Microbial Source Tracking
In the Chimacum Creek Watershed
2021-2022

Glenn Gately

Jefferson County Conservation District

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INTRODUCTION

Chimacum Creek Watershed

The Chimacum Creek watershed is in the northeastern corner of the Olympic Peninsula in eastern Jefferson County, Washington and comprises 37 square miles (Figure 1). The climate is marine with cool, dry summers and mild, wet winters. Rainfall, measured in Center, averages 29 inches per year. Average monthly rainfall ranges from 0.9 inches in July to 4.5 inches in December. Stream flow in recent years has ranged from 2 cubic feet per second (cfs) to 270 cfs. Vegetation ranges from coniferous forest in the uplands to open agricultural land in the lowlands.

Chimacum Creek's main stem originates from Delanty Lake at River Mile (RM) 13.1. From Delanty Lake to RM 11.8 at Old Eaglemount Road the stream passes through very low gradient agricultural land with organic soils. This section of stream is dry from about June to October. From RM 11.8 to RM 9.3 Chimacum Creek passes through predominantly commercial forest land, under private and state ownership. From RM 9.3 to RM 3.4 at Highway 19 in Chimacum, the stream passes through agricultural land comprised mostly of organic soils. From about RM 6.0 to RM 3.4 the gradient is extremely flat (0.0005).

Throughout the agricultural areas, residences are scattered, but from RM 2.7 downstream to RM 1.1 at Irondale Road, houses become more concentrated as Chimacum Creek passes through the towns of Chimacum, Port Hadlock, and Irondale. Downstream from RM 1.1 to its mouth in Port Townsend Bay, the gradient increases and the stream passes through a forested ravine, which offers a natural setback from residential development.

Peterson Creek joins Chimacum Creek's main stem on the west side at RM 11.1; Barnhouse Creek joins it on the east side at RM 9.0; Naylors Creek joins on the west side at RM 5.3; and Putaansuu Creek on the west side at RM 2.4. Some portions of Naylors Creek and Peterson Creek are dry during summer.

East Chimacum Creek originates in forested wetlands south of Egg and I Road. It leaves the forest at RM 5.4 and flows through mostly agricultural land with organic soils to its confluence with Chimacum Creek at RM 2.7 near the community of Chimacum.

Besides Delanty Lake, other lakes in the basin are Anderson, Beausite, Gibbs, and Peterson.

Agriculture and Water Quality

At about the turn of the 20th century most of the lowlands in the Chimacum watershed were cleared of predominantly coniferous forest and converted to pasture. To facilitate farming, much of Chimacum Creek and its tributaries were channelized, tile drains were installed, and ditches were excavated to improve drainage. Numerous dairy farms once operated in the Chimacum watershed. None remain active today; most of the former dairy farms have remained in pasture for beef cattle or converted to another form of agriculture, such as hay or vegetable crops.

Until the 1980’s when fencing began more earnestly, livestock had access to much of Chimacum Creek. Since the 1980’s, many miles of fencing have been installed along the banks of Chimacum Creek and its tributaries. Early fencing efforts commonly resulted in narrow buffers with the fence often located at "top of bank.” However, even with narrow buffers, restricting livestock access to surface water significantly improved water quality by reducing bacterial, nutrient, and sediment contamination.

Since the start of the Conservation Reserve Enhancement Program (CREP) in 2002, landowners have received rent for the land taken out of production and enrolled in the riparian buffer restoration program. Riparian forest buffer widths vary from 35 to180 feet. On small, channelized streams, minor tributaries, and ditches that have ordinary high-water levels less than 15 feet wide and that are hydrologically connected to a fish bearing stream, landowners may install a 15-foot-wide hedgerow buffer.

Through fencing and other Best Management Practices (BMPs), progress has been made in reducing fecal coliform levels in Chimacum Creek. On properties where well water or electrical power was not available to provide water for livestock, "water gaps" were installed in the fence lines to enable livestock to drink from the creek. These water gaps minimized livestock access to one or two locations on the property. This resulted in substantial reductions in bank erosion and likely reduced fecal coliform inputs.

Over the past couple of decades, the Jefferson County Conservation District (JCCD) has assisted landowners with the installation of solar-powered pumps in the stream to eliminate the need for water gaps.

Water Quality Monitoring

Fecal coliform bacteria have been used in Washington State for many years to assess the potential presence of pathogens. Fecal coliform monitoring began in Chimacum Creek in 1988. In 2019, the fecal coliform standard was replaced with a standard based on *E. coli*. However, neither fecal coliform nor *E. coli* can determine the source of the bacteria because both are excreted by *all* warm-blooded animals, including humans. Due to their high visibility, cattle have often been suspected as the major source of fecal bacteria. However, DNA monitoring, known as Microbial Source Tracking (MST), conducted in 2011-12, has shown that humans (i.e., septic systems) may be a greater source.

In the 2011-12 MST study, human DNA markers and ruminant DNA markers were used. Ruminants are animals with multiple stomachs. Cattle are ruminants, but so are sheep, goats, and deer. Since deer are prevalent throughout the watershed, the presence of ruminant markers did not positively identify livestock as a source. In the current study, markers specific to cattle were used along with human markers.

Purpose of This Study

The purpose of this study is to identify the reaches where cattle markers and human markers occur; and to determine the effectiveness of septic repairs and livestock BMPs implemented since the MST monitoring of 2011-12.

METHODS

E. Coli

E. coli samples together with MST samples were collected monthly in 4 wet months (November 2021 - February 2022) and 4 dry months (June 2022 - September 2022) at 19 stations in the Chimacum Watershed (Figure 1). *E. coli* samples were collected in new 100 mL bottles and transported in coolers with ice to Spectra Laboratory in Poulsbo, Washington. One replicate *E. coli* sample was collected at a different station each month. Samples were analyzed by a Most Probable Number (MPN) method within 24 hours of collection.

MST

MST samples were collected in sterile 250 mL bottles and transported in coolers with cold packs to the EPA laboratory in Port Orchard, Washington. Samples were filtered and frozen within 24 hours of collection. One replicate MST sample was collected each month at the same station where the *E. coli* replicate was collected. Additionally, a transfer blank containing sterilized water was taken on each sample date. MST and *E. coli* replicates are shown in appendix Table A-1.

At a later date, the samples were analyzed by quantitative Polymerase Chain Reaction (qPCR), targeting two markers used to identify human feces and two markers used to identify cattle feces. Through a process of amplification, copies were made of each marker (if present). If no marker was detected, the result was reported “ND” for non-detect. If the marker was present and the number of copies was above the “lower limit of quantification,” the result was reported as the estimated number of copies (i.e., the result was quantified). Sample results below the “lower limit of quantification” were reported as “BLOQ,” meaning below the limit of quantification. Sample results above the “lower limit of quantification” could be quantified with suitable precision and accuracy. Sample results that fell below the “lower limit of quantification” were assigned the value of BLOQ as this data is below the lowest concentration that can be quantitatively determined with suitable precision and accuracy.

RESULTS AND DISCUSSION

Interpreting the MST Data

To better interpret the MST results, if you have not already done so, please read the MST methods in the previous two paragraphs.

Ideally, MST results would have only two outcomes: ND for non-detect or a quantified number. However, with qPCR, “BLOQ” is a third possibility, and it was a common outcome in this study. BLOQs may be valid indicators of a marker’s presence, or they may be due to “noise” and falsely indicate a marker’s presence. There are two camps in deciding what to do with BLOQs. Those in one camp prefer to count them as valid indicators when other MST results from the same sampling locale are taken into consideration. Those in the

Figure 1. Map of the Chimacum Watershed showing the stations sampled in 2021-22 during the wet months of November to February and dry months of June to September.

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0 0.5 1 Miles
N

other camp prefer to treat them as NDs. In this study, BLOQs are evaluated based on the circumstances surrounding them.

MST analysis, in this study, is based on the presence of DNA from *Bacteroides* spp. bacteria, not from *E. coli* bacteria. Both kinds reside in the gastro-intestinal tracts of all warm-blooded animals and are excreted into the environment. Survival rates in the environment are different for the two kinds of bacteria. *E. coli* tend to survive better than *Bacteroides* in oxygenated water, which typically occurs in Chimacum Creek. However, DNA analysis can be conducted on dead *Bacteroides* if the DNA is still intact. One should be aware of these differences in evaluating the results.

E. Coli **Standard**

The state *E. coli* standard has two parts. For Jefferson County's streams, Part 1 requires that the geometric mean value (GMV) not exceed 100 colonies of *E. coli* bacteria in 100 milliliters of water (100 colonies/100mL). Part 2 requires that not more than 10% of the samples exceed 320 colonies/100 mL. Both parts need to be met to pass the standard. Additionally, wet months (October – May) and dry months (June – September) are analyzed separately and the standard must be met for both periods.

E. Coli **and MST Results**

E. coli failed the standard at 4 of the 19 stations sampled in the wet months (Table 1). Quantified human markers were present at 3 of the 4 failing stations (CHI/6.7, CHI/7.0, CHI/8.8) and a quantified cattle marker was present at 1 failing station (BHO/0.0).

E. coli failed the standard at 11 of the 19 stations sampled during the dry months (Table 1). Seven of these stations (CHI/6.7, CHI/7.0, CHI/7.8, CHI/8.8, EGA/0.0, NAY/0.1, NAY/0.7) were associated with quantified human markers and four (CHI/4.0, CHI/7.8, CHI/8.8, PUT/0.0) with quantified cattle markers.

In the dry months, quantified human markers were present at 4 additional stations (BHO/0.0, CHI/3.4, ECH/3.3, ECH/4.8) where *E. coli* passed the standard; a quantified cattle marker was present at 1 additional station (BHO/0.0) where the standard was passed (Table 1).

If BLOQs are counted as valid, human markers would be present at 18 of the 19 stations sampled in the dry months and present at 16 stations in the wet months. Cattle markers would be present at 13 stations in the dry months and at 13 stations in the wet months. It is likely that when a station had quantified marker(s) in the dry months that BLOQs at that station in the wet months were valid indicators. That the markers were not quantified may be due to the dilution effect of the higher flows during the wet months.

It is also likely that when a BLOQ marker occurred downstream from a station having a quantified marker, that it is a valid indicator of the marker's presence because bacteria are carried downstream. Again, dilution may be responsible because stream flow usually increases in a downstream manner. Also, bacteria and their DNA decompose as they

Table 1. *E. coli* results showing the status at stations monitored monthly in the Chimacum Watershed during the wet months of November 2021 to Februay 2022 and dry months of June to September 2022. Part 1 of the state standard requires that the geometric mean value (GMV) not exceed 100 Colonies/100 mL and Part 2 requires that not more than 10% of the samples exceed 320 Colonies/100 mL. Both parts must be met to pass the standard. MST results show the stations where human (H) and cattle (C) markers were identified. Each "H" or "C" represents a month in which the marker was identified. Bolded capital letters represent markers with quantification levels above the limit of quantification; lower case, unbolded letters represent markers with levels below the limit of quantification (BLOQ).

Station	Part 1 Wet Months	Part 1 Dry Months	Part 2 Wet Months	Part 2 Dry Months	Wet Months	Dry Months	Wet Months		Dry Months	
	GMV (Colonies/ 100 mL)	GMV (Colonies/ 100 mL)	Highest Concentration (Colonies/ 100 mL)	Highest Concentration (Colonies/ 100 mL)	Overall Status		Human Markers	Cattle Markers	Human Markers	Cattle Markers
BHO/0.0	21	94	411	166	Failed	Passed	h	**C**c	**H**hhh	**C**
CHI/0.1	30	74	88	93	Passed	Passed	hh	cc	hhhh	
CHI/1.1	18	89	35	124	Passed	Passed	hh	cc	hhh	
CHI/2.0	19	132	46	361	Passed	Failed	h	cc	hhh	
CHI/3.4	25	68	41	98	Passed	Passed	hh	**C**c	**H**h	c
CHI/5.3	38	44	58	120	Passed	Passed	hh	cc	hhh	cc
CHI/6.7	48	213	687	548	Failed	Failed	**H**hhh	ccc	**HHH**h	cc
CHI/7.0	77	186	1046	980	Failed	Failed	**H**	cc	**HHH**h	**C**c
CHI/7.8	7	447	29	2420	Passed	Failed		cc	**HH**hh	**C**
CHI/8.8	44	254	548	687	Failed	Failed	**H**h	ccc	**HHH**	**C**cc
ECH/0.2	11	73	54	153	Passed	Passed		c	hhh	
ECH/1.0	6	65	20	344	Passed	Failed				
ECH/3.3	13	82	52	156	Passed	Passed	h		**H**hh	
ECH/4.8	14	98	37	308	Passed	Passed	hhh		**HHH**h	
EGA/0.0	43	466	185	1046	Passed	Failed	hh		**HHHH**	
NAY/0.1	36	254	125	594	Passed	Failed	h		**HHH**h	
NAY/0.7	28	197	51	436	Passed	Failed			**HH**hh	
PUT/0.0	53	239	131	816	Passed	Failed	h	**C**cc	hhh	**CC**c
WVA/0.1	21	46	91	461	Passed	Failed	h	ccc	h	

travel downstream from their source. Decomposition as well as consumption by predators would decrease the quantification number.

Figure 2 shows the reaches upstream of the stations that failed the *E. coli* standard; Figure 3 shows the reaches upstream from stations where quantified human markers were present in wet or dry periods; and Figure 4 shows the reaches where quantified cattle markers were present in wet or dry months.

All data collected in this study is listed in appendix Table A-2.

Relationship of *E. Coli* to Marker Frequency

Regression of *E. coli* GMV on the human quantification number showed no correlation. Neither was there a correlation of *E. coli* GMV with the cattle quantification number. However, when *E. coli* GMV was regressed on the *frequency of occurrence* of a quantified human marker (i.e., number of months that a quantified marker was present at a station), there was a high correlation both for the wet months (p=0.002; Figure 5) and for the dry months (p=0.002; Figure 6). There was a fair correlation for the occurrence of quantified cattle markers for the dry period (p=0.07; Figure 6), but not for the wet period (p=0.69; Figure 5). The highest dry month correlation (p=0.0002) with *E. coli* was obtained when human markers and cattle markers were combined. Combining human markers and cattle markers for the wet months yielded a slightly worse correlation (p=0.007) than for the human markers alone (p=0.002; Figure 5). When BLOQs were included in the *frequency of occurrence* regressions, the correlations worsened.

Relationship of *E. Coli* to Air Temperature

E. coli concentration was positively correlated to the maximum high air temperature recorded on the day before sampling at a Bremerton weather station (p=0.06; Figure 7). Correlation to the average of the previous 3-day highs was slightly better (p=0.04; Figure 7). Previous studies conducted by JCCD have shown fecal coliform concentration to be correlated to temperature. Concentrations have been consistently higher in the summer months when stream flows are at their lowest. Low stream flow, which would concentrate bacteria, probably contributes to the correlation with temperature.

Comparison with the 2011-12 MST Study

In comparing the 2021-22 results to the 2011-12 results, several confounding factors must be considered. In 2011-12, fecal coliform bacteria were monitored, whereas in 2021-22 *E. coli* was monitored.

Also, “ruminant” markers were used in 2011-12 and cattle markers were used in 2021-22.

The MST method used in 2011-12 was PCR, but not quantitative PCR as was used in 2021-22. The non-quantitative method used in 2011-12 is probably closer to the qPCR method if the BLOQs are included in the count. In the wet months of 2011-12, human markers were present at 16 of the 19 stations. In the wet months of 2021-22, human markers were present at 15 stations if BLOQs are included (3 stations if BLOQs were not included; Figure 8). In the dry months of 2011-12, human markers were present at 17

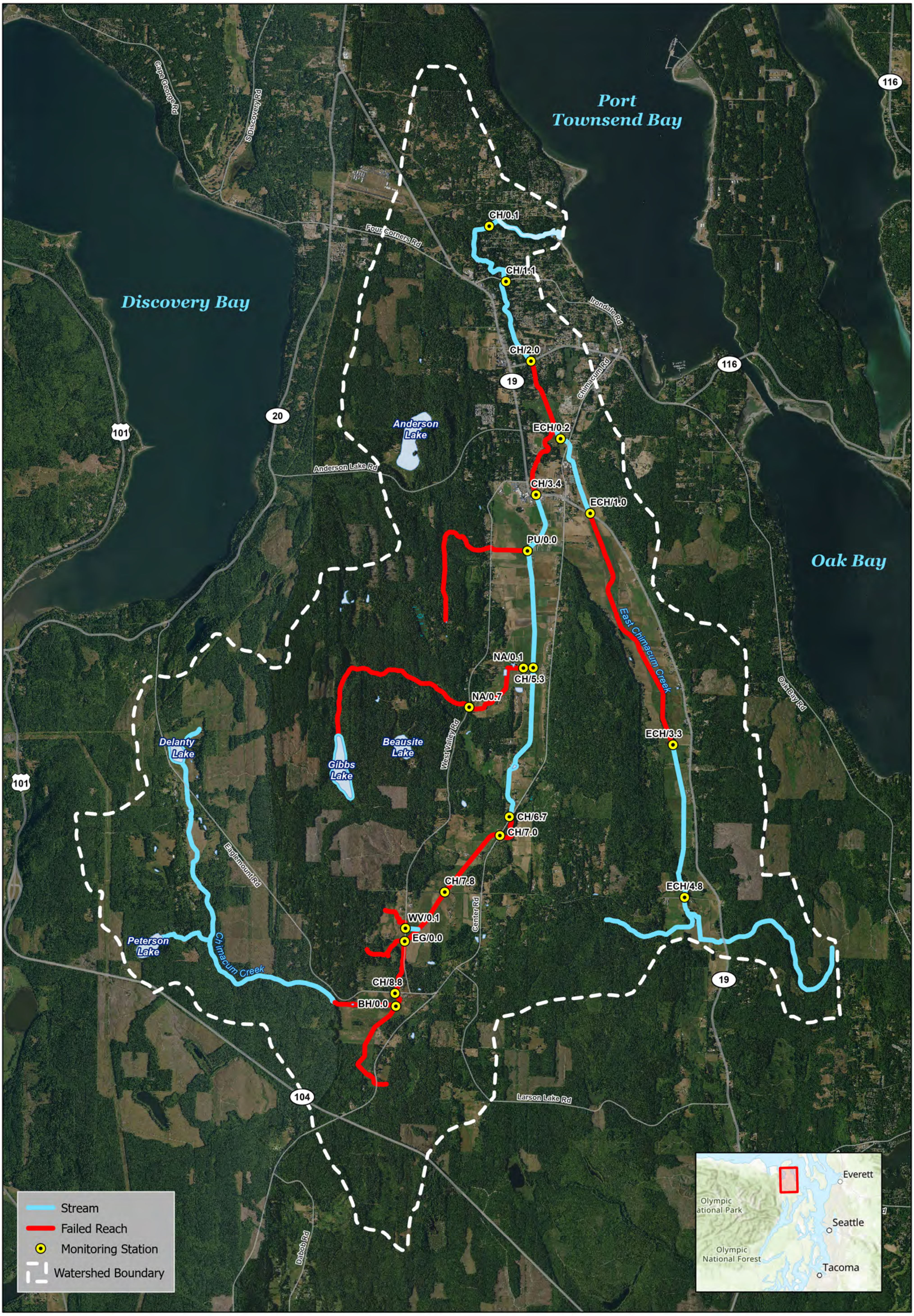

Figure 2. Map of the Chimacum Watershed showing (in red) the stream reaches in which E. coli failed the state standard in 2021-2022.

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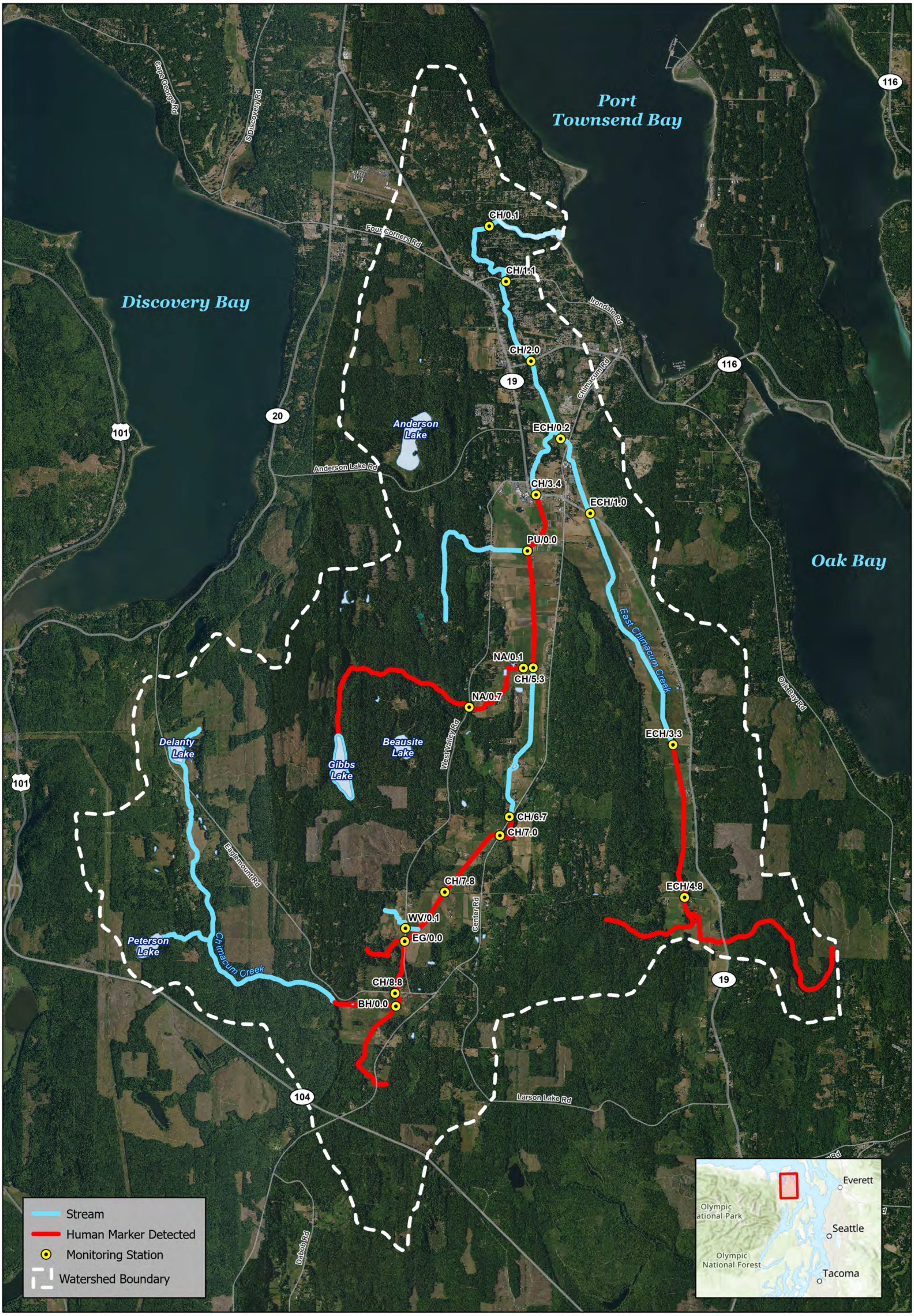

Figure 3. Map of the Chimacum Watershed showing (in red) the stream reaches in which human markers were detected in 2021-22.

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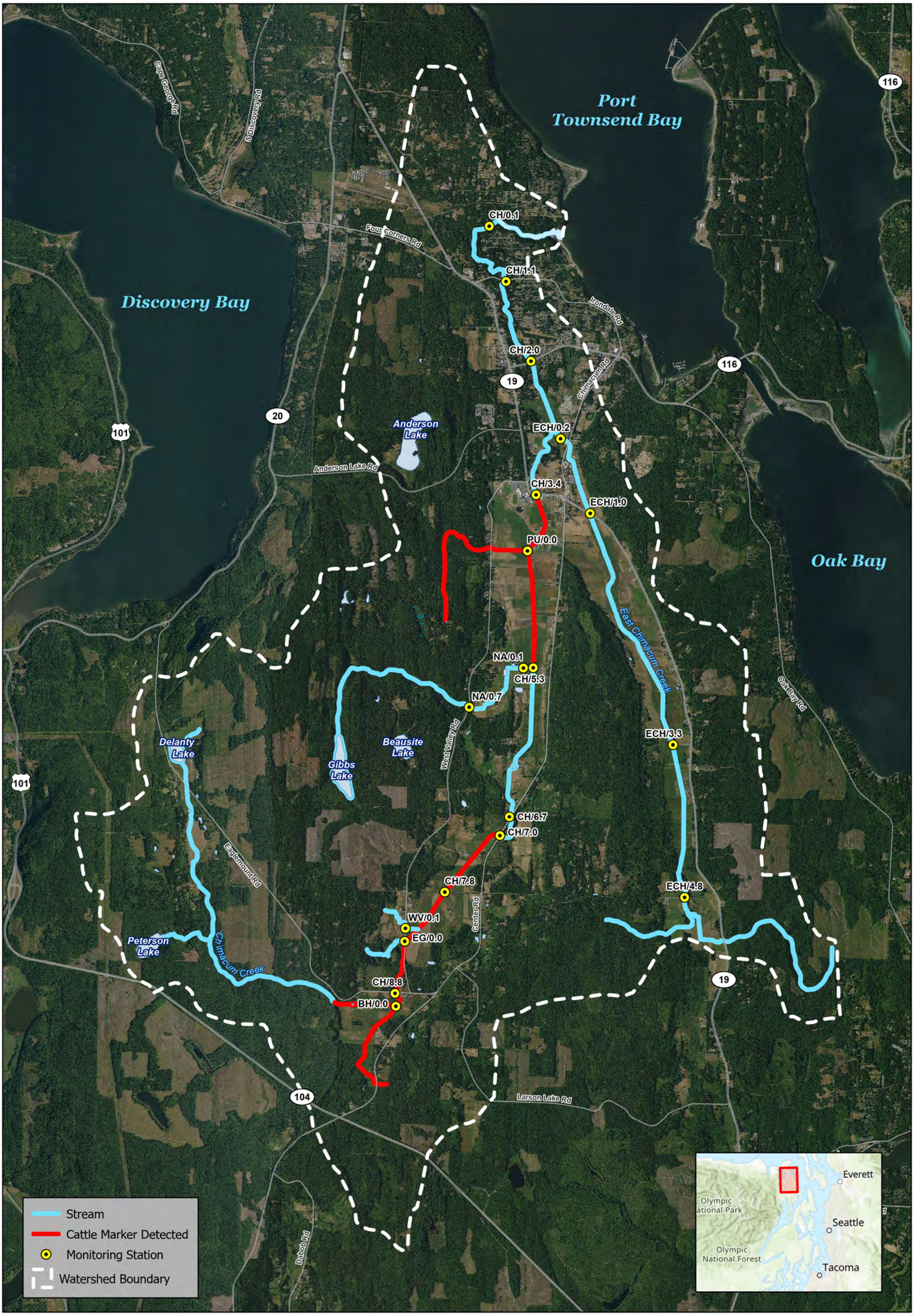

Figure 4. Map of the Chimacum Watershed showing (in red) the stream reaches in which cattle markers were detected in 2021-22.

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0 0.5 1 Miles

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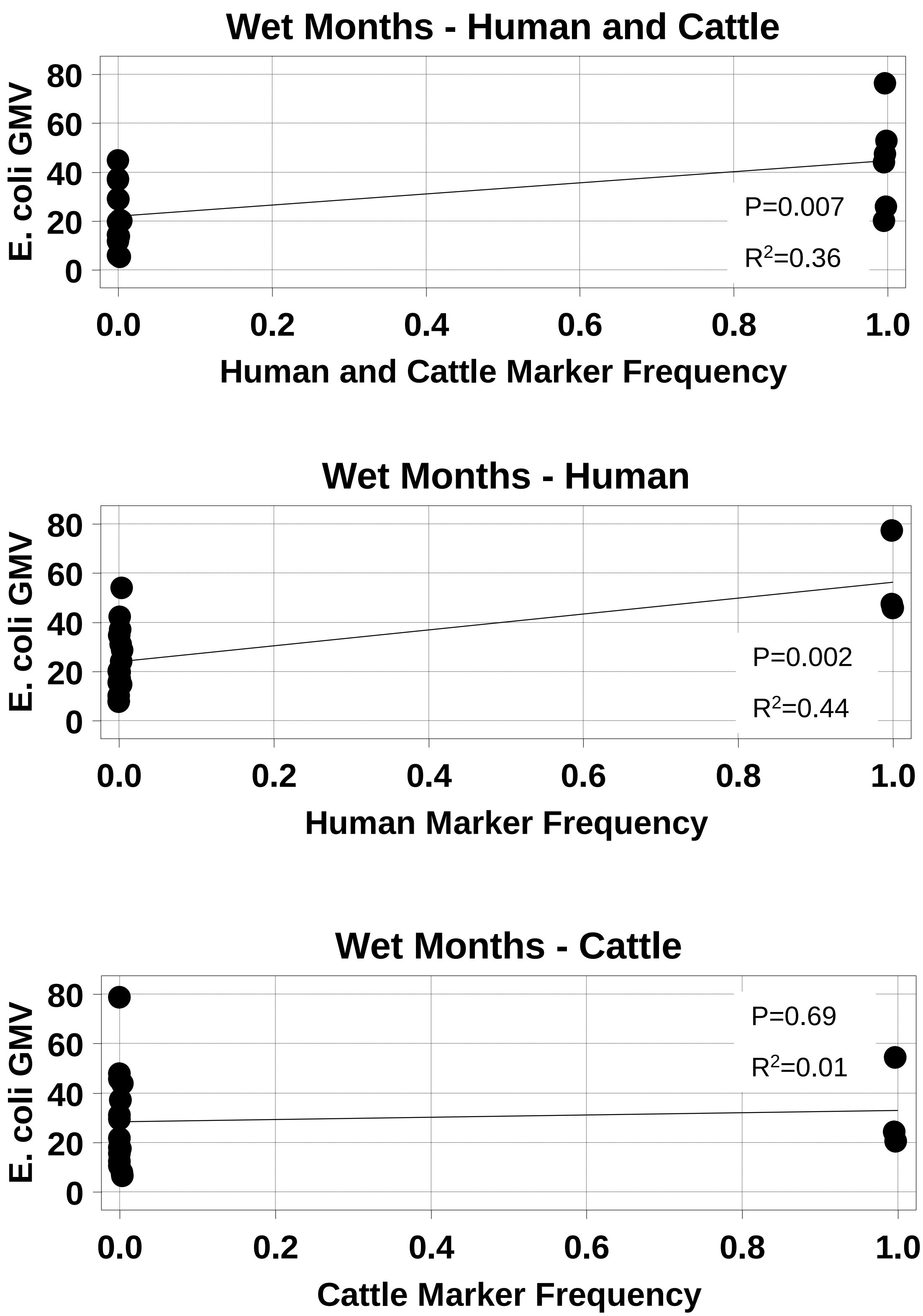


Figure 5. Regression of E. coli geometric mean values (GMV) on the frequency of human, cattle, and human and cattle markers combined in samples collected at 19 stations monitored monthly in the Chimacum Watershed from November 2021 to February 2022.

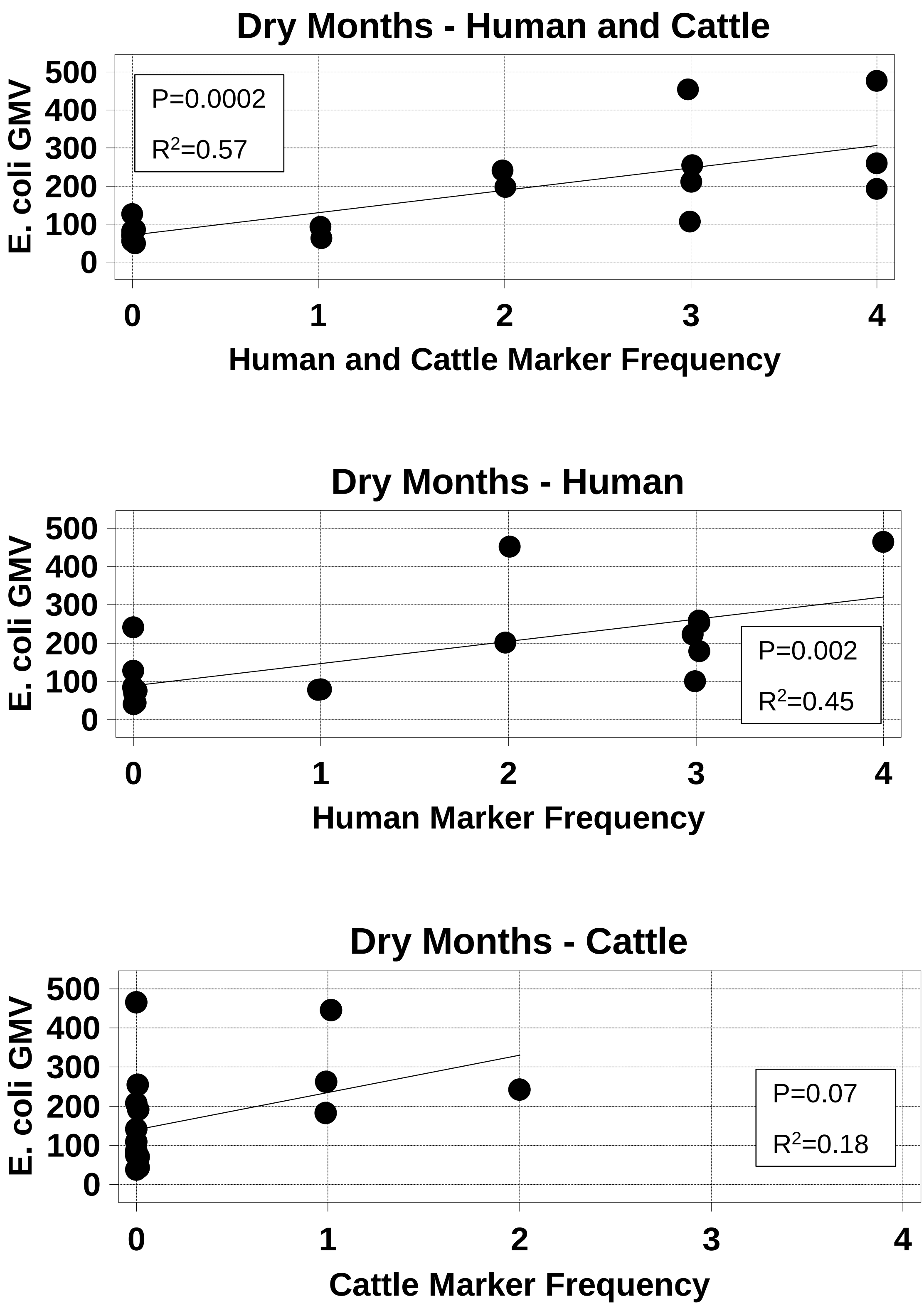
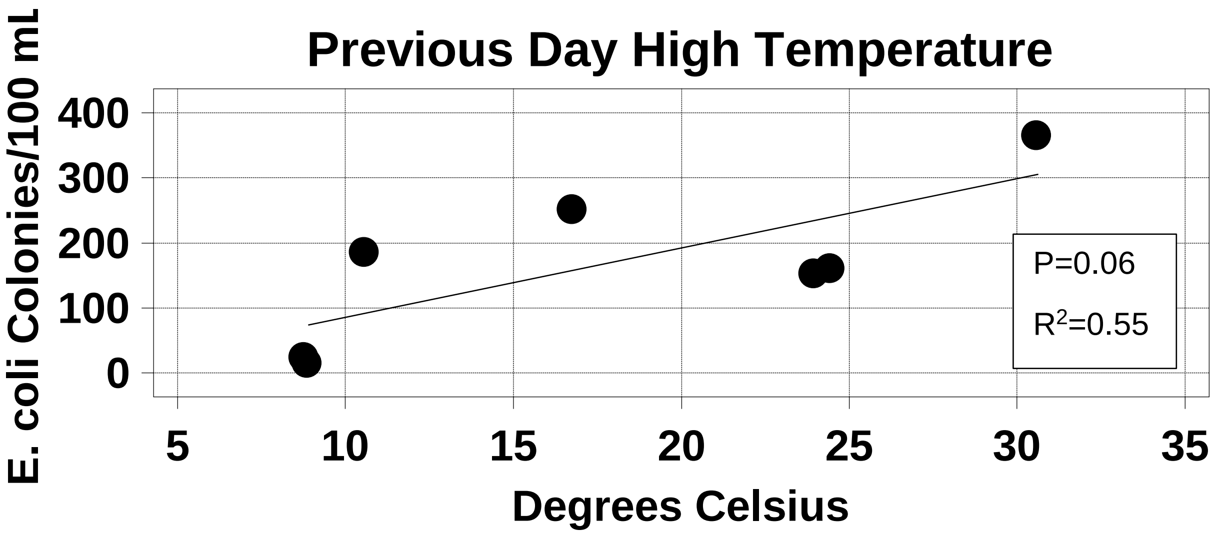


Figure 6. Regression of E. coli geometric mean values (GMV) on the frequency of human, cattle, and human and cattle markers combined in samplers collected at 19 stations monitored monthly in the Chimacum Watershed from June 2022 to September 2022.



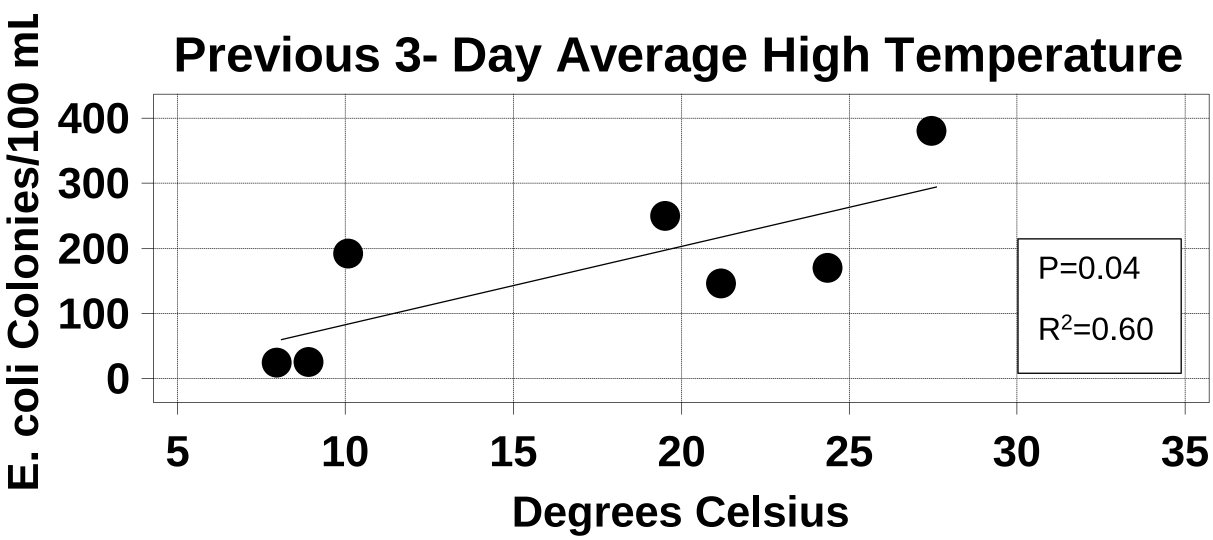
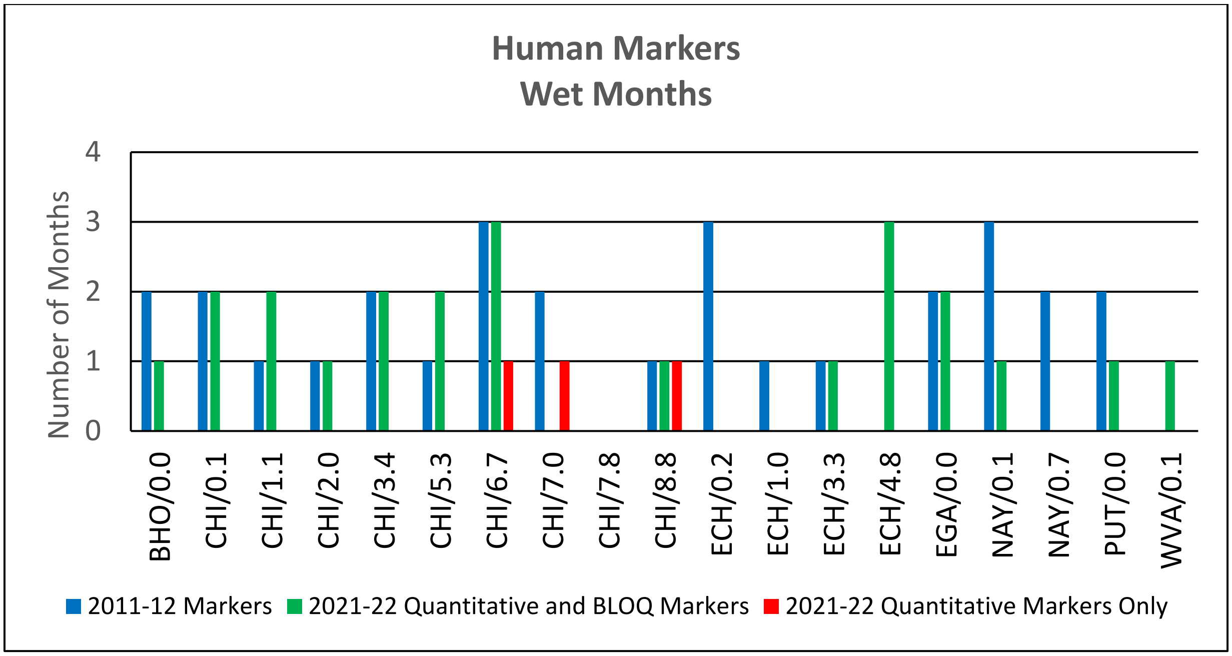


Figure 7. Regression of the average E. coli concentration of the 19 monitoring stations on the previous day's high air temperature (top) and on the average of the three previous days' high temperatures (bottom). Air temperatures were measured at Bremerton, Washington.



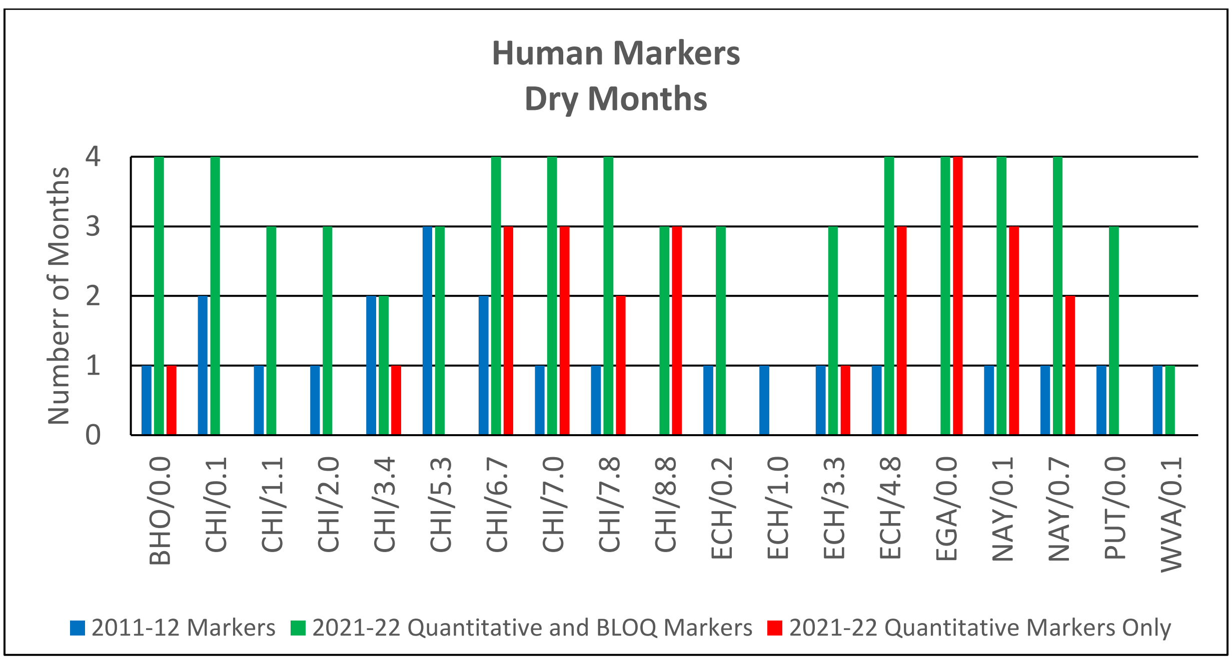


Figure 8. Comparison of the frequency of occurrence of human markers sampled monthly in 2011-12 to those sampled in 2021-22 during the wet months of November to February (top) and dry months of June to September (bottom).

stations, compared to 18 stations if BLOQs are included (11 stations if BLOQs were not included).

As previously mentioned, ruminants include other animals beside cattle. Therefore, no strong conclusions can be made when comparing the presence of cattle markers in 2021-22 to the presence of ruminant markers in 2011-12. In the wet months of 2011-12, ruminant markers were present at 4 stations. In 2021-22, cattle markers were present at 13 stations if BLOQs are included (3 stations if BLOQs are not included; Figure 9). In the dry months of 2011-12, ruminant markers were present at 5 stations. In 2021-22, cattle markers were present at 8 stations if BLOQs are included (5 stations if BLOQs are not included).

The percentage of stations passing the *E. coli* standard in 2021-22 was much higher than the percentage of stations passing the fecal coliform standard in three previous monitoring periods (Table 2). In 2021-22, 15 of the 19 stations (79%) passed the *E. coli* standard in the wet months, compared to 0% passing the fecal coliform standard in each of the previous three monitoring periods. For the dry months, 8 of 19 stations (42%) passed the *E. coli* standard in 2021-22, compared to a range of 0 to 1 of the 19 stations (0-5%) passing the fecal coliform standard in the three previous monitoring periods.

Septic repairs and agricultural BMPs implemented from 2012 to 2021 probably contributed to the increase in the number of stations passing the standard (Figure 10). It is also likely that much of the improvement is due to the change from a standard based on fecal coliform to one based on *E. coli*. In a study conducted by JCCD in June 2021 in which 23 Chimacum Creek stations were sampled for both fecal coliform and *E. coli*, 18 stations passed the *E. coli* standard, compared to only 6 stations passing the fecal coliform standard.

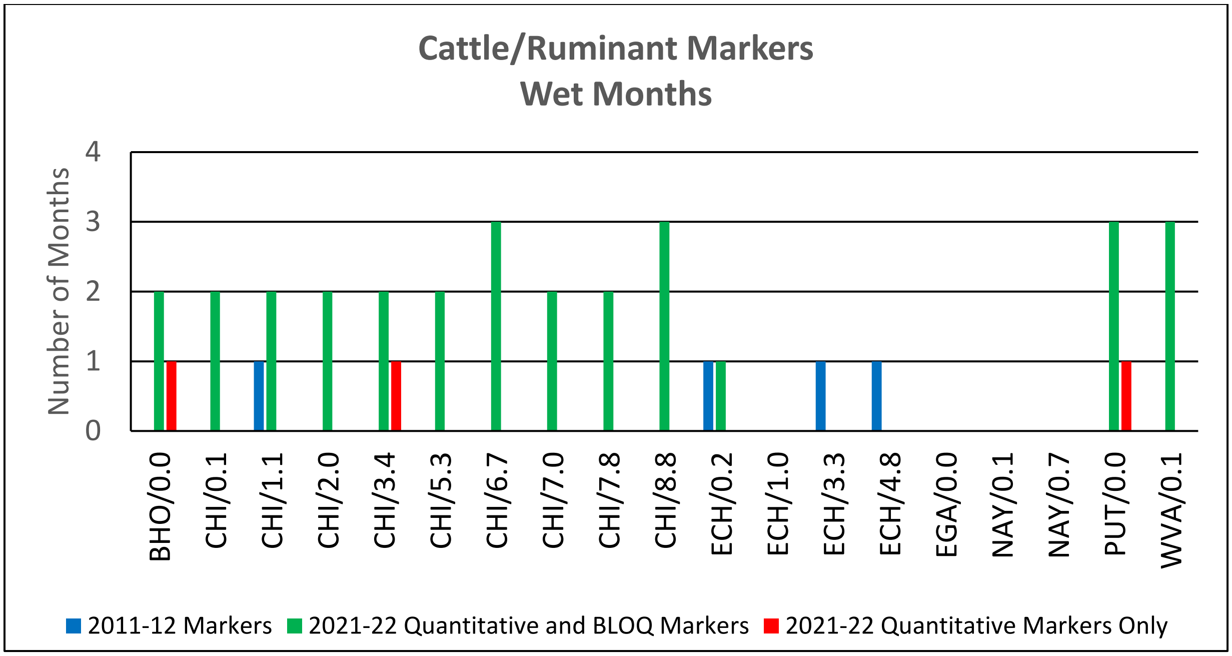
CONCLUSIONS

Human Sources

Human fecal bacteria were present in Chimacum Creek's Main Stem and East Fork, Barnhouse Creek, Naylors Creek, Putaansuu Creek, and ditches WVA and EGA. Based on the assumption that quantified markers alone are a better indicator of a marker's origin (stream reach), human sources on the Main Stem are mainly upstream from station CHI/3.4 at State Route 19; on the East Fork, upstream from station ECH/3.3 at Peat Plank Road; and upstream from the mouths of Barnhouse Creek, Naylors Creek, and ditch EGA.

Based on the assumption that including BLOQ markers with quantified markers makes for a better comparison with the unquantified results of 2011-12, the presence of human markers in 2021-22 was about the same as it was in 2011-12. Human markers were present at almost all of the 19 monitoring stations in both periods.

Quantified human markers were 3.2 times more numerous than quantified cattle markers and 2.0 times more numerous if BLOQs are included (Table 1).



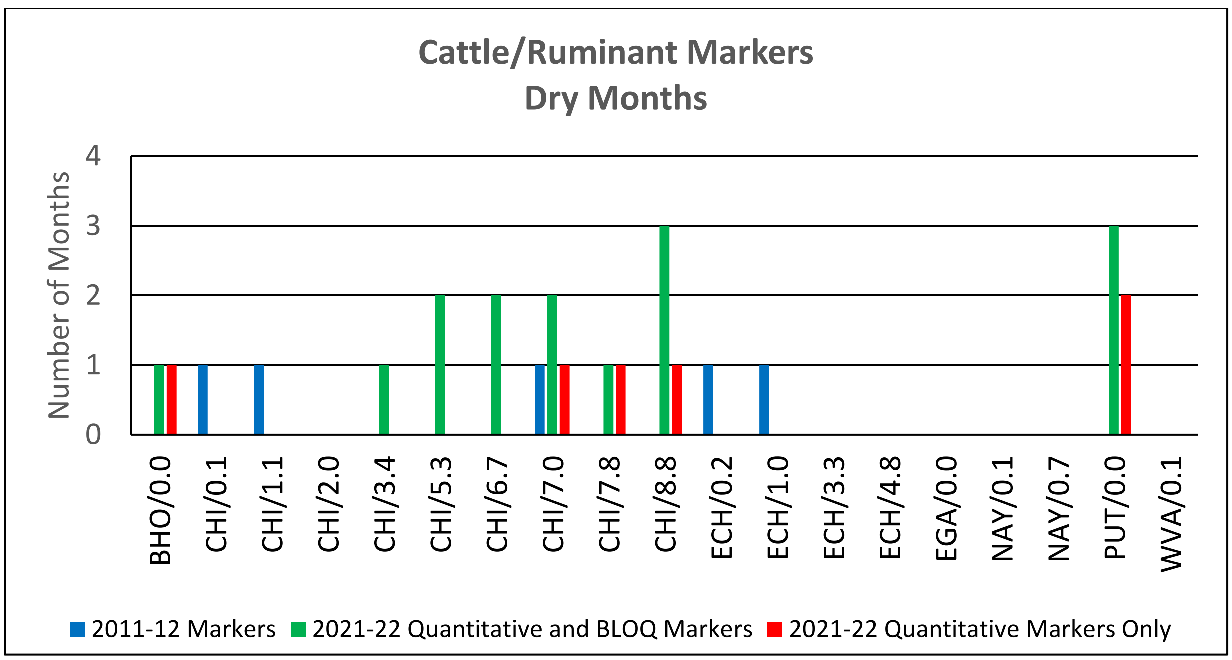


Figure 9. Comparison of the frequency of occurrence of ruminant markers sampled monthly in 2011-12 and cattle markers sampled in 2021-22 during the wet months of November to February (top) and dry months of June to September (bottom).

Table 2. Chimacum watershed monitoring stations showing station status relative to the state standard. Prior to 2020, the standard was based on fecal coliform. After 2020, it has been based on E. coli. Both standards have two parts and both parts must be met to pass the standard. Part 1 requires that the geometric mean not exceed 50 Colonies/100 mL for fecal coliform and not exceed 100 Colonies/100 mL for E. coli. Part 2 requires that not more than 10% of the samples exceed 100 Colonies/100 mL for fecal coliform and not exceed 320 Colonies/100 mL for E. coli.

Station	Fecal Coliform Standard 2007-08				Fecal Coliform Standard 2009-10				Fecal Coliform Standard 2011-12				E. coli Standard 2021-22			
	October to May[1]		June to September[2]		October to May[1]		June to September[2]		October to May[1]		June to September[1]		November to February[1]		June to September[1]	
	0% Passed		0% Passed		0% Passed		5% Passed		0% Passed		0% Passed		79% Passed		42% Passed	
	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2
CH/0.1	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Pass
CH/1.1	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Pass	Pass	Pass
CH/2.0	Fail	Fail	Fail	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Fail	Pass	Pass	Pass	Fail	Fail
CH/3.4	Fail	Fail	Fail	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Fail	Pass	Pass	Pass	Pass	Pass
CH/5.3	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Pass
CH/6.7	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail
CH/7.0	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail
CH/7.8	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Fail	Fail
CH/8.8	Fail	Fail	Fail	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail
PU/0.0	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Fail	Fail
NA/0.1	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Fail	Fail
NA/0.7	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Pass	Pass	Fail	Fail
WV/0.1	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Fail
EG/0.0	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Fail	Fail
BH/0.0	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Pass	Pass
ECH/0.2	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Pass
ECH/1.0	Pass	Fail	Fail	Fail	Pass	Fail	Pass	Pass	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Fail
ECH/3.3	Fail	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Pass
ECH/4.8	Fail	Fail	Fail	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Fail	Fail	Pass	Pass	Pass	Pass

[1] Monitoring conducted once per month.

[2] Monitoring conducted twice per month.

Figure 10. Map of the Chimacum Watershed showing location of BMP's, resolved septic complaints, and septic repairs completed from 2012-2021.

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0 0.5 1 Miles

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Cattle Sources

Cattle fecal bacteria were present in Chimacum Creek's Main Stem and East Fork, Barnhouse Creek, Putaansuu Creek, and ditch WVA. Again, assuming that quantified markers alone are a better indicator of a marker's origin, cattle sources on the Main Stem are mainly upstream from station CHI/7.0 on Center Road and upstream from the mouths of Barnhouse Creek and Putaansuu Creek. Although there is an absence of quantified cattle markers on ditch WVA, the high frequency of BLOQ cattle markers at station WVA/0.1 during the wet season indicates that cattle are a source upstream from West Valley Road during the wet season.

E. Coli

E. coli passed the standard at 15 of the 19 stations in the wet months and 8 stations in the dry months. This was a substantial improvement over the monitoring results of 2007-08, 2009-10, and 2011-12 when only one station past the fecal coliform standard. However, it is likely that much of this apparent improvement is due to the change in standards.

ACKNOWLEDGMENTS

Thanks to the Environmental Protection Agency and their Manchester Lab for providing the MST analysis and to Jefferson County Environmental Health for funding the *E. coli* analysis. Thanks to the landowners who allowed access to their property for sample collection. *E. coli* and MST samples were collected by: Joe Holtrop and Sierra Young from the Conservation District; Brad Stone, Jacquelyn Stenman, Lara Gaasland-Tatro, Michael Dawson, and Tim Weissman from Jefferson County Environmental Health. Stephanie Bailey of the Environmental Protection Agency Manchester Laboratory and Michael Dawson assisted with writing the Quality Assurance Project Plan. Stephanie Bailey and Eunice Chern conducted the MST analyses. Sierra Young, Joe Holtrop, and Stephanie Bailey reviewed this report. Kevin Hitchcock, GIS Coordinator/Administrator for Jefferson County produced the maps. Michael Dawson provided the data on septic improvements. Brian Cochrane of the Washington State Conservation Commission provided the agricultural BMP data.

APPENDIX

Table A-1. Field replicate results for MST and E. coli samples collected at stations in the Chimacum Watershed in 2021-22.

Station	Date	Time	Replicate	CowM2	CowM3	HF183	HumM2	E. Coli
ECH/0.2	11/9/2021	9:38		BLOQ	ND	ND	ND	54
ECH/0.2	11/9/2021	9:38	R1	BLOQ	ND	ND	ND	88
PUT/0.0	12/14/2021	9:32		ND	BLOQ	ND	ND	20
PUT/0.0	12/14/2021	9:34	R1	BLOQ	ND	ND	ND	20
CHI/6.7	1/11/2022	9:42		ND	BLOQ	ND	ND	
CHI/6.7	1/11/2022	9:43	R1	BLOQ	BLOQ	ND	ND	
CHI/6.7	2/8/2022	10:05		ND	ND	ND	ND	5
CHI/6.7	2/8/2022	10:05	R1	ND	ND	BLOQ	ND	8
NAY/0.1	6/14/2022	9:10		ND	ND	BLOQ	ND	62
NAY/0.1	6/14/2022	9:10	R1	ND	ND	BLOQ	ND	50
ECH/4.8	7/12/2022	9:20		ND	ND	BLOQ	ND	48
ECH/4.8	7/12/2022	9:20	R1	ND	ND	BLOQ	ND	91
CHI/1.1	8/9/2022	9:04		ND	ND	ND	BLOQ	99
CHI/1.1	8/9/2022	9:05	R1	ND	ND	ND	BLOQ	84
CHI/5.3	9/20/2022	8:43		ND	ND	ND	ND	19
CHI/5.3	9/20/2022	8:43	R1	ND	ND	BLOQ	ND	26

Table A-2. Water quality data collected for this 2021-22 MST study.

Date	Time	Station	Rep-licate	Human Marker HF183	Human Marker HumM2	Cattle Marker CowM2	Cattle Marker CowM3	E. coli Colonies /100 mL
11/9/2021	9:12	BHO/0.0		BLOQ	ND	1273	BLOQ	411
12/14/2021	9:06	BHO/0.0		ND	ND	ND	ND	11
1/11/2022	9:37	BHO/0.0		ND	ND	ND	BLOQ	
2/8/2022	9:31	BHO/0.0						2
6/14/2022	9:31	BHO/0.0		BLOQ	ND	590	781	96
7/12/2022	9:15	BHO/0.0		BLOQ	ND	ND	ND	166
8/9/2022	9:22	BHO/0.0		BLOQ	ND	ND	ND	84
9/20/2022	9:10	BHO/0.0		318	BLOQ	ND	ND	59
11/9/2021	10:55	CHI/0.1		BLOQ	ND	BLOQ	ND	88
12/14/2021	11:06	CHI/0.1		ND	ND	ND	ND	28
1/11/2022	10:00	CHI/0.1		BLOQ	ND	BLOQ	ND	
2/8/2022	10:25	CHI/0.1		ND	ND	ND	ND	11
6/14/2022	8:26	CHI/0.1		BLOQ	ND	ND	ND	71
7/12/2022	10:09	CHI/0.1		BLOQ	ND	ND	ND	68
8/9/2022	9:40	CHI/0.1		BLOQ	ND	ND	ND	68
9/20/2022	9:35	CHI/0.1		BLOQ	ND	ND	ND	93
11/9/2021	11:25	CHI/1.1		BLOQ	ND	BLOQ	BLOQ	35
12/14/2021	9:45	CHI/1.1		ND	ND	ND	ND	14
1/11/2022	9:15	CHI/1.1		ND	ND	ND	ND	
2/8/2022	9:15	CHI/1.1		BLOQ	ND	ND	BLOQ	12
6/14/2022	8:45	CHI/1.1		ND	ND	ND	ND	68
7/12/2022	9:00	CHI/1.1		BLOQ	ND	ND	ND	124
8/9/2022	9:04	CHI/1.1		ND	BLOQ	ND	ND	99
8/9/2022	9:05	CHI/1.1	R1	ND	BLOQ	ND	ND	84
9/20/2022	10:08	CHI/1.1		ND	BLOQ	ND	ND	74
11/9/2021	11:40	CHI/2.0		ND	BLOQ	BLOQ	BLOQ	46
12/14/2021	10:00	CHI/2.0		ND	ND	ND	ND	20
1/11/2022	9:25	CHI/2.0		ND	ND	BLOQ	BLOQ	
2/8/2022	9:30	CHI/2.0		ND	ND	ND	ND	8
6/14/2022	9:01	CHI/2.0		ND	ND	ND	ND	69
7/12/2022	9:15	CHI/2.0		BLOQ	ND	ND	ND	108
8/9/2022	9:25	CHI/2.0		BLOQ	BLOQ	ND	ND	361
9/20/2022	10:15	CHI/2.0		BLOQ	BLOQ	ND	ND	113
11/9/2021	10:00	CHI/3.4		BLOQ	ND	BLOQ	BLOQ	41
12/14/2021	10:25	CHI/3.4		ND	ND	ND	ND	25
1/11/2022	10:05	CHI/3.4		BLOQ	ND	BLOQ	855	
2/8/2022	10:00	CHI/3.4		ND	ND	ND	BLOQ	15
6/14/2022	9:18	CHI/3.4		ND	ND	ND	ND	98
7/12/2022	10:35	CHI/3.4		ND	ND	ND	ND	68
8/9/2022	9:35	CHI/3.4		BLOQ	ND	BLOQ	ND	69
9/20/2022	10:30	CHI/3.4		268	BLOQ	ND	ND	47
11/9/2021	8:35	CHI/5.3		ND	ND	ND	BLOQ	58

Table A-2. Water quality data collected for this 2021-22 MST study.

Date	Time	Station	Rep-licate	Human Marker HF183	Human Marker HumM2	Cattle Marker CowM2	Cattle Marker CowM3	E. coli Colonies /100 mL
12/14/2021	8:35	CHI/5.3		ND	BLOQ	ND	ND	54
1/11/2022	9:07	CHI/5.3		BLOQ	ND	BLOQ	BLOQ	
2/8/2022	8:57	CHI/5.3		ND	ND	ND	ND	17
6/14/2022	9:03	CHI/5.3		ND	ND	BLOQ	BLOQ	120
7/12/2022	8:45	CHI/5.3		BLOQ	ND	ND	BLOQ	71
8/9/2022	8:53	CHI/5.3		BLOQ	ND	ND	ND	23
9/20/2022	8:43	CHI/5.3	R1	BLOQ	ND	ND	ND	26
9/20/2022	8:43	CHI/5.3		ND	ND	ND	ND	19
11/9/2021	11:00	CHI/6.7		343	ND	BLOQ	BLOQ	687
12/14/2021	9:12	CHI/6.7		BLOQ	ND	ND	BLOQ	32
1/11/2022	9:42	CHI/6.7		ND	ND	ND	BLOQ	
1/11/2022	9:43	CHI/6.7	R1	ND	ND	BLOQ	BLOQ	
2/8/2022	10:05	CHI/6.7	R1	BLOQ	ND	ND	ND	8
2/8/2022	10:05	CHI/6.7		ND	ND	ND	ND	5
6/14/2022	9:30	CHI/6.7		BLOQ	ND	BLOQ	BLOQ	548
7/12/2022	10:20	CHI/6.7		219	ND	ND	ND	219
8/9/2022	9:55	CHI/6.7		539	ND	BLOQ	ND	121
9/20/2022	10:12	CHI/6.7		550	BLOQ	ND	ND	141
11/9/2021	9:11	CHI/7.0		290	ND	BLOQ	BLOQ	1046
12/14/2021	9:10	CHI/7.0		ND	ND	ND	ND	13
1/11/2022	9:14	CHI/7.0		ND	ND	BLOQ	BLOQ	
2/8/2022	9:21	CHI/7.0		ND	ND	ND	ND	33
6/14/2022	9:13	CHI/7.0		BLOQ	ND	BLOQ	814	980
7/12/2022	9:00	CHI/7.0		361	BLOQ	ND	ND	104
8/9/2022	9:25	CHI/7.0		339	BLOQ	BLOQ	ND	138
9/20/2022	9:14	CHI/7.0		353	BLOQ	ND	ND	86
11/9/2021	10:43	CHI/7.8		ND	ND	ND	ND	29
12/14/2021	9:01	CHI/7.8		ND	ND	ND	BLOQ	14
1/11/2022	9:33	CHI/7.8		ND	ND	BLOQ	BLOQ	
2/8/2022	9:55	CHI/7.8						1
6/14/2022	9:15	CHI/7.8		BLOQ	BLOQ	BLOQ	890	816
7/12/2022	10:00	CHI/7.8		BLOQ	ND	ND	ND	162
8/9/2022	9:35	CHI/7.8		235	BLOQ	ND	ND	2420
9/20/2022	10:05	CHI/7.8		282	BLOQ	ND	ND	125
11/9/2021	8:58	CHI/8.8		325	ND	BLOQ	BLOQ	548
12/14/2021	9:00	CHI/8.8		BLOQ	BLOQ	BLOQ	BLOQ	16
1/11/2022	9:04	CHI/8.8		ND	ND	ND	ND	
2/8/2022	9:12	CHI/8.8		ND	ND	ND	BLOQ	10
6/14/2022	9:23	CHI/8.8		ND	ND	BLOQ	BLOQ	687
7/12/2022	9:09	CHI/8.8		302	ND	ND	ND	308
8/9/2022	9:15	CHI/8.8		336	ND	BLOQ	ND	140
9/20/2022	9:05	CHI/8.8		377	BLOQ	BLOQ	461	141

Table A-2. Water quality data collected for this 2021-22 MST study.

Date	Time	Station	Rep-licate	Human Marker HF183	Human Marker HumM2	Cattle Marker CowM2	Cattle Marker CowM3	E. coli Colonies /100 mL
11/9/2021	9:38	ECH/0.2	R1	ND	ND	BLOQ	ND	88
11/9/2021	9:38	ECH/0.2		ND	ND	BLOQ	ND	54
12/14/2021	9:30	ECH/0.2		ND	ND	ND	ND	11
1/11/2022	10:03	ECH/0.2		ND	ND	ND	ND	
2/8/2022	10:05	ECH/0.2						2
6/14/2022	10:00	ECH/0.2		ND	ND	ND	ND	44
7/12/2022	10:00	ECH/0.2		BLOQ	ND	ND	ND	30
8/9/2022	9:45	ECH/0.2		BLOQ	ND	ND	ND	142
9/20/2022	9:35	ECH/0.2		BLOQ	ND	ND	ND	153
11/9/2021	10:05	ECH/1.0		ND	ND	ND	ND	20
12/14/2021	10:10	ECH/1.0		ND	ND	ND	ND	4
1/11/2022	9:40	ECH/1.0		ND	ND	ND	ND	
2/8/2022	9:45	ECH/1.0						3
6/14/2022	9:59	ECH/1.0		ND	ND	ND	ND	34
7/12/2022	10:50	ECH/1.0		ND	ND	ND	ND	344
8/9/2022	9:45	ECH/1.0		ND	ND	ND	ND	124
9/20/2022	10:45	ECH/1.0		ND	ND	ND	ND	12
11/9/2021	8:24	ECH/3.3		ND	ND	ND	ND	52
12/14/2021	8:34	ECH/3.3		ND	ND	ND	ND	1
1/11/2022	8:43	ECH/3.3		ND	ND	ND	ND	
2/8/2022	8:49	ECH/3.3		BLOQ	ND	ND	ND	45
6/14/2022	10:09	ECH/3.3		BLOQ	ND	ND	ND	118
7/12/2022	9:32	ECH/3.3		ND	ND	ND	ND	107
8/9/2022	8:48	ECH/3.3		496	ND	ND	ND	156
9/20/2022	8:30	ECH/3.3		BLOQ	ND	ND	ND	23
11/9/2021	8:43	ECH/4.8		BLOQ	ND	ND	ND	37
12/14/2021	8:45	ECH/4.8		BLOQ	ND	ND	ND	2
1/11/2022	8:51	ECH/4.8		BLOQ	ND	ND	ND	
2/8/2022	9:00	ECH/4.8		ND	ND	ND	ND	40
6/14/2022	15:00	ECH/4.8		736	ND	ND	ND	308
7/12/2022	9:20	ECH/4.8	R1	BLOQ	ND	ND	ND	91
7/12/2022	9:20	ECH/4.8		BLOQ	ND	ND	ND	48
8/9/2022	9:01	ECH/4.8		318	ND	ND	ND	184
9/20/2022	8:51	ECH/4.8		300	ND	ND	ND	34
11/9/2021	10:37	EGA/0.0		BLOQ	ND	ND	ND	185
12/14/2021	8:51	EGA/0.0		BLOQ	ND	ND	ND	32
1/11/2022	9:24	EGA/0.0		ND	ND	ND	ND	
2/8/2022	9:47	EGA/0.0		ND	ND	ND	ND	13
6/14/2022	9:05	EGA/0.0		492	ND	ND	ND	411
7/12/2022	9:55	EGA/0.0		374	BLOQ	ND	ND	270
8/9/2022	9:25	EGA/0.0		871	BLOQ	ND	ND	1046
9/20/2022	9:55	EGA/0.0		1205	522	ND	ND	408

Table A-2. Water quality data collected for this 2021-22 MST study.

Date	Time	Station	Rep-licate	Human Marker HF183	Human Marker HumM2	Cattle Marker CowM2	Cattle Marker CowM3	E. coli Colonies /100 mL
11/9/2021	8:47	NAY/0.1		ND	ND	ND	ND	125
12/14/2021	8:43	NAY/0.1		ND	ND	ND	ND	11
1/11/2022	9:13	NAY/0.1		BLOQ	ND	ND	ND	
2/8/2022	9:14	NAY/0.1		ND	ND	ND	ND	34
6/14/2022	9:10	NAY/0.1	R1	BLOQ	ND	ND	ND	50
6/14/2022	9:10	NAY/0.1		BLOQ	ND	ND	ND	62
7/12/2022	8:50	NAY/0.1		509	388	ND	ND	344
8/9/2022	9:00	NAY/0.1		7097	401	ND	ND	330
9/20/2022	8:49	NAY/0.1		1088	BLOQ	ND	ND	594
11/9/2021	10:22	NAY/0.7		ND	ND	ND	ND	51
12/14/2021	8:33	NAY/0.7		ND	ND	ND	ND	11
1/11/2022	9:07	NAY/0.7		ND	ND	ND	ND	
2/8/2022	9:30	NAY/0.7		ND	ND	ND	ND	40
6/14/2022	8:35	NAY/0.7		BLOQ	ND	ND	ND	28
7/12/2022	9:35	NAY/0.7		BLOQ	BLOQ	ND	ND	436
8/9/2022	9:00	NAY/0.7		4297	428	ND	ND	285
9/20/2022	9:34	NAY/0.7		285	BLOQ	ND	ND	436
11/9/2021	9:33	PUT/0.0		ND	ND	BLOQ	BLOQ	56
12/14/2021	9:32	PUT/0.0		ND	ND	ND	BLOQ	20
12/14/2021	9:34	PUT/0.0	R1	ND	ND	BLOQ	ND	20
1/11/2022	9:31	PUT/0.0		BLOQ	ND	ND	ND	
2/8/2022	9:43	PUT/0.0		ND	ND	BLOQ	1199	131
6/14/2022	10:35	PUT/0.0		BLOQ	ND	ND	ND	76
7/12/2022	9:50	PUT/0.0		ND	ND	BLOQ	958	194
8/9/2022	9:57	PUT/0.0		BLOQ	BLOQ	BLOQ	ND	816
9/20/2022	9:44	PUT/0.0		BLOQ	ND	BLOQ	463	272
11/9/2021	10:32	WVA/0.1		BLOQ	ND	BLOQ	BLOQ	91
12/14/2021	8:46	WVA/0.1		ND	ND	BLOQ	BLOQ	26
1/11/2022	9:18	WVA/0.1		ND	ND	BLOQ	BLOQ	
2/8/2022	9:45	WVA/0.1						4
6/14/2022	9:00	WVA/0.1		ND	ND	ND	ND	21
7/12/2022	9:50	WVA/0.1		ND	ND	ND	ND	23
8/9/2022	9:15	WVA/0.1		ND	ND	ND	ND	461
9/20/2022	9:39	WVA/0.1		BLOQ	ND	ND	ND	20
11/8/2021	15:00	PT Sewage		2402887	106598			
11/8/2021	13:30	Cow Manure- DS		ND	ND	74488	7658	
11/8/2021	14:00	Cow Manure- JB		ND	ND	4972	4896	