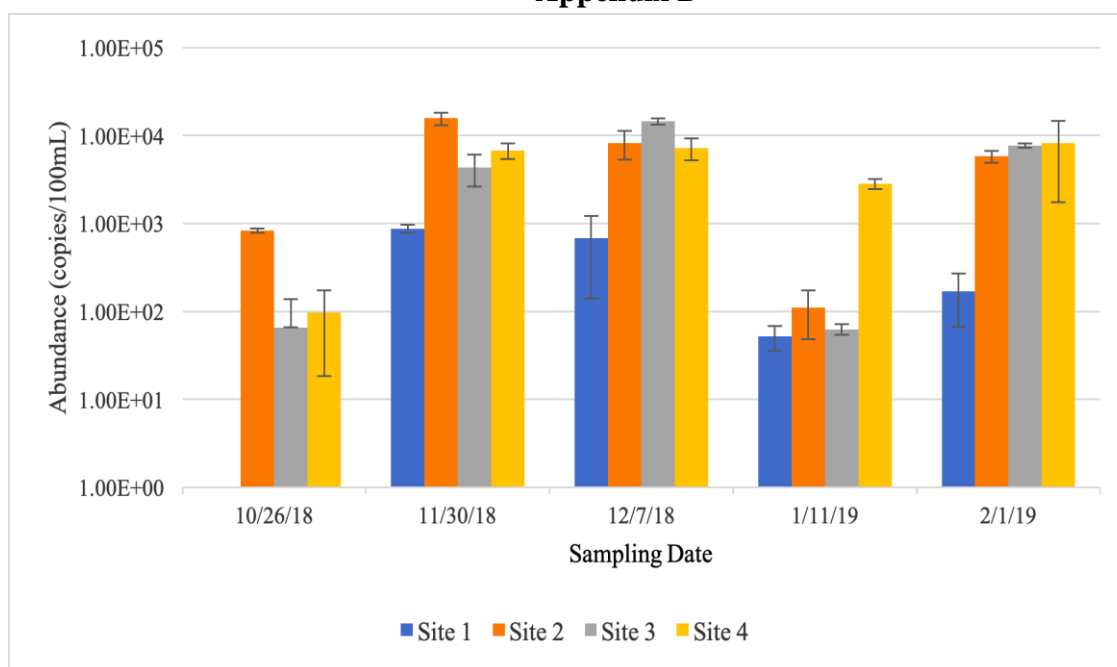


Figure 9. HF183 concentrations from October 2018 through February 2019 at each of the sampling locations.

Appendix E

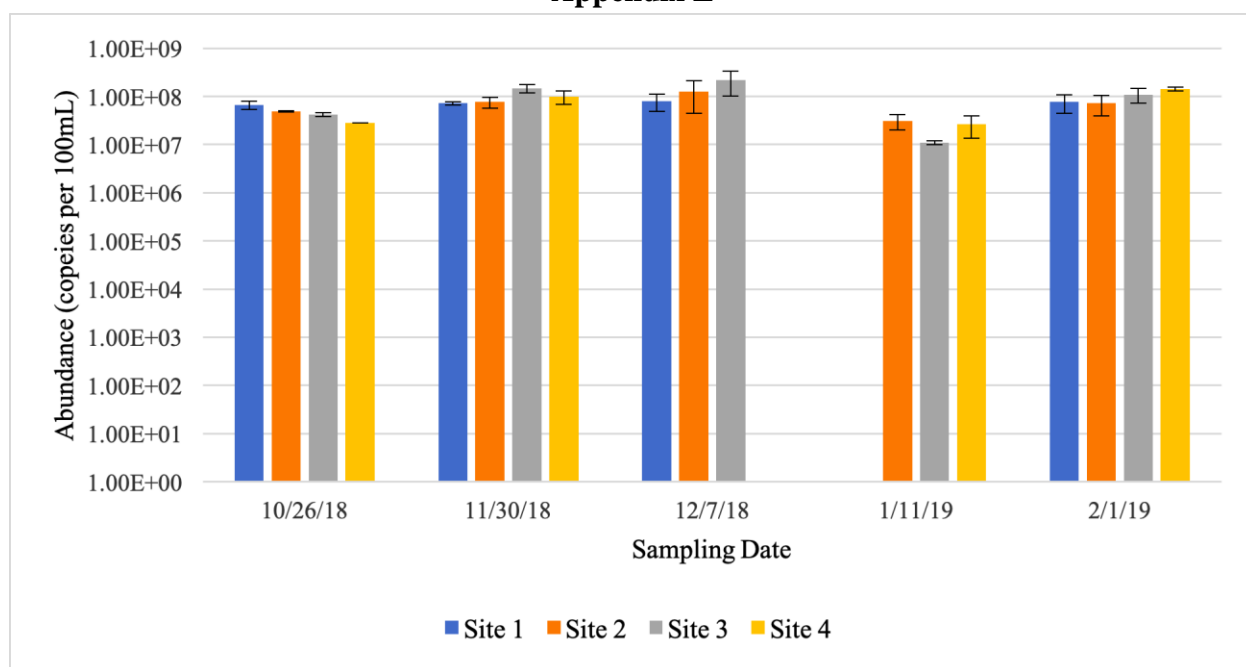


Figure 10. Total bacteria concentrations from October 2018 through February 2019 at each of the sampling locations.

Appendix F

Table 4. Presence/Absence Results for Canine, Equine, and Swine primer target.

Date	Dog			
	Location 1	Location 2	Location 3	Location 4
10/26/18	-	+	-	-
11/30/18	+	+	+	+
12/7/18	+	+	+	+
1/11/19	-	+	+	+
2/1/19	-	+	+	+

Date	Horse			
	Location 1	Location 2	Location 3	Location 4
10/26/18	-	-	-	-
11/30/18	-	-	+	-
12/7/18	+	+	+	+
1/11/19	-	-	-	+
2/1/19	-	-	-	-

Date	Pig			
	Location 1	Location 2	Location 3	Location 4
10/26/18	-	-	-	-
11/30/18	-	-	-	-
12/7/18	-	-	-	-
1/11/19	-	-	-	-
2/1/19	-	-	-	-

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