Patterns in Fecal Indicator Bacteria in the Scott River Watershed, 2007-2014

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Prepared for:
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EXECUTIVE SUMMARY

Bacterial contamination of surface waters associated with fecal waste pose a public health risk when water is consumed or humans are in close contact with the water through swimming and other activities. Water quality criteria for fecal indicator bacteria (FIB) are suggested by the U.S. EPA and in California are adopted by state water boards (North Coast Regional Water Quality Control Board, NCRWQCB) to protect public health. A number of indicator bacteria groups are used as an indicator of fecal contamination in freshwater, including *E. coli*, fecal coliform, and total coliforms.

Since 2007, the Quartz Valley Indian Reservation (QVIR) has been collecting surface water samples in the Scott River watershed to test for levels of bacterial contamination. Two sets of sites were sampled. First, baseline sites within the Quartz Valley and along the Scott River were sampled to identify specific sites and times of high bacteria levels that may indicate a risk to public health. Many of these sites are public access sites used for fishing, swimming, and cultural practices involving water contact.

The second set of sites is located in cattle grazing allotments along high-elevation streams and lakes. Two active grazing allotments and one retired grazing allotment were sampled to assess the impacts of cattle grazing on stream bacteria levels as well as to identify sites and timing of bacterial contamination that may pose risks to public health. These high-elevation sites are in U.S. Forest Service wilderness areas and are used by recreators for fishing, swimming and drinking water.

Annual patterns of bacteria levels were apparent at both sets of sites. Quartz Valley and Scott River sites had the highest *E. coli* levels (the bacterial parameter most commonly sampled at these sites) in July, though increased levels in the spring and fall were also present, likely due to rain events. At grazing allotment sites, fecal coliform levels (the bacterial parameter most commonly sampled at high elevation sites) gradually increased throughout the sampling season from June through October, peaking in October. Interannual trends in bacteria levels were less obvious, though there appears to be some increases in bacteria levels at grazing allotment sites since systematic monitoring began in 2011.

Comparisons of stream bacteria levels between actively grazed sites and retired or inaccessible ungrazed (i.e., control) sites suggest that cattle grazing is likely causing increased bacteria levels at high-elevation stream and lakes. Bacteria levels were higher at active grazing sites, both during the active grazing season as well as when cows were not present on allotments, likely due to fecal matter on the landscape from the previous grazing season. Comparisons of data at individual sites between when cows were not present on the allotments to the active grazing season show increases at all active grazing allotment sites, whereas bacteria levels at only some of the control sites increased during this same time period. Future monitoring of cattle use of specific stream reaches may help to explain the relationship between cattle use and bacteria levels in more detail.

When five samples of *E. coli* or fecal coliform were available in a 30-day period at any site, as specified by U.S. EPA and NCRWQCB criteria, we compared the geometric means and statistical threshold values to the water quality criteria for the respective bacterial indicator.

There were 24 sites that had samples collected frequently enough to conduct this comparison at least one time, most of which were sites within grazing allotments. Of the four Quartz Valley and Scott River sites, three had at least one water quality exceedance. At grazing allotment sites 12 of the 19 sites had at least one water quality exceedance. One of the five ungrazed allotment sites had at least one exceedance, whereas 11 of the 15 actively grazed allotment sites had at least one exceedance.

Many sites were sampled one to two times in a 30-day period, less frequently than the sampling schedule suggested by the water quality criteria. For sites sampled at least five times in one year between April and October, we combined all samples for the bacterial indicator sampled and calculated the geometric mean and percent of samples exceeding the statistical threshold values for that indicator bacteria. Although the results of these calculations should not be used to identify sites that do or do not exceed water quality criteria due to the difference in sampling frequency, they can be used to identify potential problem sites that warrant more frequent sampling to protect public health. We calculated statistics at 10 sites in the Quartz Valley and along the Scott River during at least one year. Some sites had sufficient data to calculate statistics for all eight years of the study. Seven of the 10 sites exceeded beach action values (the value of *E. coli* concentrations at which U.S. EPA suggests posting public health notifications for bacterial contamination) during at least one year of the study. At grazing allotment sites, both active and inactive control sites, a total of 27 sites had at least five bacteria samples during the April through October period. Of these 27 sites, 11 sites had bacteria levels above the beach action value or the single sample maximum (the value of fecal coliform concentration at which the NCRWQCB states individual samples should not exceed).

Understanding annual patterns of bacteria levels, inter-annual trends, and the spatial distribution of sites with high bacteria levels will allow for concentrating monitoring resources to times and sites with elevated public health risks. Comparison of high frequency bacteria samples to water quality criteria will assist the NCRWQCB in making decisions regarding which streams in the Scott River watershed to list as impaired by bacterial contamination. Additionally, initial analysis of the effects of cattle grazing on stream bacteria levels lays the foundation for more in-depth study of the relationship between cattle grazing and stream bacteria levels, if resources become available for these studies.

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1 INTRODUCTION

1.1 BACKGROUND

The 1964 Wilderness Act set aside specific public lands as places where "the earth and its community of life are untrammeled by man," however, cattle grazing allotments were "grand-fathered" in as long-term leases from the U.S. Forest Service (USFS) to private parties and grazing continues where it was occurring prior to the wilderness designation. Under the terms of the lease, and with monitoring by Forest Service personnel, grazing is managed so as to limit the impacts on wilderness characteristics and ecological function. Some level of disturbance is permitted and cattle presence is evident within wilderness areas as localized erosion to stream banks, areas of grazed vegetation, presence of cattle trails, and presence of cattle dung. Some wilderness users object to these observable impacts, and for some, the presence of cattle themselves is objectionable.

Quartz Valley Indian Reservation (QVIR), in cooperation with the Klamath National Forest (KNF), and the Northern California Resource Center (NCRC) have been monitoring stream bacteria concentrations associated with three cattle grazing allotments within wilderness areas in the Klamath National Forest during the months of August and September since 2012. Initial investigations began in 2007 when the QVIR began implementing their water quality-monitoring program focusing on understanding the health of the Shackleford sub-basin in the Scott River watershed and the associated impacts to Tribal cultural resources. The Shackleford grazing allotment is at the headwaters of the sub-basin, in the Marble Mountain Wilderness, and flows through the Reservation.

In 2011, members of the Tate Lab at U.C. Davis investigated the effects of cattle grazing on stream bacteria concentrations within 14 grazing allotments in Northern California, including sampling locations in the Shackleford allotment and the Mill Creek allotment, located within the Scott River Basin in the Trinity Alps Wilderness (Kromschroeder 2012, Roche et al. 2013). During the following three years QVIR, NCRC and USFS developed and implemented the current sampling program by building on the U.C. Davis study. Sampling now includes the Shackleford allotment, the Mill Creek allotment and the Kidder Creek allotment, which is a "retired" ungrazed allotment. Forest fires in 2014 prevented our sampling to continue in Shackleford and Kidder but the Mill Creek allotment was sampled.

Water samples are analyzed for total coliform, fecal coliform and/or *Escherichia coli (E.coli)* concentrations and compared to water quality objectives listed in the North Coast Regional Water Quality Control Board's (NCRWQCB) Basin Plan to determine whether presence of cattle grazing is associated with elevated bacteria in streams. As a threshold of comparison, we used the NCRWQCB REC-1 standards for fecal coliform—these fecal coliform standards are derived to protect contact recreation activities in which ingestion of water is reasonably likely, such as fishing, swimming, wading, and others.

Analysis has varied over the years and by project between fecal coliform and *E.coli* as an indicator group. During the 2014 season we collected both and will continue to collect both in the future in hopes of determining the relationship between the two indicators. Fecal coliform is

a class of total coliform and includes *E. coli* among other species. Total coliform would over estimate fecal coliform concentrations, while analysis of *E. coli* would under estimate fecal coliform. Total coliform includes *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter* bacteria genera originating from fecal or vegetative (environmental) sources. Fecal coliform includes *Escherichia, Klebsiella,* and *Citrobacter* genera. *E. coli (Escherichia coli)* is derived solely from human or animal dung. One source states that 60% to 90% of total coliforms are fecal and, 90% of fecal coliforms are *Escherichia* and typically *E. coli* (APHA 1992); however, data presented in Section 3.3 of this report indicates that at QVIR monitoring sites, only 5% of total coliforms are fecal, 2% of total coliforms are *E. coli*, and 50% of fecal coliforms are *E. coli*.

Best Management Practices (BMPs) were developed by the USFS for grazing allotments and annual checklists completed by USFS personnel are performed on randomly selected allotments. Through our water quality sampling efforts we hope to understand if current BMPs and checklists are adequate in assessing the impacts to water quality from cattle grazing.

In addition to monitoring streams within USFS grazing allotments, we have been monitoring streams within the Quartz Valley and sites along the Scott River for fecal indicator bacteria (FIB) as part of on-going water quality monitoring efforts (QVIR 2008, 2009, 2011, 2012, 2013). Annual and seasonal variations in bacteria concentrations have allowed the Environmental Program to prepare for tribal notifications of the health threats associated with popular swimming holes in the Scott River and Quartz Valley tributaries, as well as the wilderness lakes.

1.2 STUDY GOALS

The purpose of this study is to describe the patterns and concentrations of fecal indicator bacteria sampled in streams in the Scott River watershed during the initial eight-year monitoring period. We split the samples into two distinct groups (wilderness grazing allotment sites and baseline monitoring sites in the Quartz Valley and Scott River). For each group of sites, we 1) described seasonal and annual variation in bacteria concentrations; 2) calculated site-specific metrics to compare bacteria concentrations to water quality objectives; 3) suggested changes to past monitoring efforts to concentrate resources toward monitoring sites with high bacteria levels. Additionally, we compared grazed and ungrazed sites, as well as times of active grazing and times of no cattle present, to assess the impact of grazing on stream bacteria concentrations in grazing allotments.

1.3 DESCRIPTION OF STUDY AREA

Bacteria samples were collected throughout the 814 square mile Scott River watershed, located in northwest California (Figure 1, Figure 2, Table 1, and Table 2). Watershed elevation ranges from 8540 feet at China Mountain to 1460 feet at the confluence of the Scott River and Klamath River. Surface flows in the Scott River watershed are influenced by the Mediterranean climate regime, in which most precipitation occurs during the winter and summers are characterized by drought. Large discharge events occur due to winter rains and spring snowmelt. Land use in the watershed includes cattle grazing, agriculture, mining, logging, and urban development.

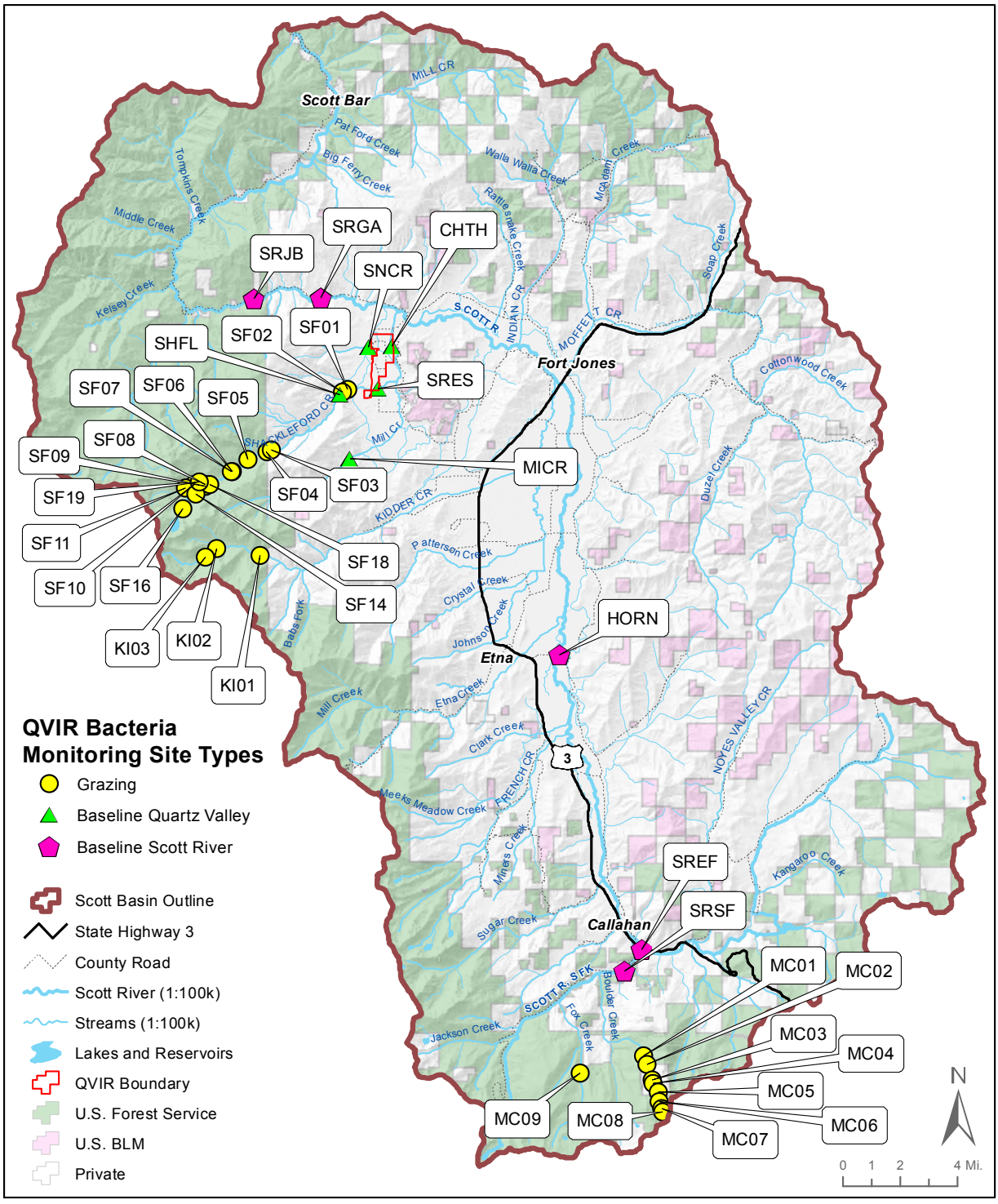

Figure 1. Map of all bacteria sampling locations in the Scott River watershed where more than five samples were collected during the eight-year study period.

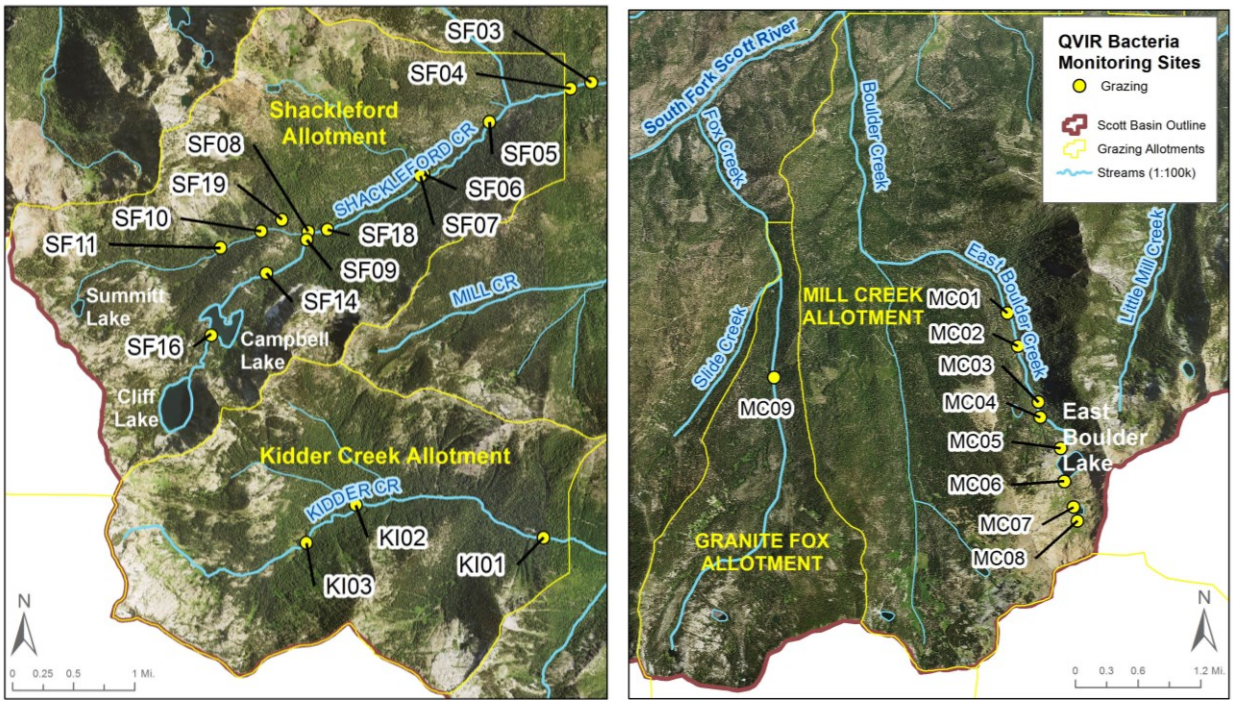

Figure 2. Map of bacteria sampling sites within U.S. Forest Service grazing allotments in the Scott River watershed where more than five samples were collected during the eight-year study period.

Table 1. Site code, description, management, and location for bacteria sampling sites with at least five samples collected during the study period in the Quartz Valley and Scott River

Baseline monitoring sites: Quartz Valley				
Site Code	**Site Description**	**Management**	**Latitude**	**Longitude**
SRES	Shackleford Creek at Quartz Valley	USA Indian Trust	41.59333	-122.97500
CHTH	Shackleford Creek near mouth	Private	41.61528	-122.96550
MICR	Mill Creek above Shackleford Creek	Private	41.55791	-122.99454
SHFL	Shackleford Creek at falls	Private	41.59055	-123.00080
SNCR	Sniktaw Creek	USA Indian Trust	41.61473	-122.98150

Baseline monitoring sites: Scott River				
Site Code	**Site Description**	**Management**	**Latitude**	**Longitude**
SRSF	Scott River South Fork	USFS	41.29569	-122.80900
SREF	Scott River East Fork	USFS	41.30664	-122.79740
HORN	Scott River at Horn Lane	Private	41.45741	-122.85248
SRGA	Scott River at Gage	Private	41.64000	-123.01380
SRJB	Scott River at Jones Beach	USFS	41.63944	-123.05910

Table 2. Site code, description, location and grazing status for bacteria sampling sites with at least five samples collected during the study period in Forest Service grazing allotments in the Scott River headwaters.

Shackleford Allotment: Marble Mountain Wilderness

Site Code	Site Description	Latitude	Longitude	Grazing Status
SF01	Shackleford Cr. below Shackleford Falls and swimming hole	41.59292	-122.99569	active
SF02	Shackleford Cr. above Shackleford Falls and swimming hole	41.59173	-122.99964	active
SF04	Shackleford Cr. above trailhead and corral	41.56136	-123.05067	active
SF03	Shackleford Cr. below corral and culvert	41.56210	-123.04741	active
SF05	Shackleford Cr. at gate below meadow complex, directly below rock wall	41.55739	-123.06350	active
SF06	Long High Cr. above confluence with Shackleford Cr. and above trail crossing	41.55118	-123.07390	active
SF07	Shackleford Cr. above confluence with Long High Cr.	41.55097	-123.07440	active
SF08	Shackleford Cr. above Campbell Lake tributary (directly above log crossing)	41.54436	-123.09220	active
SF09	Isolates Campbell Lake tributary before confluence with Shackleford Cr.	41.54341	-123.09240	active
SF10	Shackleford Cr. below Log Lake Meadow complex and slightly above lake	41.54439	-123.09960	active
SF11	Shackleford Cr. above Log Lake	41.54243	-123.10600	active
SF14	Campbell Lake tributary below Campbell lake (swimming and dispersed camping) and above confluence with unnamed tributary	41.53941	-123.09877	active
SF16	Campbell Lake tributary above inlet for Campbell Lake	41.53205	-123.10750	active
SF18	Isolates Emerald Tributary, ungrazed without heavy recreation	41.54459	-123.08910	inactive
SF19	Isolates unnamed tributary above confluence with Shackleford Cr., near trail split for Calf Lake	41.54576	-123.09630	active

Kidder Creek Allotment: Marble Mountain Wilderness

Site Code	Site Description	Latitude	Longitude	Grazing Status
KI01	Kidder Creek above first unnamed tributary crossing Kidder Creek Trail from the wilderness boundary	41.50808	-123.05490	inactive
KI02	Kidder Creek above Kidder Lake tributary	41.51194	-123.08460	inactive
KI03	Kidder Creek adjacent to Hays Meadow	41.50744	-123.09240	inactive

Mill Creek Allotment: Trinity Alps Wilderness

Site Code	Site Description	Latitude	Longitude	Grazing Status
MC01	East Boulder Cr. below trail head and camping	41.25240	-122.79600	active
MC02	East Boulder Cr. below first meadow	41.24780	-122.79409	active
MC03	East Boulder Cr. above confluence	41.23999	-122.79030	active
MC04	Unnamed tributary above confluence with East Boulder Cr.	41.23790	-122.79000	active
MC05	East Boulder Cr. at the outlet of East Boulder Lake, below meadow complex	41.23361	-122.78625	active
MC06	East Boulder Cr. above inlet for East Boulder Lake	41.22897	-122.78560	active
MC07	East Boulder Cr. below outlet for Upper Lake	41.22540	-122.78390	active
MC08	East Boulder Cr. above inlet for Upper Lake	41.22347	-122.78320	active
MC09	Fox Creek at road, no grazing in this drainage	41.24351	-122.83900	inactive

Figure 3. Photographs: (A) cattle grazing in lakeside meadow, (B) Campbell Lake within the Shackleford Grazing Allotment, and (C) a llama assisting QVIR pack sampling gear into the backcountry.

Mill Creek Allotment: Trinity Alps Wilderness

Site Code	Site Description	Latitude	Longitude	Grazing Status
MC01	East Boulder Cr. below trail head and camping	41.25240	-122.79600	active
MC02	East Boulder Cr. below first meadow	41.24780	-122.79409	active
MC03	East Boulder Cr. above confluence	41.23999	-122.79030	active
MC04	Unnamed tributary above confluence with East Boulder Cr.	41.23790	-122.79000	active
MC05	East Boulder Cr. at the outlet of East Boulder Lake, below meadow complex	41.23361	-122.78625	active
MC06	East Boulder Cr. above inlet for East Boulder Lake	41.22897	-122.78560	active
MC07	East Boulder Cr. below outlet for Upper Lake	41.22540	-122.78390	active
MC08	East Boulder Cr. above inlet for Upper Lake	41.22347	-122.78320	active
MC09	Fox Creek at road, no grazing in this drainage	41.24351	-122.83900	inactive

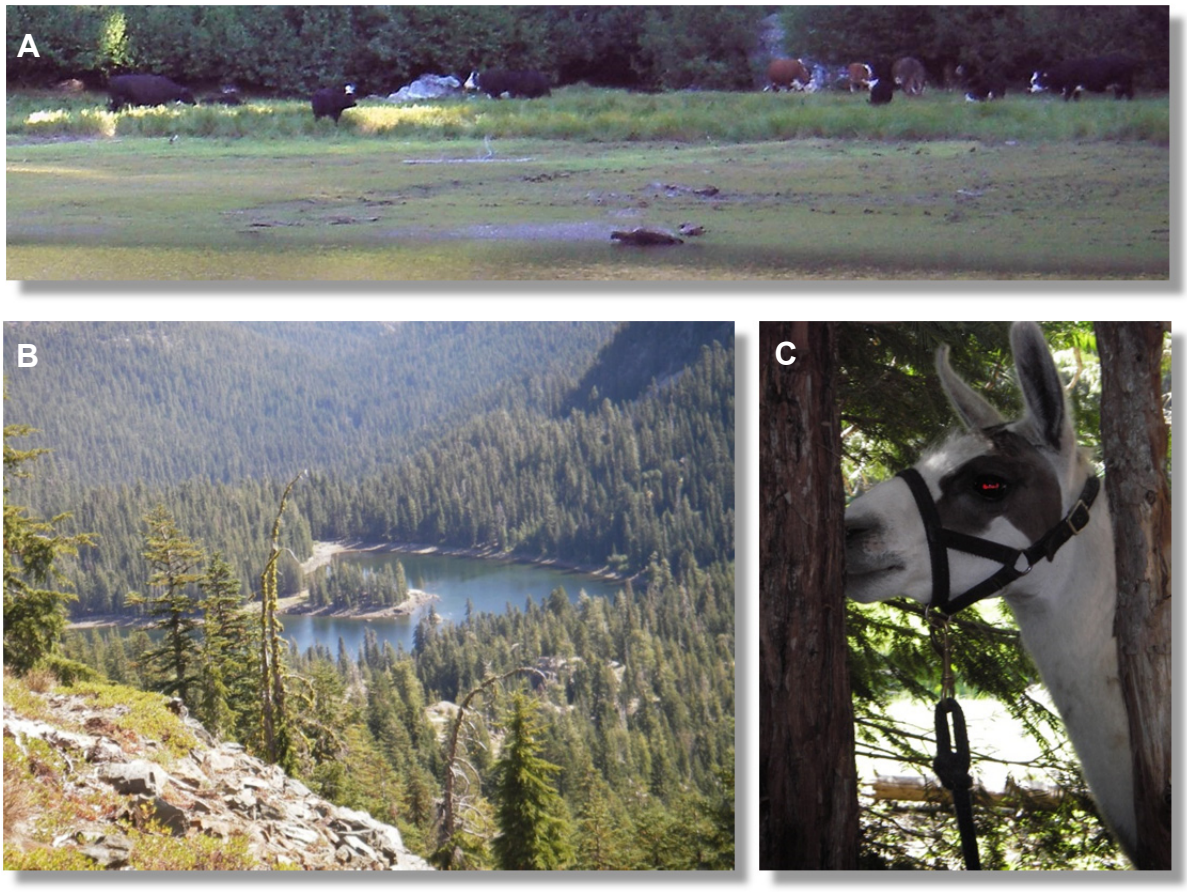

Figure 3. Photographs: (A) cattle grazing in lakeside meadow, (B) Campbell Lake within the Shackleford Grazing Allotment, and (C) a llama assisting QVIR pack sampling gear into the backcountry.

2 METHODS

2.1 SAMPLING METHODS

Fecal indicator bacteria samples were collected at over 55 sites (Appendix A) from 2007 to 2014 from streams in the Scott River watershed. Samples were analyzed for one to three bacterial indicators (*E. coli*, fecal coliform, or total coliform) depending on the site and the year (Figure 4). Sampling was performed by three crews of two technicians each working simultaneously in each allotment, using a clean catch method, as described in the sampling protocol provided at the end of this document (Appendix B). In 2011, sampling at grazing allotments (as described below) was conducted by the Tate Lab (Kromschroeder 2012).

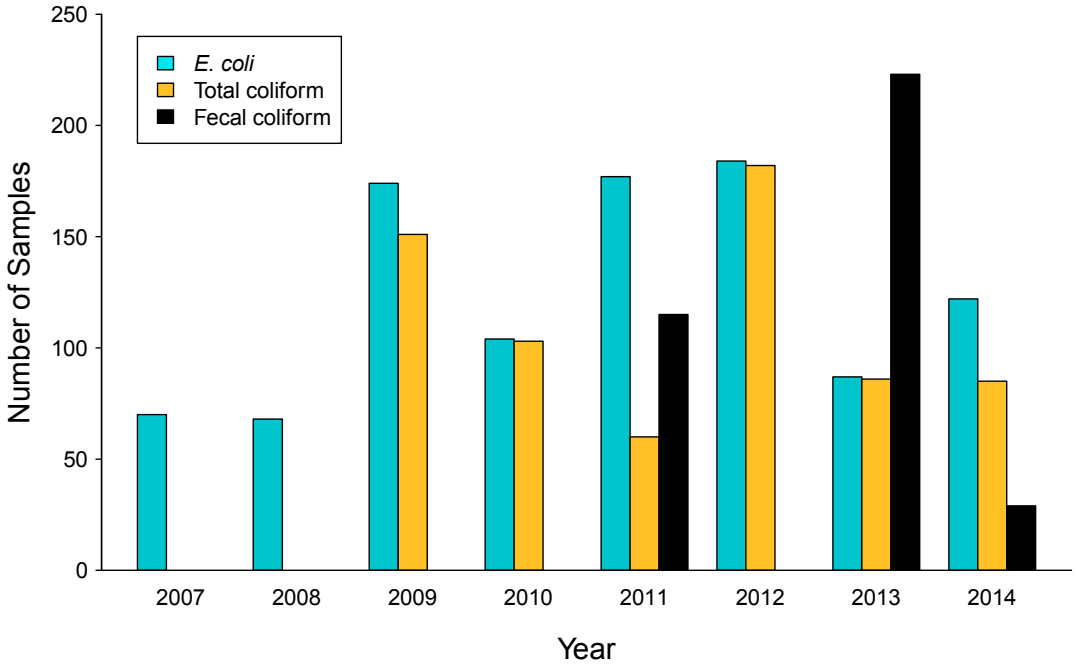

Figure 4. Number of fecal indicator bacteria samples by sample type and year for all sites in this study.

Two sets of data were collected during the eight-year sampling period. Baseline monitoring sites in the Quartz Valley and Scott River were monitored beginning with *E. coli* monitoring in 2007 (Figure 5). Additional sites and FIB indicators were added in 2009. Sites were generally sampled from April through October, with additional sample collection in the winter and early spring during 2009 and 2010. Total coliform and *E. coli* were sampled at most sites during most years, while fecal coliform was only sampled during 2013 at these baseline monitoring sites.

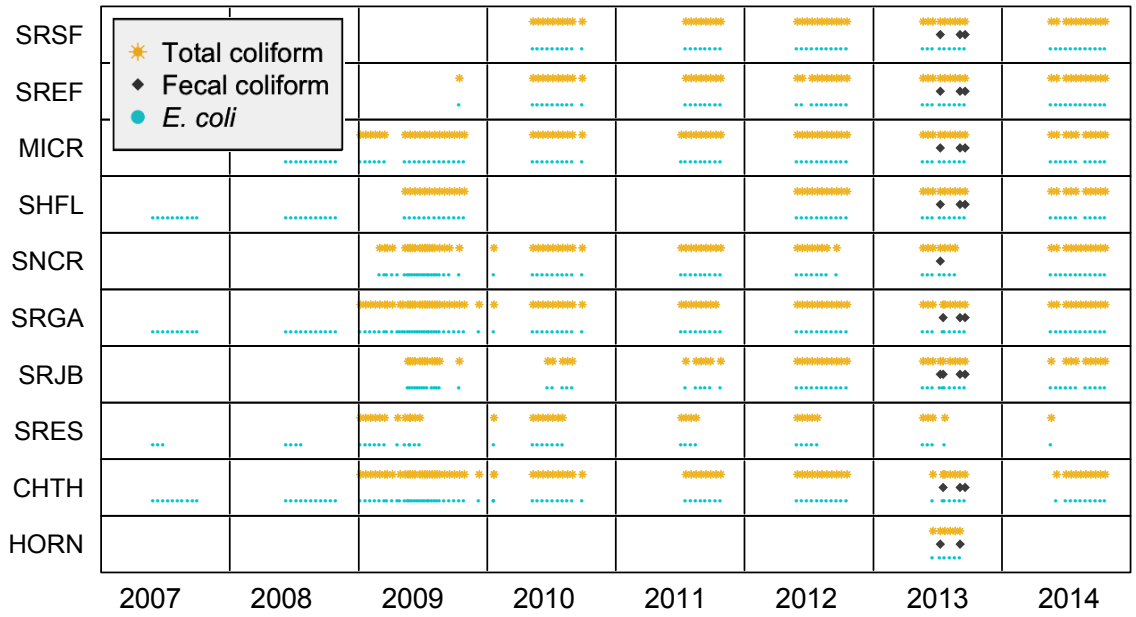

Figure 5. Sample timing by date and indicator bacteria type for baseline monitoring sites in the Quartz Valley and Scott River at sites with more than five samples present.

Bacteria samples were collected in grazing allotments in the Scott River headwaters. Three allotments were sampled, with the majority of the sampling occurring between 2011 and 2014, when six to 23 sites were sampled annually. Prior to 2011, only one to four sites were sampled annually, all within the Shackleford allotment(Figure 6, Figure 7, and Figure 8). In 2011, samples were collected monthly from June through November at most sites within the Shackleford and Mill Creek allotments. In 2012, samples were collected weekly from mid-August through September in the previously sampled allotments, as well as in the Kidder Creek allotment, which is no longer actively grazed. Additional samples were collected at some sites in 2012. In 2013, weekly samples were collected for five weeks in early summer, and again for five weeks in late summer at all sites, and analyzed for fecal coliform only. In 2014, samples were collected at only six sites, all within the Mill Creek allotment, according to the same sampling schedule as 2013. Samples were not collected from the Shackleford or Kidder Creek allotments in 2014 due to wildfires.

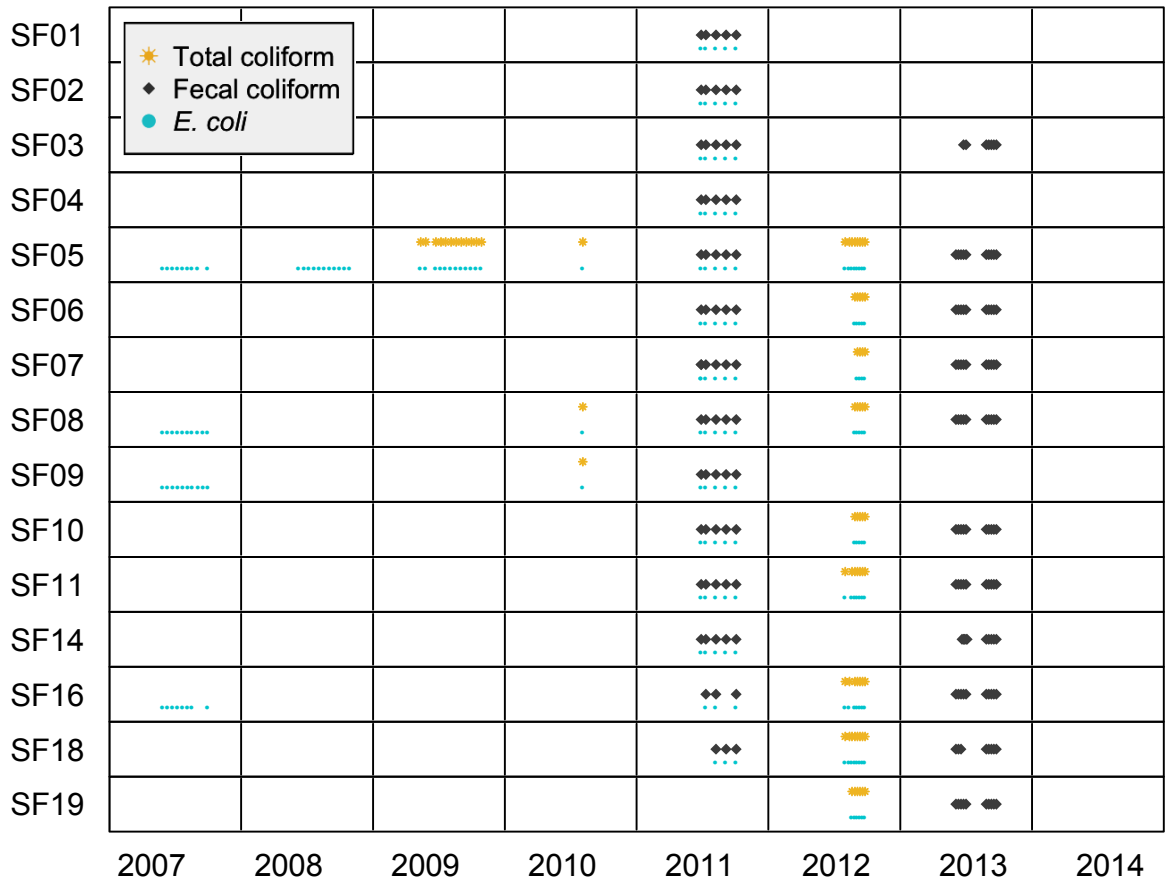

Figure 6. Sample timing by date and indicator bacteria type at sampling sites in the Shackleford Creek Grazing allotment at sites with more than five samples present.

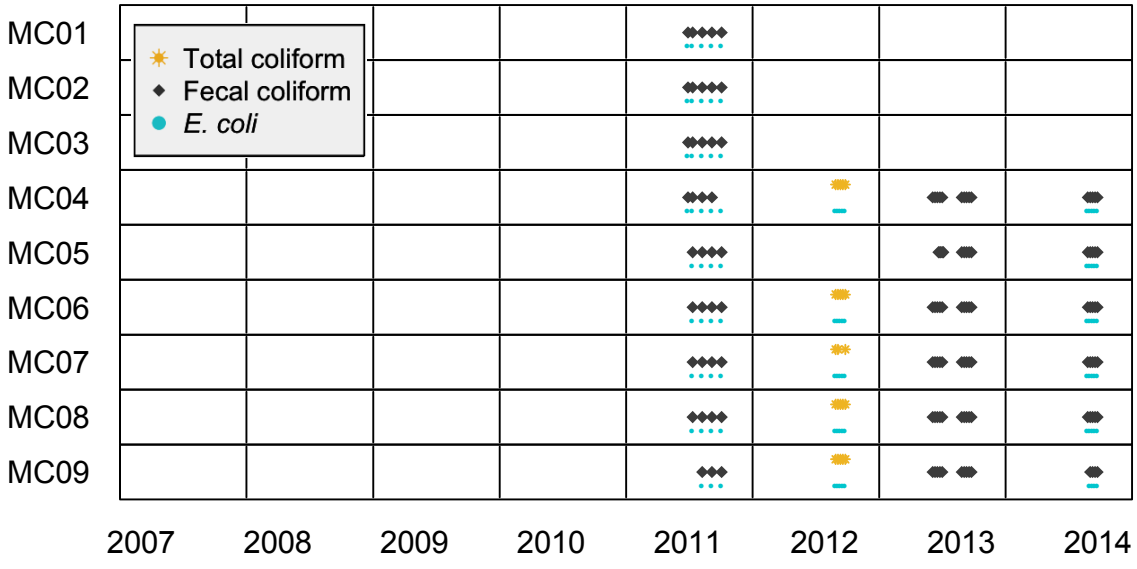

Figure 7. Sample timing by date and bacteria type at sampling sites in the Mill Creek Grazing allotment.

Figure 8. Sample timing by date and indicator bacteria type at sampling sites in the Kidder Creek Grazing allotment (retired allotment; no grazing during study period).

2.2 SAMPLE COLLECTION AND LABORATORY ANALYSIS

A vertical, depth-integrated stream water collection was made at the stream channel thalweg. Water was collected in sterilized, acid-washed one liter sample containers, which were immediately stored on ice. All samples were analyzed for fecal coliform, total coliform and/or *E. coli* within eight hours of field collection.

Most samples were analyzed at the Quartz Valley Indian Reservation's state certified bacteria laboratory using IDEXX Colilert/Quantity Trays 2000 System, following Standard Method number 9223 B, the Enzyme Substrate Coliform Test. Sample data is expressed as a density or concentration using the units cfu/100 ml, which is the most probable number of colony forming units per 100 ml of sample.

Samples from the grazing allotments in 2011 were determined by direct one step membrane filtration and incubation on selective agar following standard method SM9222D at the U.C. Davis Tate Laboratory (Kromschroeder 2012).

2.3 DATA ANALYSIS

We compiled data from eight years of sampling in the Scott River watershed. The complete dataset is included as Electronic Appendix E. We categorized samples by study type ("bg" for Scott River and Quartz Valley sites, grazing for grazed allotment sites, ungrazed for allotment sites without active grazing, and "ss" for samples taken as part of a special study). Here, we report on the first three data categories, and exclude special studies due to different sampling strategies and goals. We included only one sample per site per day in our analysis, so when more than one sample was present, we labeled the second sample as a duplicate in the database. We excluded all duplicates and blanks from the analysis. Data entered as <1 was forced to 1, and data entered as >2419.6, the maximum number of countable cells per plate, was forced to 2420.

We calculated descriptive statistics for bacteria samples taken from the grazing allotments in the Scott River headwaters. We calculated the mean, standard deviation, median, and maximum values for each allotment (Shackleford, Mill Creek, and Kidder Creek) by year. Calculations were made for *E. coli,* total coliform, and fecal coliform, when more than one sample was present per year.

We examined temporal trends in the FIB data. To assess annual variation in FIB levels, we plotted medians, interquartile ranges, arithmetic means and geometric means of *E. coli* and total coliform by month at the baseline monitoring sites in the Quartz Valley and Scott River. We calculated geometric means by adding 1 to all input values before multiplying the values and taking the n^{th} root of the product, and then subtracted 1 from the final product. The extra step of adding 1 to each number and subtracting 1 from the product accounts for zeros in the dataset.

We assessed interannual trends in FIB levels by comparing *E. coli* levels at the three sites (MICR, SRGA, and CHTH) that were sampled each year from 2008 to 2014 during the same summer and autumn dates. We compared levels during June through October each year when sampling was conducted on the same date at each site as to not introduce bias from seasonal variation.

We assessed temporal trends in fecal coliform and *E. coli* from grazing allotment sites by month from June through October, using data from all grazing allotment sites and years. To examine annual variation, we compared *E. coli* levels at five sites in the Mill Creek allotment (MC04, MC06, MC07, MC08, MC09) in 2011, 2012, and 2014 during August and September, when all five of these sites were sampled at least one time per month. For fecal coliform, we compared the sites listed above, as well as MC05, which also had fecal coliform data during three years. We compared medians, ranges, arithmetic and geometric means at these sites for 2011, 2013, and 2014 as an indicator of annual variation in bacteria concentrations in the grazing allotments.

Some samples included laboratory analysis for more than one FIB parameter, enabling comparisons between *E. coli*, fecal coliform, and total coliform concentrations. We used two techniques to assess the relationship between the FIB parameters. The first was to construct scatterplots on a log-log scale, with a Locally Estimated Scatterplot Smoothing (LOESS) regression curve (Helsel and Hirsch 2002) fit as a visual aid. The LOESS smoother is useful for visually examining the overall relationship between two parameters when there are many data points, considerable scatter, and the relationship is non-linear. The second technique was to calculate the ratios between FIB parameters (i.e., *E. coli*:fecal coliform).

We assessed the effect of cattle grazing on stream bacteria levels. We compared grazed sites to ungrazed sites (retired allotments or sites inaccessible to cattle) by plotting medians and ranges of fecal coliform samples on these two site types, both before and after active grazing. We plotted medians and ranges of fecal coliform at each site while cattle were actively grazing and while cattle were not actively grazing.

We compared bacteria levels to regulatory benchmarks to identify sites posing potential public health risks. Water quality criteria recommendations and water-board standards suggest collecting and analyzing a minimum of five bacteria samples in a 30-day period (U.S. EPA 2012, NCRWQCB 2011). The U. S. EPA criterion suggests using *E. coli* as the primary indicator of fecal contamination of freshwater, whereas the NCRWQCB suggests using fecal coliform. Although regulatory agencies suggest using the five samples with the highest bacteria levels during the 30-day period, there were no cases when more than five samples were collected during a 30-day period in our data set. We calculated the geometric mean as described above any time that five samples of *E. coli* or fecal coliform taken within a 30-day period. We calculated the percent of samples during that same period that exceeded the benchmarks set forth by the U.S. EPA and NCRWQCB (Table 3).

Table 3. Benchmarks and threshold values for *E. coli* and fecal coliform from U.S. EPA 2012 standards and the NCRWQCB Basin Plan

Abbre-viation	Definition	Threshold Value (cfu/100ml)	Illness Rate	Bacterial Indicator	Source
BAV	Beach action value	190	32/1000	*E. coli*	U.S. EPA (2012)
BAV	Beach action value	235	36/1000	*E. coli*	U.S. EPA (2012)
STV	Statistical threshold value	320	32/1000	*E. coli*	U.S. EPA (2012)
STV	Statistical threshold value	410	36/1000	*E. coli*	U.S. EPA (2012)
SSM	Single sample maximum	400	NA	Fecal coliform	NCRWQCB (2011)

We marked the "exceeded" category with a "yes" if: 1) the geometric mean for *E. coli* was more than 100 cfu/100 ml; 2) the geometric mean for fecal coliform was over 50 cfu/100 ml; or 3) any of the benchmarks in Table 3 were exceeded by more than 10% of samples at a site for any given year. Because *E. coli* is a sub-set of fecal coliform, it may be more appropriate to compare *E. coli* to the fecal coliform standard of 50 cfu/100 ml, or even a lower number.

We calculated similar metrics as above for bacteria samples collected from April through October. Because a majority of the bacteria sampling was conducted less frequently than five samples in 30 days, we calculated seasonal geometric means and percent of samples exceeding threshold and benchmark values (Table 3) at each site and each year when a minimum of five samples were taken for either *E. coli* or fecal coliform. We also combined samples from each site across all years and calculated geometric means and percent of samples exceeding threshold values. Although data from these sites should not be compared directly to water quality criteria recommendations due to variable sampling frequencies, the data can be used to identify potential sites of contamination where more sampling should be conducted in the future.

3 RESULTS AND DISCUSSION

3.1 COMPARING BACTERIA LEVELS TO REGULATORY BENCHMARKS

Bacteria samples were not routinely collected at Scott River and Quartz Valley sites frequently enough to directly compare the geometric means and statistical threshold values to the U.S. EPA standards. In 2009, higher frequency sampling of *E. coli* was conducted at four sites for portions of the year that resulted in five samples collected within a 30-day period. We were able to compare *E. coli* levels to regulatory benchmarks once at SRJB, and three times each at SRGA, CHTH, and SNCR, resulting in a total of 10 comparisons. Geometric means exceeded 100 cfu/100 ml at 50% of the comparisons, and the statistical threshold value was exceeded at 60% of the comparisons, resulting in seven threshold exceedances (Table 4).

Table 4. Geometric means (GM) and percent of samples that exceeded regulatory benchmarks* for *E. coli* in 2009 at Quartz Valley and Scott River sites based on five samples collected within a 30-day period.

Site	Year	Date of 1st sample	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)	Exceeded?
SRJB	2009	21 May	5	47	0%	0%	0%	0%	
SRGA	2009	22 Apr	5	159	40%	40%	40%	40%	yes
SRGA	2009	27 May	5	73	20%	20%	20%	20%	yes
SRGA	2009	01 Jul	5	40	20%	20%	0%	0%	
SNCR	2009	13 May	5	153	40%	40%	20%	20%	yes
SNCR	2009	17 Jun	5	331	80%	80%	40%	40%	yes
SNCR	2009	21 Jul	5	562	80%	80%	80%	80%	yes
CHTH	2009	13 May	5	25	0%	0%	0%	0%	
CHTH	2009	17 Jun	5	128	40%	20%	0%	0%	yes
CHTH	2009	21 Jul	5	63	40%	20%	20%	0%	yes

* For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators) or if the BAV, or STV threshold is exceeded by 10% or more of the samples, we considered that site year as having a standard exceeded.

Bacteria sampling at grazing allotment sites was often conducted more frequently, which resulted in five samples collected in a 30-day period in many cases. In 2012, high frequency sampling for *E. coli* was conducted at 16 grazing allotment sites, five of which were ungrazed control sites (Table 5). Sampling was conducted beginning on August 28th (with the exception of SF11 and SF19, in which sampling began on August 20th), and continued approximately weekly, resulting in five samples collected during 30 days or less. The 2012 samples were collected during the active grazing season. The statistical threshold value was exceeded at one ungrazed site and three grazed sites, while the geometric means never exceeded 100 cfu/100 ml in 2012. High frequency bacteria sampling is important in the grazing allotment sites, where there is high temporal variability in *E. coli* levels as demonstrated through exceedance of statistical threshold values rather than geometric means. Swimmers and wilderness area users relying on these streams for drinking water should be aware that variability in bacteria levels is common, and sampling results may not capture all spikes.

Table 5. Geometric means (GM) and percent of samples that exceeded regulatory benchmarks* for *E. coli* in 2012 at grazing allotment sites based on five samples collected within a 30-day period.

				E. coli							
Site	Year	Date of 1st sample	Grazing status	Cows on?	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)	Exceed -ed?
SF18	2012	28 Aug	ungrazed	no	5	20	20%	20%	20%	20%	yes
MC09	2012	28 Aug	ungrazed	no	5	27	0%	0%	0%	0%	
KI01	2012	28 Aug	ungrazed	no	5	2	0%	0%	0%	0%	
KI02	2012	28 Aug	ungrazed	no	5	1	0%	0%	0%	0%	
KI03	2012	28 Aug	ungrazed	no	5	6	0%	0%	0%	0%	
MC04	2012	28 Aug	grazed	yes	5	71	0%	0%	0%	0%	
MC06	2012	28 Aug	grazed	yes	5	14	20%	20%	0%	0%	
MC07	2012	28 Aug	grazed	yes	5	48	20%	20%	20%	0%	yes
MC08	2012	28 Aug	grazed	yes	5	33	0%	0%	0%	0%	
SF05	2012	28 Aug	grazed	yes	5	14	0%	0%	0%	0%	
SF06	2012	28 Aug	grazed	yes	5	3	0%	0%	0%	0%	
SF08	2012	28 Aug	grazed	yes	5	2	0%	0%	0%	0%	
SF10	2012	28 Aug	grazed	yes	5	5	0%	0%	0%	0%	
SF11	2012	20 Aug	grazed	yes	5	15	20%	20%	20%	20%	yes
SF16	2012	28 Aug	grazed	yes	5	3	0%	0%	0%	0%	
SF19	2012	20 Aug	grazed	yes	5	62	20%	20%	20%	20%	yes

* For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators) or if the BAV or STV threshold is exceeded by 10% or more of the samples, we considered that site year as having a standard exceeded.

In 2013 sampling occurred both before grazing and during grazing at active grazing sites, and during similar dates at the ungrazed sites. We calculated exceedances for fecal coliform only (no *E. coli* data were collected in 2013 at the grazing allotment sites) at 18 sites prior to the grazing season, with the first sample in the set of five having been collected between June 4th and June 19th. No exceedances occurred during this time. The second set of samples were collected weekly beginning on August 27th. No exceedances occurred at the ungrazed sites. At the actively grazed sites, geometric means exceeded 50 cfu/100 ml at eight sites, with an additional site having a single sample exceeding the single sample maximum value of 400 cfu/100 ml, resulting in a total of nine exceedances, or 60% of grazed sites during the active grazing season (Table 6).

Table 6. Geometric means (GM) and percent of samples that exceeded regulatory benchmarks* for fecal coliform in 2013 based on five samples collected within a 30-day period.

					Fecal coliform			
Site	Year	Date of 1st sample	Grazing status	Cows present	n	GM	SSM	Exceeded?
MC09	2013	04 Jun	ungrazed	no	5	2	0%	
KI01	2013	04 Jun	ungrazed	no	5	3	0%	
KI02	2013	04 Jun	ungrazed	no	5	2	0%	
KI03	2013	04 Jun	ungrazed	no	5	1	0%	
MC04	2013	04 Jun	grazed	no	5	10	0%	
MC05	2013	19 Jun	grazed	no	5	2	0%	
MC06	2013	04 Jun	grazed	no	5	3	0%	
MC07	2013	04 Jun	grazed	no	5	2	0%	
MC08	2013	04 Jun	grazed	no	5	4	0%	
SF05	2013	04 Jun	grazed	no	5	3	0%	
SF06	2013	04 Jun	grazed	no	5	1	0%	
SF07	2013	04 Jun	grazed	no	5	3	0%	
SF08	2013	04 Jun	grazed	no	5	8	0%	
SF10	2013	04 Jun	grazed	no	5	7	0%	
SF11	2013	04 Jun	grazed	no	5	2	0%	
SF14	2013	19 Jun	grazed	no	5	1	0%	
SF16	2013	04 Jun	grazed	no	5	6	0%	
SF19	2013	04 Jun	grazed	no	5	2	0%	
SF18	2013	27 Aug	ungrazed	no	5	10	0%	
MC09	2013	27 Aug	ungrazed	no	5	13	0%	
KI01	2013	27 Aug	ungrazed	no	5	7	0%	
KI02	2013	27 Aug	ungrazed	no	5	3	0%	
KI03	2013	27 Aug	ungrazed	no	5	3	0%	
MC04	2013	27 Aug	grazed	yes	5	766	80%	yes
MC05	2013	27 Aug	grazed	yes	5	57	0%	yes
MC06	2013	27 Aug	grazed	yes	5	37	0%	
MC07	2013	27 Aug	grazed	yes	5	95	20%	yes
MC08	2013	27 Aug	grazed	yes	5	19	0%	
SF03	2013	27 Aug	grazed	yes	5	9	0%	
SF05	2013	27 Aug	grazed	yes	5	196	20%	yes
SF06	2013	27 Aug	grazed	yes	5	4	0%	
SF07	2013	27 Aug	grazed	yes	5	68	0%	yes
SF08	2013	27 Aug	grazed	yes	5	9	0%	
SF10	2013	27 Aug	grazed	yes	5	163	0%	yes
SF11	2013	27 Aug	grazed	yes	5	47	20%	yes
SF14	2013	27 Aug	grazed	yes	5	55	0%	yes
SF16	2013	27 Aug	grazed	yes	5	49	0%	
SF19	2013	27 Aug	grazed	yes	5	141	20%	yes

* The single sample maximum (SSM) for fecal coliform is set at 400 cfu/100 ml. If the GM is above 50 cfu/100 ml for fecal coliform or if more than 10% of samples are above the SSM limit, we considered that site as having a standard exceeded.

In 2014 only five sites were sampled five times within 30 days, all within the Mill Creek grazing allotment, because a wildfire prevented access to sites within the Kidder Creek and Shackleford allotments. All sites were active grazing sites, sampled while cows were present on the allotment (Table 7). Bacteria samples from 2014 were analyzed for both *E. coli* and fecal coliform. Two of the five sites exceeded regulatory benchmarks for *E. coli*, while all five sites exceeded the benchmarks for fecal coliform. Regulatory standards for fecal coliform are set by the NCRWQCB and have lower limits for geometric means than the standards recommended by the U.S. EPA (2012) for *E. coli*, even though *E. coli* levels are generally about half of fecal coliform levels (Figure 27).

Table 7. Geometric means (GM) and percent of samples that exceeded regulatory benchmarks* for *E. coli* and fecal coliform in 2014 based on five samples collected within a 30-day period.

				E. coli					**Fecal coliform**			
Year	Site	Date of 1st sample	Grazing status	Cows present	n	GM	STV (3.2%)	STV (3.6%)	n	GM	SSM	Exceed -ed?
2014	MC04	26 Aug	grazed	yes	5	38	20%	20%	5	83	20%	yes
2014	MC05	27 Aug	grazed	yes	5	7	0%	0%	5	75	20%	yes
2014	MC06	28 Aug	grazed	yes	5	41	0%	0%	5	77	0%	yes
2014	MC07	29 Aug	grazed	yes	5	129	40%	40%	5	324	60%	yes
2014	MC08	30 Aug	grazed	yes	5	18	0%	0%	5	45	20%	yes

* For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. In 2014, the percent of samples exceeding BAV were the same as the percent of samples exceeding STVs. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The single sample maximum (SSM) for fecal coliform is set at 400 cfu/100 ml. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators), over 50 cfu/100 ml for fecal coliform, or if any of the BAV, STV, or SSM data is exceeded at a rate of 10% or more of the samples, we considered that site year as having a standard exceeded.

3.2 BACTERIA CONCENTRATIONS BY SITE AND YEAR

3.2.1 OVERALL TRENDS IN BACTERIA LEVELS IN RELATION TO BENCHMARKS

We calculated the geometric means and the percent of samples which exceeded water quality recommendations at a total of 37 sites over eight years, resulting in 136 individual site-year comparisons of *E. coli* levels and 68 site-year comparisons of fecal coliform levels. In considering all sites and years, the highest seasonal levels of *E. coli* occurred in the Quartz Valley, though these high levels occurred at one site only (SNCR, see section 3.2.2 below). Scott River sites had the highest seasonal median *E. coli* levels, followed closely by the Mill Creek grazing allotment sites (Figure 9). The lowest seasonal median *E. coli* levels occurred in the Kidder Creek grazing allotment.

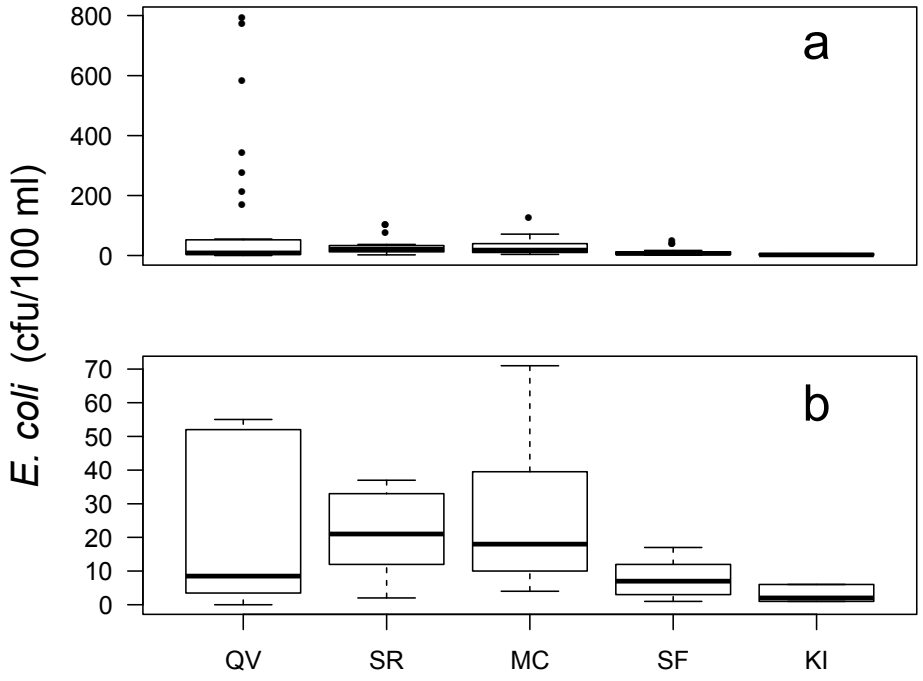

Figure 9. Median and range of *E. coli* seasonal geometric means at sites in each of the five study areas from 2007 to 2014. Panel A includes outliers; panel B does not show outliers for closer examination of medians and ranges. (QV= Quartz Valley, SR = Scott River, MC= Mill Creek Allotment, SF = Shackleford Allotment, and KI= Kidder Creek Allotment).

Fecal coliform samples were primarily collected in grazing allotments at high elevation sites. We did not calculate site-specific metrics for fecal coliform at the baseline monitoring sites because there were no sites with five or more samples collected during the primary water contact season (Figure 5). Medians of the site-year geometric means were similar for the Mill Creek and Shackleford Creek allotments, though higher seasonal levels of fecal coliform occurred in the upper quantile of the data in the Mill Creek allotment than in the Shackleford Creek allotment (Figure 10). Seasonal medians of fecal coliform were lowest at the ungrazed Kidder Creek allotment.

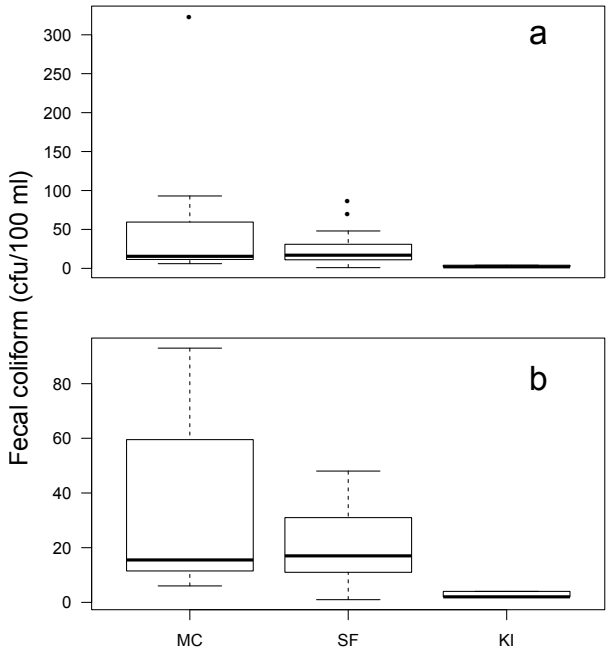

Figure 10. Median and range of fecal coliform levels at sites in each of the three grazing allotments from 2007 to 2014. Panel A includes outliers; panel B does not show outliers for closer examination of medians and ranges. (MC= Mill Creek Allotment, SF = Shackleford Allotment, and KI= Kidder Creek Allotment).

The site-specific geometric mean and percent of samples exceeding regulatory benchmarks at each site are likely lower in the analysis of April through October data than what they would be if more frequent sampling occurred throughout the summer. In many cases, samples were collected every two weeks throughout the summer, and we calculated the April through October geometric mean of all of these samples, so we include both times of lower and higher bacteria levels. At sites in the Quartz Valley and Scott River, there is a seasonal pattern in *E. coli* levels, where median sample concentrations in July (median = 36 cfu/100 ml) are more than twice that of May (median = 16 cfu/100 ml) and nearly triple the September median (median = 12 cfu/100 ml, Figure 34). Similarly, median samples at grazing allotments were three times higher in October than in June and July, and about a third higher in October than in August and September (Figure 36). If samples were collected weekly during the time of highest bacteria levels, we may see rates double or triple of those reported in the following calculations.

3.2.2 BACTERIA LEVELS IN THE QUARTZ VALLEY AND SCOTT RIVER

E. coli levels in the Quartz Valley were generally below regulatory benchmarks, with the exception of one site (Figure 11). Annual water-contact season (April through October) geometric means were below 100 cfu/100 ml during all sampling years at four sites (Figure 11). Sniktaw Creek (SNCR) was the exception, where annual geometric means were above 100

cfu/100 ml for every sampling year, with recent years having geometric means near 800 cfu/100 ml (Figure 12). Although seasonal geometric means at CHTH were always below the benchmark, they were higher than the other three Quartz Valley sites, and more than 10% of samples exceeded beach action values during two years of sampling (Figure 13, Table 8).

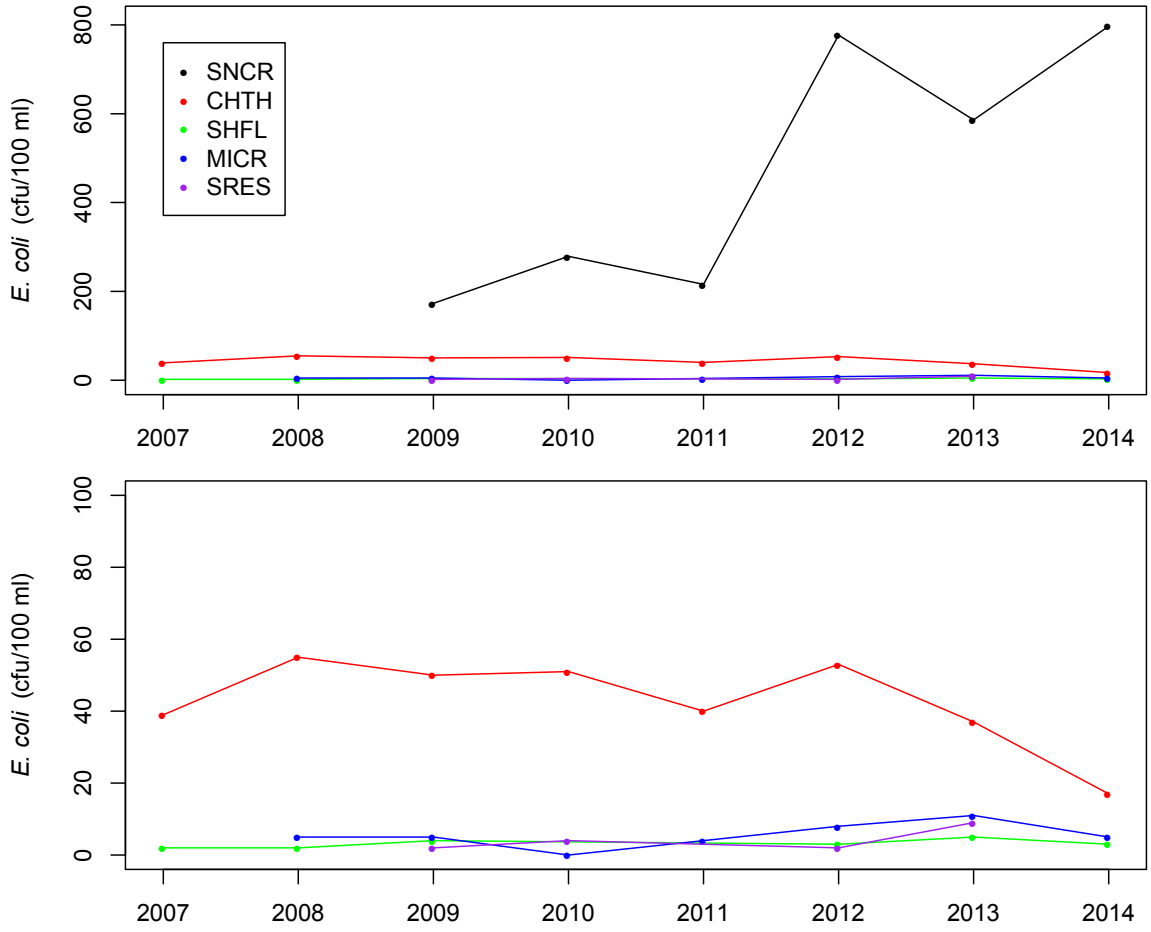

Figure 11. Geometric means of *E. coli* concentrations by year at sampling sites in the Quartz Valley (means are based on samples collected from April–October). The bottom panel is a zoomed-in version of the data, excluding the site on Sniktaw Creek (SNCR).

Table 8. Number of *E. coli* samples (n), geometric means (GM), and percent of samples from April through October exceeding regulatory standards at individual sites by year in the Quartz Valley.

		E. coli					
Year	Site	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)
2007	SHFL	10	2	0%	0%	0%	0%
2007	CHTH	10	39	20%	0%	0%	0%
2008	SHFL	11	2	0%	0%	0%	0%
2008	MICR	11	5	0%	0%	0%	0%
2008	CHTH	11	55	0%	0%	0%	0%
2009	SHFL	13	4	0%	0%	0%	0%
2009	SNCR	20	173	50%	50%	35%	35%
2009	MICR	13	5	0%	0%	0%	0%
2009	CHTH	23	50	26%	17%	13%	9%
2009	SRES	5	2	0%	0%	0%	0%
2010	SNCR	10	279	60%	60%	60%	60%
2010	MICR	10	0	0%	0%	0%	0%
2010	CHTH	10	51	0%	0%	0%	0%
2010	SRES	7	4	0%	0%	0%	0%
2011	SNCR	9	216	56%	44%	22%	11%
2011	MICR	9	4	0%	0%	0%	0%
2011	CHTH	8	40	0%	0%	0%	0%
2012	SHFL	11	3	0%	0%	0%	0%
2012	SNCR	8	777	100%	100%	75%	75%
2012	MICR	11	8	0%	0%	0%	0%
2012	CHTH	11	53	9%	9%	9%	9%
2012	SRES	5	2	0%	0%	0%	0%
2013	SHFL	9	5	0%	0%	0%	0%
2013	SNCR	7	586	71%	71%	71%	71%
2013	MICR	9	11	0%	0%	0%	0%
2013	CHTH	7	37	0%	0%	0%	0%
2014	SHFL	11	3	0%	0%	0%	0%
2014	SNCR	12	797	100%	92%	75%	75%
2014	MICR	11	5	0%	0%	0%	0%
2014	CHTH	10	17	0%	0%	0%	0%
All	SRES	33	4	3%	3%	3%	3%
All	SHFL	65	3	0%	0%	0%	0%
All	SNCR	66	346	70%	67%	53%	52%
All	MICR	74	0	0%	0%	0%	0%
All	CHTH	90	42	10%	6%	4%	3%

* Beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators.

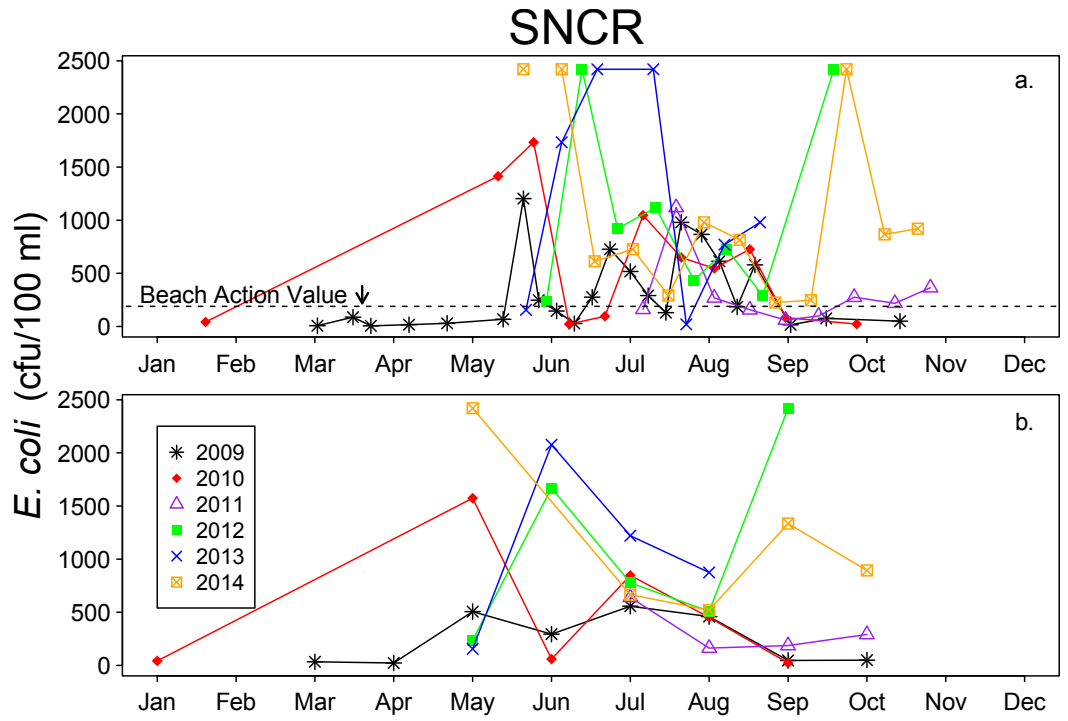

Figure 12. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data at Sniktaw Creek.

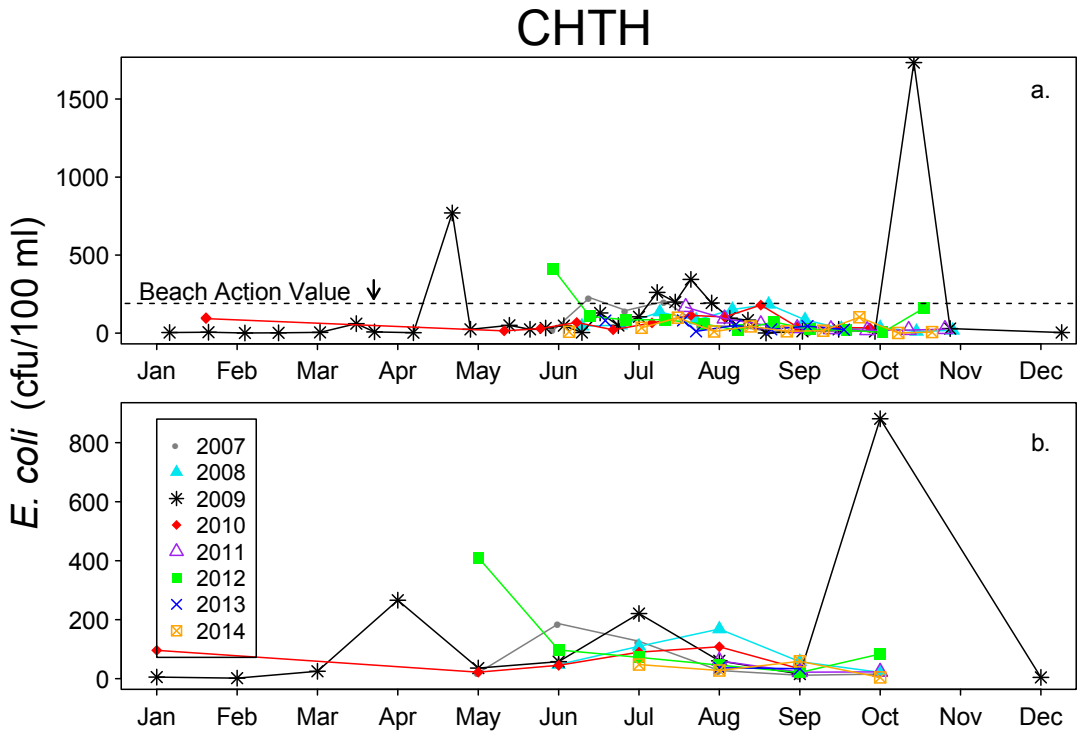

Figure 13. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data from samples taken near the mouth of Shackleford Creek.

Sites along the Scott River exceeded bacterial benchmarks during some years. Seasonal geometric means were below the 100 cfu/100 ml benchmark, with the exception of one site (Figure 14). Scott River at Horn Lane (HORN) was sampled six times in 2013, with resulting *E. coli* concentrations suggesting that there may be local bacteria contamination (Figure 17). It would be informative to continue sampling at this site to see if the high levels of *E. coli* are a persistent problem here, however sampling has been discontinued due to private property access constraints. No other sites had seasonal *E. coli* geometric means above 100 cfu/100 ml, but all sites had over 10% of their samples above beach action values during at least one sampling year (Figure 16, Figure 15, Figure 18, Figure 19, and Table 9).

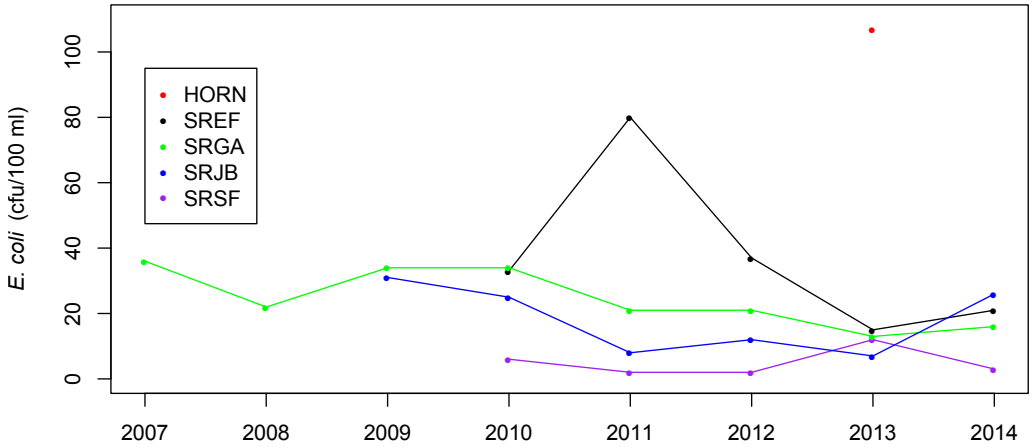

Figure 14. Geometric means of *E. coli* concentrations by year at sampling sites along the Scott River (means are based on samples collected from April–October).

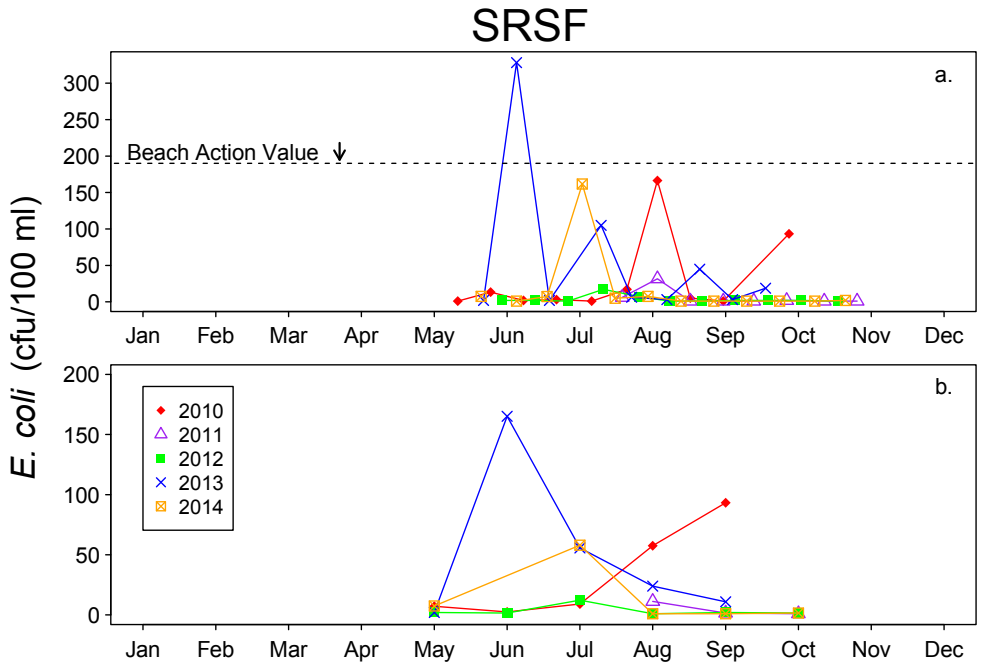

Figure 15. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data at the South Fork of the Scott River sampling location.

Table 9. Number of *E. coli* samples (n), geometric means (GM), and percent of samples from April through October exceeding regulatory standards at individual sites by year at Scott River sites.

		E. coli					
Site	Year	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)
SRGA	2007	10	36	10%	10%	10%	10%
SRGA	2008	12	22	0%	0%	0%	0%
SRGA	2009	24	34	17%	17%	13%	13%
SRJB	2009	14	31	0%	0%	0%	0%
SRGA	2010	10	34	0%	0%	0%	0%
SRJB	2010	5	25	0%	0%	0%	0%
SREF	2010	10	33	10%	10%	0%	0%
SRSF	2010	10	6	0%	0%	0%	0%
SRGA	2011	9	21	11%	0%	0%	0%
SRJB	2011	6	8	17%	0%	0%	0%
SREF	2011	8	80	13%	0%	0%	0%
SRSF	2011	8	2	0%	0%	0%	0%
SRGA	2012	11	21	9%	0%	0%	0%
SRJB	2012	11	12	0%	0%	0%	0%
SREF	2012	10	37	0%	0%	0%	0%
SRSF	2012	11	2	0%	0%	0%	0%
SRGA	2013	9	13	0%	0%	0%	0%
SRJB	2013	10	7	0%	0%	0%	0%
SREF	2013	9	15	11%	11%	0%	0%
SRSF	2013	9	12	11%	11%	11%	0%
HORN	2013	6	107	17%	17%	0%	0%
SRGA	2014	12	16	8%	0%	0%	0%
SRJB	2014	11	26	18%	0%	0%	0%
SREF	2014	12	21	0%	0%	0%	0%
SRSF	2014	12	3	0%	0%	0%	0%
SRGA	All	97	24	8%	5%	4%	4%
SRJB	All	58	16	5%	0%	0%	0%
SREF	All	50	32	8%	6%	2%	2%
SRSF	All	50	3	2%	2%	2%	0%

* Beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators.

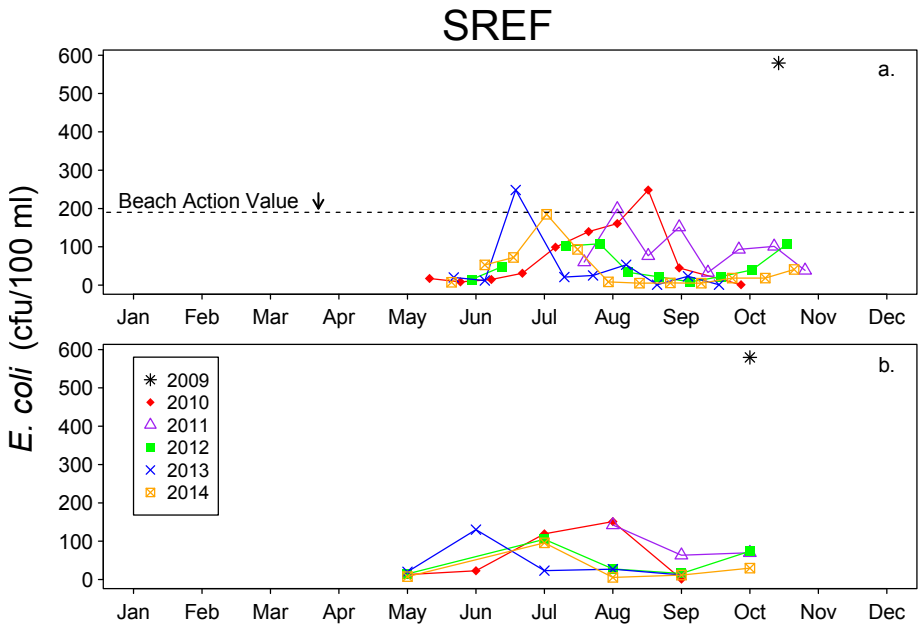

Figure 16. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data at the East Fork Scott River sampling site.

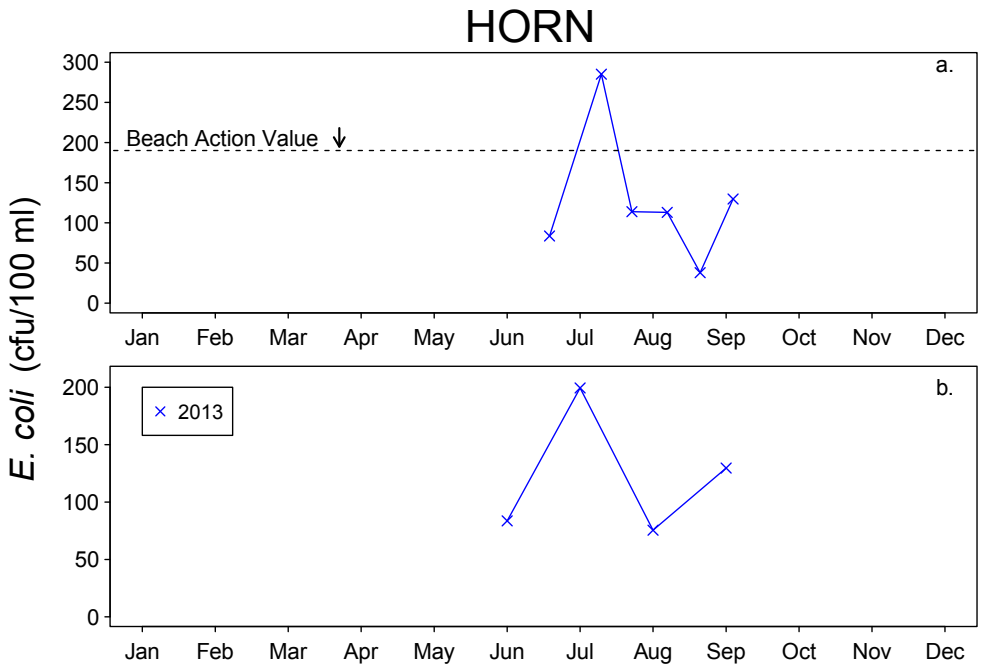

Figure 17. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with data from 2013 near Horn Lane on the Scott River.

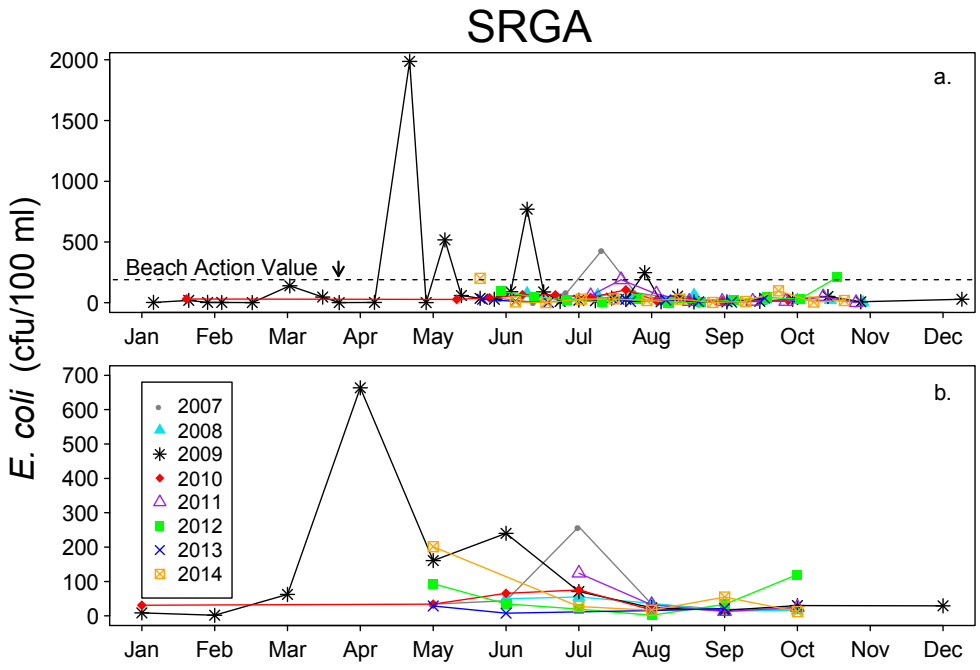

Figure 18. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data near the USGS gage on the Scott River.

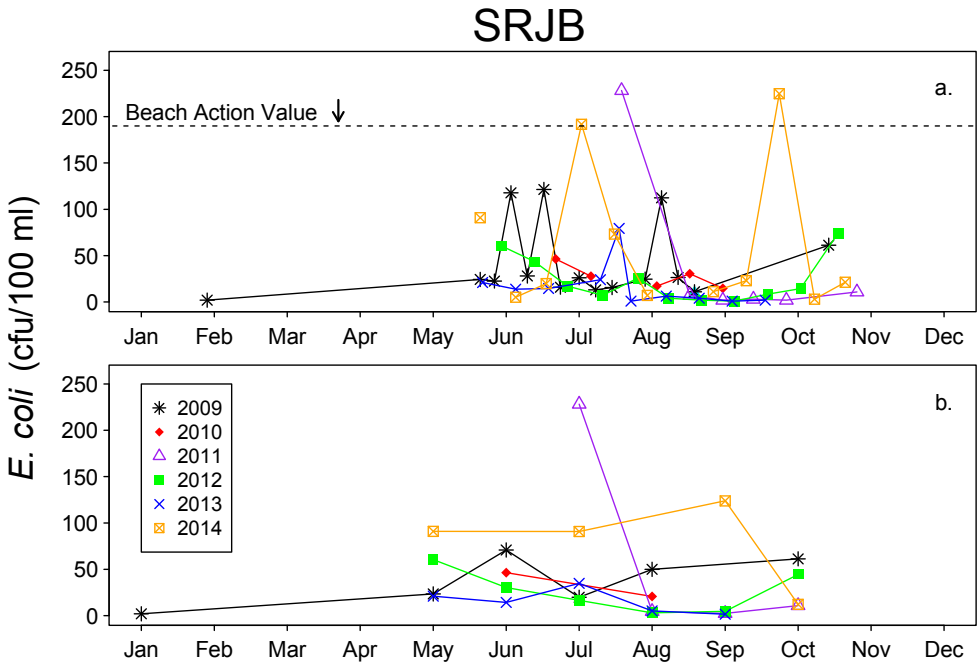

Figure 19. *E. coli* concentrations from individual samples (panel a.) and monthly means of *E. coli* concentrations (panel b.), with each line showing a different year of data at Jones Beach on the Scott River.

3.2.3 INDIVIDUAL SITE EXCEEDANCES AT GRAZING ALLOTMENT SITES

Geometric means of *E. coli* and fecal coliform concentrations in the grazing allotment sites were generally below 100 cfu/100 ml when all samples from April through October were combined (Figure 20, Figure 21). The highest geometric mean for *E. coli* and fecal coliform within the Shackleford allotment occurred in 2011 at site SF10, with geometric means of 53 and 88 cfu/100 ml, respectively (Table 10). The median of geometric means for *E. coli* and fecal coliform within the Shackleford allotment were seven and 17, respectively. A total of six *E. coli* samples exceeded beach action values and five fecal coliform samples exceeded the single sample maximum in the Shackleford allotment over the sampling period. More than 10% of the *E. coli* and fecal coliform samples at SF11 and SF19 exceeded threshold values when data from all years were combined (Figure 22, Figure 23).

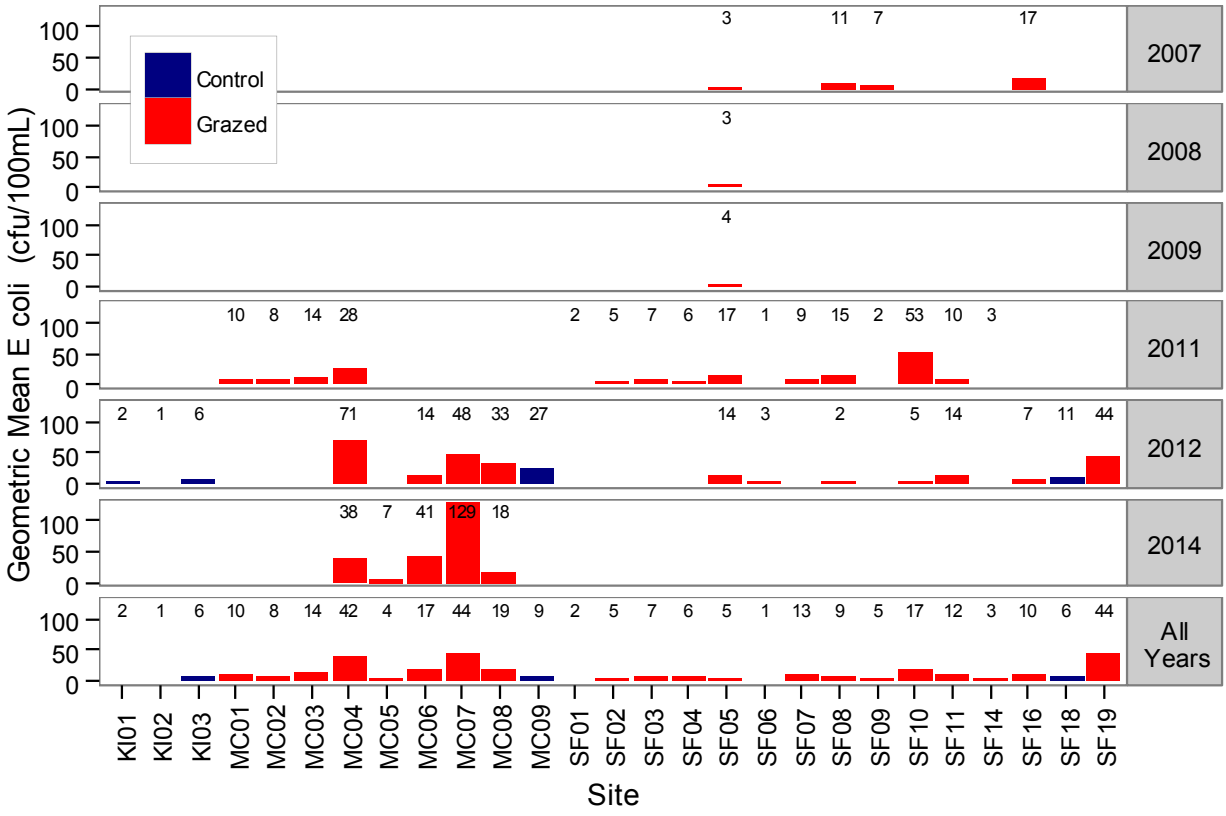

Figure 20. Comparison of geometric mean of *E. coli* levels at grazed and ungrazed study sites from 2007 to 2014 using samples from April through October when available. Blue bars show sites where no active grazing occurs anytime in the year. Red bars show site with seasonally active grazing. If no bar is present, no data (or less than five samples) was collected during that site during that year.

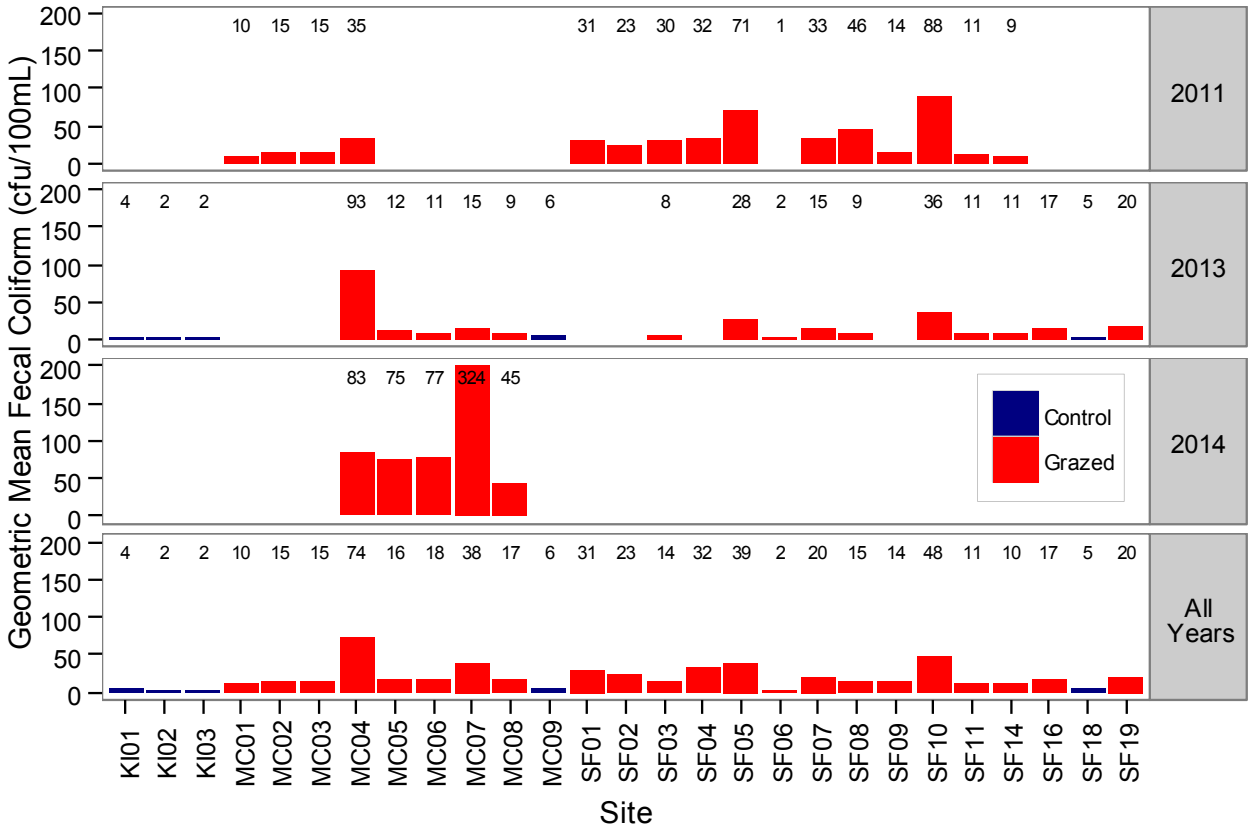

Figure 21. Comparison of geometric mean of fecal coliform levels at grazed and ungrazed study sites from 2007 to 2014 using samples from April through October when available. Blue bars show sites where no active grazing occurs anytime in the year. Red bars show site with seasonally active grazing. If no bar is present, no data (or less than five samples) was collected during that site during that year.

Table 10. Number of *E. coli* and fecal coliform samples (n), geometric means (GM), and percent of samples from April through October exceeding regulatory benchmarks* at individual sites by year in the Shackleford Creek grazing allotment (SF).

		E. Coli						**Fecal coliform**		
Year	Site	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)	n	GM	SSM
2007	SF05	9	3	0%	0%	0%	0%	0	–	–
2007	SF08	10	11	0%	0%	0%	0%	0	–	–
2007	SF09	10	7	0%	0%	0%	0%	0	–	–
2007	SF16	8	17	0%	0%	0%	0%	0	–	–
2008	SF05	11	3	0%	0%	0%	0%	0	–	–
2009	SF05	12	4	8%	0%	0%	0%	0	–	–
2011	SF01	5	2	0%	0%	0%	0%	5	31	0%
2011	SF02	5	5	0%	0%	0%	0%	5	23	0%
2011	SF03	5	7	0%	0%	0%	0%	5	30	0%
2011	SF04	5	6	0%	0%	0%	0%	5	32	0%
2011	SF05	5	17	0%	0%	0%	0%	5	71	0%

2011	SF06	5	1	0%	0%	0%	0%	5	1	0%
2011	SF07	5	9	0%	0%	0%	0%	5	33	0%
2011	SF08	5	15	0%	0%	0%	0%	5	46	0%
2011	SF09	5	2	0%	0%	0%	0%	5	14	0%
2011	SF10	5	53	0%	0%	0%	0%	5	88	20%
2011	SF11	5	10	20%	20%	0%	0%	5	11	20%
2011	SF14	5	3	0%	0%	0%	0%	5	9	0%
2012	SF05	8	14	0%	0%	0%	0%	0	–	–
2012	SF06	5	3	0%	0%	0%	0%	0	–	–
2012	SF08	5	2	0%	0%	0%	0%	0	–	–
2012	SF10	5	5	0%	0%	0%	0%	0	–	–
2012	SF11	7	14	14%	14%	14%	14%	0	–	–
2012	SF16	7	7	14%	14%	14%	14%	0	–	–
2012	SF18	8	11	13%	13%	13%	13%	0	–	–
2012	SF19	6	44	17%	17%	17%	17%	0	–	–
2013	SF03	0	–	–	–	–	–	7	8	0%
2013	SF05	0	–	–	–	–	–	10	28	10%
2013	SF06	0	–	–	–	–	–	10	2	0%
2013	SF07	0	–	–	–	–	–	10	15	0%
2013	SF08	0	–	–	–	–	–	10	9	0%
2013	SF10	0	–	–	–	–	–	10	36	0%
2013	SF11	0	–	–	–	–	–	10	11	10%
2013	SF14	0	–	–	–	–	–	10	11	0%
2013	SF16	0	–	–	–	–	–	10	17	0%
2013	SF18	0	–	–	–	–	–	8	5	0%
2013	SF19	0	–	–	–	–	–	10	20	10%
All	SF01	5	2	0%	0%	0%	0%	5	31	0%
All	SF02	5	5	0%	0%	0%	0%	5	23	0%
All	SF03	5	7	0%	0%	0%	0%	12	14	0%
All	SF04	5	6	0%	0%	0%	0%	5	32	0%
All	SF05	46	5	2%	0%	0%	0%	15	39	7%
All	SF06	10	1	0%	0%	0%	0%	15	2	0%
All	SF07	9	13	0%	0%	0%	0%	15	20	0%
All	SF08	21	9	0%	0%	0%	0%	15	15	0%
All	SF09	16	5	0%	0%	0%	0%	5	14	0%
All	SF10	10	17	0%	0%	0%	0%	15	48	7%
All	SF11	12	12	17%	17%	8%	8%	15	11	13%
All	SF14	5	3	0%	0%	0%	0%	15	10	0%
All	SF16	18	10	6%	6%	6%	6%	13	17	0%
All	SF18	11	6	9%	9%	9%	0%	11	5	0%
All	SF19	6	44	17%	17%	17%	17%	10	20	10%

*For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The single sample maximum (SSM) for fecal coliform is set at 400 cfu/100 ml. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators), over 50 cfu/100 ml for fecal coliform, or if any of the BAV, STV, or SSM data is exceeded at a rate of 10% or more of the samples, we considered that site year as having a standard exceeded.

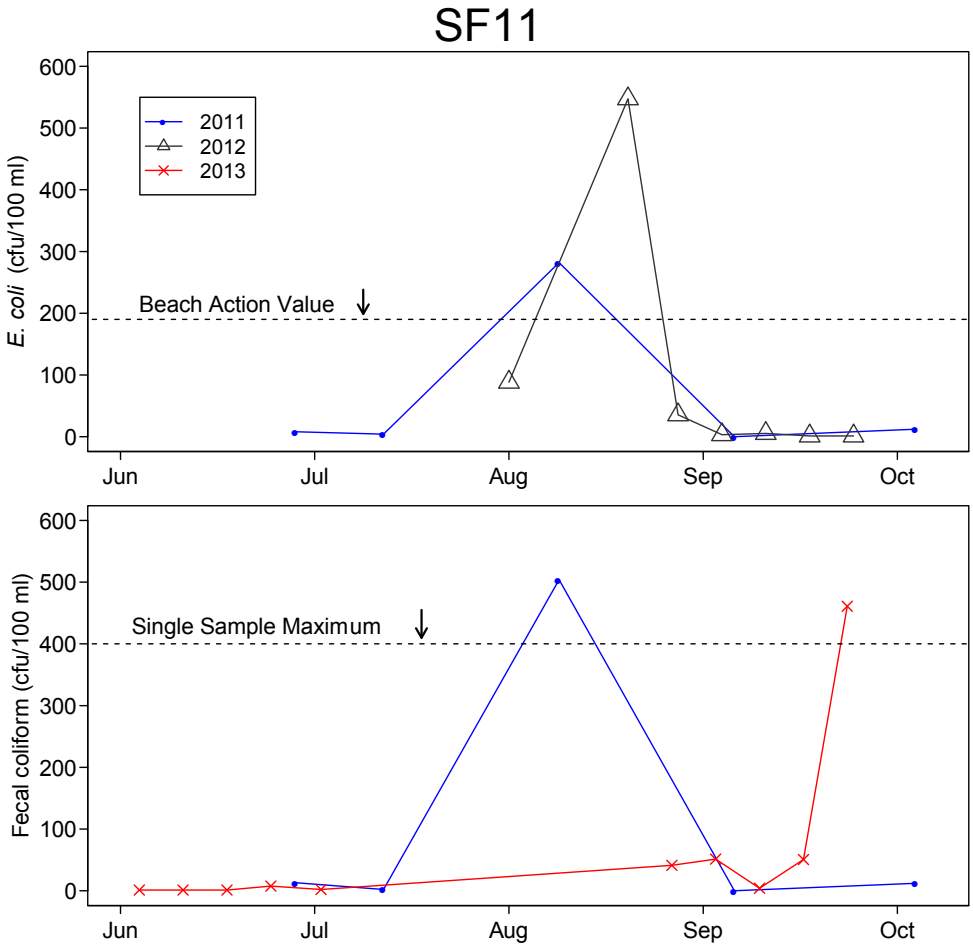

Figure 22. *E. coli* and fecal coliform concentrations from individual samples by date at SF11 in the Shackleford Creek grazing allotment, with each line showing a different year of data.

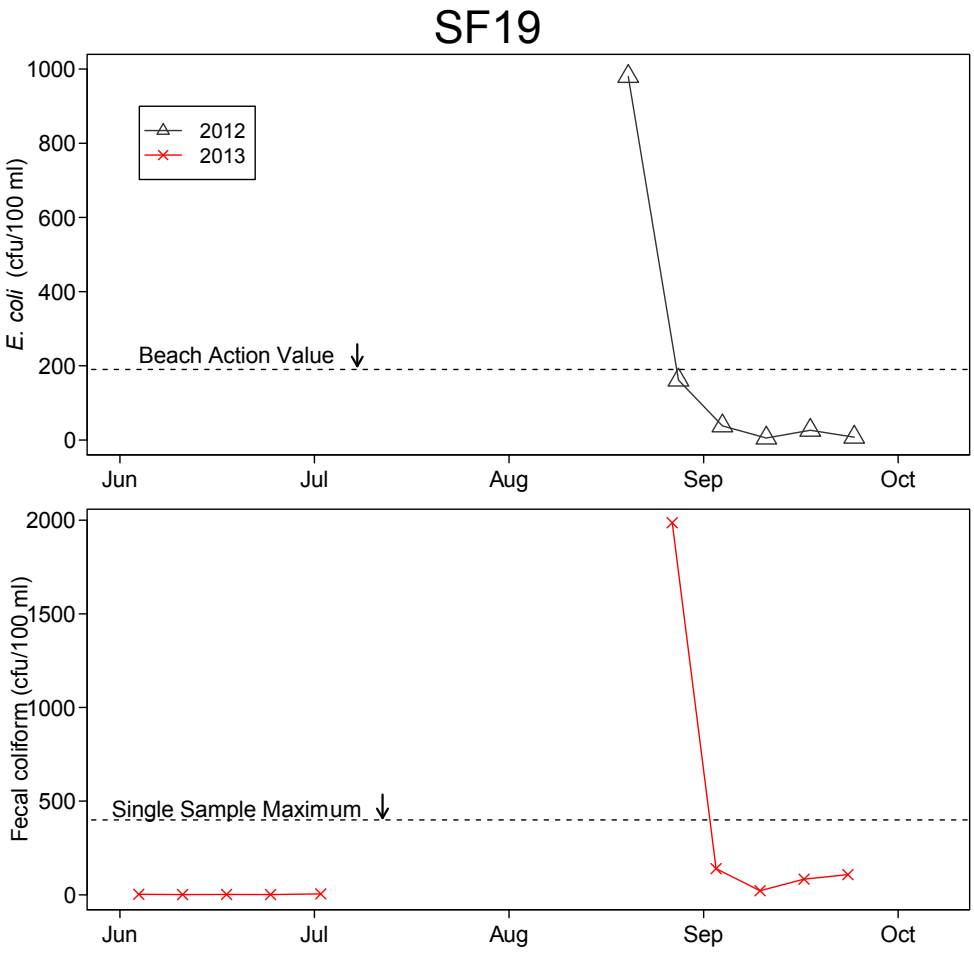

Figure 23. *E. coli* and fecal coliform concentrations from individual samples by date at SF19 in the Shackleford Creek grazing allotment, with each line showing a different year of data.

Season-long geometric means were below 100 cfu/100 ml at sites within the Mill Creek allotment, with the exception of MC07 in 2014, where the geometric mean for *E. coli* was 129 cfu/100 ml and the geometric mean for fecal coliform was 324 cfu/100 ml (Table 11). Median geometric means for *E. coli* and fecal coliform were 18 and 16 cfu/100 ml respectively in the Mill Creek allotment. A total of nine *E. coli* samples exceeded beach action values and 14 fecal coliform samples exceeded the single sample maximum in the Mill Creek allotment over the sampling period. More than 10% of the *E. coli* and fecal coliform samples at MC04 and MC07 exceeded threshold values, and over 10% of fecal coliform samples exceeded the threshold value at MC08 when data from all years were combined (Figure 24, Figure 25, and Figure 26).

Stream bacteria concentrations at sites within the ungrazed Kidder Creek allotment were consistently low. Geometric means for E. coli were never above 6 cfu/100 ml, and geometric means for fecal coliform ranged from 2-4 cfu/100 ml (Table 12).

Table 11. Number of *E. coli* and fecal coliform samples (n), geometric means (GM), and percent of samples from April through October exceeding regulatory benchmarks* at individual sites by year in the Mill Creek grazing allotment (MC).

		E. coli						**Fecal coliform**		
Year	Site	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)	n	GM	SSM
2011	MC01	5	10	0%	0%	0%	0%	5	10	0%
2011	MC02	5	8	0%	0%	0%	0%	5	15	0%
2011	MC03	5	14	0%	0%	0%	0%	5	15	0%
2011	MC04	5	28	40%	40%	0%	0%	4	35	25%
2012	MC04	5	71	0%	0%	0%	0%	0	–	–
2012	MC06	5	14	20%	20%	0%	0%	0	–	–
2012	MC07	5	48	20%	20%	20%	0%	0	–	–
2012	MC08	5	33	0%	0%	0%	0%	0	–	–
2012	MC09	5	27	0%	0%	0%	0%	0	–	–
2013	MC04	0	–	–	–	–	–	10	93	40%
2013	MC05	0	–	–	–	–	–	10	12	0%
2013	MC06	0	–	–	–	–	–	10	11	0%
2013	MC07	0	–	–	–	–	–	10	15	10%
2013	MC08	0	–	–	–	–	–	10	9	0%
2013	MC09	0	–	–	–	–	–	10	6	0%
2014	MC04	5	38	20%	20%	20%	20%	5	83	20%
2014	MC05	5	7	0%	0%	0%	0%	5	75	20%
2014	MC06	5	41	0%	0%	0%	0%	5	77	0%
2014	MC07	5	129	40%	60%	40%	40%	5	324	60%
2014	MC08	5	18	0%	0%	0%	0%	5	45	20%
All	MC01	5	10	0%	0%	0%	0%	5	10	0%
All	MC02	5	8	0%	0%	0%	0%	5	15	0%
All	MC03	5	14	0%	0%	0%	0%	5	15	0%
All	MC04	15	42	20%	20%	7%	7%	19	74	31%
All	MC05	9	4	0%	0%	0%	0%	19	16	5%
All	MC06	14	17	7%	7%	0%	0%	19	18	0%
All	MC07	14	44	29%	21%	21%	14%	19	38	26%
All	MC08	14	19	7%	7%	0%	0%	19	17	11%
All	MC09	12	9	0%	0%	0%	0%	17	6	0%

* For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The single sample maximum (SSM) for fecal coliform is set at 400 cfu/100 ml. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators), over 50 cfu/100 ml for fecal coliform, or if any of the BAV, STV, or SSM data is exceeded at a rate of 10% or more of the samples, we considered that site year as having a standard exceeded.

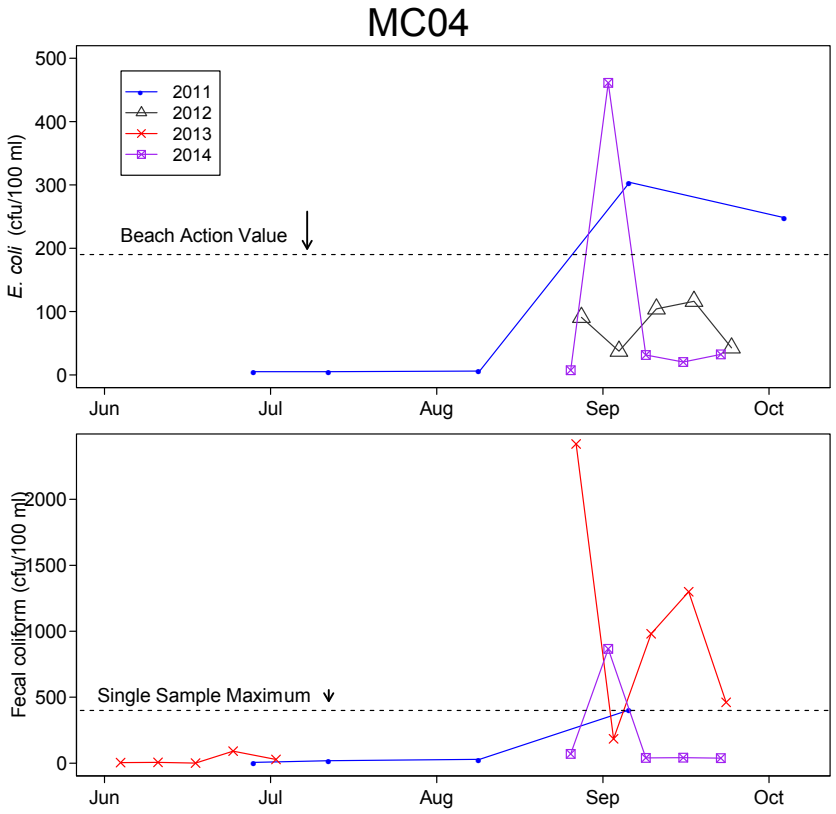

Figure 24. *E. coli* and fecal coliform concentrations from individual samples by date at MC04 in the Mill Creek grazing allotment, with each line showing a different year of data.

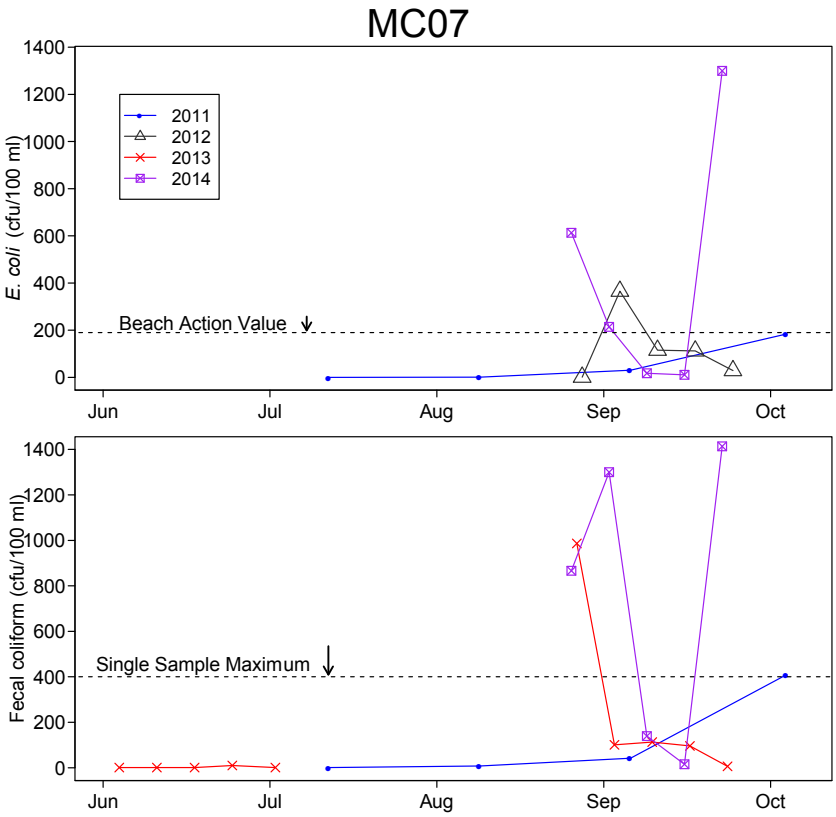

Figure 25. *E. coli* and fecal coliform concentrations from individual samples by date at MC07 in the Mill Creek grazing allotment, with each line showing a different year of data.

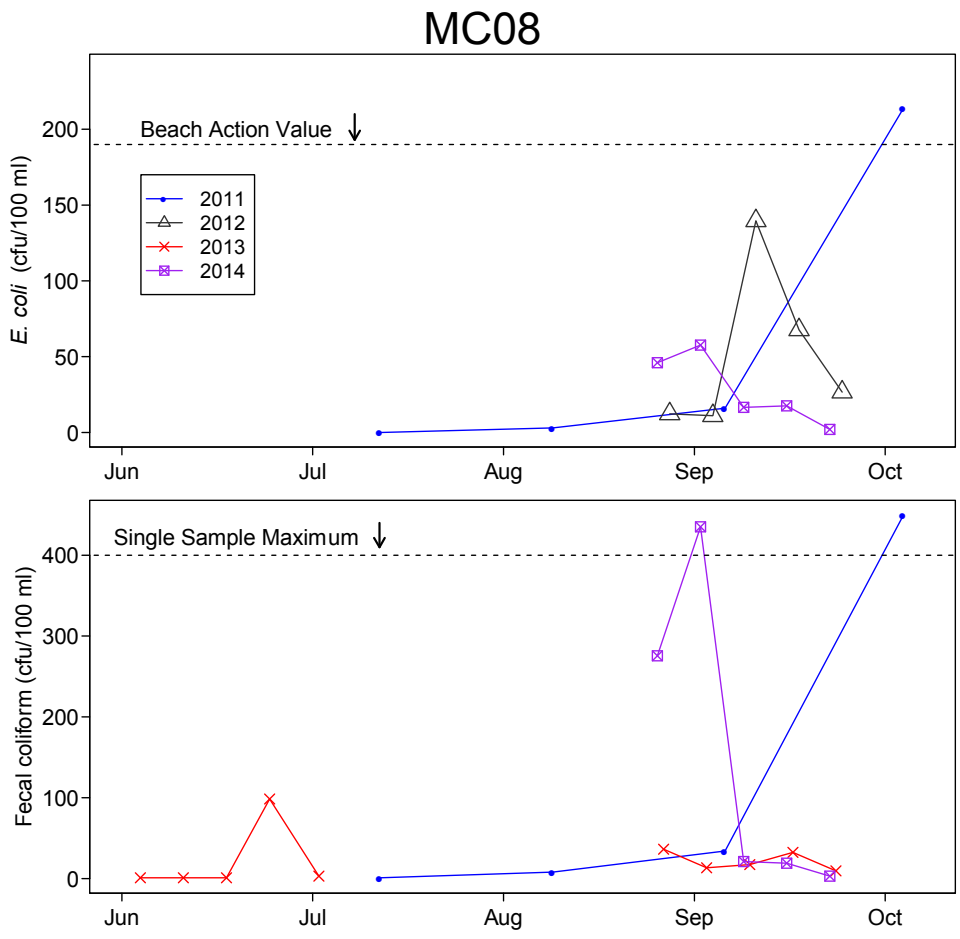

Figure 26. *E. coli* and fecal coliform concentrations from individual samples by date at MC08 in the Mill Creek grazing allotment, with each line showing a different year of data.

Table 12. Number of *E. coli* and fecal coliform samples (n), geometric means (GM), and percent of samples from April through October exceeding regulatory benchmarks* at individual sites by year in the Kidder Creek grazing allotment (KI).

		E. coli						**Fecal coliform**		
Year	Site	n	GM	BAV (3.2%)	BAV (3.6%)	STV (3.2%)	STV (3.6%)	n	GM	SSM
2012	KI01	5	2	0%	0%	0%	0%	0	–	–
2012	KI02	5	1	0%	0%	0%	0%	0	–	–
2012	KI03	5	6	0%	0%	0%	0%	0	–	–
2013	KI01	0	–	–	–	–	–	10	4	0%
2013	KI02	0	–	–	–	–	–	10	2	0%
2013	KI03	0	–	–	–	–	–	10	2	0%
All	KI01	5	2	0%	0%	0%	0%	10	4	0%
All	KI02	5	1	0%	0%	0%	0%	10	2	0%
All	KI03	5	6	0%	0%	0%	0%	10	2	0%

* For *E. coli*, beach action values (BAV) are set at 190 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 235 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The statistical threshold value (STV) for *E. coli* are set at 320 cfu/100 ml using an illness rate of 3.2% of primary contact recreators, or 410 cfu/100 ml using an illness rate of 3.6% of primary contact recreators. The single sample maximum (SSM) for fecal coliform is set at 400 cfu/100 ml. If the GM is above 100 cfu/100 ml for *E. coli* (the U.S. EPA standard using a suggested illness rate of 3.2% of primary contact recreators), over 50 cfu/100 ml for fecal coliform, or if any of the BAV, STV, or SSM data is exceeded at a rate of 10% or more of the samples, we considered that site year as having a standard exceeded.

3.3 RELATIVE CONCENTRATIONS OF FECAL INDICATOR BACTERIA TYPES

E. coli, fecal coliform, and total coliform were all positively correlated with each other, but there was substantial scatter in the relationships (Figure 27, Figure 28, Figure 29). The median ratios of *E. coli* to fecal coliform was 0.5 (i.e., 50% of fecal coliform was *E. coli*) (Figure 27 right panel), while the median ratio of *E. coli* to total coliform was 0.02 (Figure 28, right panel). The median ratio of both *E. coli* to fecal coliform (Figure 27 right panel) and *E. coli* to total coliform (Figure 28 right panel) appeared to be higher at the Mill Creek Allotment (MC) than the Shackleford Allotment (SF) or baseline sites. The relationship between *E. coli* and total coliform (Figure 28) was more variable than between *E. coli* and fecal coliform (Figure 27). Fecal coliform and total coliform were only collected together at baseline sites in 2013, where the median ratio of fecal coliform to total coliform was 0.05 (Figure 29).

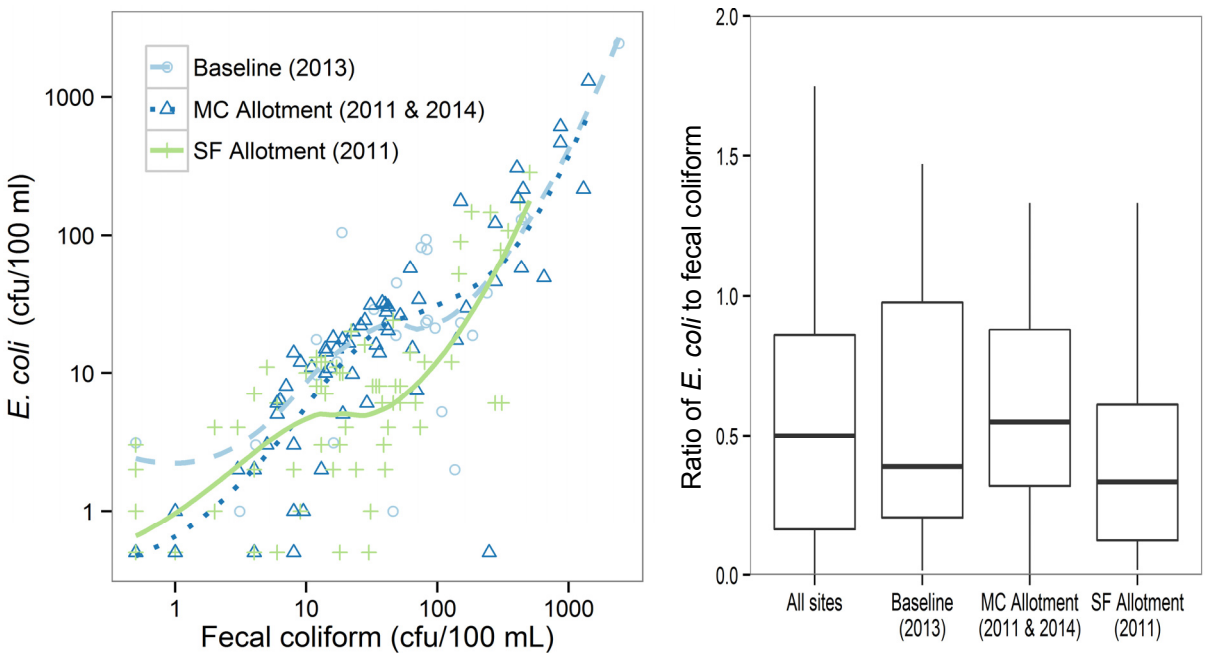

Figure 27. Scatterplot of *E coli* concentration vs. fecal coliform concentration (left panel) and boxplot of ratio of *E. coli* concentration to fecal coliform concentration (right panel) for all samples (all dates and sites except special studies) where both parameters were analyzed. Ratios not calculated from samples with non-detect or maximum (2419.6) values for either parameter. LOESS smoother shown as visual aid.

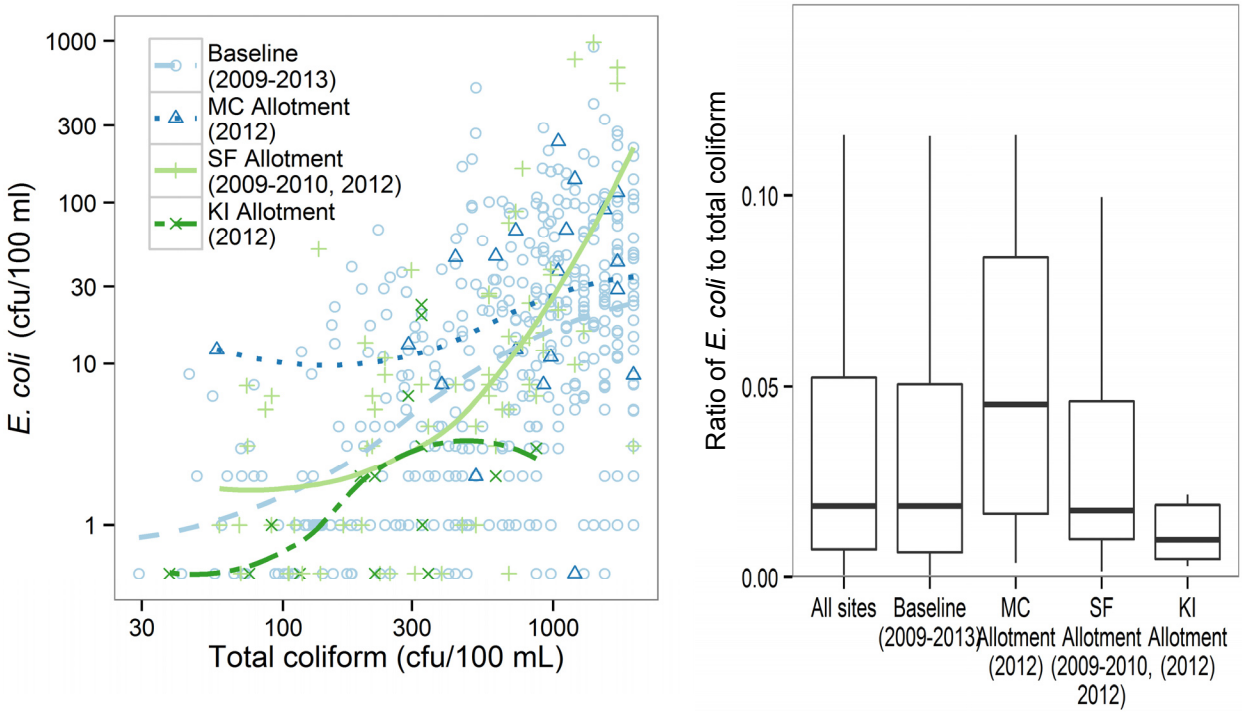

Figure 28. Scatterplot of *E coli* concentration vs. total coliform concentration (left panel) and boxplot of ratio of *E. coli* concentration to total coliform concentration (right panel) for all samples (all dates and sites except special studies) where both parameters were analyzed. Ratios not calculated from samples with non-detect or maximum (2419.6) values for either parameter. LOESS smoother shown as visual aid.

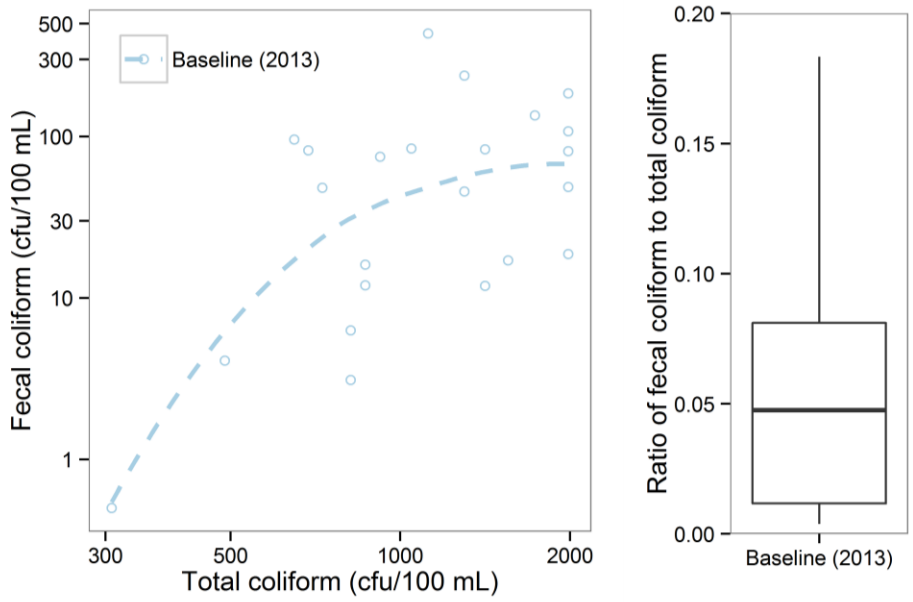

Figure 29. Scatterplot of fecal coliform concentration vs. total coliform concentration (left panel) and boxplot of ratio of fecal coliform concentration to total coliform concentration (right panel) for all samples (all dates and sites except special studies) where both parameters were analyzed. Ratios not calculated from samples with non-detect or maximum (2419.6) values for either parameter. LOESS smoother shown as visual aid.

3.4 EFFECTS OF CATTLE GRAZING ON BACTERIA LEVELS

Cows were present on the Mill Creek and Shackleford grazing allotments for approximately three months each summer-fall season. Date of first cattle entry onto the allotments varied by year and ranged from July 6th to early August (Table 13). Cows were removed from the allotments between October 1st and October 30th. Both allotments were grazed at or near the permitted cow-calf pair capacity, with 75 to 80 cow-calf pairs on the Shackleford Creek allotment and 165 cow-calf pairs on the Mill Creek allotment.

Median and ranges of bacteria concentrations were higher during the permitted grazing season than during the times that cows were not on allotments (Figure 30). There was also an increase in fecal coliform levels at sites without grazing, though the increase was larger at actively grazed sites. No pre-grazing season data existed for *E. coli* at ungrazed sites. On ungrazed sites, fecal coliform levels increased from a mean of 2.3 cfu/100 ml to 12.5 cfu/100 ml, a five-fold increase from the season where cows were not on active grazing allotments to the grazing season. On actively grazed sites, fecal coliform levels increased from a mean of 14.5 cfu/100 ml to 175 cfu/100 ml, a 12-fold increase from the season where cows were not on active grazing allotments to the grazing season. These data suggest that some of the increase in bacteria levels may be due to seasonal variation not associated with cattle grazing, although the much larger increase at the grazed sites points toward cattle grazing as a likely cause of increased bacteria levels on the grazed allotments. Higher levels of bacteria on active grazing allotments during the non-grazing season may be due to the effects of cows from the previous season.

Table 13. Number of cow-calf pairs per allotment and year with date cows were turned onto allotment and date they were removed by. Both actual dates and permitted dates are listed. Where a range of dates were given, the earliest date for turn-on was used and the latest date for turn-off.

		Actual			*Permitted*		
Allotment	**Year**	**Turn-on**	**Turn-off**	**Cow-calf pairs**	**Turn-on**	**Turn-off**	**Cow-calf pairs**
Shackleford	2007	15 Jul	15 Oct	80	15 Jul	15 Oct	80
	2008	15 Jul*	16 Oct	76	15 Jul	15 Oct	80
	2009	15 Jul	5 Oct	80	15 Jul	1 Oct	80
	2010	27 Jul	15 Oct	80	15 Jul	1 Oct	80
	2011	1 Aug**	30 Oct	80	15 Jul	1 Oct	80
	2012	15 Jul	2 Oct	80	15 Jul	1 Oct	80
	2013	15 Jul	1 Oct	80	15 Jul	1 Oct	80
	2014	7 Jul	1 Oct	75	15 Jul	1 Oct	80
Mill Creek	2011	1 Aug**	17 Oct	165	15 Jul	5 Oct	165
	2012	15 Jul	5 Oct	165	15 Jul	5 Oct	165
	2013	6 Jul	5 Oct	165	15 Jul	5 Oct	165
	2014	7 Jul	5 Oct***	165	15 Jul	5 Oct	165

* 53 Pairs turned out 15 July; 23 more turned out on 5 August
** Date specified was "early August"
*** Half of the cows came off by 9 September

Medians and upper quantiles of the fecal coliform data increased at every sampling site in active grazing allotments from times of no cattle present to times of active grazing (Figure 31 and Figure 33). Sites with both large and small changes in bacteria levels pre- and post- cattle could be monitored for level of use intensity by cattle in the future to test for associations between bacteria increases and degree of cattle use.

Ungrazed control sites showed mixed changes to fecal coliform levels during the season that cows were not present to times that cows were present on the active grazing sites (Figure 32). The medians and upper quantiles of the data increased at three of the five sites. At one site (KI01) the median increased, but the upper quantile of the data decreased, and another site (KI02) had a decrease in both the median and the upper quantile of the data. Changes to fecal coliform levels at ungrazed sites were generally much smaller than changes at grazing sites (note y-axis scaling) (Figure 31 and Figure 32).

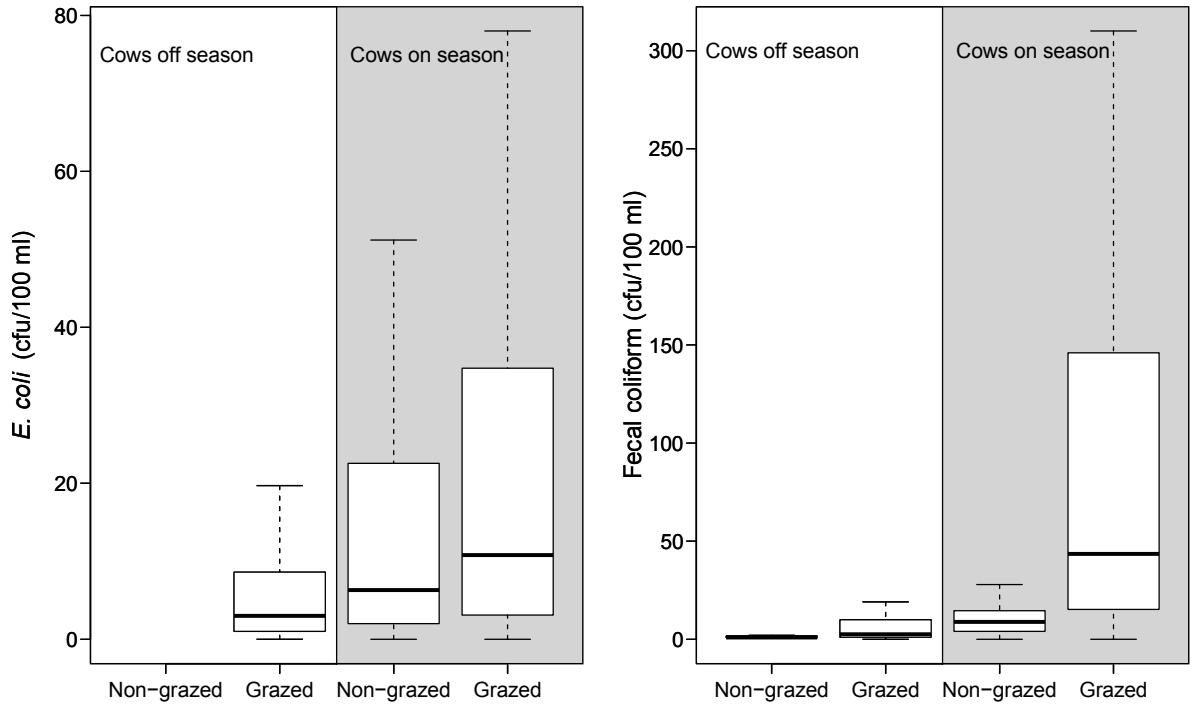

Figure 30. Medians and ranges of *E. coli* and fecal coliform at grazed and ungrazed sites including samples from all grazing allotment sites during all years that samples were collected, both when cows were not actively grazing (white background) and when cows were present on the allotments (grey background). Data outliers are not shown.

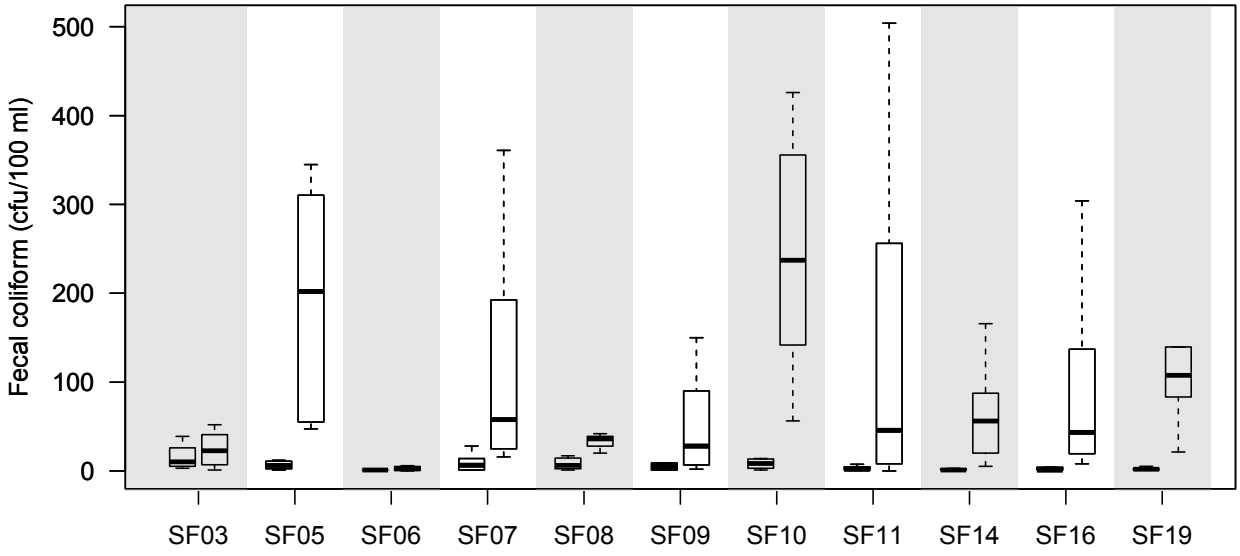

Figure 31. Medians and ranges of fecal coliform at the Shackleford Creek allotment sites when cows were not present (left box in each pair) and when cow were present (right box in each pair) on grazing allotments using all data available for each site (2011 and 2013).

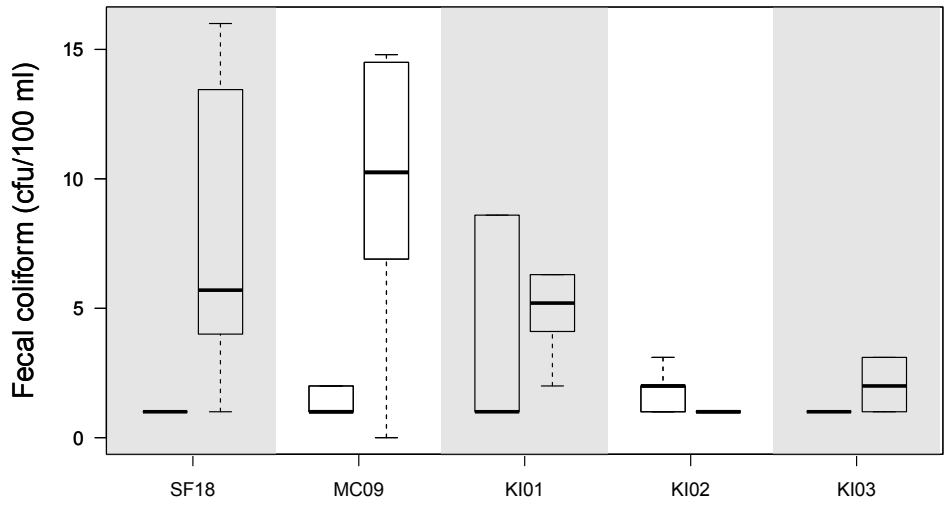

Figure 32. Medians and ranges of fecal coliform at ungrazed control sites (inactive allotments) in all three allotments (Shackleford Creek, Mill Creek, Kidder Creek) for the season that cows were not present (left box in each pair) on the active grazing allotments in this study and when cow were present (right box in each pair) on active grazing allotments in this study using all data available for each site (2011, 2013, and 2014).

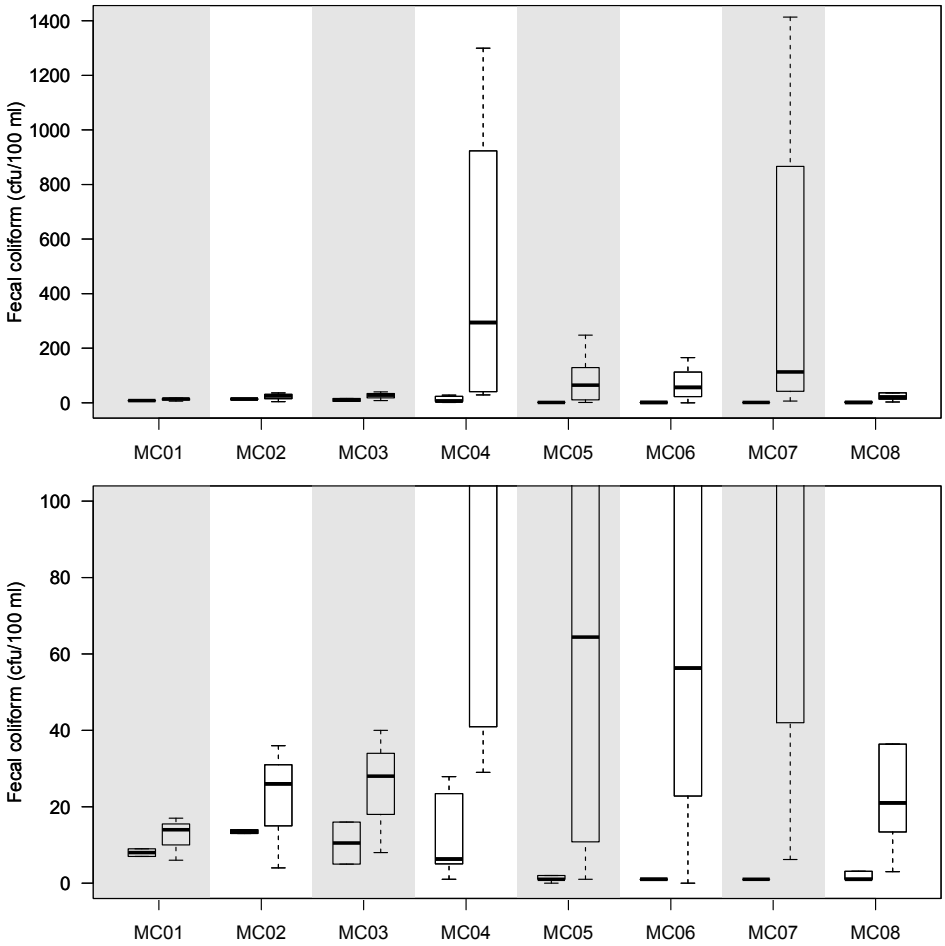

Figure 33. Medians and ranges of fecal coliform at the Mill Creek allotment sites when cows were not present (left box in each pair) and when cow were present (right box in each pair) on grazing allotments using all data available for each site (2011, 2013, and 2014). The bottom plot re-scales the x-axis so that the detail can be seen at sites with lower fecal coliform concentrations.

Cattle grazing was likely responsible for some of the increase seen in bacteria levels from pre-grazing dates to active grazing dates. The higher over-all bacteria levels on actively grazed allotment sites, and the greater magnitude increase from pre-grazing to active grazing dates, support this conclusion. Grazing is likely not responsible for all of the increases in bacteria levels, because ungrazed sites also showed an increase from pre-grazing sample dates, to active grazing sample dates, even though no cows were present at these sites at any time of the year. Some seasonal variation in bacteria concentrations is likely due other factors such as precipitation, snowmelt, wildlife, plant growth, and human use. Additional survey efforts, outlined in the final section of this report, could allow for a more thorough analysis of the degree of cattle effects on bacteria concentrations at high elevation streams and lakes of the Scott River watershed.

3.5 TEMPORAL TRENDS IN FECAL INDICOR BACTERIA

3.5.1 SCOTT RIVER AND QUARTZ VALLEY MONITORING SITES

E. coli concentrations followed a seasonal pattern in the Scott River and Quartz Valley streams. *E. coli* concentrations were lowest during the winter months, and increased in the spring, peaking in July (Figure 34). The monthly means for *E. coli* at all sites followed a similar pattern, with the exception of the April mean of 315 cfu/100 ml, which was the result of a small sample size for April, where the mean was based on only nine samples, all taken during 2009. Two very high values, both samples taken from April 22, drove the high mean, which was reduced to 12 cfu/100 ml when these outliers were removed.

Total coliform, which is the other FIB that was frequently sampled during the eight-year study period at Scott River and Quartz Valley sites, followed less obvious seasonal patterns, though was still likely influenced by time of year (Figure 34). Because total coliform concentrations were often higher than the total countable colonies per plate (2419.6 cfu/100 ml), the upper quantile of the data is cut off and calculated means are likely lower than true means, though medians should be the same. Unlike *E. coli*, total coliform follows a bimodal distribution, with high levels both in the early spring and late summer. This could be due to different processes that are associated with high total coliform levels. First, runoff in the spring could promote bacterial runoff from the watershed that has been building on the landscape over the winter months. Once initial run-off has occurred, total coliform levels may again drop either due to fewer terrestrial inputs, as habitat and forage increases after snow melt, and/or high spring flows diluting those inputs. Total coliform levels likely rise again in the late summer when flows are low, allowing for little dilution of total coliform inputs and/or increased terrestrial usage as air temperatures peak and vegetation outside of the riparian zone diminishes.

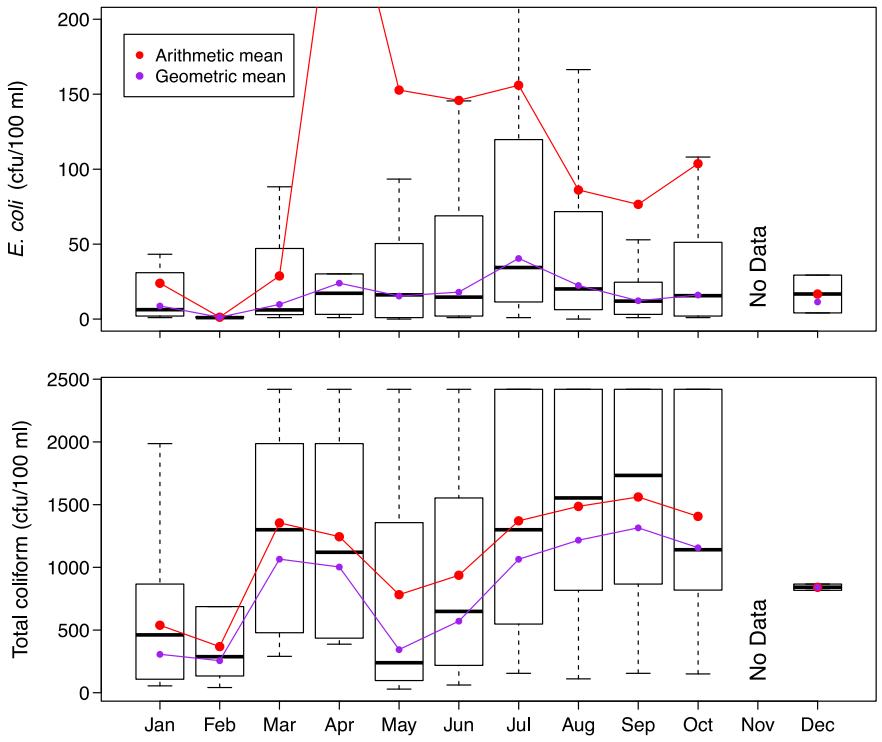

Figure 34. *E. coli* and total coliform in colony forming units per 100 ml of sample (cfu/100 ml) at monitoring sites in the Quartz Valley and Scott River by month from 2007 to 2014 using all samples collected. Box extents show the interquartile range of the data and the mid-line is the median. Outliers are not shown.

Neither *E. coli* nor total coliform showed strong variation among years, when comparing the three sites with consistent data among years (Figure 35). When considering the arithmetic mean alone, we see an increase in *E. coli* concentration in 2009, but when considered alongside the median and geometric mean, it became evident that this higher mean is influenced by one high data point (1733 cfu/100 ml on 14 Oct 2009 at CHTH). Although this sample is very relevant to public health, it may not alone be a good indicator of annual trends. When a high arithmetic mean exists compared to the geometric mean, it suggests more variation in the data. This variation in bacteria levels is an important point to consider for public health, and may be of interest to investigate further through high frequency sampling.

Total coliform concentrations were lower in 2010 than in 2009, though the following years were consistently between those levels. Continuing sampling across the same suit of sites, and during similar dates in the future will allow for a larger data set to be used in assessing annual variation in bacteria concentrations. This expanded data could be used to assess the environmental or management conditions that may be driving the bacteria concentrations from year to year.

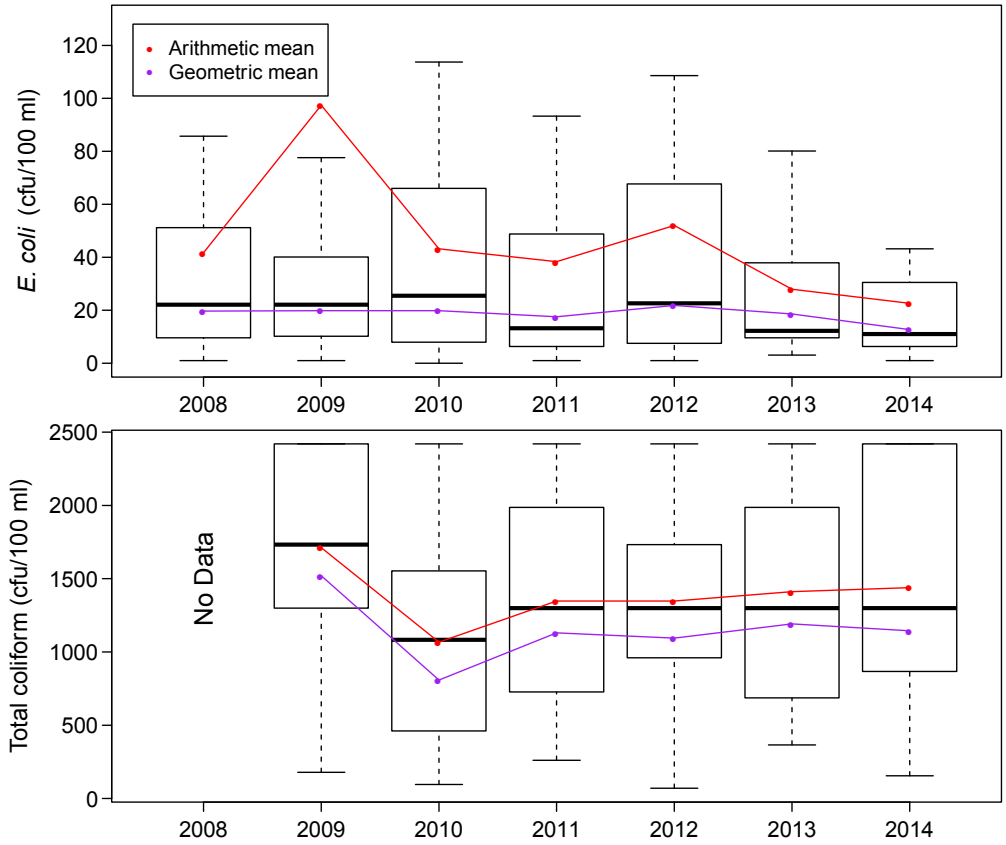

Figure 35. *E. coli* and total coliform by year at MICR, SRGA, and CHTH, the three baseline monitoring sites in the Quartz Valley and Scott River Valley that had data from similar dates during 2008 through 2014. Box extents show the interquartile range of the data and the mid-line is the median. Outliers are not shown.

3.5.2 HEADWATER STREAMS IN FOREST SERVICE GRAZING ALLOTMENTS

E. coli and fecal coliform, the two bacterial metrics commonly sampled in streams within headwater grazing allotments, followed seasonal patterns that were similar for both metrics. Samples collected from June through October had low means and medians in June and July, and increased in August, with a larger increase being reflected in the arithmetic mean and range in data, indicating that a few samples having high bacteria concentrations drove this trend (Figure 36). Fecal indicator bacteria means and medians remained higher through October, and the upper quantile of the data continued to rise into October, indicating that a larger range, including higher concentrations in some bacteria samples, were observed later in the season. The late timing of increased bacteria levels is likely due to multiple factors. Cattle are not present on allotments until after July 1st with the exact date variable. The sites are high elevation within the allotments and the subsequent late snowmelt and base-flow period of these streams would drive concentrations of bacteria. The timing of fall rains will effect concentrations in October and September by increasing the non-point source run-off of bacteria. Lastly, the forage diminishes rapidly in late summer forcing cattle and other wildlife to graze within riparian zones.

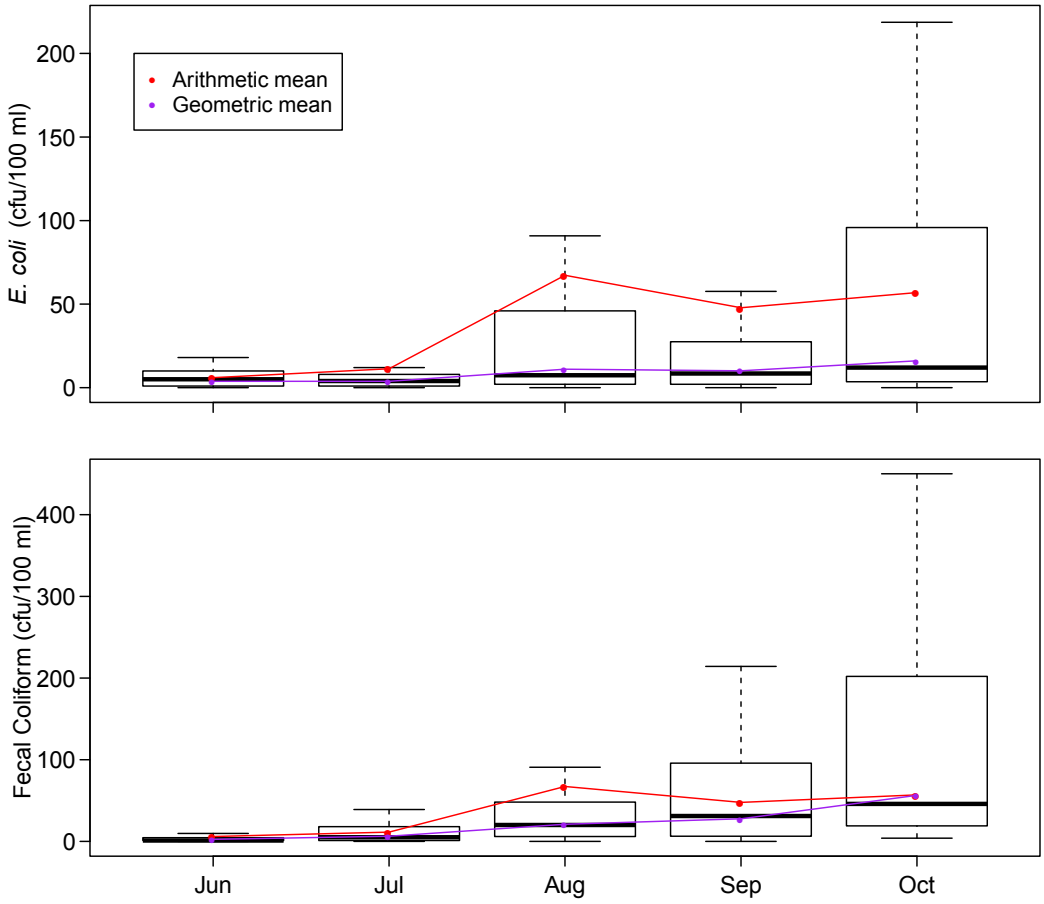

Figure 36. *E. coli* and fecal coliform in colony forming units per 100 ml of sample (cfu/100 ml) from all samples at grazing allotments in the Scott River headwaters by month from 2007 to 2014. Box extents show the interquartile range of the data and the mid-line is the median. Outliers are not shown.

Concentrations of bacterial indicators were higher in recent sampling years than in 2011, the first year in which wide spread sampling occurred at grazing allotment sites (Figure 37). Annual arithmetic means of *E. coli* concentrations and the annual range in fecal coliform concentrations increased across years, suggesting a possible increase in bacteria levels in these streams. Alternatively, 2011 may have been a year of lower than average bacteria concentrations (Table 14). Each water year since 2011 has been progressively dryer, with most recent years being declared droughts by the state of California. Reduced stream flow likely results in less dilution, increasing bacteria concentrations. Because 2014 data were only available within the Mill Creek allotment (and only six of the nine sites within that allotment), this pattern may not be representative of all allotments in this report.

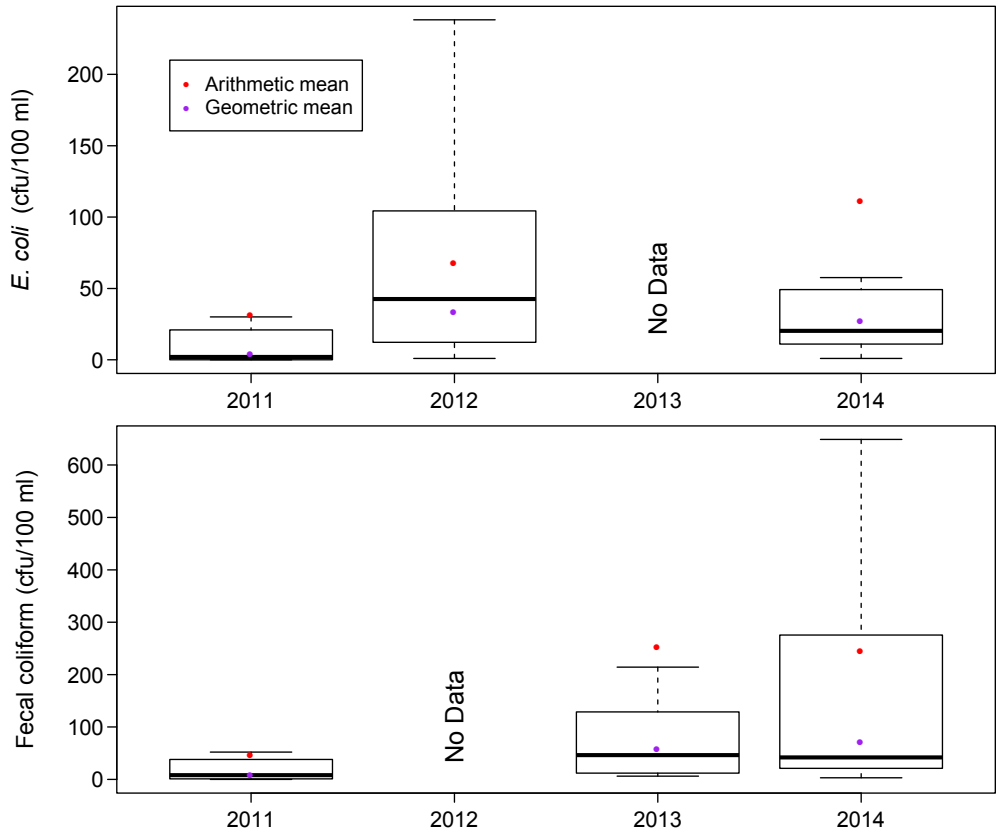

Figure 37. *E. coli* and fecal coliform by year at sites in the Mill Creek grazing allotment in the Scott River headwaters where data was available at the same sites during similar date for each year shown. Box extents show the interquartile range of the data and the mid-line is the median. Outliers are not shown.

Table 14. Mean (± standard deviation), median, and maximum values for the three fecal indicator bacteria considered in this study by grazing allotment and year, when data were present. FC is fecal coliform and TC is total coliform.

Allot-ment	Year	Mean			Median			Max		
		FC	*E. coli*	TC	FC	*E. coli*	TC	FC	*E. coli*	TC
SF	2007	–	33(±54, n=37)	–	–	5	–	–	179	–
	2008	–	3(±3, n=11)	–	–	3	–	–	9	–
	2009	–	21(±62, n=12)	444(±647, n=12)	–	1	216	–	219	2420
	2010	–	41(±47, n=3)	561(±177, n=2)	–	41	561	–	75	687
	2011	56(±102, n=77)	19(±45, n=77)	–	17	6	–	504	282	–
	2012	–	102(±322, n=55)	737(±655, n=56)	–	7	596	–	1986	2420
	2013	90(±311, n=105)	–	–	6	–	–	2420	–	–
	2014	–	–	–	–	–	–	–	–	–
MC	2011	24(±47, n=38)	16(±31, n=39)	–	8	5	–	225	152	–
	2012	–	68(±83, n=25)	1185(±702, n=23)	–	43	2420	–	365	1046
	2013	132(±387, n=60)	–	–	10	–	–	2420	–	–
	2014	247(±392, n=29)	111(±266, n=29)	–	42	20	–	1414	1300	–
KI	2012	–	4(±7, n=15)	291(±214, n=15)	–	2	291	–	23	866
	2013	6(±14, n=30)	–	–	1	–	–	70	–	–
All sites/years		91(±276, n=339)	43(±130, n=303)	735(±673, n=108)	9	7	387	2420	1300	2420

4 SUGGESTIONS FOR FUTURE MONITORING

A number of sites in the Quartz Valley and on the Scott River warrant more frequent sampling. Sniktaw Creek (site SNCR) had bacteria levels of concern throughout the year and among years. Shackleford Creek (site CHTH) near the mouth generally had April through October geometric means of *E. coli* below 100 cfu/100 ml, but many individual samples exceeded beach action values. These sites are used for cultural activities that involve water contact, and CHTH is also used for swimming and fishing. More frequent sampling and subsequent posting of high bacteria levels may serve to protect public health and allow for a better understanding of temporal variation in bacteria concentrations. All sites on the Scott River had occasional samples that exceeded beach action values. Most exceedances occurred at the Scott River gage site (SGRA). Increased sampling and subsequent posting of high bacteria levels should occur at any high-use recreational areas to protect public health, especially during times of high public use or cultural activities.

Data collected from 2007 to 2014 suggests that cattle grazing increased bacteria levels in wilderness area streams in the Scott River watershed. Some slight changes to the data collection protocol and collection of additional variables to quantify the degree of cattle use may be helpful in understanding finer temporal and spatial scales of stream bacteria levels and the relative impact from cattle grazing. A variety of methods could be instigated to collect quantitative data on cattle use. Transects could be established upstream of sampling locations and number of cow pies intersecting transects could then be quantified. The area of stream bank that is affected by cattle could be measured. Motion sensing cameras could be deployed at a few sample sites during the grazing season to quantify the number and timing of cow visits at sites. Quantitative data of cattle use will allow for analysis of the degree of cattle use and levels of stream bacteria. Sample collection should continue both prior to and during active cattle grazing when resources allow. Sample collected prior to cattle turn-on should occur as close to cattle turn on as possible, to eliminate seasonal variation in bacteria levels not associated with grazing.

The role of precipitation events, which wash organic matter including fecal contamination from the terrestrial landscape into surface waters, should be further investigated along with the effects of grazing. It may be helpful to monitor bacteria levels daily following precipitation events to understand how long bacteria levels remain elevated.

DNA source-tracking is a relatively new technology which allows attribution of *E. coli* to specific animal species (Griffith et al. 2010). QVIR is currently updated its Sampling Analysis Plan (SAP) to include DNA source-tracking and plans to begin pilot sampling for DNA in the 2015 sampling season (QVIR 2015).

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6 ACKNOWLEDGMENTS

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APPENDIX A: COMPLETE LIST OF ALL SITES SAMPLED

Table A1. List of all sites sampled for fecal indicator bacteria in the Scott River watershed from 2007 to 2014. Site names describe site location and are left blank when unavailable; n is the number of sampling events during the 8-year period, and site-type refers to long-term Quartz Valley/Scott River sites (bg), grazing allotments sites (ungrazed or grazed), and special studies (ss).

Site Code	Site Name	n	Site Type	Latitude	Longitude
Scott River and Quartz Valley Baseline Sites:					
BKBG		1	bg		
BKBG1	Up-stream of trib	3	bg		
BKBG2	Downstream of trib	3	bg		
CHTH	Shackelford Creek nr mouth (Charlie Thom's)	100	bg	41.61528	-122.96550
DEEP	Deep Creek	0	bg	41.66500	-123.11220
ELLR	Scott River under Black's Bridge	4	bg	41.51929	-121.14838
HORN	Scott River near Horn Lane	6	bg	41.45741	-122.85248
LWSF		1	bg		
MICR	Mill Creek	80	bg		
SCBF		1	bg		
SCRX		1	bg	41.59055	-123.00080
SCTG		1	bg	41.61473	-122.98150
SHFL	Shackleford Creek at Falls	65	bg		
SNCR	Sniktaw Creek	70	bg	41.30664	-122.79740
SRAK		1	bg	41.59333	-122.97500
SREF	Scott River East Fork	50	bg	41.64000	-123.01380
SRES	Shackleford Creek at Quart Valley	41	bg		
SRGA	Scott River Gaging Station	107	bg	41.63944	-123.05910
SRGF		1	bg	41.29569	-122.80900
SRJB	Scott River Jones Beach	59	bg	41.59806	-121.00310
SRSF	Scott River South Fork	50	bg	41.61528	-122.96550
UPSN	Upper Sniktaw Creek	2	bg	41.66500	-123.11220
Kidder Creek Allotment:					
KI01	Kidder Ck above first unnamed tributary from the	15	ungrazed	41.50808	-123.05490
KI02	Kidder Ck above Kidder Lk tributary	15	ungrazed	41.51194	-123.08460
KI03	Kidder Ck adjacent to Hays Meadow	15	ungrazed	41.50744	-123.09240
Mill Creek Allotment:					
MC01	East Boulder Creek below trailhead	5	grazed	41.25240	-122.79600
MC02	East Boulder Creek below first meadow	5	grazed	41.24780	-122.79409
MC03	East Boulder Creek above confluence	5	grazed	41.23999	-122.79030
MC04	Tributary above confluence with East Boulder Creek	25	grazed	41.23790	-122.79000
MC05	East Boulder below E. Boulder Lk outlet	19	grazed	41.23361	-122.78625

MC06	E. Boulder CK above E. Boulder Lk inlet	24	grazed	41.22897	-122.78560
MC07	E. Boulder Ck below Upper Lk outlet	24	grazed	41.22540	-122.78390
MC08	E. Boulder Ck above Upper Lk inlet	24	grazed	41.22347	-122.78320
MC09	Fox Ck at road	22	ungrazed	41.24351	-122.83900
Shackleford Creek Allotment:					
SF01	Shackleford below Falls	5	grazed	41.59292	-122.99569
SF02	Shackleford above falls	5	grazed	41.59173	-122.99964
SF03	Shackleford below corral	12	grazed	41.56210	-123.04741
SF04	Shackleford above trailhead	5	grazed	41.56136	-123.05067
SF05	Shackleford Ck at gate below meadows	56	grazed	41.55739	-123.06350
SF06	Long High Ck above Shackleford confluence	20	grazed	41.55118	-123.07390
SF07	Shackleford Ck above Long High Ck confluence	20	grazed	41.55097	-123.07440
SF08	Shackleford Ck above Campbell Lk tributary	31	grazed	41.54436	-123.09220
SF09	Campbell Lake tributary before confluence with	16	grazed	41.54341	-123.09240
SF10	Shackleford Ck below Log Lk Meadow complex	20	grazed	41.54439	-123.09960
SF11	Shackleford Ck in Log Lake Meadow below trail	22	grazed	41.54243	-123.10600
SF12	Shackleford above Bull Meadow confluence	2	grazed	41.54092	-123.18970
SF13	Bull Meadow Tributary above confluence with	2	grazed	41.54092	-123.10820
SF14	Campbell Lake tributary below Campbell Lake	15	grazed	41.53941	-123.09877
SF15	Isolate unnamed tributary before confluence with	3	grazed		
SF16	Campbell Lk trib above Campbell Lk inlet	28	grazed	41.53205	-123.10750
SF17	Cliff Lake outlet	3	grazed	41.52770	-122.88800
SF18	Emerald Ck	19	ungrazed	41.54459	-123.08910
SF19	Tributary above Shackleford Ck confluence by Calf Lk	16	grazed	41.54576	-123.09630
Sites from Special Studies:*					
KANG	Kangaroo Creek	1	ss	41.33861	-122.70580
SC07	Scott River longitudinal study	15	ss		
SCGF	Scott River at Gold Flat	2	ss		
SCIS	Scott River at Indian Scotty	2	ss		
SCJO	Scott River at Johnson's Bar	2	ss		
SCSP	Scott River at Sugar Pine	2	ss	41.69614	-123.05055
SCTC	Scott River at Tompkins	2	ss		
SHRI_CAN	Shasta River Canyon	1	ss		
SHRI_SAL	Shasta River Salmon Heaven	1	ss		

* Sites listed under special studies were part of longitudinal studies not reported on in this analysis, but included in Electronic Appendix E. Some sites listed as "bg" were also included in these longitudinal studies, but sites were not listed under both Site-Type headings.

APPENDIX B: STANDARD OPERATING PROCEEDURES FOR SAMPLE COLLECTION

Document B1. Standard operating procedure for collection of surface water samples in the Scott River watershed. The original document was prepared by Crystal Robinson, QVIR Environmental Director, approved by Janis Gomes, USEPA Project Officer, and implemented by Andrea Collins, QVIR Environmental Biologist.

Scope and Application

1.1 This standard operating procedure must be followed when collecting and storing surface water samples for laboratory analysis.

1.2 Samples must be collected in such a way that no foreign material is introduced into the sample and no material of interest escapes from the sample prior to analysis.

2.0 Personnel Qualifications

2.1 All field samplers will be pre-trained in all sampling and equipment procedures by an experienced sampler before beginning the sampling procedure.

2.2 All personnel will be responsible for complying will all quality assurance/quality control requirements as outlined in the QVIR QAPP.

3.0 Summary of Sample Collection Procedure

3.1 Acquire certified sample containers from Laboratory.
3.1.1 Order 100 ml bottles from IDEXX and perform quality control (see QVIR Lab Manual)
3.1.2 Call lab and order sample bottles

3.2 Do all necessary preparation prior to sampling.

3.3 Assemble all equipment (See 6. Equipment and Supplies Checklist).

3.4 Collect all QA samples.

3.5 Perform field analyses.

3.6 Obtain samples using dip sampler if necessary and certified clean collection bottle.

3.7 Store nutrient samples at 4ºC and bacteria samples at 10ºC

3.8 Submit samples to laboratory (Refer to Sample Submission SOP).

4.0 Grab Sampling Procedure – Nutrients, Chlorophyll a and Phaeophytin a

4.1 Streams are always sampled upstream from any manmade structure such as a bridge.

4.2 Lakes are sampled at their outlet.

4.3 Collect from the same sampling site each time.

4.4 Check last year's field notes or GPS log for exact sampling location.

4.5 Immerse the thermometer or YSI handheld in the water and leave immersed five minutes before reading temperature. Avoid disturbing the bottom with the thermometer at the sample site.

4.6 Label bottle with a unique site code (geographic area name and stream or lake name), date, time, water temperature and sampler's initials. Include whether it is a grab or composite sample. Label bottle before immersion using a black permanent marker or pre-printed labels. If using pre-printed labels affix with clear plastic packaging tape to avoid them getting wet. Aquatic Research Inc., contracted lab, provides only certified clean containers.

4.7 Use latex gloves when handling bottles during sampling. Fingers contain contaminants such as nitrates. Bug repellents or sunscreen are particularly troublesome as contaminants. Once the gloves are on, be careful not to touch your face, the ground, or anything but the bottles.

4.8 The sample should be taken from flowing, not stagnant water, facing upstream positioned in the thalwag.

4.9 Be sure to immerse the bottle completely, 10 cm (4 inches) deep, with mouth of bottle pointing upstream, so no water flows over your hand into the bottle. Remove the cap under water. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Water samples should be collected 6-12 inches below the water surface. Fill bottle at least half full, replace cap loosely, remove from water and shake. Pour out rinse water downstream of sample point. Pour some rinse water over inside of cap. Do not touch bottle mouth or inside of cap. Partially fill the bottle, cap, shake, and rinse three times.

4.10 Collect the sample on the fourth immersion. Use the same procedure as before but fill bottle completely. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If necessary, squeeze the bottle slightly as the cap is tightened so no air remains in bottle. If stream is too shallow to immerse bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.

4.11 Collect one "duplicate" sample every two weeks (sampling frequency). Sample sites chosen for duplicate sampling are selected at random among sites sampled. When a duplicate sample is selected for the site, repeat procedures as with normal stream samples. The duplicate is the second sample when two samples are collected. Duplicates document repeatability of individual sample collections and reproducibility of laboratory results.

4.12 Place sample immediately in a Ziploc bag in the cooler after collection. Do not expose sample bottles to the sun. Fill out the field data sheet, noting any unusual conditions such as wind or rain. Measure air temperature (shaded) and record. Dispose of latex gloves.

4.13 Samples are analyzed in the lab. Keep samples cool while transporting. Ziploc bags (double bagged) filled with snow work well if frozen icepacks are unavailable for transport from the field. Store at 4 ºC but do not freeze. Include a separate Ziploc bag containing the completed Chain of Custody form. Ship to the lab in a picnic cooler with frozen icepacks via FedEx or UPS overnight. Do not ship so the sample arrives on a weekend. If necessary, keep samples refrigerated for arrival weekdays. Hand delivery to the lab is preferred; or arrange for a contact to pick up the samples.

5.0 Grab Sampling Procedure – Total Coliforms and *E. coli*

5.1 Streams are always sampled upstream from any manmade structure such as a bridge.

5.2 Lakes are sampled at their outlet.

5.3 Collect from the same sampling site each time.

5.4 Check last year's field notes or GPS log for exact sampling location.

5.5 Immerse the thermometer or YSI handheld in the water and leave immersed five minutes before reading temperature. Avoid disturbing the bottom with the thermometer at the sample site.

5.6 Label bottle with location (geographic area name and stream or lake name), date, time, water temperature and sampler's initials. Label bottle before immersion using a black permanent marker or pre-printed labels. QVIR Bacteria Lab, State Certified Lab, purchases only certified sterile, 100 mL, sealed containers from IDEXX.

5.7 Use latex gloves when handling bottles during sampling. Fingers contain contaminants such as nitrates. Bug repellents or sunscreen are particularly troublesome as contaminants. Once the gloves are on, be careful not to touch your face, the ground, or anything but the bottles.

5.8 The sample should be taken from flowing, not stagnant water, facing upstream positioned in the thalwag.

5.9 Be sure to immerse the bottle completely, 10 cm (4 inches) deep, with mouth of bottle pointing upstream, so no water flows over your hand into the bottle. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Water samples should be collected 6-12 inches below the water surface. Fill bottle, to the 100ml line indicated, on **first immersion,** pour off the excess and cap. Do not under fill or over fill, do not redunk. If too much water is poured off, redo sample with new 100 ml container.

5.10 Do not touch bottle mouth or inside of cap. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If stream is too shallow to immerse bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.

5.11 Collect one "duplicate" sample every two weeks (sampling frequency). Sample sites chosen for duplicate sampling are selected at random among sites sampled. When a duplicate sample is selected for the site, repeat procedures as with normal stream samples. The duplicate is the second sample when two samples are collected. Duplicates document repeatability of individual sample collections and reproducibility of laboratory results.

5.12 Samples are analyzed in the QVIR Bacteria lab. Keep samples cool while transporting. Store at 10 ºC but do not freeze. Hand-deliver to the lab. See Lab SOP.

6. Equipment/Supplies

Equipment that is necessary for the collection of surface water samples includes:

6.1 Wilderness First Aid Pack
6.2 Water Filter
6.3 Camel Packs
6.4 Ice Packs
6.5 Coolers
6.6 Sample Bottles
6.7 Sun Block
6.8 Leatherman
6.9 Waders & Boots
6.10 Camera
6.11 Note Pad & Pencil
6.12 Calculator
6.13 Data Sheets
6.14 Meter Measuring Tape/ 4 Utility Clamps
6.15 YSI Handheld
6.16 Aqua Calc & Rod
6.17 Turbidity Meter
6.18 Tape measure (25 ft.)
6.19 Latex gloves
6.20 Ziploc bags
6.21 GPS Unit
6.22 Field Notebook
6.23 2 Waterproof (Sharpie) pens and 2 black ink writing pens
6.24 Water or Gatorade
6.25 Air temperature thermometer
6.26 Trash bag

7. Procedure for Nutrients, Chlorophyll a and Phaeophytin a

7.1 Two weeks prior to sampling – order bottles from Aquatic Research Inc. for sampling.

7.2 Create sampling bottle labels, label sample bottles, place in cooler.

7.3 Fill out mailing label.

7.4 Be sure Blue Ice packs are freezing.

7.5 Calibrate YSI handheld according to protocol.

7.6 Fill tatum: write-in-the-rain data sheets (Flow & Surface Water per site), pencils, calculator, field notebook, thermometer (NIST),

7.7 Pack truck, complete gear checklist (See Section 5)

7.8 Arrive at first sampling site, make sure all instrumentation is in shade.

7.9 Collect flow according to USDA protocol, record on flow datasheet.

7.10 Just upstream of flow site, place YSI probe in water to stabilize in the thalwag, where the water samples will be taken (see discharge data sheet to locate thalwag). The probes should be ~ 6-12 inches below water surface. Record results on surface water datasheet.

7.11 Collect nutrient samples according to protocol (Section 4). Place samples in a Ziploc bag in cooler. Record sample collected and time of collection.

7.12 Collect Total Coliforms and *E.coli* samples according to protocol (Section 5). Place samples in cooler. Record sample collected and time of collection.

7.13 Take air temperature inside riparian canopy (if possible), record.

7.14 Wilderness samples will be packed into a Ziploc bag and placed inside another Ziploc filled with Blue Ice. Upon reaching the car, at the trailhead, samples will be placed inside the cooler w/fresh Blue Ice prior to collecting samples at the trailhead.

7.15 Once all samples are collected, return to office, open cooler and replace all ice packs with fresh Blue Ice from office freezer. Put used Blue Ice from sampling day in freezer, to re-freeze. The samples sit overnight in the cooler.

7.16 Arrive to office the next day, replace Blue Ice with fresh from freezer.

7.17 Complete Chain of Custody (COC) Forms as each sites samples are packed into the cooler. Copy COC from, file at QVIR, send original COC in a Ziploc bag in the cooler with the samples.

7.18 Using packing tape secure lid on cooler, place FedEx label on the handle (luggage tag style labels). Drop off at Yreka Mail Box and Package Service in Yreka by 1:30 pm.

Comments:

- If there is no current, create a current artificially by pushing the bottle forward horizontally.
- For shallow waters such as streams springs, seeps or other types of discharges, attempt to sample the water without touching any solids.
- if flows are too deep, wide or fast samples may be taken from a well-mixed area at the water's edge.

APPENDIX C: LABORATORY STANDARD OPERATING PROCEEDURES

Document C1. Standard operating procedure for laboratory analysis of *E. coli* and total coliform from surface water samples collected in the Scott River watershed. The original document was prepared by the Quartz Valley Indian Reservation Environmental Protection Department.

Standard Operating Procedure for:

Escherichia coli and Total Coliform using the IDEXX Quanti-Tray/2000 System with Colilert reagent

Quartz Valley Indian Reservation
Environmental Protection Department

Prepared by: _____ Date: _____
QVIR Quality Assurance and Lab Director

Reviewed by:_____ Date:_____
Environmental Laboratory Accreditation Program

Table of Contents

1 Identification of the test method

Escherichia coli using the IDEXX Quanti-Tray/2000 System with Colilert reagent (Standard Methods, 9223 B.)

2 Applicable matrix or matrices

This method is suitable for use with surface water samples.

3 Detection Limit

The detection limit for this analysis is 1 Most Probable Number (MPN) per 100mL of sample.

4 Scope of the test method

This standard operating procedure describes the test method for the collection and analysis of water samples for the enumeration of *Escherichia coli* (*E. coli*) and Total coliform bacteria.

5 Summary of test method

Surface water samples are collected in 120ml shrink-banded, sterile IDEXX bottles. An undiluted water sample will be analyzed from the sample collected. The Colilert® reagent is added directly to the 100 ml undiluted sample. Both are mixed thoroughly to dissolve the reagent. The sample is transferred to Quanti-Trays®/2000 and sealed using the Quanti-Tray sealer. Samples are incubated at 35.0 ± 0.5º C for 24 hours. Results are reported as MPN/100mL.

6 Definitions

6.1 Analytical batch: The set of samples processed at the same time

6.2 Control cultures: For each lot of medium, check analytical procedures by testing with known positive and negative control cultures. For example, *E.coli* is a positive control for this analysis and *Staphylococcus aureus* is a negative control.

6.3 Field duplicate (FD): Two samples taken at the same time and place under identical circumstances and that are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage as well as laboratory procedures.

6.4 Laboratory reagent blank (LRB): An aliquot of sterilized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

6.5 Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation or storage procedures.

7 Interferences

Water samples containing humic or other material may be colored. If there is background color, compare inoculated trays to a control tray containing only water (SM, 9223 A.)

8 Health and safety

The analysis involves handling of freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

The Colilert® reagent is not hazardous according to the manufacturer's material safety data sheet. The manufacturer does recommend wearing gloves and safety glasses while using this reagent and washing hands after use.

9 Personnel qualifications

Laboratory and field personnel shall have a working knowledge of this analytical procedure and will have received training from an QVIR employee knowledgeable of the proper sample analysis procedures.

10 Equipment and supplies

10.1 Sterile, shrink-wrapped 100ml IDEXX bottles.

10.2 Quanti-Tray Sealer®: catalog number WQTS2X-115. IDEXX Laboratories, Inc., Westbrook, ME

10.3 Incubator

11 Reagents and standards

11.1 Colilert® reagent: for 100 ml samples, catalog number WP200. IDEXX Laboratories, Inc., Westbrook, ME.

11.2 Quanti-Tray®/2000: 100 trays containing 97 wells each, part number WQT-2K. IDEXX Laboratories, Inc., Westbrook, ME

12 Sample collection, preservation, shipment and storage

12.1 Arrive at site and record site number, date and time.

12.2 Immerse the thermometer or YSI handheld in the water and leave immersed five minutes before reading temperature. Avoid disturbing the bottom with the thermometer at the sample site.

12.3 Label bottle with location (geographic area name and stream or lake name), date, time, water temperature and sampler's initials. Label bottle before immersion using a black permanent marker or pre-printed labels. QVIR Bacteria Lab, State Certified Lab, purchases only certified sterile, 100 ml, sealed containers from IDEXX.

12.4 Use latex gloves when handling bottles during sampling. Fingers contain contaminants such as nitrates. Bug repellents or sunscreen are particularly

troublesome as contaminants. Once the gloves are on, be careful not to touch your face, the ground, or anything but the bottles.

12.5 The sample should be taken from flowing, not stagnant water, facing upstream positioned in the thalwag.

12.6 Be sure to immerse the bottle completely, 10 cm (4 inches) deep, with mouth of bottle pointing upstream, so no water flows over your hand into the bottle. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Water samples should be collected 6-12 inches below the water surface. Fill bottle, to the 100ml line indicated, on **first immersion,** pour off the excess and cap. Do not under fill or over fill, do not redunk. If too much water is poured off, redo sample with new 100 ml container.

12.7 Do not touch bottle mouth or inside of cap. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If stream is too shallow to immerse bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.

12.8 Collect one "duplicate" sample every two weeks (sampling frequency). Sample sites chosen for duplicate sampling are selected at random among sites sampled. When a duplicate sample is selected for the site, repeat procedures as with normal stream samples. The duplicate is the second sample when two samples are collected. Duplicates document repeatability of individual sample collections and reproducibility of laboratory results.

12.9 Samples are analyzed in the QVIR Bacteria lab. Keep samples cool while transporting. Store at 4 ºC until analysis, but do not freeze. The maximum holding time is 6 hours.

12.10 For each sample, the location number, bottle numbers used and time collected will be recorded in the field sample log.

12.11 The samples will be kept in the possession of QVIR personnel who both collect and analyze the samples.

13 Quality control

13.1 **Accuracy:** Initial analyst demonstration of capability and for each new lot of Quanti-Tray/2000, analyze the following:

Check each new lot of Colilert.

- Shine the ultraviolet lamp on the media snap packs. If the lot is fluorescent it will be discarded.
- Dissolve one packet in 100 ml distilled water. Do not incubate. Check for fluorescents.
- Analyze sterile reagent water blank with each batch of samples to verify that there is a negative result from 24-28 hours
- Gravimetrically check each new lot of sterile, transparent, non-fluorescing 100-ml vessels to ensure the 100-mL fill line is accurately represented on the vessel.

Quanti-Cult Procedure

a) Good laboratory practices will be used for this procedure.
b) Pre-heat incubator to bring temperature up to 35ºC.
c) Pre-warm rehydration fluid vials to 35º-37ºC. Use blue autoclavable foam vial holder to hold vials.
d) Discard blue cap from rehydration fluid vial.
e) Remove organism vial from pouch (vial with colorless cap).
f) Transfer colorless cap onto pre-warmed rehydration vial and discard vial containing the desiccant.
g) Place rehydration vials into the foam vial holder. Invert and place in incubator for 10 minutes at 35º-37ºC.
h) Fill four sterile IDEXX 100 ml. bottles with distilled water to fill line. Label three bottles with each bacteria name and one bottle "control". Place in incubator until a temperature of 35º ± 0.5ºC is reached.
i) Remove vial from holder. Hold vial upside down and tap cap gently to mix. Remove cap and look at inside surface to ensure that no un-dissolved black particles are present. Inoculate an additional 10 minutes if present.
j) Add entire contents of each appropriate bacteria vial to pre-warmed 100 ml. labeled bottles.
k) Add Colilert reagent to sample bottles including control. Place in incubator and follow incubation instructions outlined in section 7. Do not place in Quanti-Trays.
l) See figure 5.1 for Quanti-Cult datasheet

Results

The following results should be observed:

Organism	**Result**
Escherichia coli	Yellow wells, fluorescence
Klebsiella pneumoniae	Yellow wells, no fluorescence
Pseudomonas aeruginosa	Clear wells, no fluorescence
Method Blank	Clear wells, no fluorescence

m) Disposal
   i. All materials must be autoclaved prior to disposal and workspaces thoroughly disinfected.
   ii. Dispose of media in accordance with Good Laboratory Practices.

Bibliography

IDEXX, "Colisure Granulated Test Kit" product instructions. Number 06-03553-00, undated.
IDEXX, "Quanti-Tray" product instructions. Number 06-02030-07, undated.

IDEXX, "Quanti-Tray Sealer Model 2X User Manual." Number 06-03128-02, undated.
EPA Region 9 Laboratory Standard Operating Procedure guidance. "Colilert, Colilert-18 and Colisure Total Coliform and E.coli Water Analysis". Revised July 30, 1998.
Standard Method, "9223 B. Enzyme Substrate Test'" 20th ed., rev. 1998.

13.2 **Precision:** the analyst should analyze:

a. Field duplicates: one field duplicate per every 10 samples or 10%, randomly selected, taken at the same time

b. Laboratory duplicates (LD): two replicates taken from the same collection bottle. Analyze at least one LD for every 10 samples collected.

c. Laboratory reagent blank (LRB): analyze one LRB per sample batch.

13.3 Calculate Relative Percent Deviation (RPD). (Section 16.2)

14 Calibration and standardization

There are no calibration or standardization procedures for this method.

15 Procedure

See Appendix A for the manufacturer's instructions.

15.1 The 100ml duplicate water sample is shaken well just prior to preparation for analysis. Samples over the 100 ml mark must not be poured to volume. If there is at least 1" of headspace, the sample may be shaken and excess volume taken out with a sterile pipet. If there is insufficient headspace (<1") for proper mixing, do not pour off and discard a portion of the sample. Rather, pour the entire sample into a larger sterile container, mix properly, and proceed with the analysis.

15.2 Open a Colilert ampule and pour contents into either the diluted sample or undiluted sample. Repeat for the remaining sample.

15.3 Mix thoroughly, making sure the Colilert reagent is completely dissolved.

15.4 Follow manufacturer's instructions for preparation of Quanti-Tray/2000 and use of the Quanti-Tray Sealer.

15.5 Allow bubbles to settle or dissipate. Failure to do this may result in the wells filling or sealing improperly.

15.6 Record the sample's site code on the back of the well for identification purposes.

15.7 Record the lot number of the reagents and the wells used on the bench sheet in the comments section.

15.8 Incubate at 35.0 ± 0.5ºC for 24 hours.

15.9 Count the number of small and large positive wells and refer to the MPN table to find the most probable number for Total coliform.

15.10 E. coli results are obtained by placing the wells under a black light and counting the number of fluorescent wells. Refer to the MPN table to determine the E. coli concentration.

15.11 Report results on the bench sheet.

15.12 The completed bench sheet should be reviewed by the analyst, the laboratory director and the QA manager.

16 Data acquisition, calculations, and reporting

16.1 For each sample analyzed, including quality control samples, record the number of small and large positive wells and the MPN in the appropriate places on the bench sheet (see below). Calculate precision for duplicate analyses using equation 1.

16.2 Equation 1. Precision (as RPD) = $\frac{(A - B) \times 100\%}{(A + B)/2}$

Where: A = MPN from aliquot A and
B = MPN from aliquot B

17 Computer hardware and software

Word: This document and attached bench sheet are prepared using Microsoft Word. The Word document file name for this SOP is: Standard Operating Procedures for Surface Water_E.coli.doc

18 Method performance

The QVIR lab must successfully analyze at least one set of PT material once every 12 months for each method for which it is certified. The only method that the QVIR lab is certified for is an enzyme substrate method using Colilert. The test must contain 10 samples; all shipped at the same time, and must be lyophilized, dehydrated or in aqueous state. The test should contain total coliforms, fecal coliforms, *E. coli,* non-coliforms and at least one blank. An acceptable result is a correct analysis of 9 of the 10 samples, with no false negative results. The WS (Water Supply) Micro Standards and the WP (Water Pollution) Micro Standards from Wibby Environmental should both be used.

18.1 Store PT standards in the refrigerator at ~ 4ºC. Remember to record the temperature daily until samples are used.

18.2 Follow the date of analysis requirements provided by Wibby Environmental.

18.3 When ready to use, place the samples in the incubator for 2 hours at ~ 35ºC.

18.4 Homogenize the samples by shaking vigorously prior to beginning sample analysis

18.5 If using the Water Pollution standards, be sure to complete sample dilutions before proceeding with analysis.

18.6The Wibby Environmetnal WP Microstandard contains total Coliforms, Fecal Coliforms and E.coli within the EPA/NELAC specified concentration range of 20-2400 CFU/100ml
18.7 Report results to Wibby Environmental within the specified dates.
18.8 Include results in yearly laboratory report.

19 Data assessment and acceptable criteria for quality control measures

19.1 The analyst should review all data for correctness (e.g., use of MPN table).
19.2 Precision values are calculated for pairs of duplicate analyses.
19.3 Record the precision values as RPD on the bench sheet.
19.4 The desired precision is ± 20% (RPD).
19.5 The desired detection limit is 1 MPN/100mL
19.6 The completed bench sheet is reviewed by the analyst's supervisor or the QVIR Lab Director

20 Corrective actions for out-of-control or unacceptable data

20.1 The results for precision and blank data are compared to the acceptable values for this analysis; ± 20% and 1 MPN/100mL, respectively.
20.2 If a precision value exceeds 20% then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control duplicate analysis. The UCL = 20%." Note: "UCL" is the Upper Control Limit (i.e., 20%).
20.3 If a blank value exceeds 1 MPN/100mL then the analyst should write in the comments section of the bench sheet: "These data are associated with a blank value that exceeds the detection limit of 1 MPN/100mL."
20.4 The samples cannot be reanalyzed because the sample volume will be depleted after the initial analysis.
20.5 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

21 Waste management

The wastes generated in this method are not hazardous. The water can be discarded in the laboratory sink. Quanti-Trays are hand delivered to *Basic Lab* in Redding, CA were they are autoclaved and then discarded with the trash.

22 References

22.1 IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of: Colilert®, Quanti-Tray®/2000, and Quanti-Tray Sealer®.
22.2 Standard Methods for the Examination of Water and Wastewater. Method 9223 B., APHA, 21st Edition, 2005.

23 Reporting

23.1 Procedure for notification of clients for drinking water positives:
It is required to formally notify water suppliers for Total or E. Coli positive test results as follows:

a) Formally document notification- see attachment data sheet
b) Notify an officially designated contact person with the water supplier.
c) Arrange for re-sampling within 24 hours. Important: the notification must be with a "live voice" The message should not be left on an answering machine when notifying the water supplier.

23.2 A final report will be written by the Project Coordinator and QC Officer as well as sent to any funders, local Boards, EPA, and other interested parties. The final report will include the table and graphs that were developed for the web site and media, and it will describe the program's goals, methods, quality control results, data interpretation, and recommendations. Following notification of the Tribal Council, the QVIR Environmental Protection Department would then inform the North Coast Regional Water Quality Control Board staff and work cooperatively with that agency for abatement of problems.

24 Tables, diagrams, flowcharts and validation data

24.1 See Appendix A for MPN tables and Quanti-Tray/2000 instructions.
24.2 See below for the bench sheet. The analyst should make a copy of this form for each batch of samples analyzed.
24.3 The following validation procedures will be established throughout the project: Equipment will be calibrated at the start of the season and checked before each collection; blind field replicates will be submitted to the laboratory, which will also analyze lab duplicates, blanks, and Quanti-Cult new lot checks, chain of custody will be maintained; field sheets and data entry will be checked by the QC Officer; descriptive statistics and graphs will be produced.

Quanti-Tray®

Insert & MPN Table

Quanti-Tray Certificate of Sterility

This certifies that the enclosed Quanti-Trays have been sterilized with ethylene oxide.
For further information or documentation, contact IDEXX Laboratories, Inc.

IDEXX Laboratories
One IDEXX Drive, Westbrook, Maine 04092 USA

Phone 1-800-321-0207
Fax 207-856-0630

IDEXX

06-02030-09

Quanti-Tray®

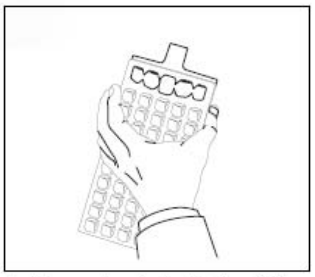
Introduction

IDEXX Quanti-Trays are designed to give quantitated bacterial counts of 100 ml samples using IDEXX Defined Substrate Technology* reagent products. Add the reagent/sample mixture to a Quanti-Tray, seal it in a Quanti-Tray Sealer and incubate per the reagent directions. Then count the number of positive wells and use the MPN table attached to determine the Most Probable Number (MPN).

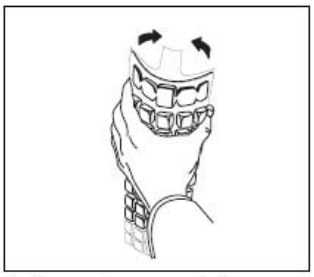
Contents

This package contains 100 sterile, 51-well Quanti-Trays.

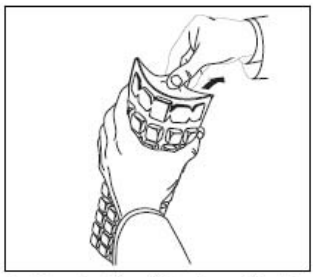
User Instructions



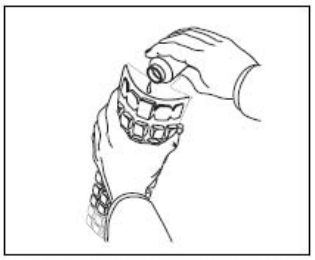
1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.



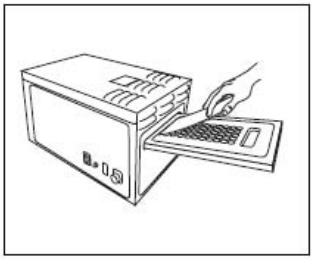
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm.



3. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.



4. Pour the reagent/sample mixture directly into the Quanti-Tray avoiding contact with the foil tab. Allow foam to settle.



5. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier.

6. Seal according to Sealer instructions.
7. Incubate according to reagent directions.
8. Count positive wells and refer to the MPN table on the back of this instructions sheet to find the Most Probable Number (MPN).
9. Dispose of media in accordance with Good Laboratory Practices.

For Technical Assistance, visit www.idexx.com/water, or in the U.S. and Canada, call 1-800-321-0207 or 207-856-0496

IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine 04092 USA

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.8	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.9	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.8	325.5	344.8	365.4	387.3	410.6	435.2

*Quanti-Tray is either a trademark or a registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries. Covered by U.S. Patent Numbers 4,925,789 ; 5,429,933 ; 5,518,892. Other patents pending.

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.8	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.8	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

*Quanti-Tray is either a trademark or a registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries. Covered by U.S. Patent Numbers 4,925,789 ; 5,429,933 ; 5,518,892. Other patents pending.

Quartz Valley Indian Reservation
Environmental Protection Program
Quartz Valley, CA
Escherichia coli IDEXX System

Analyst: _____ Project: _____

Date analyzed: _____

Data Reviewed By:	

Incubator Data: Start Day/Time: _____ Start Temperature (ºC): _____

End Day/Time: _____ End Temperature (ºC):_____

Sample Data		Large Well Positive Count		Small Well Positive Count		Most Probable Number (MPN/100ml) * [Mean of A + B]
		Replicate		Replicate		
Sample Identification	Date Collected	A	B	A	B	

Comments:
_____
_____

*See MPN tables.

Quartz Valley Indian Reservation
Environmental Protection Program
Quartz Valley, CA
Total Coliform IDEXX System

Analyst: _____ Project: _____

Date analyzed: _____

Data Reviewed By:	

Incubator Data: Start Day/Time: _____ Start Temperature (ºC): _____

End Day/Time: _____ End Temperature (ºC):_____

Sample Data		Large Well Positive Count		Small Well Positive Count		Most Probable Number (MPN/100ml) * [Mean of A + B]
		Replicate		Replicate		
Sample Identification	Date Collected	A	B	A	B	

Comments:

_____

_____

*See MPN tables

Quartz Valley Indian Reservation Microbiology Laboratory
Log for formally notifying client of a Total or E. coli
Positive Test Result

Name of Water Supplier	Phone Number	Date of Sample	Date of Notification	Time of notification	Date scheduled for Re-Sample	Staff Initials Performing Notification