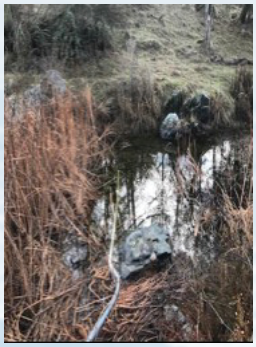
Quantification of Sources of Fecal Pollution at Mule Creek



Amy Zimmer-Faust
John Griffith
Steve Weisberg

Southern California Coastal Water Research Project

SCCWRP Technical Report #1186

Quantification of Sources of Fecal Pollution at Mule Creek

Amy Zimmer-Faust, John Griffith, Steve Weisberg

Southern California Coastal Water Research Project, Costa Mesa, CA

April 2021
Technical Report 1186

ACKNOWLEDGEMENTS

The report was prepared for the Central Valley Regional Water Quality Control Board and the California Department of Corrections and Rehabilitation.

EXECUTIVE SUMMARY

Mule Creek, located in Amador County, California has a history of fecal indicator bacteria levels in exceedance of state bacterial water quality standards. Mule Creek State Prison sits adjacent to Mule Creek and due to its proximity, the facility and surrounding grounds have the potential to impact Mule Creek's water quality.

This study had three primary objectives: 1) Determine the extent to which runoff from the prison affects microbiological water quality in Mule Creek, 2) Identify sources within the prison that may be contributing to fecal bacteria levels within the creek, and 3) Identify fecal sources upstream of the prison that may also be affecting Mule Creek water quality. To address the three objectives, samples were collected on 22 days at sites within prison grounds and in Mule Creek, at sites upstream and downstream of prison boundaries, during active rainfall, post-storm, and dry weather conditions.

Water quality in Mule Creek was affected by prison runoff, but not on all sampling days. For roughly one-third of the sampling days, *E. coli* concentrations were higher upstream than downstream of the prison facility. For another third, the concentration increase moving past the prison property was sufficient to cause a downstream water quality standard exceedance where the upstream sample was in compliance. For the remaining third, there was an increase moving downstream, but no difference in water quality compliance status between the upstream and downstream sites.

The runoff from prison grounds appears to be mostly from deer and birds, rather than from human fecal sources. Testing for genetic markers of human fecal material was conducted and human genetic marker was detected in only two out of 64 samples collected on the prison property, with these two detections both near the limit of detection. In contrast, nearly every sample contained genetic markers at high levels for birds and ruminants (e.g., cow, elk, or deer). Deer and birds were frequently observed on prison grounds, supporting these findings.

Upstream sources of fecal contamination also appear to be from birds and deer. In addition, genetic markers of cow fecal material were detected in samples upstream, whereas markers for cow were largely absent in runoff from the prison property.

TABLE OF CONTENTS

INTRODUCTION

Fecal indicator bacteria (FIB), including fecal coliform, *E. coli*, and *Enterococcus,* are the basis of current water quality objectives (USEPA 2012). However, FIB do not come exclusively from human sources. They also originate in the feces of other warm-blooded animals (e.g., cow, dog, bird). Therefore, FIB data can provide information about the presence and magnitude of water quality impairments, but these data do not provide information about the source of pollution, making effective and efficient mitigation of fecal pollution sources challenging.

To identify the sources of fecal contamination, microbial source tracking (MST) methods allow for the measurement of DNA sequences that are associated with specific fecal sources. MST-based results can be used to gauge the presence and extent of different fecal contamination sources and sensitive and specific methods have been developed targeting common sources, including human, cows, birds, and dogs (Griffith et al. 2013). These methods are widely applied, with the EPA recently promulgating an approved method for the most commonly used human marker, HF183 (USEPA 2019). The California State Water Resources Control Board has also created a manual for implementing microbial source tracking tools in state waters (Griffith et al. 2013). More recently, select quantitative polymerase chain reaction (qPCR)-based MST assays have been adapted to digital PCR. The application of digital PCR to MST has been shown to provide enhanced sensitivity and increased tolerance to inhibitory substances (Cao et al. 2015).

At Mule Creek, sources causing elevated fecal bacteria levels are unknown and of concern to the surrounding community and public health officials. Elevated fecal coliform levels, frequently in exceedance of state standards, were previously measured at different points within and surrounding the Mule Creek State Prison, which is located within close proximity to Mule Creek. However, no definite sources of fecal contamination were identified.

In this study, water samples within Mule Creek State Prison grounds and within adjacent Mule Creek were collected and processed for traditional FIB and MST genetic markers targeting ruminant (which captures signals from deer and cow), cow-only, avian, and human sources by digital PCR using previously published assays.

STUDY DESIGN AND METHODS

General Approach

The purpose of this study was to characterize the microbiological quality of the Mule Creek prison discharges and any effects the prison has on water quality in Mule Creek. The study aimed to address three main questions:

1. What is the microbial water quality of effluents from Mule Creek prison and to what extent do they influence water quality in the creek?
2. What sources of fecal sources are part of any prison bacterial contribution?
3. What other sources of fecal bacteria are contributing to creek exceedances?

Six sites were identified and sampled during three different flow conditions to address the study questions. The Mule Creek state prison facility has an onsite stormwater collection system that flows to a perimeter ditch that collects runoff and drains to two outfalls. The stormwater from these two outfalls then travels through culverts that connect to vegetated stormwater conveyance channels before being discharged to Mule Creek. Four sites within the prison boundaries were selected to capture flows from the prison. These included the two outfall locations, capturing the main discharges leaving the prison and entering the perimeter ditch (sites MCSP5 and MCSP6) and an additional two sites located at the end of the two stormwater conveyance channels, which capture flows leaving the prison before they are discharged to the creek (MCSP2 and MCSP3). In addition, two sites were sampled within Mule Creek to identify changes in water quality from upstream to downstream of the prison (MCSP1 and MCSP4) (Figure 1).

Study Question 1: What is the microbial water quality of effluents from Mule Creek State Prison and to what extent do they influence water quality in the creek?

This question was addressed by collecting water samples from within prison discharges and from Mule Creek upstream and downstream of the prison, examining two outcomes: a) Does the concentration of *E. coli* increase moving downstream, which would suggest that potential inputs from the prison are impacting Mule Creek water quality, and b) How does the microbial load from prison discharges compare to stream pollutant levels upstream and downstream of the prison.

Task 1: Compare E. coli concentrations upstream and downstream of Mule Creek State Prison.

Water samples were collected from the two creek stations (MCSP1 and MCSP4; Figure 1) and processed for *E. coli*. Samples were collected from both locations during the three flow conditions described in Table 1. Samples were collected in wet weather, both during active rainfall and when rainfall has stopped but overland stormwater runoff continues. Samples were also collected during dry weather.

Table 1. Flow Conditions targeted for sample processing

Flow Condition	Description	Sample Timing
Wet Weather: Rain	-Samples collected during active rainfall	-Target storms with predicted rainfall of > 0.3 in of precipitation in 24 hours.
Post-wet weather	-Samples collected at the culmination of rainfall	-Samples collected within 24 hours post-rain event
Dry Weather	-Samples collected during dry weather conditions	-Between April 1- Oct 1

Task 2: Compare mass loadings at four locations.

Mass *E. coli* loads were evaluated in addition to concentrations in order to compare the extent of contributions from Mule Creek upstream vs. from the prison (onsite) vs. from the Mule Creek adjacent vegetated stormwater channels, which capture flows leaving the prison.

Mass loading calculations were based on flow measurements at the upstream and downstream boundaries and the two prison outfalls (MCSP5 and MCSP6) and *E. coli* concentration data. Flow measurements were collected at four of the six sites shown in Figure 1; blue stars indicate sites with flow measurements. It was assumed that additional flow contributing between sites MCSP5 and MCSP6 and sites MCSP2 and MCSP3 is negligible; thus, flow measurements taken at Sites MCSP5 and MCSP6 were used to characterize flows leaving the prison.

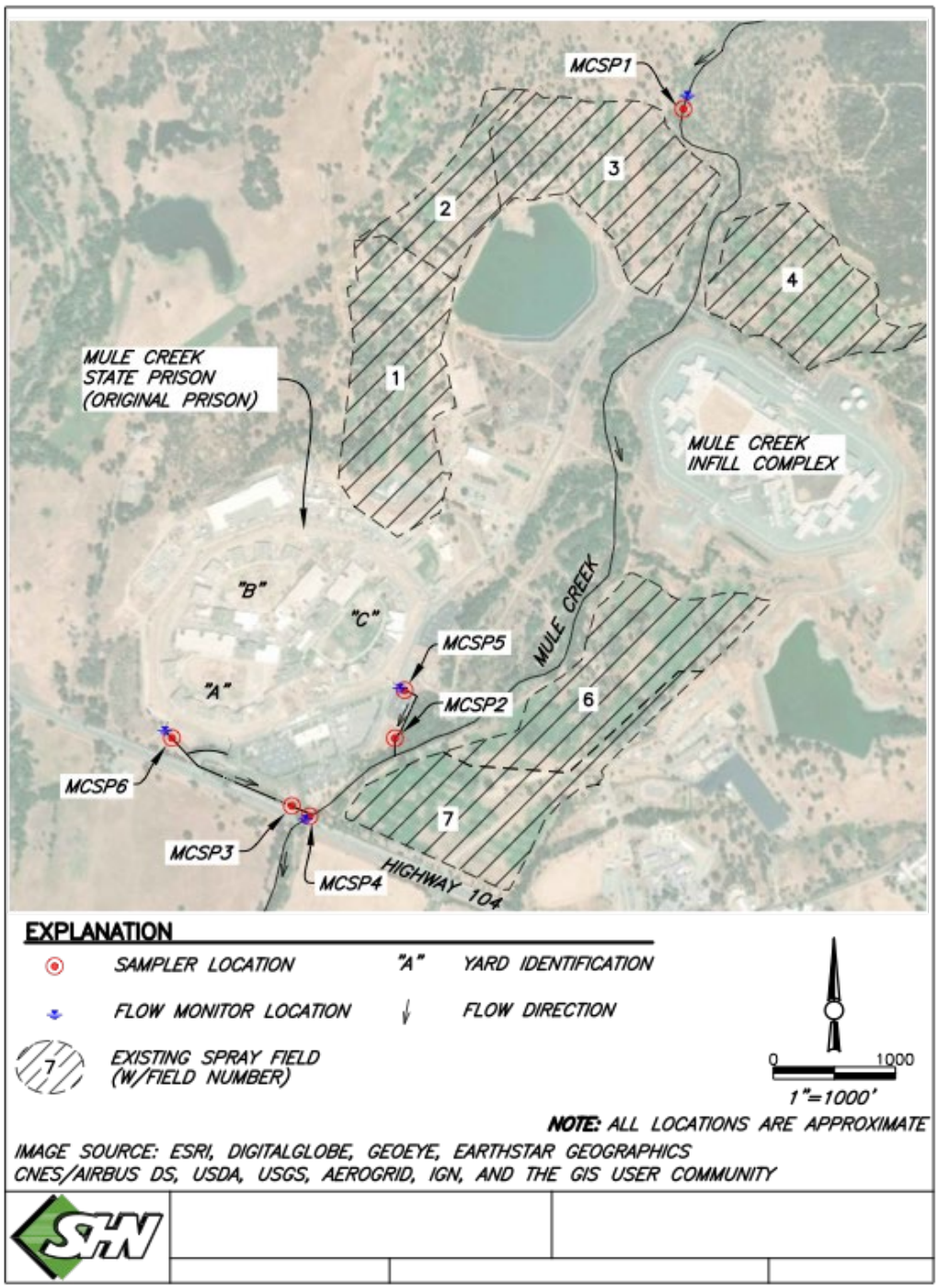


Figure 1. Map of Mule Creek. Sampling locations are denoted with red circles and locations of flow meters are denoted with blue lines. MCSP1 is located directly upstream of prison boundaries and MCSP4 is located at the downstream end of prison boundaries.

Study Question 2: What fecal sources are part of any prison contribution?

Study question 2 addresses source identification, which involved performing assays to quantify human, ruminant, and avian microbial source tracking (MST) markers at sites on prison grounds under the three flow conditions specified (Table 1). Potential sources that were evaluated include leakages from the sanitary system into the stormwater collection system and birds and deer found within MCSP grounds.

Task 1: Investigate human sources within prison grounds.

Human marker (HF183) was measured in samples collected at the two outlets from the perimeter ditch (MCSP5 and MCSP6) to characterize discharges from the prison and from sites MCSP2 and MCSP3 to capture any human sources that may be running off from the surrounding prison grounds.

Task 2: Investigate avian sources within prison grounds.

Avian marker was measured in samples at the two outlets from the perimeter ditch (MCSP5 and MCSP6) and at the two outlets from the stormwater conveyance channels (MCSP2 and MCSP3) to characterize potential avian inputs occurring within prison grounds as well as any additional inputs occurring within the stormwater conveyance channels. Previous visual inspections of the area have identified bird nesting structures within the prison and birds defecating in the peripheral ditch surrounding the prison grounds.

Task 3: Investigate ruminant sources within prison grounds.

Ruminant marker was measured in samples at the two outlets from the perimeter ditch (MCSP5 and MCSP6) and at the two outlets from the stormwater conveyance channels (MCSP2 and MCSP3) to characterize potential ruminant (including deer) inputs occurring within prison grounds as well as any additional inputs occurring within the stormwater conveyance channels. Deer have been previously observed within prison grounds.

Study Question 3: What other fecal sources are contributing to creek exceedances of microbial water quality criteria?

The third study question addressed source identification of upstream fecal sources. Although the compliance point for the Mule Creek State Prison is at the point of discharge from the prison facility, understanding upstream sources is useful information for determining prison facility contributions and for prioritizing other sources, to the extent they are present, moving forward.

Potential upstream sources that were investigated include cattle, deer, septic systems, and birds. Previous inspections of Mule Creek have identified properties on septic systems and properties with cattle upstream of the prison facility. In addition, the prison applies treated wastewater effluent to fields adjacent to Mule Creek and upstream, in close proximity, of prison grounds.

Task 1: Investigate human sources to Mule Creek, upstream of prison grounds.

Upstream and downstream Mule Creek water samples (MCSP1 and MCSP4) were analyzed for human marker (HF183) to characterize potential inputs from septic systems located upstream of the prison and to allow comparison between potential upstream and prison inputs.

Task 2: Investigate avian sources to Mule Creek, upstream of prison grounds.

Water samples were collected upstream and downstream of Mule Creek (MCSP1 and MCSP4) and analyzed for avian marker. These samples were used to characterize potential inputs from avian sources upstream of the prison grounds and to allow comparison between potential upstream and prison inputs of avian fecal contamination.

Task 3: Investigate cattle sources to Mule Creek, upstream of prison grounds.

Water samples were collected upstream and downstream of the prison (MCSP1 and MCSP4) and analyzed for markers targeting ruminants (cow, deer, or elk) and cow-only. These samples were intended to identify potential inputs from cow and other ruminants located upstream of prison grounds. In order to evaluate if concentrations increase or decrease along a downstream gradient, upstream and downstream cow and ruminant marker levels were also compared.

Detailed Methods

Sampling Methods

Time-spaced composite samples were collected autonomously using ISCO 6712 samplers and sterilized Teflon tubing mounted to the channel bottom for water quality analyses. However, there were instances when the auto-sampler did not trigger, and in these cases a grab sample was substituted. Statistical analyses (ANOVA) were conducted comparing grab and composite samples combined versus only composite samples and grab versus composite samples. Both comparisons were completed for each target (*E. coli* and the MST markers) and there were no statistical differences (all P-values > 0.2) for any site or flow condition. Thus, for completeness, in this report all samples collected, including when a grab sample was substituted, have been included.

Channel velocity measurements were taken using a Global Water Flow Probe within a surveyed cross-section at the flow locations indicated (Figure 1). Discharge was then estimated using continuous stage data via a stage-discharge rating curve.

Appropriate pre-labeled and sterilized sample containers were used for all sample collection. Following sample collection, sample bottles were stored immediately on ice and transported to the lab, protected from light and under chain of custody documentation, for sample processing.

Filtration and fecal indicator bacteria measurement methods were performed by subcontractors, Alpha laboratories, in collaboration with SCCWRP. DNA extraction, digital PCR, and QC/QA checks were performed by SCCWRP.

Laboratory Analysis Methods

FIB Cultivation (E. coli)

Samples were analyzed for *E. coli* using the chromogenic substrate method (Colilert Quantitray 2000™ system [IDEXX, Westbrook, ME]), as per the manufacturer's instructions. Initially, two dilutions covering a 10,000-fold range of concentration were applied and used for range-finding. As sample concentrations were better characterized, the number of dilutions was reduced. All *E. coli* results are calculated and reported in MPN per 100 mL.

Filtration, Extraction, and Sample Processing for MST Markers

Filtration was performed following the California Source Tracking Manual (Griffith et al. 2013).

Briefly, 50-100 mL of water was filtered in triplicate on a vacuum manifold through 47 mm diameter, 0.4 µm polycarbonate filters (Millipore Type HTTP, Millipore, Bedford, MA) to capture bacterial DNA. Each filter was placed in an individual 2-mL polypropylene screw cap tube, containing ZR BashingBead lysis matrix high density beads and 1-mL DNA/RNA Shield solution (Zymo Research, Costa Mesa, CA). Filters were stored at 4°C in DNA/RNA Shield solution (Zymo Research, Irvine, CA) to preserve nucleic acids during sample transport, and shipped to SCCWRP for sample analysis. The limit of detection for the digital PCR assays was approximately 45 copies/100 mL, which is equivalent to 3 positive droplets above the baseline threshold.

Bacterial DNA was extracted using the Zymo Microbiomics DNA Miniprep commercially available DNA purification kit (Zymo Research Corp, Irvine, CA). MST assays utilized targeted human (HF183), avian (GFD), and cattle fecal sources (Rum2Bac and CowM3). All MST assays have been previously published and were included in the California Microbial Source Identification Manual (Griffith et al. 2013), apart from the GFD avian marker. However, the GFD marker has been published (Green et al. 2011) and validated previously in freshwaters (Ahmed et al. 2016).

2019 Pilot Mule Creek Sampling

The sampling described in this report was preceded by a pilot effort conducted between March and July 2019. The sampling approach was the same as described above, but as flow meters and autosamplers were not yet installed, those data are segregated into Appendix B.

RESULTS AND DISCUSSION

Summary of samples collected and Data QA/QC

Samples were collected during 7 active rainfall events, 7 post-storm events, and 8 dry weather events (Table 2). A limited number of samples were collected from the sites located at the ends of the stormwater conveyance channels during dry weather (MCSP2 and MCSP3) due to a lack of flow. All collected samples were processed successfully for *E. coli* and corresponding microbial source tracking markers (Table 3).

Controls were included as follows. A filter blank that consisted of sterile PBS was filtered alongside each set of samples. An extraction blank was included in each set of samples extracted and was subjected to all steps in the extraction protocol. For each 96-well plate run, a minimum of one extraction and one filter blank, per batch, were processed along with six NTC reactions. DNA from a halophilic archaeon, *Natronomonas pharaonic* (ATCC 35678/DSM 2160), was added to the lysis buffer prior to extraction as an external extraction and inhibition control.

There was no evidence of inhibition in the samples tested and all laboratory and sample processing negative controls were negative for genetic markers. Appropriate positive controls were included as well on each 96-well plate, which were positive for the corresponding genetic marker. In addition, a sample collected from the onsite wastewater treatment plant at Mule Creek State Prison was analyzed for the HF183 human marker. A concentration of 1.8 e7 per 100 mL was detected, similar to the concentration detected from any typical sewage treatment plant, meaning that HF183 is an appropriate indicator for the Mule Creek State Prison population.

Table 2. Number of samples collected and processed for MST markers and *E. coli* between December 2019-June 2020.

Site	Site ID	Rain (n=7)	Post-Rain (n=7)	Dry Weather (n=8)	Overall
Upstream Boundary	MCSP1	7	7	8	**22**
Downstream Boundary	MCSP4	7	7	7	**21**
Secondary Outfall (SO)	MCSP5	6	5	7	**18**
Main Outfall (MO)	MCSP6	7	6	8	**21**
Swale (Downstream SO)	MCSP2	6	6	0	**12**
Swale (Downstream MO)	MCSP3	5	6	2	**13**

Table 3. Quality control report

Site	*E. coli*		Microbial Source Tracking Markers	
	Sampling success	Processing success	Sampling success	Processing success
MCSP1	100%	100%	100%	100%
MCSP4	100%	100%	100%	100%
MCSP5	100%	100%	100%	100%
MCSP6	100%	100%	100%	100%
MCSP2	100%	100%	100%	100%
MCSP3	100%	100%	100%	100%

Microbiological Results:

Study Question 1: What is the microbial water quality of effluents from Mule Creek State Prison and to what extent do they influence water quality in the creek?

Task 1: Compare E. coli concentrations upstream and downstream of Mule Creek State Prison.

E. coli concentrations (both arithmetic and geometric means) were compared upstream (MCSP1) and downstream (MCSP4) of the prison boundary for the three flow conditions, rain, post-rain, and dry weather.

Increasing *E. coli* concentrations were observed moving downstream past the prison both during dry weather and rain events. However, no statistically significant upstream to downstream differences were present for any of the three flow conditions (Tables 4 and 5). During post-storm events, *E. coli* concentrations were similar upstream to downstream when both average and geometric means were compared. *E. coli* results for each site and event are further illustrated in the Supplemental Material (Figures A1 and A2).

Water quality results were also compared to relevant water quality standards for *E. coli*: the geomean threshold of 100 MPN per 100 mL and the statistical threshold value of 320 MPN per 100 mL, which was used in place of a single sample threshold. Geometric means were above the 100 MPN threshold at both upstream and downstream locations during wet weather, both during rain and post-storm conditions. In dry weather, the upstream site (MCSP1) was not in exceedance, while the downstream site (MCSP4) was in exceedance of the 100 MPN threshold (Table 5). When single sampling events were compared to the bacterial objective of 320 MPN per 100 mL, more frequent exceedances occurred during wet weather at both the upstream and downstream site. During dry weather, the two exceedance events at the downstream site (MCSP4) were the last two events of the season where samples were collected under low to no-flow conditions (Table 6).

Table 4. Arithmetic mean *E. coli* concentrations at the upstream (MCSP1) and downstream (MCSP4) creek sites for each flow condition.

Site	E. coli Mean (+/- SD) [MPN per 100 mL]			
	Rain (n=7)	Post-Rain (n=7)	Dry Weather (n=8)	Overall (n=22)
Upstream boundary	1790 (2438)	1969 (3296)	151 (179)	1251 (2354)
Downstream boundary	3558 (3573)	2466 (3769)	676 (865)	2233 (3130)

Table 5. Geometric mean *E. coli* concentrations at the upstream (MCSP1) and downstream (MCSP4) creek sites for each flow condition.

Site	*E. coli* Geometric mean [MPN per 100 mL]			
	Rain (n=7)	Post-Rain (n=7)	Dry Weather (n=8)	Overall (n=22)
Upstream boundary	294	337	94	203
Downstream boundary	1977	587	323	721

Table 6. Number of events where *E. coli* > 320 MPN/100 mL bacterial objective

Site	Rain (n)	Post-Rain (n)	Dry Weather (n)	Overall (%)
Upstream boundary	3	4	1	37%
Downstream boundary	6	4	2	57%

Task 2: Compare mass loadings at four locations.

Total *E. coli* load per sampling event was compared between the upstream, downstream, and prison discharge locations. Calculations were based on flow measurements at the upstream and downstream boundaries and the two prison outfalls (MCSP5 and MCSP6) and *E. coli* concentrations. Ideally, composite samples were collected over a 6-hour time period; however, samples were sometimes taken over a shorter time period (less than 6 hours) or a grab sample was substituted due to a technical issue with the auto-sampler. For these events, a consistent time period was applied for comparison of equivalent load between the six sites. For all events, calibrated flow data was collected from each site and paired with the event time to calculate a total volume captured per site and event.

Trends in flow rates between events were analyzed. In general, total flow decreased moving downstream past the prison, likely due to infiltration within this stream reach. The exception to this was the two largest rain events, which occurred on 3/16/2020 and 4/6/2020. Average flow rates for each event at the upstream location (MCSP1) are presented in Table 7.

Trends in *E. coli* load per event were similar to trends observed for concentration. Total loads increased moving downstream during both rain and dry weather conditions and, in contrast, were similar upstream to downstream during post-storm conditions (Table 8).

The magnitude of contributions from the prison grounds to creek *E. coli* levels was also evaluated by comparing load per event at the upstream and downstream locations versus *E. coli* load from the prison discharges. During dry weather conditions, prison loads were evaluated based on concentrations solely from the perimeter ditch, since samples were rarely collected in the stormwater conveyance channels due to lack of flow. Overall, the prison outfalls contributed to the downstream *E. coli* load, particularly during rain conditions where the biggest upstream to downstream differences were observed. However, downstream *E. coli* loads were, in large part, a function of *E. coli* contributed from upstream of the prison (Table 8). There may also be additional inputs, particularly during active rainfall, that weren't captured in this study. The upstream to downstream differences observed weren't always accounted for by the load introduced by solely the prison sites.

Table 7. Average flow rate (cubic feet per sec) for each event sampled measured at the upstream site (MCSP1).

Condition	Date	Average flow (cfs)
Rain	12/23/19	6.48
	1/16/20	6.40
	3/16/20	30.34
	3/25/20	9.38
	4/6/20	44.21
	5/12/20	6.60
	5/18/20	6.88
Post-Storm	1/17/20	6.69
	1/27/20	7.10
	3/17/20	26.00
	4/7/20	24.88
	5/13/20	6.55
	5/19/20	6.74
Dry	4/20/20	7.85
	4/23/20	7.39
	4/27/20	6.91
	4/30/20	6.90
	5/5/20	6.50
	5/28/20	6.86
	6/4/20	6.78
	6/10/20	6.60

Table 8. Mean *E. coli* loads in MPN per event for each site and condition.

Site	Site ID	*E. coli* Mean [MPN per Event]		
		Rain	Post-Storm	Dry
Upstream Boundary	MCSP1	2.63E+11	1.49E+11	5.61E+09
Downstream Boundary	MCSP4	1.02E+12	1.34E+11	8.02E+09
Secondary Outfall	MCSP5	1.44E+09	2.21E+08	1.40E+07
Main Outfall	MCSP6	1.23E+10	1.28E+09	8.14E+08
Swale (Downstream SO)	MCSP2	1.27E+09	2.01E+08	
Swale (Downstream MO)	MCSP3	1.71E+10	6.65E+08	

Study Question 2: What fecal sources are part of any prison contribution?

Task 1: Investigate human sources within prison grounds.

Fecal sources within the prison grounds were evaluated. First, human sources were investigated at the four sites sampled within prison grounds. Overall, human marker (HF183) was detected in a total of two samples, both detections occurring during rain events (Figure 2). Concentrations were near the limit of detection and well below 500 copies per 100 mL (Table 9). While the State Water Board has not yet defined a regulatory threshold for HF183, 500 copies per 100 mL is presently being used by the San Diego Regional Water Quality Control Board for deciding when follow-up investigation of human fecal sources is warranted. The threshold is based on QMRA modeling results estimating human health risk associated with HF183 concentrations in surface waters (Boehm et al. 2020).

The two human marker detections did not display a spatial or temporal pattern and do not appear to be connected to specific prison operational parameters. Taken together, these results make it unlikely that there is a consistent human source, such as leaky infrastructure, coming from the prison. The two low-level human marker detections at this site may be a result of wastewater running off and entering the creek, where it is subject to dilution and decay, or possibly a result of cross reactivity. HF183 human marker has been previously reported to cross react with deer (for examples, see Layton et al. 2013; Nguyen et al. 2018).

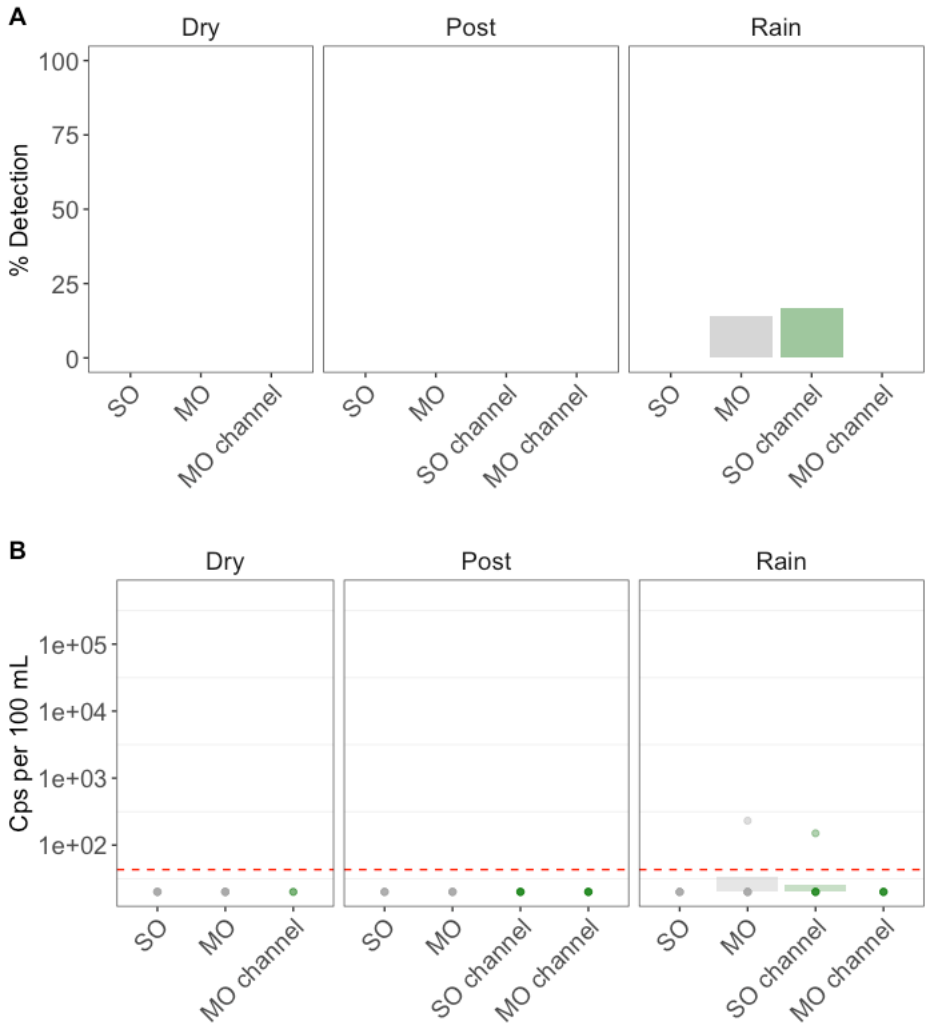


Figure 2. Human marker (HF183) results at the four sites within prison grounds for the three flow conditions. MO is the main outfall (MCSP6). SO is the secondary outfall (MCSP5). SO channel is the site at the end of the SO SW conveyance channel (MCSP2) and MO channel is the site at the end of the MO SW conveyance channel (MCSP3). A) Human marker detection frequency. B) Human marker average concentrations (bars) and individual measurements (dots) taken at each sampling date. The red dashed line represents the limit of detection.

Task 2: Investigate avian sources within prison grounds.

Birds, which have been observed nesting in and around the prison, were also investigated as a potential source within prison grounds. Bird marker (GFD) was detected frequently, between 20 and 100% of the time at the four prison sites overall. Increasing concentrations and frequency of detection were observed at the two sites at the end of the stormwater conveyance channels (MO channel and SO channel) versus the two sites within the perimeter ditch (MO and SO) during wet weather (Figure 3), with the bird marker detected between 83-100% of the time from the stormwater conveyance channel sites. In dry conditions, the bird marker was detected in the main outfall frequently (50% of the time); and in 1 of 2 samples collected from the MO stormwater conveyance channel. As hypothesized, birds are contributing both within the prison grounds and

within the stormwater conveyance channels. Bird is a source of fecal material within prison grounds, with concentrations increasing as discharges flow from the prison property and connect with Mule Creek. A confirmatory bird marker (Gull2) was also analyzed. The results parallel the results found by the GFD bird marker and are presented in the Supplementary Material (Table A1).

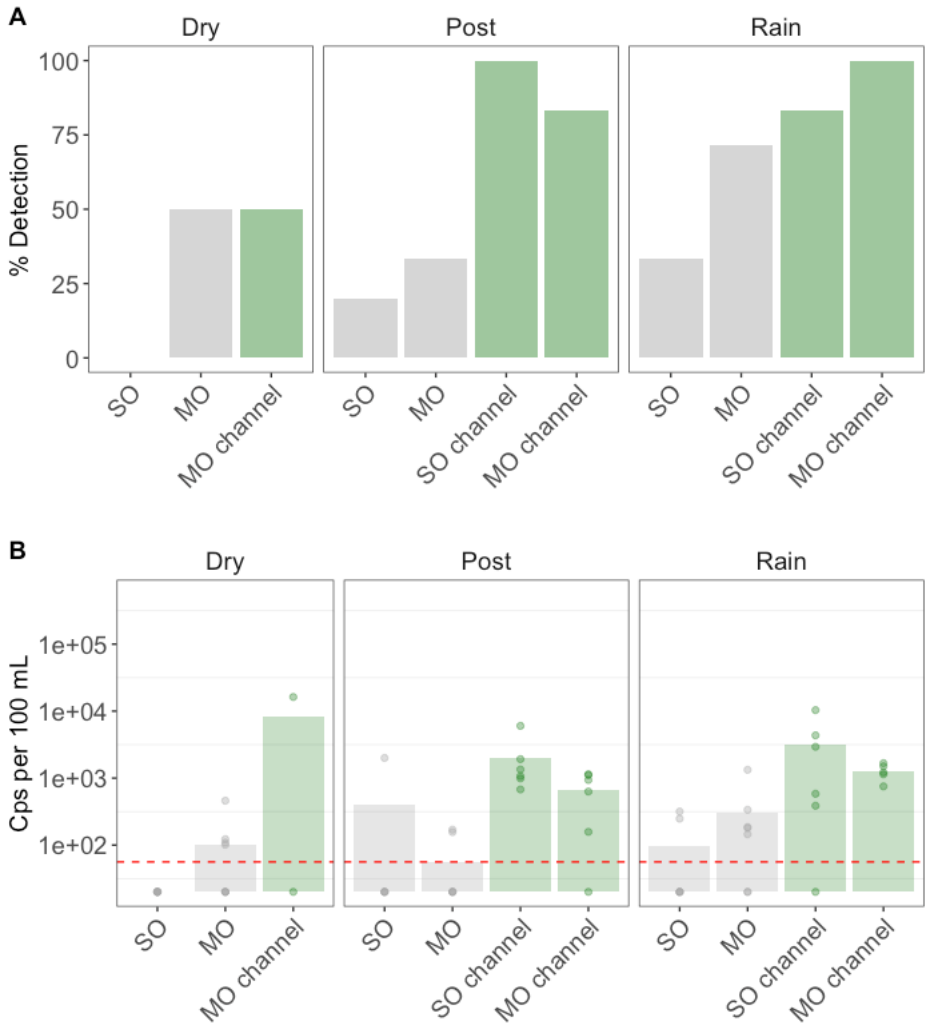


Figure 3. Bird marker (GFD) results at the four sites within prison grounds for the three flow conditions. MO is the main outfall (MCSP6). SO is the secondary outfall (MCSP5). SO channel is the site at the end of the SO SW conveyance channel (MCSP2) and MO channel is the site at the end of the MO SW conveyance channel (MCSP3). A) Bird marker detection frequency. B) Bird marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

Task 3: Investigate ruminant sources within prison ground

Ruminant, particularly deer, have been observed in and around the prison and were also investigated as a possible source within prison grounds. The ruminant marker was detected frequently in wet weather, in between 50 and 85% of samples collected at the end of the two stormwater conveyance channels (Figure 4; green bars) and in 66-100% of samples from the two prison outfalls (Figure 4; gray bars).

The ruminant marker was also detected frequently during dry weather at the two prison perimeter ditch sites (100% of the time at the main outfall and 29% at the secondary outfall) (Figure 4; grey bars). The stormwater conveyance channels rarely flowed during dry weather, and the ruminant marker was detected in one of two dry weather samples from the MO channel. These results suggest that ruminants are present within the prison grounds during both the wet and dry season and are a likely and consistent source of fecal bacteria to the creek.

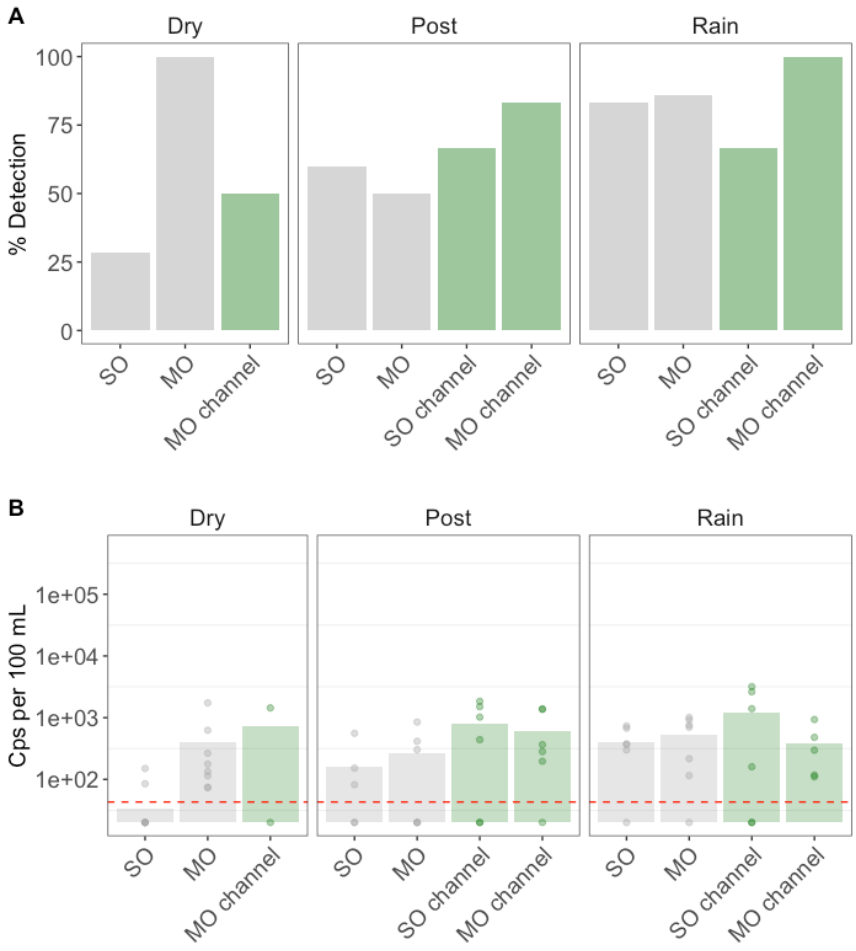


Figure 4. Ruminant marker (Rum2Bac) results at the four sites within prison grounds for the three flow conditions. MO is the main outfall (MCSP6). SO is the secondary outfall (MCSP5). SO channel is the site at the end of the SO SW conveyance channel (MCSP2) and MO channel is the site at the end of the MO SW conveyance channel (MCSP3). A) Ruminant marker detection frequency. B) Ruminant marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

Study Question 3: What other fecal sources are contributing to creek exceedances of microbial water quality criteria?

Task 1: Investigate human sources to Mule Creek, upstream of prison grounds.

The focus of study question 3 was to evaluate what fecal sources are part of any upstream contribution. Upstream and downstream Mule Creek water samples (MCSP1 and MCSP4) were analyzed for human marker (HF183) to characterize potential inputs from septic systems and to allow comparison between potential upstream and prison inputs.

Overall, the human marker was detected infrequently and only during wet weather conditions at the two Mule Creek sites. Human marker was detected in a total of two samples at the site located upstream of the prison (MCSP1). One sample was a rain event and one sample was a post-storm event. At the downstream site (MCSP4), human marker was detected in a total of three samples, all during wet weather conditions (Figure 5). Consistent with the human marker detections within prison grounds, concentrations measured in the creek were near the limit of detection and under the 500 copies per 100 mL threshold (Table 9). These results make it unlikely that there is a consistent human source entering the creek, upstream of the prison. There are also no known human sources located upstream of site MCSP1. The human marker detected at this site may be a result of treated wastewater effluent running off and entering the creek or possibly a result of cross reactivity. Deer and other ruminant have been observed upstream of prison grounds, and the ruminant marker was detected frequently and at elevated concentrations at this location.

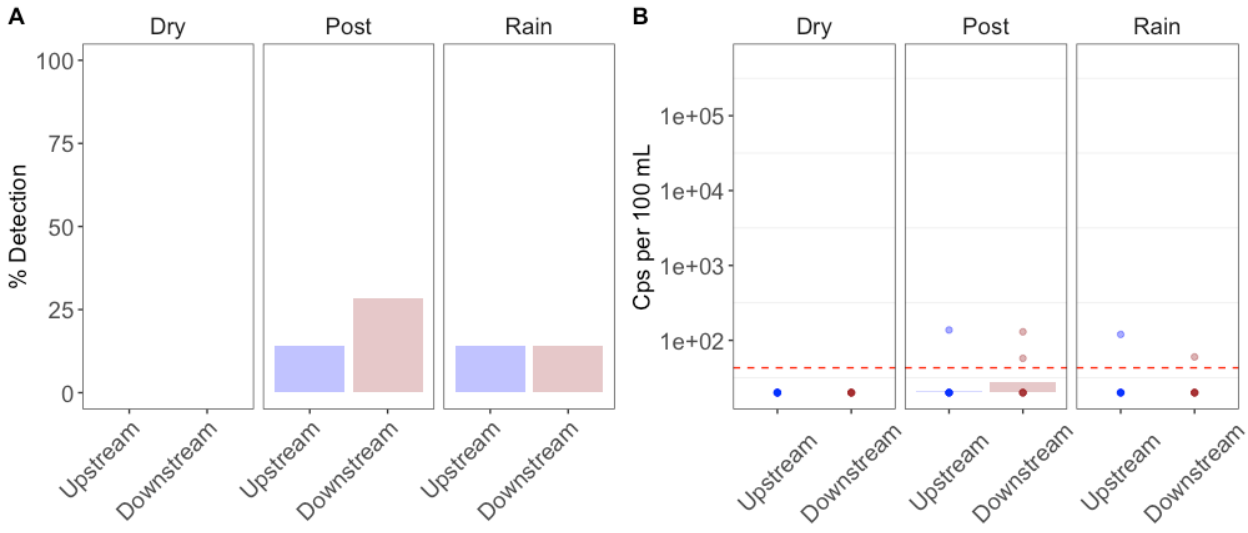


Figure 5. Human marker (HF183) results at the two creek for the three flow conditions. Upstream is the site located upstream of the prison boundary (MCSP1) and downstream is the site located just downstream of the prison boundary (MCSP4). A) Human marker detection frequency. B) Human marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

Task 2: Investigate avian sources to Mule Creek, upstream of prison grounds.

Water samples were collected upstream and downstream of Mule Creek (MCSP1 and MCSP4) and analyzed for avian marker. The avian marker was detected in 100% of samples collected at the upstream site and concentrations of bird marker were similar between the three flow conditions at the upstream location (Figure 6), suggesting that bird is a consistent source upstream of the prison, regardless of season or flow pattern.

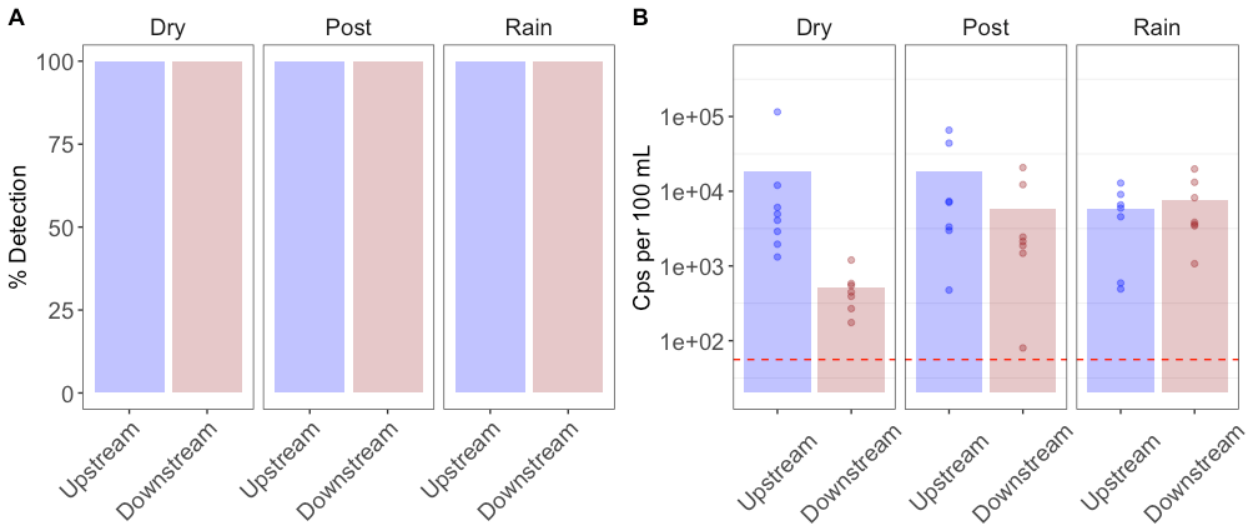


Figure 6. Bird marker (GFD) results at the two creek for the three flow conditions. Upstream is the site located upstream of the prison boundary (MCSP1) and downstream is the site located just downstream of the prison boundary (MCSP4). A) Bird marker detection frequency. B) Bird marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

Task 3: Investigate cattle sources to Mule Creek, upstream of prison grounds

Water samples collected upstream and downstream of the prison (MCSP1 and MCSP4) were analyzed for the ruminant marker (Rum2Bac) and a marker specific to cows (CowM3). These samples were intended to identify potential inputs from cattle or other ruminants (including deer) located upstream of the prison grounds.

The ruminant marker was detected frequently, in all three flow conditions, at both the upstream and downstream locations (Figure 7). The cow marker was detected less frequently, roughly 50% of the time in wet conditions and 25% of the time in dry conditions (Figure 8). These results suggest that ruminant more broadly, including cows, deer, and elk, are part of the upstream contributions to Mule Creek, and that cows are part of this contribution particularly during wet weather.

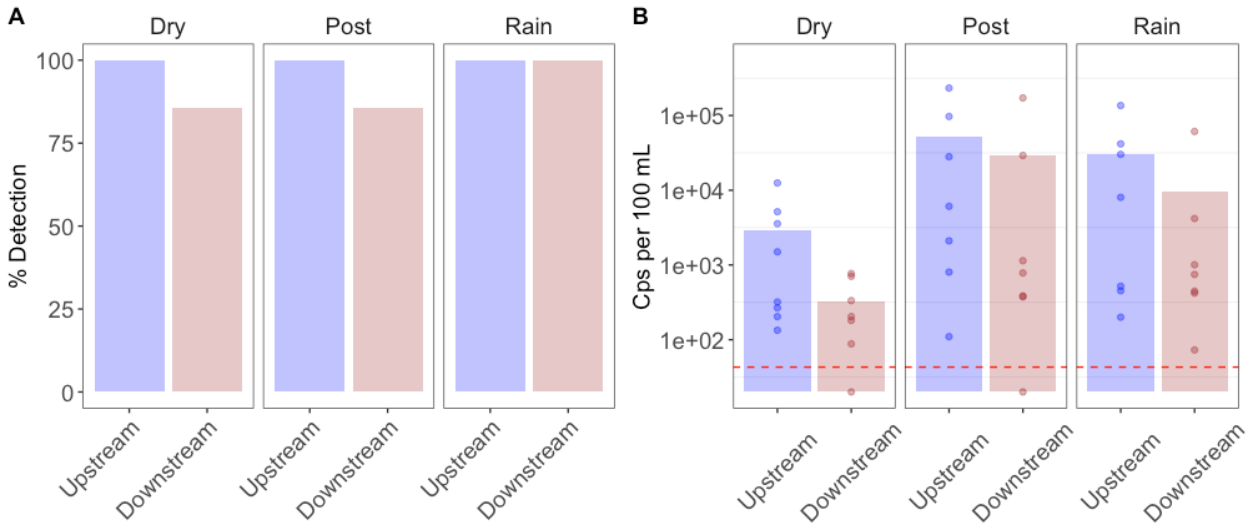


Figure 7. Ruminant marker (Rum2Bac) results at the two creek sites for the three flow conditions. Upstream is the site located upstream of the prison boundary (MCSP1) and downstream is the site located just downstream of the prison boundary (MCSP4). A) Ruminant marker detection frequency. B) Ruminant marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

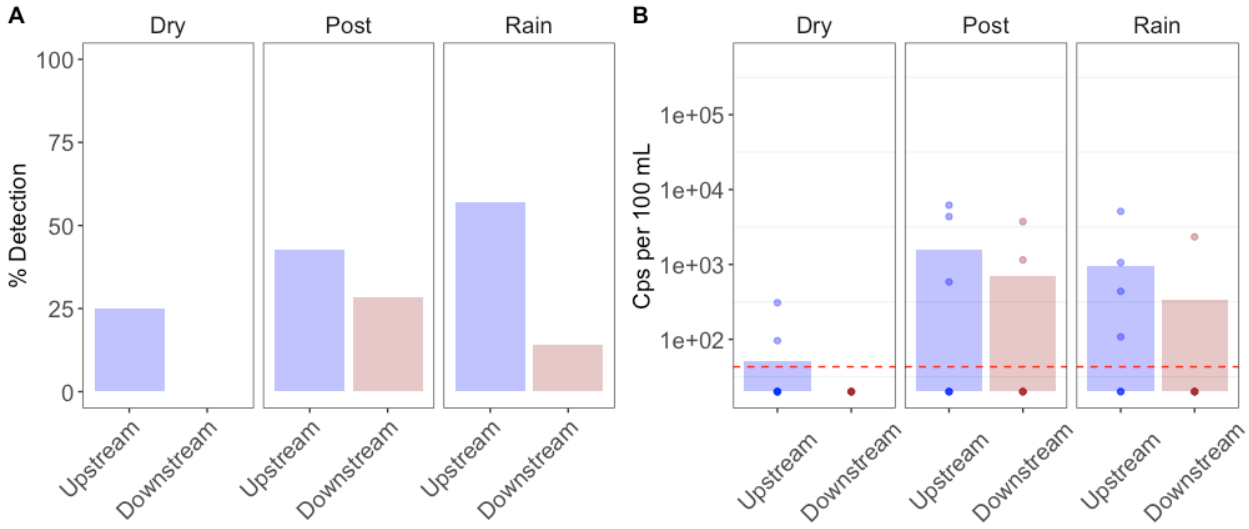


Figure 8. Cow marker (CowM3) results at the two creek sites for the three flow conditions. Upstream is the site located upstream of the prison boundary (MCSP1) and downstream is the site located just downstream of the prison boundary (MCSP4). A) Cow marker detection frequency. B) Cow marker average concentrations (bars) and individual measurements (dots). The red dashed line represents the limit of detection.

Table 9. HF183 human marker concentrations when detected (n=7 samples).

Site	Site ID	Condition	Date	Concentration [Cps/100 mL]
Upstream Boundary	MCSP1	Post-Rain	3/17/2020	138
Upstream Boundary	MCSP1	Rain	4/6/2020	120
Downstream Boundary	MCSP4	Post-Rain	3/17/2020	130
Downstream Boundary	MCSP4	Rain	4/6/2020	60
Downstream Boundary	MCSP4	Post-Rain	5/19/2020	58
Swale (Downstream SO)	MCSP2	Rain	4/6/2020	150
Main Outfall	MCSP6	Rain	5/12/2020	231

Comparison to 2019 Mule Creek Pilot Sampling Efforts

Results from the 2019 pilot sampling efforts were similar to those from the 2020 full study (Appendix B). Like in 2020, increasing *E. coli* levels were observed moving downstream, past the prison boundaries, but not on every sampling date. Increases moving downstream occurred on seven of the eleven sampling dates, with only one of these events leading to a downstream water quality standard exceedance where the upstream sample was in compliance.

Similar trends in terms of fecal pollution sources were also observed. Deer and birds, but not human, were identified as potential fecal sources with the prison. Ruminant genetic marker was detected frequently within prison boundaries during the pilot efforts. Bird marker was detected, but less often and at lower concentrations. Human genetic marker was only detected in one out of 45 samples and at low concentrations. Birds, deer, and cows were also identified using genetic markers as the most likely sources of fecal bacteria to Mule Creek upstream of the prison.

CONCLUSIONS

Runoff from the prison facility affects Mule Creek bacterial water quality some of the time. In 68% of samples, *E. coli* concentrations were higher downstream versus upstream of the prison facility. In the remaining 32% of samples, there was a reduction in *E. coli* levels moving past the prison boundaries, with this pattern occurring at least twice during each flow condition.

Animals (including birds and deer), and not human, were identified as the primary source of fecal pollution within prison grounds. Genetic markers of bird and ruminant were measured frequently, and at elevated concentrations within prison grounds. In contrast, the genetic marker of human fecal material (HF183) was detected infrequently, in 2 out of 64 samples from within prison grounds and at low levels, at concentrations near the limit of detection.

Animals (including birds, cow, and deer), and not human, were identified as the primary source of fecal pollution upstream of prison grounds. Genetic markers of bird, cow, and ruminant were measured frequently and at elevated concentrations upstream of the prison facility. In contrast, the genetic marker of human fecal material (HF183) was detected infrequently, in 2 out of 22 samples from upstream of the prison facility and at low levels, at concentrations near the limit of detection.

Similar trends were observed during the pilot sampling completed in 2019. During the pilot efforts, increases in *E. coli*, from upstream to downstream of the prison, were observed, but not on every sampling date. Increases were observed 64% of the time, in comparison to 68% of the time during the 2020 full study. Moreover, sources of fecal pollution both within prison grounds and upstream of the prison facility were found to originate from primarily animal hosts (including cow, deer, and birds), and not human, consistent with results from the full study.

REFERENCES

Ahmed, W., V.J. Harwood, K. Nguyen, K., S. Young, K. Hamilton, and S. Toze. 2016. Utility of *Helicobacter* spp. associated GFD markers for detecting avian fecal pollution in natural waters of two continents. Water Research, 88 (1) 613-622.

Boehm, A.B. and J.A. Soller. 2020. Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination. Microb. Risk Anal. 16, 100139.

Green, H.C., L.K. Dick, B. Gilpin, M. Samadpour, and K.G. Field. 2011. Genetic markers for rapid PCR-Based identification of gull, Canada goose, duck, and chicken fecal contamination in water. Applied and Environmental Microbiology. 78 (2), 503-510.

Griffith, J.F., B.A. Layton, A.B. Boehm, P.A. Holden, J.A. Jay, C. Hagedorn, C.D. McGee, and S.B. Weisberg. 2013. The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches. Technical Report 0804. Southern California Coastal Water Research Project. Costa Mesa, CA.

Layton, B.,Y. Cao, D.L. Ebentier, K. Hanley, et al. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. Water Res., 47, pp. 6897-6908

Nguyen, K.H., C. Senay, S. Young, B. Nayak, A. Lobos, J. Conrad, V.J. Harwood. 2018. Determination of wild animal sources of fecal indicator bacteria by microbial source tracking (MST) influences regulatory decisions. Water Research, 144, 424-434.

USEPA. 2012. Recreational Water Quality Criteria. 2012. Office of Water. Washington, DC. EPA 820-F-12-058

USEPA. 2019. Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay. Office of Water. Washington, DC. EPA 821-R-19-002

APPENDIX A: MST GENETIC MARKER AND *E. COLI* CONCENTRATIONS

Mean genetic marker concentrations for each site and flow condition are detailed below in Tables A1 and A2. The human marker was detected too infrequently to calculate summary statistics, so human marker results are not included. *E. coli* concentrations per site and event for wet and dry weather conditions are illustrated in Figures A1 and A2.

Table A1. Average bird marker concentrations by site and condition. ND = marker not detected. NT = samples not taken due to lack of flow.

Site	Site ID	Rain	Post-Rain	Dry Weather
		Mean [Copies per 100 mL]		
Avian Marker (GFD)				
Upstream Boundary	MCSP1	5729	18689	18562
Downstream Boundary	MCSP4	7602	5857	517
Secondary Outfall	MCSP5	95	401	ND
Main Outfall	MCSP6	311	55	100
Swale (Downstream SO)	MCSP2	3102	2005	NT
Swale (Downstream MO)	MCSP3	1257	663	8134
Gull Marker (Gull2)				
Upstream Boundary	MCSP1	ND	ND	ND
Downstream Boundary	MCSP4	2575	312	ND
Secondary Outfall	MCSP5	3518	382	ND
Main Outfall	MCSP6	3653	906	ND
Swale (Downstream SO)	MCSP2	2131	541	NT
Swale (Downstream MO)	MCSP3	2444	447	ND

Table A2. Average ruminant and cow marker concentrations by site and condition. ND = marker not detected. NT = samples not taken due to lack of flow.

Site	Site ID	Rain	Post-Rain	Dry Weather
		Mean [Copies per 100 mL]		
Ruminant Marker (Rum2Bac)				
Upstream Boundary	MCSP1	31021	52471	2959
Downstream Boundary	MCSP4	9726	29126	326
Secondary Outfall	MCSP5	409	159	34
Main Outfall	MCSP6	522	262	400
Swale (Downstream SO)	MCSP2	1230	802	NT
Swale (Downstream MO)	MCSP3	388	599	721
Cow Marker (CowM3)				
Upstream Boundary	MCSP1	960	1591	51
Downstream Boundary	MCSP4	335	698	ND
Secondary Outfall	MCSP5	ND	ND	ND
Main Outfall	MCSP6	ND	ND	ND
Swale (Downstream SO)	MCSP2	ND	ND	NT
Swale (Downstream MO)	MCSP3	ND	ND	ND

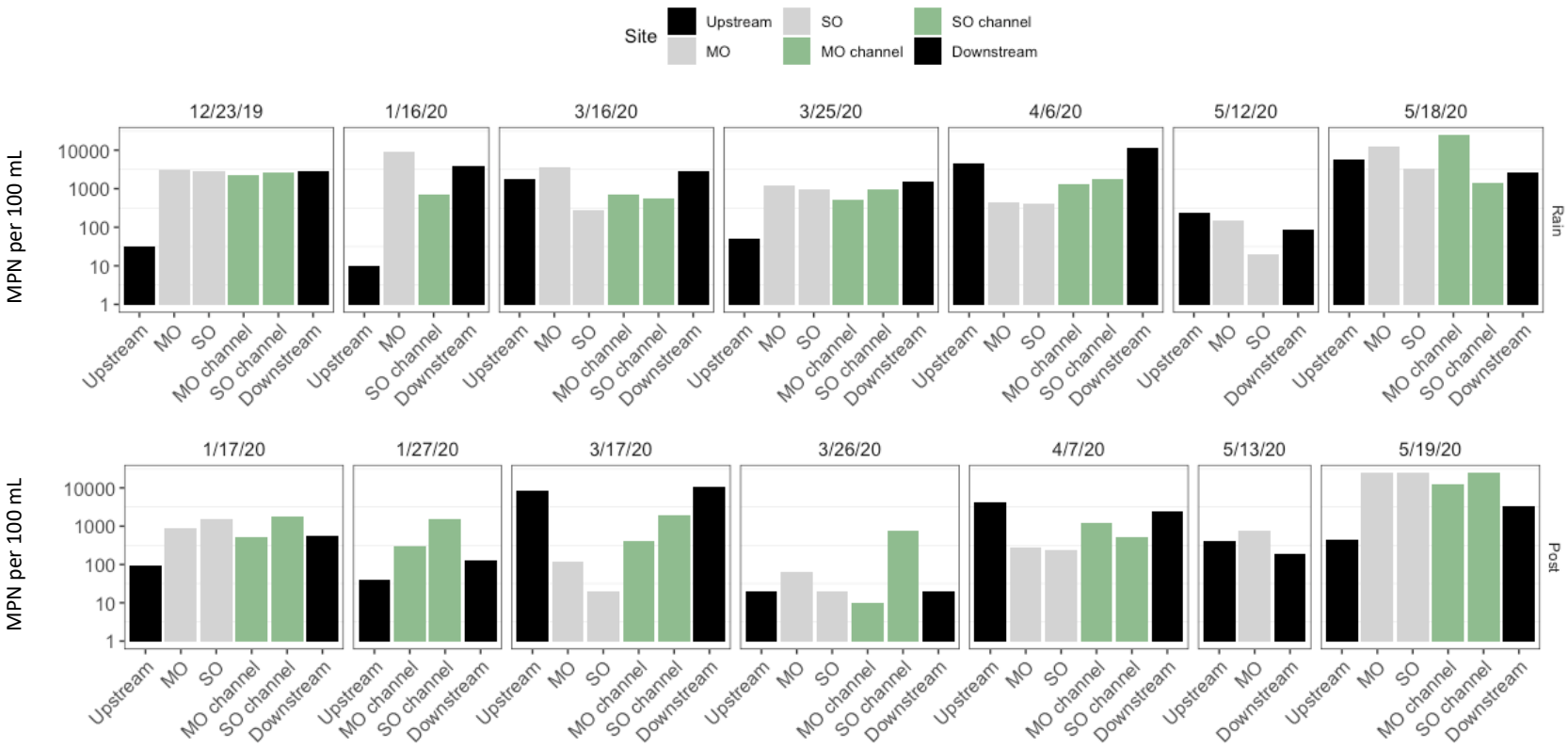


Figure A1. *E. coli* results (MPN per 100 mL) per site and event during wet weather conditions. Active rainfall is the top barplot and post-storm conditions are the bottom barplot. Site is along the x-axis and concentration is along the y-axis in MPN per 100 mL.

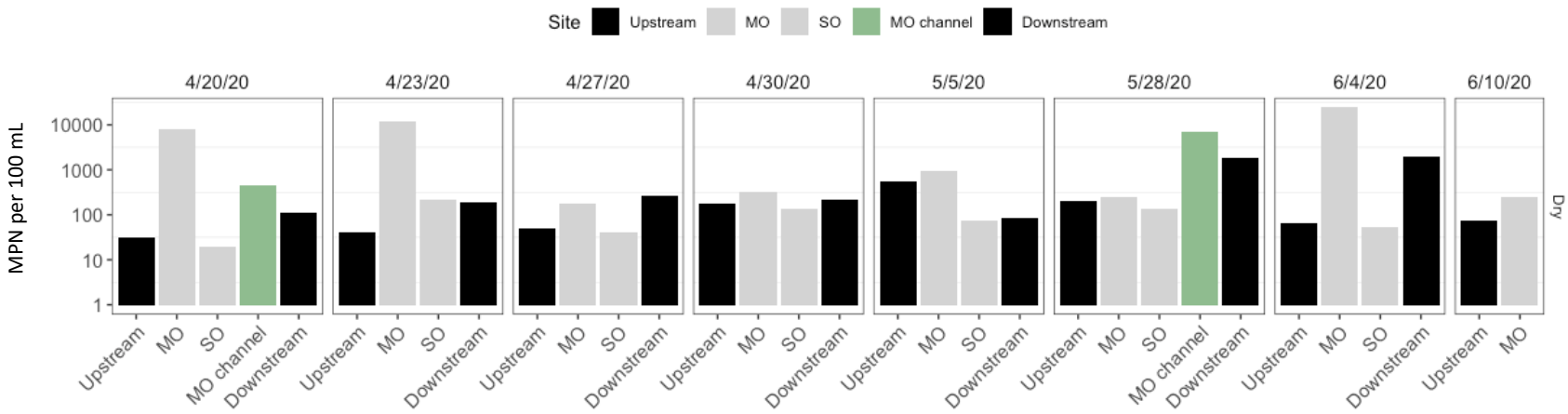


Figure A2. *E. coli* results (MPN per 100 mL) during dry weather conditions. Active rainfall is the top barplot and post-storm conditions are the bottom barplot. Site is along the x-axis and concentration is along the y-axis in MPN per 100 mL.

APPENDIX B: 2019 MULE CREEK PILOT SAMPLING

Summary of samples collected

Samples were collected during 3 active rainfall events, 4 post-storm events, and 5 dry weather events (Table B1). Limited samples were collected from the sites located at the ends of the stormwater conveyance channels (MCSP2 and MCSP3) due to a lack of flow. All collected samples were processed successfully for *E. coli* and corresponding microbial source tracking markers.

Table B1. Number of samples collected and processed for MST markers and *E. coli* between March 2019-July 2019.

Site	Site ID	Rain (n=3)	Post-Rain (n=4)	Dry Weather (n=5)	Overall
Upstream Boundary	MCSP1	3	4	4	**11**
Downstream Boundary	MCSP4	3	4	4	**11**
Secondary Outfall (SO)	MCSP5	2	4	3	**9**
Main Outfall (MO)	MCSP6	2	4	5	**11**
Swale (Downstream SO)	MCSP2	1	1	0	**2**
Swale (Downstream MO)	MCSP3	1	0	0	**1**

Microbiological Results:

Fecal Indicator Bacteria

On average, *E. coli* concentrations were higher at the downstream location for all three flow conditions (Table B2). However, *E. coli* concentrations did not increase moving downstream for all sampling dates. Increases moving downstream occurred on seven of the eleven sampling dates, with only one of these events leading to a downstream water quality standard exceedance where the upstream sample was in compliance.

Water quality results were compared to relevant water quality standards for *E. coli*. The statistical threshold value of 320 MPN per 100 mL was used in place of a single sample threshold. Exceedances occurred during both rain and dry weather conditions. During dry weather, the two exceedance events at the downstream site (MCSP4) were the last two events of the season where samples were collected under low to no-flow conditions (Table B3).

Table B2. Arithmetic mean *E. coli* concentrations at the upstream (MCSP1) and downstream (MCSP4) creek sites for each flow condition.

Site	E. coli Mean [MPN per 100 mL]			
	Rain (n=3)	Post-Rain (n=4)	Dry Weather (n=5)	Overall
Upstream boundary	560	107	546	390
Downstream boundary	738	141	1393	759

Table B3. Number of events where *E. coli* > 320 MPN/100 mL bacterial objective

Site	Rain (n)	Post-Rain (n)	Dry Weather (n)	Overall (%)
Upstream boundary	1	0	3	36%
Downstream boundary	1	0	2	27%

Genetic Marker Results

Ruminant marker was detected frequently and at elevated levels at sites both within prison grounds and within Mule Creek (Table B3). Ruminant marker was detected in 81% of samples from the upstream location and in 64% of samples at the downstream boundary. Within prison grounds, the ruminant marker was detected in 3 out of 3 samples collected from the stormwater conveyance channels (MCSP2/MCSP3). Concentrations ranged from 53-4,150 copies per 100 mL for these three samples. The ruminant marker was not detected from either of the prison perimeter ditch sites (MCSP5/MCSP6).

Bird marker was detected infrequently and at low concentrations within Mule Creek, in 18% of samples collected at the upstream location and in 9% of samples at the downstream boundary. However, for samples collected during the pilot study only the secondary bird marker (Gull2) was analyzed.

Human genetic marker was only detected in one sample, out of 45 total water samples collected. This detection occurred at MCSP3 (the secondary outfall stormwater conveyance channel) during a rain event on 5/16/2019 with a concentration of 75 copies per 100 mL.

Table B4. Average ruminant marker concentrations at the Mule Creek upstream (MCSP1) and downstream (MCSP4) sites for each flow condition.

Site	Ruminant marker mean [Copies per 100 mL]			
	Rain (n=3)	Post-Rain (n=4)	Dry Weather (n=5)	Overall
Upstream boundary	351	849	2505	1315
Downstream boundary	286	154	ND	134