Final Report

Project Title: Microbial Source Tracking (MST) at Bacteria-impaired Waters of the Lahontan Region

State Water Resource Control Board Contract Number: 13-054-160

Contract Period: 4/7/2014 – 4/30/2016

Contract Amount: $295,200

Date Submitted: March 24, 2016

Principal Investigators:

Roland Knapp, Ph.D., Center for Eastern Sierra Aquatic Microbial Ecology, Sierra Nevada Aquatic Research Laboratory, University of California, 1016 Mount Morrison Road, Mammoth Lakes, CA 93546 (roland.knapp@lifesci.ucsb.edu)

Craig Nelson, Ph.D., Center for Microbial Oceanography, University of Hawai'i at Mānoa, 1950 East-West Road, Honolulu, HI 96822 (craig.nelson@hawaii.edu)

Contract Manager:

Mary Fiore-Wagner, California Regional Water Quality Control Board - Lahontan Region, 2501 Lake Tahoe Blvd., South Lake Tahoe, CA 96150

TABLE OF CONTENTS

Appendices

EXECUTIVE SUMMARY

Project Scope

This study had three primary objectives:

(1) Provide a detailed description of spatial and temporal patterns of fecal indicator bacteria (FIB) concentrations in impaired stream reaches in the eastern Sierra Nevada portion of the Lahontan Region (Mono and Inyo counties), with reference to EPA *E.coli*-based water quality criteria. These data were collected by personnel from the Sierra Nevada Aquatic Research Laboratory.

(2) Use microbial source tracking (MST) assays that are based on quantitative real-time polymerase chain reaction (qPCR) methods to identify the relative contribution of humans versus ruminant animals (including cattle) to fecal contamination in impaired stream reaches in the eastern Sierra Nevada that were sampled under Objective 1.

(3) To determine the generality of the finding that in the Mono-Inyo County study area cattle are a primary driver of FIB levels, analyze FIB dataset collected from a large portion of the Lahontan Region (by Lahontan personnel) to identify possible landscape-scale and site-specific drivers of FIB concentrations at this broader scale. Use MST results to provide additional insights.

Findings and Interpretations

Results from sampling conducted during the current contract period clearly indicate that streams in several areas in Mono and Inyo counties show high levels of fecal coliform contamination, as determined by fecal indicator bacteria (FIB). For Bridgeport Valley, our results are consistent with those of previous sampling efforts that indicated high levels of fecal coliform bacteria *E. coli* in Swauger, Buckeye, and Robinson creeks, and the East Walker River, and support their listing as "impaired" under Section 303(d) of the Clean Water Act. Results from the current study also indicate that the listing as "impaired" of lower Mammoth Creek, upper Owens River, lower Rock Creek, lower Pine Creek, lower Horton Creek, North Fork Bishop Creek, and South Fork Bishop Creek may be warranted.

The patterns of fecal contamination for the study streams are now well-described, but it is also important to identify the primary sources of this contamination. Results from broadly and narrowly-targeted microbial source tracking (MST) assays indicated that ruminant-derived fecal contamination, including that from cattle, was common in the study streams and often at high concentrations. In contrast, human-derived fecal contamination was relatively rare and concentrations were generally low. In addition, the concentration of ruminant-derived Bacteroidales bacteria (as quantified by the BacCow MST assay) was the strongest predictor of *E. coli* concentrations (as quantified by the membrane filtration assay). Although these results clearly show that cattle are a primary source of fecal contamination in the study streams, data on the importance of other potential fecal sources is lacking. This is particularly relevant in areas in which multiple fecal sources are possible, and future studies should apply a broader assortment of MST assays to better describe their relative contributions.

To assess the generality of our finding that in the Mono-Inyo County study area cattle are an important driver of fecal contamination of streams, we analyzed a dataset of FIB results from more than 3,300 samples collected from streams across much of the Lahontan Region by personnel from the Lahontan Regional Water Quality Control Board. Results of the analysis were very similar to those reported previously for streams in Inyo and Mono counties. Specifically, the strongest predictors of FIB concentrations at sampling sites were the presence/absence of livestock (primarily cattle) immediately upstream at the time of sampling, sampling date, and sampling time. Other variables, including the amount of upstream human development, the amount of upstream meadow habitat, and elevation, had weaker but still significant effects on FIB concentrations.

INTRODUCTION

One of the primary causes of water quality impairment is the presence of pathogens associated with human and animal feces. Such feces can originate from a wide variety of sources, including sewage treatment facilities, septic tanks, farms, rangeland livestock, pets, and wildlife. Fecal-associated pathogens in waters can cause illnesses in humans, including those associated with bacteria (e.g., *Escherichia coli*, *Enterococcus*, and *Campylobacter*), protozoans (e.g., *Giardia* and *Cryptosporidium*), and viruses (e.g., rotaviruses). Some of these microorganisms can be pathogenic even at very low concentrations, and these low concentrations can make their detection difficult. Therefore, water quality monitoring often relies on detecting bacteria that are common in vertebrate feces and that can provide useful indicators of the presence of fecal material and associated pathogens. The most commonly tested-for fecal indicator bacteria (FIB) include fecal coliforms, *E. coli*, and *Enterococcus*. For recreational waters, early water quality criteria were based on fecal coliform bacteria (U.S. Environmental Protection Agency 1976) but are being replaced by criteria based on *E. coli* and *Enterococcus* (U.S. Environmental Protection Agency 1986).

Under the federal Clean Water Act, the U.S. Environmental Protection Agency (USEPA) is charged with developing water quality criteria, but this authority and implementation/enforcement of these criteria can be delegated to individual states. In California, this is the responsibility of the State Water Resources Control Board (State Water Board) and nine Regional Water Quality Control Boards (Regional Water Boards). The State Water Board adopts and enforces standards and policies at the statewide level and Regional Water Boards do the same at regional/local levels. The Lahontan Regional Water Quality Control Board (Lahontan Water Board) manages the Lahontan Region that covers the area of eastern California in which the current study was conducted. In the Lahontan Region, the current FIB objective is still based on fecal coliform bacteria, with a water quality standard of 20 colony-forming units (CFU) per 100 mL. Based on updated bacterial water quality criteria developed by the USEPA (U.S. Environmental Protection Agency 1986, 2012), the State Water Board is currently revising the statewide FIB standard for recreational waters. The most recent USEPA water quality criteria are 100 or 126 *E. coli* CFU per 100 mL (U.S. Environmental Protection Agency 2012).

The primary focus of the current contract was to assess FIB concentrations and sources in a subset of streams in the eastern Sierra Nevada in which FIB levels commonly exceed both the current fecal coliform standard and the EPA *E. coli*-based criteria. Some of the study streams are officially listed as “impaired” under Section 303(d) of the Clean Water Act, and others meet the criteria of “impaired” but have not yet been listed. For clarity, in this report streams in both categories as referred to as “impaired.” As part of this contract, we (Center for Eastern Sierra Aquatic Microbial Ecology – CESAME) also conducted a landscape-level analysis of FIB results from sites across a large portion of the Lahontan Region. As such, in this report we describe analyses and results focused on three primary objectives:

(1) Provide a detailed description of spatial and temporal patterns of FIB concentrations in impaired stream reaches in the eastern Sierra Nevada portion of the Lahontan Region (Mono and Inyo counties), with specific reference to recent EPA *E.coli*-based water quality criteria.

(2) Use microbial source tracking (MST) assays that are based on quantitative real-time polymerase chain reaction (qPCR) methods to identify the relative contribution of humans versus ruminant animals (including cattle) to FIB concentrations in impaired stream reaches in the eastern Sierra Nevada that were sampled under Objective 1.

(3) To determine the generality of the finding that in the Mono-Inyo County study area cattle are a primary driver of FIB concentrations, analyze FIB results from samples collected from a large portion of the Lahontan Region (by Lahontan personnel) to identify possible landscape-scale and site-specific drivers of FIB concentrations at this broader scale. Use MST results to provide additional insights.

OBJECTIVES 1 & 2
CHARACTERIZATION OF FIB CONCENTRATIONS AND FECAL SOURCES – CESAME-COLLECTED DATA

Methods

Study area description

The study area is located at the base of the eastern escarpment of the southern Sierra Nevada and includes both Sierra Nevada and Great Basin ecoregions. Elevations of the sampling sites range from 1259 m (lower Bishop Creek) to 2393 m (upper Mammoth Creek), and the area is characterized by cold winters and warm to hot summers. Precipitation amounts are highest near the crest of the Sierra Nevada, and decrease rapidly east of the crest. Most precipitation falls as winter snow, and precipitation events during summer are typically associated with convective thunderstorms. During the two-year duration of the study (2014-2015) the area was in the midst of an extreme drought, with total annual precipitation generally <50% of the long-term average. In each of the four areas that are the focus of the current study (Bridgeport Valley, Long Valley, Round Valley, and Bishop Creek), the upper stream reaches are under the jurisdiction of the U.S Forest Service (Humboldt-Toiyabe National Forest or Inyo National Forest), and the lower reaches are generally private lands or are owned by the Walker River Irrigation District (WRID), Los Angeles Department of Water and Power (LADWP), or Bishop Paiute Tribe. All sampling sites were open to the public.

To characterize FIB concentrations in the study streams and the fecal sources, we repeatedly sampled 39 sites located on 12 streams in Mono County and northern Inyo County, California (Figure 1-4, Appendix A). All of the streams drained watersheds originating in the Sierra Nevada, and most were relatively small with base flow discharges of 0.05 to 4 $m^3 \cdot s^{-1}$. Sampling sites were generally located in the immediate vicinity of land uses that were potential contributors of FIB to the study streams (human developments, cattle grazing, campgrounds, etc.). When possible, stream reaches immediately above these land uses were also sampled. Coordinates of each sampling site were determined using a geographic information system (ArcGIS 10.2).

Collection of water samples

During 2014 and 2015, samples were collected from all sites monthly from May to October. All sampling was conducted during baseflow or near-baseflow conditions. Water samples were collected by hand in mid-stream, approximately 3 cm below the water surface and upstream of the collector. For each sample, a new pair of disposable gloves was used by the collector. Prior to use, the 1000 mL polypropylene sample bottles were autoclaved to ensure they were sterile. Sample bottles were filled to within 1-2 cm of the rim, capped, and immediately placed into a cooler with ice packs and transported to the Sierra Nevada Aquatic Research Laboratory (SNARL) for analysis. The time between sample collection and arrival at SNARL was always ≤ 6 hours (range 0.2-5.2, average = 2.2).

Culturing of fecal indicator bacteria

FIB were cultured from the samples using standard membrane filtration methods, specifically "Standard Methods" for fecal coliform bacteria (9222D) and *E. coli* (9222G; American Public Health Association et al. 1998). To process a sample, the 1000 mL sample bottle was first shaken vigorously to mix the contents, and then 1-4 subsamples were removed from the sample using a sterile serological pipette. Each subsample was placed into a separate filtration unit, and pulled through a 0.45 µm mixed cellulose ester membrane filter (Millipore HAWG) using vacuum filtration (< 250 mm Hg). Subsample filtration volumes ranged from 5 to 100 mL; the number and volume of subsamples were based on the bacteria culturing results from previous samples from a site and based on observations made during sample collection (e.g., presence or absence of cattle upstream), with the goal of obtaining 20-60 CFUs per filter. Following filtration, each filter was transferred face-up to a petri dish containing a filter pad and 2.0 mL of m-FC Broth with Rosolic Acid (Millipore MHA000P2F). A lid was placed on the petri dish, and the dish was inverted, placed into a waterproof container, and submerged in a water bath where it was incubated for fecal coliform bacteria: 22-26 hours at 44.5 ± 0.2 °C. All samples were processed within two hours of arriving at the laboratory (range = 0.3-2.0, average = 1.1) and incubation of each filter began within 30 minutes after filtration. Filtration "blanks" were run regularly during sample processing to ensure that rinsing procedures were sufficient to remove all bacteria in previous samples from the filtration unit. Blanks were created by filtering 100 mL of autoclaved deionized (Milli-Q) water using the same methods as described above for field samples. For each batch of samples, every tenth filter and the first and last filter was a blank. In addition, one of the subsamples in each batch was run in duplicate to evaluate within-subsample variation in FIB counts.

At the conclusion of the fecal coliform incubation period, filters were removed from the water bath and fecal coliform CFUs were counted under a low-power binocular microscope. Fecal coliform CFUs were distinguished from non-fecal coliforms by their characteristic blue color. For each sample, the filter with a CFU count that most closely matched the 20-60 CFU target was selected for subsequent *E. coli* culturing. To do this, the filter was removed from the m-FC media and transferred to a sterile petri dish containing nutrient agar with 4-methylumbelliferyl-β-D-glucuronide (NA-MUG; BD Difco 223100). The dish was then placed into a waterproof container and incubated in a water bath for 4 hours at 35 ± 0.5 °C as described above. Following incubation, *E. coli* CFUs were enumerated under a 6W 365nm long wave-length ultraviolet light source (UVP 95-0006-02). *E. coli* CFUs were distinguished from non-*E. coli* CFUs by their distinctive blue fluorescence.

Quality assurance and quality control practices

All samples were collected, processed, and analyzed, and all data were reviewed and managed, in accordance with all relevant provisions of the project's Quality Assurance Project Plan (membrane filtration: SNARL-CESAME.QAPP.V1.2012) and Standard Operating Procedures (qPCR: Appendix C).

Spatial and temporal patterns in FIB concentrations

As summarized in the Introduction, for recreational waters the current FIB water quality standard in the Lahontan Region is 20 fecal coliform CFU per 100 mL, and the 2012 EPA water quality criteria are 100 or 126 *E. coli* CFU per 100 mL. To allow comparison of our FIB results against the current standard and the EPA criteria, we present all of our FIB results as counts of *E. coli* CFU per 100 mL. Counts based on *E. coli* provide a more accurate description of the concentration of fecal-derived bacteria than do results based on fecal coliforms because some bacteria categorized as fecal coliforms

are actually not derived from feces (U.S. Environmental Protection Agency 1986). In addition, counts of fecal coliform and *E. coli* CFU per 100 mL from individual samples are highly correlated (r = 0.95) and the slope of their relationship is not significantly different from 1 (Knapp and Nelson 2015).

To describe the temporal patterns in *E. coli* concentrations across each of the four focal areas, for each site we plotted a time series for the 2014-2015 sampling period. Filters that produced no FIB colonies were given a CFU value of zero. To provide as long-term a perspective as possible for each site, when FIB data were available from previous time periods (i.e., collected under previous contracts) those data were included in the time series. Project data were stored in a SQL relational database (Microsoft Access v. 2013) and geographic information system (ArcGIS v. 10.2).

Microbial source tracking

To continue our efforts to describe the relative contribution of ruminant and human sources to fecal bacteria in impaired stream reaches in the study area, under this contact we applied five MST assays (two general bacterial assays and three source-specific assays; Table 2) to 273 samples collected from impaired stream reaches and adjacent reaches in the Mono-Inyo County study area by CESAME personnel. Collection locations and dates for all 273 samples are provided in Appendix B.

Bacterial cells were collected from water samples by filtering 150-800 mL of water (median = 800 mL) from the 1000 mL sample using the methods described above for the membrane filtration assays. One filtration blank was collected on every date on which samples were processed. Following filtration, all filters were placed into microcentrifuge tubes and stored at -40 °C until analysis. Filters selected for analysis were collected by CESAME personnel during the 2013-2014 field seasons (115 and 158, respectively), and represented a broad diversity of land uses.

A description of MST standard operating procedures is provided in Appendix C, and these procedures are summarized here. DNA was extracted from filters using MoBio PowerSoil® DNA Isolation Kits (MoBio 12888). All samples were analyzed using a suite of five targeted 5' exonuclease quantitative polymerase chain reaction (qPCR)-based MST assays (Table 2). Two of these assays targeted general bacterial groups or individual species (Bacteroidales, and *Escherichia* including *Escherichia coli*). The remaining three assays targeted two specific subgroups of Bacteroidales associated with animal sources of fecal contamination: ruminants and humans. The only ruminants in the project area are mule deer, cattle, domestic sheep, and domestic goats. Assays were conducted using widely established methods including those approved by the USEPA (summarized in Boehm et al. (2013) and references in Table 2). The source-specific assays we used (Table 2) are those recommended following thorough testing for sensitivity and specificity (Boehm et al. 2013, Layton et al. 2013, Raith et al. 2013). Sensitivity is the ability of an assay to detect the target bacteria (and by extension, its vertebrate source) when it is present in a sample. Specificity is the ability of an assay to discriminate the target bacteria from bacteria of other potential sources. Therefore, assays with high sensitivity detect the target bacteria when it is present, and those with high specificity identify as negative those samples lacking the target bacteria. Names of source-specific assays used in this report are those by which each assay was originally described in the peer-reviewed scientific literature. The ruminant assay (BacCow; Kildare et al. 2007) was originally developed as a cow-specific assay but was subsequently discovered to cross-react with feces/bacteria from other ruminants (Boehm et al. 2013, Raith et al. 2013). Therefore, it is now classified as a ruminant-specific assay. The two human assays (BacHum: Kildare et al. 2007; HF183: Haugland et al. 2010, Green et al. 2014) differ somewhat in their sensitivity and specificity, with BacHum being highly sensitive but not 100% specific to human feces, and HF183 less sensitive but 100% human-specific

(Layton et al. 2013). Because these differences are complimentary, it is generally recommended that samples be analyzed using both assays (Boehm et al. 2013, Layton et al. 2013).

Statistical Analyses

Analysis of landscape-scale drivers of MST-derived fecal bacteria concentrations. Results from a previous analysis indicated that the most important land-use predictor of *E. coli* concentration (as measured using membrane filtration methods) was the presence of cattle upstream of a sampling site (Knapp and Nelson 2015). The intensity of upstream human development was a much weaker predictor. To further test the hypothesis that within our Mono-Inyo County study area the presence of cattle is a primary driver of fecal concentrations in the study streams, we developed similar statistical models in which MST-derived concentrations of each of the targeted bacteria were used as response variables, instead of the *E. coli* concentration as determined from membrane filtration. MST results from a total of 273 samples analyzed under the current contract were included in the analysis. The final data set included landscape predictors and MST results from 79 sites located on 20 streams. Because this dataset was substantially smaller than that used in the FIB analysis developed under the previous contract (Knapp and Nelson 2015), the number of predictor variables was reduced from 11 to seven (Table 2); no new variables were added.

Several of the predictor variables describe the extent or presence/absence of a particular land use in the vicinity of each sampling location (i.e., presence of upstream lakes, area of high-intensity land use, and presence of livestock). As in Knapp and Nelson (2015), these variables were calculated for a "sector" that circumscribes the area in the immediate vicinity of a sampling location, regardless of watershed boundaries. Sectors were created using the ArcGIS Sectors tool. Each sector was centered on a sampling site, oriented upstream, and had a radius of 1.5 km and an angle of 90° (Appendix D). The 1.5 km radius was chosen based on results from studies of bacteria attenuation conducted in similar montane habitats (Willden 2006), and FIB results for the study area that suggested similar high attenuation rates. The majority of sectors fell entirely or almost entirely within the watershed that contained the associated sampling site.

All statistical analyses were conducted using R version 3.2.2 (R Development Core Team 2015) and the R package *glmmADMB*. We used multivariate generalized linear models to quantify the strength of associations between predictor variables and MST-derived fecal bacteria concentrations (BacCow, EC23S857, GenBac3; Table 1). In all analyses we were primarily interested in the effects of the landscape variables but included other covariates to reduce the chances of confounding effects caused by not including important predictors. Our general regression analysis approach followed the protocol of Zuur et al. (2009, Section 4.2.3). Our approach, which included a model with both fixed and random effects (see below), allowed us to account for between-sample dependencies and thereby include every sample as a separate record in the analysis. This allows for a much more informative and statistically powerful analysis than is possible using a simpler approach in which MST results are averaged for each sampling site and only the averaged values are included in the analysis. The response variable in this analysis was BacCow copies per 100 mL (BACCOW).

The regression analysis started with a generalized linear mixed effects model that contained all categorical and continuous predictor variables (Table 2). A square-root transformation was applied to the BACCOW variable to reduce the leverage of extremely high values, and the transformed data were assumed to approximate a zero-inflated Poisson or zero-inflated negative binomial distribution. SITEID and YEAR were included as random effects in the model to account for consistent differences in MST

concentrations between sites, and the lack of independence in intra-site results due to repeated sampling of the same location through time. The starting model was as follows:

(1) *BACCOW ~ LAKE + LANDUSE23 + COW + DAY + TIME | YEAR/SITEID*

The model was analyzed using each of four different distributions (Poisson, zero-inflated Poisson, and two forms of a zero-inflated negative binomial distribution); the distribution that provided the best fit to the data was assessed using AIC.

To find the optimal fixed effect structure for Model 1, we sequentially dropped the least significant variable and refit the model until all remaining fixed-effect variables were significant ($P \leq 0.05$). To evaluate whether model fit could be further improved by including non-linear terms for the continuous variables in the final model, we added quadratic terms for DAY and TIME (i.e., DAY^2, $TIME^2$).

To determine whether the predictor variables in Model 1 also had significant effects on the number of GenBac3 and EC23S857 (*Escherichia*) copies, we developed similar models for each of these response variables. In each case, we evaluated the models using the same four distributions, and after identifying the distribution that provided the best fit to the data we identified the optimal fixed-effect structure.

Results

During the 2014-2015 study period, a total of 539 samples were collected from 43 sites on 12 streams, and analyzed for FIB by CESAME personnel. At nearly all sites, collections were made on a monthly basis during May-October 2014 and February-October 2015. In addition, under the current contract a total of 273 samples were analyzed using five MST qPCR assays. These samples were collected in May-October 2014 under the current contract or April-October 2013 under the previous contract.

Membrane filtration quality control measures

Results from membrane filtration blanks demonstrated the adequacy of our sterile techniques when culturing FIB. Of the 197 blanks that were incubated, *E. coli* colonies were observed on only four filters and included only a single CFU on three of those filters and two CFUs on the fourth filter. Therefore, the between-sample rinsing protocol was nearly always sufficient to remove bacteria from the filtration unit. Membrane filtration duplicates indicated the repeatability of FIB counts. Subsamples from 62 samples were run in duplicate, and the number of *E. coli* colonies on duplicate filters was very similar (Model II (reduced major axis) regression: $R^2 = 0.98$, $P << 0.0001$; slope = 1.0, 95% confidence interval = 0.96-1.04).

Microbial source tracking

General patterns. Results from five MST assays applied to 273 CESAME samples are provided in Appendix B. The concentrations of *Escherichia* (including *E. coli*; EC23S857 assay), total Bacteroidales (GenBac3 assay), and ruminant Bacteroidales (BacCow assay) were found in concentrations ranging generally over five orders of magnitude, from 10^1 to 10^6 copies per 100 mL. These concentrations match those provided in our previous contract report (Knapp and Nelson 2015), indicating general comparability of these two sets of MST data. Almost all samples were positive for GenBac3, with mean and median values exceeding 10,000 copies per 100 mL in both 2013 and 2014 samples. *Escherichia* copy concentrations were roughly two orders of magnitude lower, with 25-40% of the samples below

limits of detection in both years. BacCow exhibited a strong binomial distribution, with roughly half of the samples below limits of detection in both years and positive samples averaging roughly 1,000 copies per 100 mL. Among 131 samples in which both total and ruminant Bacteroidales were detected, the lognormal mean and median proportional ruminant contribution (BacCow/GenBac3) was 5%, and was highly correlated to absolute BacCow copy number (r = 0.76, P < 0.0001). The human-specific Bacteroidales assays (BacHum and HF183) produced positive results in only 20 samples, ranging from 2 to 1,253 copies per 100 mL. Positive results were detected from multiple sites on Robinson Creek, Bishop Creek, and Mammoth Creek, and for these streams detections occurred on multiple dates. Five of the 20 samples were positive for both human-specific assays. Membrane filtration-based *E. coli* concentrations strongly and significantly predicted qPCR-based *Escherichia* gene copy concentrations ([$\log_{10}$ + 1] transformed data: r = 0.76, $P < 10^{-10}$), an important validation that the qPCR *Escherichia* assay is detecting similar organisms as those detected by the *E. coli* membrane filtration assay. Landscape-scale drivers of ruminant-derived Bacteroidales, total Bacteroidales, and *Escherichia* copy numbers are described in the following section; results from the human-specific assays could not be related to landscape parameters because of the low detection rate.

Landscape-scale drivers of MST-derived fecal bacteria concentrations. For the BacCow model, the fit of Model 1 under four different distributions indicated that the zero-inflated negative binomial (binom) distribution provided the best fit to the data. Of the five predictor variables included in the model, all but LANDUSE23 (a measure of human development intensity upstream of the sampling site) had significant effects on the number of BacCow copies (Table 3). The presence of cattle upstream at the time of sampling (COW) had by far the strongest effect on BACCOW; when cattle were present upstream of the sampling site, the number of BacCow copies was much higher than when cattle were absent. Day of the year (DAY) and sampling time (TIME) also had highly significant positive effects on the number of BacCow copies detected (Table 3). The presence of upstream lakes had a significant negative effect on the number of BacCow copies. Collectively, these results are very similar in both magnitude and direction to those identified previously as important predictors of FIB levels in the study streams (Knapp and Nelson 2015), and provide another indication that in the Mono-Inyo County study area cattle appear to be a major driver of fecal bacteria concentrations in streams.

In the final GenBac3 (total Bacteroidales) and EC23S857 (*Escherichia*) MST models, the presence of cattle upstream of the sampling location was by far the strongest predictor of the number of GenBac3 and EC23S857 copies. The GenBac3 model also included significant effects of LAKE (negative) and DAY (positive); in the EC23S857 model no other predictors were significant.

FIB and MST patterns in impaired stream reaches

A primary objective of the current contract was to describe the patterns of fecal contamination in impaired stream reaches in the Mono-Inyo County study area, using data obtained from both membrane filtration and microbial source tracking assays. The following provides a detailed summary of patterns and likely sources of fecal contamination for the study streams located in Bridgeport Valley, Long Valley, Round Valley, and the Bishop Creek watershed. Terms, such as “low”, “moderate”, and “high” are used to describe the overall concentrations of FIB and MST markers, and are intended to provide general descriptions that integrate across all samples collected across different seasons. Sample-specific concentrations for all sites are provided in the associated figures (Figure 5-12).

Bridgeport Valley. Bridgeport Valley is traversed be several streams that are currently listed as "impaired." These streams (Swauger Creek, Buckeye Creek, Robinson Creek, East Walker River) flow through extensive areas used for cattle grazing (Figure 1), and previously-collected membrane filtration and MST data have suggested that cattle are a major source of fecal contamination (Knapp and Nelson 2015).

Our four sampling sites on Swauger Creek are (from upstream to downstream) SWA.02, SWA.06, SWA.05, and SWA.08 (Figure 1). SWA.02 is located near the headwaters of Swauger Creek in an area characterized by very low-density residential development (septic systems provide waste water treatment) and occasional grazing by domestic sheep. SWA.06 is at the upstream end of Huntoon Valley, and is downstream of a moderate-density residential development (also with septic systems for waste water treatment), and some properties have horse pastures. The area also appears to be grazed by domestic sheep seasonally, and perhaps by some cattle. SWA.05 is at the downstream end of Huntoon Valley, an area subject to heavy cattle grazing of flood-irrigated pastures. SWA.08 is downstream of an extensive beaver dam complex, and a U.S. Forest Service housing compound and associated horse pasture. All four sites on Swauger Creek showed strong seasonality in FIB concentrations, with low *E. coli* CFU counts in winter-spring and relatively high levels in summer-fall (Figure 5).

- *E. coli* levels in SWA.02 were generally low, rarely exceeding 50 CFU per 100 mL (Figure 5) and never exceeding 100 CFU per 100 mL. MST assays indicated low concentrations of *Escherichia* (including *E. coli*) and ruminant markers (EC23S857 and BacCow, respectively), and no detections of human markers (BacHum, HF183; Appendix B).
- SWA.06 showed substantially higher *E. coli* concentrations; levels regularly exceeded 50 CFU per 100 mL but rarely exceeded 100 CFU per 100 mL (Figure 5). MST assays indicated occasional high concentrations of *Escherichia* and ruminant markers, but no detections of human markers (Appendix B).
- SWA.05 was the most contaminated of the Swauger Creek sites, with *E. coli* CFUs commonly near or exceeding 100 CFU per 100 mL during summer months and occasionally exceeding 400 CFU per 100 mL (Figure 5). In 2015, *E. coli* concentrations at this site were lower than in 2013 and 2014, probably due to much lower cattle stocking densities in 2015 due to severe drought conditions and the resulting lack of water for flood irrigation. MST assays commonly indicated high concentrations of *Escherichia* and ruminant markers, but no detections of human markers.
- SWA.08 had much lower levels of fecal contamination than did the upstream SWA.05, but elevated *E. coli* CFU counts were still evident (Figure 5). *E. coli* concentrations regularly exceeded 30 CFU per 100 mL, but only rarely exceeded 100 CFU per 100 mL. MST assays indicated occasional moderate concentrations of *Escherichia* and ruminant markers, and very low concentrations of human markers on one occasion.

In summary, Swauger Creek shows consistently high levels of fecal contamination in the vicinity of Huntoon Valley, with cattle as a likely source. The relatively high levels of contamination at the lower end of Huntoon Valley (SWA.05) even in 2015 when cattle stocking densities were very low suggests that significantly reducing levels of fecal contamination at this site may not be possible without controls on cattle access to the stream and immediate vicinity. The source of elevated *E. coli* levels at the upper end of Huntoon Valley remain somewhat uncertain, with MST assays sometimes indicating low or no contribution by ruminant or human sources even when *E. coli* CFU counts were relatively high. The

presence of horses upstream suggests that this may be a useful site at which to test samples for the presence of horse-derived MST markers. SWA.08 presents another opportunity to employ the horse MST assay, as well as a beaver MST assay.

Buckeye Creek was sampled at four locations: BUC.03, BUC.04, BUC.05, and BUC.08 (Figure 1). BUC.03 is located immediately downstream of Buckeye Hot Springs, an area heavily used by human bathers. BUC.03 is upstream of the portion of Bridgeport Valley that is intensively grazed by cattle, but cattle grazing occurs several kilometers upstream in Big Meadow. A U.S. Forest Service (USFS) campground is also located upstream. BUC.04 and BUC.05 are immediately adjacent to each other at Highway 395 (on North Branch and Middle Branch of creek, respectively), and are immediately downstream of flood-irrigated cattle-grazed pastures. BUC.08 is located on WRID property, and is also immediately downstream of areas subject to intensive cattle grazing. All four sites on Buckeye Creek show strong seasonality in FIB concentrations (Figure 6), with peak *E. coli* concentrations during summer and fall.

- BUC.03 has low levels of fecal contamination, with *E. coli* concentrations rarely exceeding 40 CFU per 100 mL and never exceeding 100 CFU per 100 mL (Figure 6). MST assays indicate low-to-moderate concentrations of *Escherichia* and ruminant markers, but no detection of human markers (Appendix B).
- BUC.04, BUC.05, and BUC.08 all show similar and high levels of fecal contamination, with *E. coli* levels commonly exceeding 100 CFU per 100 mL in mid-summer, and occasionally exceeding 400 CFU per 100 mL (Figure 6). MST assays indicate very high concentrations of *Escherichia* and ruminant markers. Human markers were detected only rarely and at very low concentrations (Appendix B).

In conclusion, Buckeye Creek shows consistently high levels of fecal contamination downstream of areas intensively grazed by cattle, and MST results indicate that cattle are a likely source. Controlling livestock access to the creek may, by itself, be insufficient to prevent the documented fecal contamination. Because flood irrigation practices spread water broadly across the area, and return-flows to the creek occur and may contain high levels of fecal contamination, reducing fecal contamination in this area may require a two-pronged approach of strategically controlling cattle access to riparian areas while simultaneously minimizing contaminated irrigation return flows.

Robinson Creek was sampled at five locations: RBS.03, RBS.05, RBS.07, RBS.08, and RBS.10. RBS.03 is located immediately below Lower Twin Lake (Figure 1). RBS.05 is located downstream of several USFS campgrounds and a residential development. RBS.07 and RBS.08 are immediately adjacent to each other at Highway 395 (on North Branch and South Branch of creek, respectively), and are immediately downstream of flood-irrigated cattle-grazed pastures. RBS.10 is located on WRID property, and is also immediately downstream of areas subject to intensive cattle grazing. All five sites on Robinson Creek show strong seasonality in FIB concentrations (Figure 7), with peak *E. coli* concentrations during summer and fall.

- RBS.03 has very low levels of fecal contamination, with *E. coli* concentrations never exceeding 5 CFU per 100 mL (Figure 7). MST assays never detected *Escherichia*, ruminant, or human markers (Appendix B).
- RBS.05 shows higher but still relatively low fecal contamination, with *E. coli* concentrations generally not exceeding 30 CFU per 100 mL (Figure 7). MST assays

occasionally detected *Escherichia* and ruminant markers, but human markers were never detected (Appendix B).

- RBS.07, RBS.08, and RBS.10 show very high levels of fecal contamination, with *E. coli* concentrations commonly exceeding 100 CFU per 100 mL and occasionally exceeding 400 CFU per 100 mL (Figure 7). MST assays indicated very high concentrations of *Escherichia* and ruminant markers. Human markers were detected on two occasions, both in samples collected from RBS.08 in 2013, but concentrations were relatively low (Appendix B).

In summary, Robinson Creek shows consistently high levels of fecal contamination downstream of areas intensively grazed by cattle, and MST results indicate that cattle are a likely source. Low-level human-derived fecal contamination was detected in one of 11 samples from RBS.08, suggesting that this contribution is rare and relatively insignificant. Human-derived fecal contamination occurred at a much higher level in samples collected in 2012 (Knapp and Nelson 2015). As is the case for Buckeye Creek, reducing the cattle-derived fecal contamination in Robinson Creek may require more than simply controlling livestock access to riparian areas, due to flood irrigation practices that may result in contaminated return-flows to the creek.

The East Walker River watershed was sampled at four locations: VIR.04, GRE.40, EWK.06, and EWK.08 (Figure 1). GRE.40 is located on Green Creek and the watershed upstream of this site is relatively undeveloped, characterized by dispersed recreation including several USFS campgrounds. VIR.04 is located on a reach of Virginia Creek that parallels Highway 395 and is immediately downstream of a moderate-density residential/commercial development. EWK.06 is located several kilometers below the confluence of Virginia and Green creeks and downstream of extensive flood-irrigated cattle-grazed pastures. EWK.08 is located on WRID property, and is immediately downstream of the town of Bridgeport and of extensive areas subject to intensive cattle grazing. Sampling sites in the East Walker River watershed generally show strong seasonality in FIB concentrations (Figure 8), with peak *E. coli* concentrations during summer and fall.

- GRE.40 shows low levels of fecal contamination year-round, and *E. coli* concentrations rarely exceed 20 CFU per 100 mL (Figure 8). Only a single sample was analyzed using MST assay, and this sample showed relatively low concentrations of *Escherichia* markers and no ruminant or human markers (Appendix B).
- VIR.04 shows moderate levels of fecal contamination, with *E. coli* concentrations commonly above 20 CFU per 100 mL but rarely above 100 CFU per 100 mL (Figure 8). MST data are available for only a single sample but indicate a low-to-moderate concentration of *Escherichia* markers and no ruminant or human markers (Appendix B).
- EWK.06 and EWK.08 show very high levels of fecal contamination, with *E. coli* concentrations in mid-summer commonly exceeding 200 CFU per 100 mL (Figure 8). MST assays show very high levels of *Escherichia* and ruminant markers, but human markers were not detected (Appendix B).

In summary, sites in the East Walker River watershed located immediately downstream of areas subject to intensive cattle grazing show consistently high levels of fecal contamination, and MST results indicate that cattle are a likely source. As is the case elsewhere in Bridgeport Valley, reducing this fecal contamination may require controlling livestock access to surface waters in combination with managing flood irrigation practices that result in contaminated return-flows to the river.

Long Valley. Long Valley is traversed by several major streams, and previous sampling indicated moderate-to-high levels of fecal contamination in Mammoth Creek and the upper Owens River above Crowley Reservoir. To better describe this fecal contamination, we sampled three sites on Mammoth Creek (MAM.30, MAM.40, and MAM.50) and the Owens River downstream of the Mammoth Creek-Owens River confluence (OWE.40; Figure 2). MAM.30 is located in the town of Mammoth Lakes, and the upstream watershed is characterized by high-density residential development. MAM.40 is located immediately below Highway 395 and is heavily utilized by recreationists. MAM.50 is located immediately below Chance Ranch, which is flood irrigated and heavily grazed by cattle. On the Chance Ranch, access to Mammoth Creek is limited by corridor fencing, but cattle have direct access to the creek in the upper portion of the ranch. The fenced riparian corridor may be grazed in some years, but details are unknown. OWE.40 is in an area heavily utilized by cattle and recreationists. Fenced irrigated pastures exist on both sides of the river and cattle in pastures often have direct river access. Sampling sites in Long Valley generally show strong seasonality in FIB concentrations, with peak *E. coli* concentrations during summer and fall (Figure 9).

- MAM.30 shows moderate levels of fecal contamination, with *E. coli* concentrations regularly above 50 CFU per 100 mL but rarely exceeding 100 CFU per 100 mL (Figure 9). MST assays indicate low-to-moderate concentrations of *Escherichia* markers for a majority of samples, low concentrations of ruminant markers on three occasions, and low concentrations of human markers on two occasions (Figure 9). Given the absence of cattle upstream of MAM.30, the presence of BacCow markers may indicate the presence of low levels of fecal contamination from other ruminants, such as deer. However, given the low concentrations this could also be the result of cross-amplification of Bacteroidales from other sources.
- MAM.40 shows moderate-to-high levels of fecal contamination, with *E. coli* concentrations often in the 50-100 CFU per 100 mL range. During summer 2014, concentrations commonly exceeded 100 CFU per 100 mL, but such exceedances did not occur in 2013 or 2015 (Figure 9). MST analyses indicate moderate concentrations of *Escherichia* and ruminant markers. In addition, low concentrations of human markers were detected on one sampling date.
- MAM.50 shows a similar pattern of fecal contamination to MAM.40, with most samples having *E. coli* concentrations between 50 and 100 CFU per 100 mL, but regularly exceeding 100 CFU per 100 mL (Figure 9). MST assays indicate moderate-to-high concentrations of *Escherichia* and ruminant markers. In addition, low concentrations of human markers were detected on two sampling dates.
- OWE.40 shows high levels of fecal contamination, with *E. coli* concentrations commonly exceeding 100 CFU per 100 mL (Figure 9). MST assays indicate relatively high concentrations of *Escherichia* markers, but variable concentrations of ruminant markers. No human markers were detected. The concentration of ruminant markers was lower than expected given generally high *E. coli* levels obtained from membrane filtration assays and the presence of many cattle in the area.

In summary, Mammoth Creek and the upper Owens River show moderate-to-high levels of fecal contamination, and although cattle are likely to be important sources of contamination at some sites (MAM.50, OWE.40), MST results suggest that other sources are also possible, including wildlife such as

deer. Analysis of additional samples with MST assays targeting ruminant, human, and other sources of contamination (e.g., dogs) will be necessary to better resolve this issue.

Round Valley. Round Valley is traversed by Horton Creek, Pine Creek, and Rock Creek. Results from previous sampling indicted that reaches above the cattle-grazed portion of the main valley were relatively free of fecal contamination but that reaches leaving the downstream portion of the valley after flowing through flood irrigated pastures grazed by cattle and horses had the potential for high FIB levels. To describe patterns of fecal contamination, we sampled one site on the lower end of each creek at the point where each creek leaves Round Valley (HOR.70, PIN.50, ROC.80; Figure 3). The area upstream of each site is characterized by flood-irrigated pastures and intensive grazing by cattle and some horses. Sampling sites in Round Valley show relatively weak seasonality in FIB levels, with peak *E. coli* concentrations extending from late-spring to late-fall (Figure 10).

- HOR.70 and PIN.50 have very high levels of fecal contamination, with *E. coli* concentrations regularly exceeding 100 CFU per 100 mL and levels >300 CFU per 100 mL were recorded on numerous occasions (Figure 10). In contrast to results from membrane filtration assays, MST assays typically generally showed low-to-moderate concentrations of *Escherichia* and ruminant markers and no human markers were detected (Appendix B).
- ROC.80 showed low-to-moderate *E. coli* concentrations in 2014 (generally <25 CFU per 100 mL) but much higher concentrations in 2015 (often >100 CFU per 100 mL; Figure 10). Only two samples were analyzed using MST assays; both samples were collected in 2014 and indicated relatively low concentrations of *Escherichia* markers and no ruminant or human markers were detected (Appendix B).

In conclusion, although membrane filtrations assays indicated that streams in Round Valley have high *E. coli* concentrations, limited results from MST analyses showed low concentrations of *Escherichia* and ruminant markers in most samples. Additional study (i.e., analysis of additional samples with ruminant and human MST assays as well as a horse assay) would be necessary to determine the sources of fecal contamination at these sites.

Bishop Creek. As Bishop Creek leaves the Sierra Nevada it flows in a single channel. Near the outlying areas of the town of Bishop, the creek is divided into the North Fork and South Fork which both eventually empty into the Bishop Canal. Both forks flow through a mix of residential neighborhoods and pasture lands, and the South Fork also flows through a commercial area in downtown Bishop and through the city park (Figure 4). Pasture lands owned by the Los Angeles Department of Water and Power (LADWP) are typically grazed by cattle and some horses, and those owned by the Bishop Paiute Tribe are grazed by cattle, sheep, and horses. In residential areas, a network of small ditches diverts water from Bishop Creek into “backyard” streams and ponds. The complex mixture of land ownership and land uses makes it difficult to unambiguously determine the sources of fecal contamination. Previous sampling of Bishop Creek has indicated high levels of fecal contamination in its lower reaches, and MST analyses suggested that ruminants were a more important source of contamination than were humans (Knapp and Nelson 2015). Under the current contract, we intensively sampled both forks of Bishop Creek to better describe patterns of fecal contamination and fecal sources.

We sampled two sites on the main stem of Bishop Creek located above the outlying residential areas (BIS.10, BIS.15), six sites on South Fork Bishop Creek (BIS.20, BIS.30, BIS.40, BIS.50, BIS.60, BIS.90 [A-1 Ditch]), and seven sites on North Fork Bishop Creek (BIS.21, BIS.31, BIS.41, BIS.51, BIS.52 [B-1

Drain], BIS.53, BIS.55; Figure 4). Sampling sites on Bishop Creek generally show little or no seasonality in FIB concentrations (Figure 11, 12).

- BIS.10 and BIS.15 on upper Bishop Creek generally had low levels of fecal contamination, with *E. coli* concentrations typically <10 CFU per 100 mL (Figure 11). MST assays applied to samples from BIS.10 indicated low concentrations of *Escherichia* markers and no ruminant or human markers were detected (Appendix B).
- On the South Fork Bishop Creek, BIS.20 also generally had low levels of fecal contamination, with *E. coli* concentrations typically ≤30 CFU per 100 mL; however, one sample had an *E.coli* concentration >100 CFU per 100 mL (Figure 11). MST assays failed to detect any *Escherichia*, ruminant, or human markers.
- All sites downstream of BIS.20 had much higher levels of fecal contamination, with *E. coli* concentrations at all lower sites often >100 CFU per 100 mL and many samples had *E. coli* levels above 200 CFU per 100 mL (Figure 11). *E. coli* levels at sites BIS.50, BIS.60, and BIS.90 were particularly high. MST assays indicated moderate concentrations of *Escherichia* and ruminant markers at BIS.30 and BIS.40, and high concentrations of both markers at BIS.50 and BIS.60. Human markers were detected in only one sample from the South Fork sites (BIS.60), and concentrations in this sample were low. No MST analyses were conducted using samples from BIS.90 (Appendix B).
- Located at the top of the North Fork Bishop Creek, BIS.21 had *E. coli* concentrations that were generally <30 CFU per 100 mL, and MST assays also indicated a lack of *Escherichia*, ruminant, or human markers in most samples (Figure 12; Appendix B).
- BIS.31 and BIS.41 showed moderate-to-high *E. coli* concentrations, and MST assays also indicated moderate-to-high concentrations of *Escherichia* and ruminant markers. Low concentrations of human markers were detected at BIS.31 on two sampling dates and at BIS.41 on one sampling date (Figure 12; Appendix B).
- BIS.51 and BIS.52 (B-1 Drain) showed the highest *E. coli* concentrations on the North Fork Bishop Creek. At both of these sites, during summer months *E. coli* concentrations always exceeded 100 CFU per 100 mL, and commonly exceeded 200 CFU per 100 mL. Concentrations of *Escherichia* and ruminant markers were also high at both sites. Low concentrations of human markers were detected at BIS.51 on three sampling dates (Figure 12; Appendix B).
- BIS.53 (Bishop Canal) and BIS.55 typically had relatively low concentrations of fecal contamination, and MST assays applied to samples from BIS.53 generally failed to detect *Escherichia* or ruminant markers. No MST analyses have yet been conducted using samples from BIS.55 (Figure 12; Appendix B).

In summary, the middle and lower reaches of both the south and north forks of Bishop Creek show high levels of fecal contamination. Given the high contact rates of people with contaminated water in this drainage (swimming, backyard water features), the potential for water-borne illness is likely to exist. The results from MST assays indicate that ruminants are a much more important source of bacterial contamination than are humans. Because cattle are ubiquitous throughout the middle and lower watershed where bacterial contamination is highest (and other ruminants, such as deer and sheep, were not observed during sampling), the available evidence indicates that domestic cattle are the predominant controllable source of bacteria in lower Bishop Creek. Regardless, the source of low-level human fecal contamination on the North Fork may be significant from a public health standpoint, and

should be investigated further. In the future, analyzing samples using an assay that is more narrowly targeted at cattle may help to quantify their contribution relative to that of other ruminants. Use of assays targeting waterfowl (especially below the Bishop City Park), dogs, horses, and beavers could provide additional information regarding the influence of these other potential sources.

OBJECTIVE 3
LANDSCAPE -SCALE DRIVERS OF FIB CONCENTRATIONS – LAHONTAN-COLLECTED SAMPLES

Between 2009 and 2014, Lahontan personnel collected more than 3,000 water samples from streams across the Lahontan region (Figure 13), and analyzed them for fecal coliform and *E. coli* bacteria using membrane filtration assays. As part of the current contract, we agreed to analyze this dataset with a goal of identifying the primary drivers of FIB concentrations across this broad area. In addition, for 63 samples collected by Lahontan personnel from sites across the northern Lahontan Region, we used microbial source tracking methods to describe the relative contribution of ruminants versus humans as sources of fecal contamination.

Methods

Data set development

Project data were obtained from six Excel files provided by Contract Manager Mary Fiore-Wagner. Workbooks contained fecal coliform (FC) and *E. coli* CFU data from samples collected between 2009 and 2014. Individual worksheets from each workbook were exported to comma-separated value (csv) files and manipulated in R (version 3.2.2; R Core Development Team 2015). Data were merged into a single table, and duplicate records, lab duplicates, lab QA/QC samples, and lab blanks were removed. We also removed data for which record-specific comments indicated a potentially problematic result (e.g., bacteria culturing plates for which accurate counts were not possible). A separate table of station codes (i.e., sampling sites) with associated x-y coordinates (i.e., latitude, longitude) was developed and joined to the FC dataset. After removing records for which the presence/absence of upstream livestock was not recorded (>1,000 records), the final database contained 3,383 samples collected from 131 sites. Additional details on the development of this dataset are provided in Appendix E, and Appendix F lists provides a description of the sampling sites.

For each sampling location in the final dataset, we calculated several predictor variables to describe landscape characteristics. These variables included elevation, road density, lake presence, amount of developed land cover, and amount of meadow land cover, and were calculated using a "sector" that circumscribes the area in the immediate upstream vicinity of a sample site (for details see Knapp and Nelson 2015). Each sector was centered on a sampling site, oriented upstream, had a radius of 1.5 km and an angle of 90° (Appendix D).

The following variables were included in the analysis (see also Table 4):

- SITEID: A unique nine-digit alphanumeric code identifying each sampling location, or station ("Station Code" in the original Lahontan worksheets).
- YEAR, DATE, and TIME: Sampling year and sampling day (i.e., day of the year) were included to account for yearly and seasonal variation in FC concentrations due to factors such as inter-year differences in precipitation amounts, seasonal variation in human use, and seasonal variation in livestock grazing intensity that is not captured by the livestock presence/absence variable (e.g., stocking densities are often lowest early and late in the grazing season). Sampling time was included because of possible diel variation in FC concentrations due to inactivation of bacteria by sunlight(Whitman et al. 2004) or temperature (Howell et al. 1996).

- STOCK: The presence or absence of livestock in a sector at the time of sample collection. When stock were noted as present at a site at the time of sample collection, 84% of observed livestock were cattle, 13% were both cattle and goats, 1% were cattle and horses, and <1% were sheep; for the remaining ~2% of records no information was provided regarding the type of livestock present. Livestock presence was determined visually for all sites, and this was modified for a few sites based on site knowledge by Lahontan staff.
- ELEVATION: Elevation of the sample site, obtained from 30-m DEMs (digital elevation models)
- LAKE, DEVELOPED, DEVELOPEDHIMED, MEADOW, ROAD: These five variables were calculated for the area within a 1.5 km radius upstream-oriented sector (described above).
    - LAKE: The presence or absence of lakes on the sampled stream within the associated sector. LAKE was included because previous analyses (Knapp and Nelson 2015) indicated that FC concentrations were always very low immediately below lakes even when FC concentrations were relatively high immediately above the water body. This might be due to dilution of the incoming FC, settling and/or death of FC in the water body, or some combination of these or other factors.
    - DEVELOPED: The cell (30m x 30m) count of developed open space, and low, medium and high intensity development (cell values 21-24) within each sector calculated from the 2014 National Land Cover Data Set (NLCDS).
    - DEVELOPEDHIMED: The cell (30m x 30m) count of medium and high intensity development (cell values 23 and 24) within each sector calculated from the 2014 National Land Cover Data Set (NLCDS).
    - MEADOW: The cell count of NLCD "meadow" land cover types which included herb, hay, crop, woody wetlands, and emergent herbaceous wetlands (= cell values of 71, 81, 82, 90, and 95). Because livestock are generally grazed in meadow habitats and a visual assessment of livestock presence/absence made at the time of sample collection will not always accurately reflect recent livestock presence upstream, this variable provided another measure of potential livestock use in the vicinity of the sample site.
    - ROAD: Total length of roads within each sector, calculated from 2014 U.S. Census Bureau TIGER/Line shapefiles. These files include both primary roads (paved) and secondary roads (paved and unpaved). Road length was included as an indicator of the intensity of human development and/or activity, which may affect FC concentrations.

Statistical Analysis

FC and *E. coli* concentrations (CFU per 100 mL) were highly correlated ($r = 0.988$, $P < 0.0001$; see also Knapp and Nelson (2015; pages 6, 7, 14) that showed that in the Mono-Inyo County study streams FC and *E. coli* concentrations were related nearly 1:1). Given that the current FIB water quality standard

in the Lahontan region is based on FC bacteria, we focused our analysis on the FC results (variable name = FC100). We used multivariate generalized linear and generalized additive models to quantify the strength of associations between predictor variables and FC concentrations. In all analyses we were primarily interested in the effects of the landscape variables but included other variables to reduce the chances of confounding effects caused by not including important predictors. Our general regression analysis approach followed the protocol of Zuur et al. (2009; Section 4.2.3). All statistical analyses were conducted using R and the R packages *usdm*, *nlme*, and *mgcv*. Our approach, which included both fixed and random effects (see below), allowed us to account for between-sample dependencies and thereby include every sample as a separate record for the analysis. As described for the modeling conducted under Objectives 1 and 2, this is a more powerful approach than averaging FC result for each sampling site and including only the averaged values in the analysis.

Prior to the analysis, we evaluated the continuous predictor variables for collinearity by calculating variance inflation factors (VIF) and Pearson correlation coefficients. ROAD and DEVELOPED were the only variables with VIF >3. In addition, several other variables were highly correlated, including ROAD and DEVELOPED (r = 0.91), DEVELOPEDHIMED and DEVELOPED (r = 0.49), and DEVELOPEDHIMED and ROAD (r = 0.47). Entering each of these three variables (ROAD, DEVELOPED, DEVELOPEDHIMED) separately into a preliminary generalized linear model indicated that a model including DEVELOPED had slightly higher explanatory power than models including the other two variables, so we dropped both ROAD and DEVELOPHIMED from the analysis, resulting in VIF values <2 for all remaining variables and correlations less than 0.30.

The regression analysis started with a generalized linear model that contained all categorical and non-collinear continuous predictor variables (Table 4). A $\log_{10}(Y + 1)$ transformation was applied to the FC100 variable to meet assumptions of normality (transformed variable name = LFC100). The starting model was as follows:

(1) LFC100 ~ ELEVATION + STOCK + LAKE + DEVELOPED + MEADOW + DATE + TIME

A key assumption underlying regression analysis is that residuals are homogenous. We assessed the validity of this assumption for Model 1 by plotting the standardized residuals versus fitted values and versus each individual predictor variable. Hetereogenity was detected and was at least partly due to consistent differences between sampling sites and between years. Therefore, in the next iteration of the model we included YEAR and SITEID as random effects in the model; this allowed us to account for anticipated between-year differences due to different sample sites visited between years, and the lack of independence in intra-site results due to repeated sampling of the same location through time. Including SITEID and YEAR as random effects instead of as fixed effects has two important advantages: (1) it allows general conclusions to be made, not only conclusions restricted to the sampling sites and years, and (2) it reduces the number of estimated parameters and thereby increases statistical power to detect effects.

To implement these changes we developed two new models, each of which included both fixed and random effects and are therefore referred to as mixed effects models. In one model the random effect term was simply SITEID, in the other model SITEID was nested within YEAR. These models were as follows:

(2) LFC100 ~ ELEVATION + STOCK + LAKE + DEVELOPED + MEADOW + DATE + TIME | SITEID

(3) LFC100 ~ ELEVATION + STOCK + LAKE + DEVELOPED + MEADOW + DATE + TIME | YEAR/SITEID

Likelihood ratio tests indicated that Models 2 and 3 provided much better fits to the data than did Model 1, and that Model 3 provided a significantly better fit than did Model 2, although the difference between the two models was small.

The next step in the modeling process was to find the optimal fixed effect structure for Model 3. We sequentially dropped the least significant variable and refit the model until all remaining fixed effect variables were significant ($P \leq 0.05$). Using this reduced model, we again assessed the homogeneity of residuals as described above and detected some evidence of patterns in the residuals for two of the continuous predictor variables, DATE and TIME. To evaluate whether model fit could be further improved by using non-linear terms, we fit a generalized additive mixed effects (GAM) model in which the linear DATE and TIME terms were replaced by terms that used smoothing splines. Both terms had effective degrees of freedom >1, indicating that the terms were non-linear and that the smoothers improved model fit.

Microbial Source Tracking

Samples were collected by Lahontan personnel from the northern Lahontan Region in 2014-2015 and filtered at the Lahontan Water Board laboratory in South Lake Tahoe. Lahontan personnel shipped 63 frozen filters to our laboratory on September 1, 2015. These samples were analyzed using the same qPCR assays and methods described elsewhere in this report (Objectives 1 & 2: Methods – Microbial Source Tracking; Appendix C). A list of MST samples and the sites from which they were collected is provided in Appendix G.

Results

Landscape-scale drivers of FIB concentrations

A summary of FC results for all sampled sites is provided in Appendix F; the full dataset used in this analysis was provided in digital form to Contract Manager Mary Fiore-Wagner. The majority of sampling sites had average (i.e., geometric mean) FC concentrations of less than 20 colonies (mpn or cfu) per 100 mL (Figure 14). However, several sample sites were characterized by substantially higher average FC concentrations, including some that exceeded 100 CFU per 100 mL. This included sites on the Susan River (637SUSB01) in the Honey-Eagle Lake subbasin and sites on Griff Creek (634GRFB01, 634GRFB10) in the Lake Tahoe subbasin; the east tributary of Griff Creek (634GETB01) had the highest FC concentration in the entire study area. Four sites on the South Fork of Bishop Creek (603BSP004, 603BSP005,603BSP006, 603BSP008) in the Crowley Lake subbasin also had high FC concentrations.

The final regression model met the assumption of homogeneity of residuals, provided a good fit to the data, and explained 38% of the variation in FC concentrations (adjusted $R^2 = 0.38$). Significant predictors of FC concentration were (in order of their importance) STOCK, DATE, TIME, MEADOW, DEVELOPED, and ELEVATION (Table 5). LAKE did not have a significant effect on FC concentration and was dropped during the variable selection procedure used to find the optimal fixed structure. Of the five continuous predictor variables retained in the final model, the effects of MEADOW, DEVELOPED, and ELEVATION were linear (Table 5a) and those DATE and TIME were significantly non-linear (Table 5b). The results of the final GAM regression model are shown graphically in a series of plots (Figure 15). Each plot describes the relationship between one of the significant predictor variables and per-sample FC

concentration, after accounting for the effects of all other significant predictor variables. The plotted terms are based on partial residuals, and the y-axis is standardized to have an average value of zero.

The predictor variable STOCK describes the presence or absence of livestock (primarily cattle, but occasionally horses, sheep, and goats) upstream of the sampling sites, and had the strongest effect on FC concentration of any of the variables included in the model. After accounting for the effects of all other significant variables, FC concentrations were substantially higher in the presence of livestock compared to when livestock were absent (Table 5a, Figure 15a).

The variables DATE and TIME were also strongly associated with FC concentration. The predictor variable DATE indicated the number of days since January 1 and described seasonal trends in FC concentrations not accounted for by the other variables. The effect of DATE was highly significant and nonlinear (Table 5b), being lowest in spring and fall and highest in mid-summer (Figure 15b). TIME describes the time of day at which a sample was collected, and also had a highly significant non-linear effect on FC concentrations (Table 5b). After accounting for the effects of all other significant predictor variables, FC concentrations were highest in early and late morning and somewhat lower mid-morning (Figure 15c). Although this could indicate a causative relationship (driven by temporal patterns of cattle activity, viability of bacteria, etc.), it could also be an artifact of when particular sites were sampled. For example, if a collection of highly contaminated sites were consistently sampled in early or late-morning this could produce the TIME effect shown in Figure 15c. No estimate for either DATE or TIME is provided in Table 5b because the estimates for continuous variables are based on the slope of the line describing the effect of a predictor variable on the response variable; given that the effect of DATE and TIME is non-linear, the slope of this effect cannot be described with a single number.

The predictor variables MEADOW, DEVELOPED, and ELEVATION also had significant effects on FC concentration, but their importance was less than that of STOCK, DATE, and TIME (Table 5). The area of meadow land cover (MEADOW) and developed land cover (DEVELOPED) upstream of the sampling site were both positively associated with FC concentration (Figure 15d, e). Sample site elevation (ELEVATION) had a significant and negative effect on FC concentration, indicating that after accounting for all other significant variables sample sites at lower elevations tended to have higher FC concentrations than those at higher elevations (Figure 15f).

Microbial source tracking

Results from five MST assays applied to 63 samples collected by Lahontan personnel are provided in Appendix G. Ribosomal subunit gene copies from *Escherichia* and both total and ruminant Bacteroidales (GenBac3 and BacCow, respectively) were found in concentrations ranging generally over five orders of magnitude, from 10^1 to 10^6 copies per 100 mL sample, matching distributions from our previous contract report. Almost all Lahontan samples were positive for GenBac3, with mean and median values of approximately 2,000 copies per 100 mL. As with the CESAME samples, *Escherichia* copy concentrations were roughly two orders of magnitude lower, with 25-40% of the samples below limits of detection. BacCow exhibited a strong binomial distribution, with roughly half of the samples below limits of detection in both years and positive samples averaging roughly 1,000 copies per 100 mL. Among 29 samples in which total and ruminant Bacteroidales were both detected, the lognormal mean and median proportional ruminant contribution was 15% and was highly correlated with absolute BacCow copy number ($r = 0.78$, $P < 0.0001$). The human-specific Bacteroidales assays (BacHum, HF183) exhibited a positive result in only one sample (632MLBB01 on 7/7/2014; BacHum). The fact that 29 samples had detectable levels of ruminant markers and only one had detectable levels of human

markers indicates that for the sampled sites, ruminants (including cattle) are a much more important contributor to fecal pollution that are humans. Of the 63 samples, *Escherichia* markers were detected in 42 samples, indicating the presence of *E. coli*. *E. coli* concentrations obtained from the membrane filtration assay and *Escherichia* copy numbers obtained from the MST assay were relatively weakly correlated (for [$\log_{10}$ +1] transformed data: $r = 0.29$, $P = 0.02$). This is in contrast to the strong correlation for these two variables in the CESAME-collected samples.

MST samples for this portion of the study were collected by Lahontan staff, and we have no familiarity with these sites or associated watersheds, nor with conditions at the time of sample collection. In the absence of this critical contextual information, we are not comfortable providing detailed discussion of potential fecal sources. However, for those watersheds that were sampled relatively intensively (Markleeville Creek/Millberry Creek, Trout Creek, and Griff Creek) some general patterns are worthy of mention. Samples collected from Markleeville Creek showed relatively high FIB levels, and MST results indicated high concentrations of ruminant markers. Human markers were never detected. As such, available data suggests that fecal contributions from ruminants are substantial and are a much more important contributor to this fecal contamination than are humans. Results for Millberry Creek, a tributary to Markleeville Creek, are more ambiguous. Although FIB concentrations in samples sometimes reached relatively high levels, ruminant markers were never detected, and human markers were detected only once. Therefore, the source of fecal contamination at this site remains unclear. Samples collected from Griff Creek showed relatively high FIB concentrations, and this was corroborated by similarly high concentrations of *Escherichia* MST markers. Although human markers were never detected in Griff Creek samples, ruminant markers were detected regularly at low-to-moderate concentrations. Because the Griff Creek watershed is not grazed by cattle or domestic sheep (Mary Fiore-Wagner, personal communication), this might point to fecal contributions by deer. Although we suspect that deer densities are generally too low to allow deer fecal contributions to reach significant levels, very low stream flows could magnify this contribution because of a lack dilution. In cases such as Millberry and Griff creeks, using additional MST assays targeting other potential sources (birds, dogs, horses, etc.) may be helpful in identifying the sources of observed fecal contamination.

DISCUSSION

Characterization of FIB concentrations and fecal sources: CESAME-collected data

Results from sampling conducted during the current contract period clearly indicate that streams in several areas in Mono and Inyo counties show high levels of fecal contamination. For Bridgeport Valley, our results are consistent with those of previous sampling efforts that indicated high levels of *E. coli* in Swauger Creek, Buckeye Creek, Robinson Creek, and the upper East Walker River, and support their listing as “impaired” under Section 303(d) of the Clean Water Act. Results from the current study also indicate that the listing as “impaired” of lower Mammoth Creek, upper Owens River, lower Rock Creek, lower Pine Creek, lower Horton Creek, and the north and south forks of Bishop Creek may be warranted.

In a previous report (Knapp and Nelson 2015), we showed that the presence of cattle upstream of a sampling location was the strongest predictor of *E. coli* concentrations (CFU per 100 mL), providing evidence that cattle were likely a primary driver of fecal contamination in the Mono-Inyo County study streams. Results obtained during the current contract period provide additional support for this link. Results from statistical modeling presented in this report indicate that the concentration of ruminant-derived Bacteroidales bacteria (as quantified by the BacCow MST assay) was the strongest predictor of

E. coli concentrations (as quantified by the membrane filtration assay). The importance of cattle as a primary driver of fecal pollution in the study streams is further supported by two additional results. First, temporal patterns of *E. coli* concentrations in the study streams generally match those of cattle presence/absence and abundance. Specifically, in areas where cattle are grazed only in summer months (Bridgeport Valley, Long Valley), *E. coli* concentrations were low when cattle were absent (winter, early-spring, and late-fall) and much higher when cattle were present (late-spring to early-fall). In areas where cattle grazing occurs during most or all months of the year (Round Valley, Bishop Creek), *E. coli* concentrations showed less seasonal variation and were high year around. Second, concentrations of Bacteroidales bacteria derived from ruminants (including cattle) were far higher than those derived from humans.

Although these results clearly show that cattle are an important source of fecal contamination in the study streams, information on the importance of other fecal sources is lacking. This is particularly relevant in areas in which multiple fecal sources are possible. For example, our results indicate that fecal contamination in Bishop Creek is strongly associated with cattle and only weakly with humans. However, these waters could also be affected by feces from horses, pets (especially dogs), and wildlife (especially deer, beaver, and waterfowl). Future studies should apply a broader assortment of MST assays in an effort to better describe the relative contributions of these potential sources of fecal contamination. Attention should also be focused on resolving the issue seen in samples from several sites in which membrane filtration assays indicated high *E. coli* concentrations but MST assays showed relatively low concentrations of *Escherichia*, ruminant, and human markers. This was evident particularly in samples collected from the upper Owens River (OWE.40) and Round Valley (ROC.80, PIN.50, and HOR.70). Despite these shortcomings in the available information, current results strongly implicate cattle as a major source of fecal contamination, and strategies to reduce this contamination should be considered. Implementing improved cattle management practices will be important for bringing impaired waters into compliance with current and proposed Lahontan Water Board and State Water Board standards for FIB, and such efforts should not be delayed by the lack of complete information on the contribution of all potential fecal sources.

Characterization of FIB concentrations and fecal sources: Lahontan-collected data

Results from the statistical analysis of the Lahontan-collected FIB dataset were similar in many regards to the results from a similar analysis applied to our Mono-Inyo County FIB dataset and described in a previous report (Knapp and Nelson 2015). In both analyses, livestock presence/absence (including cattle), day of year, and time of day were the most important predictors of FIB concentrations. This suggests that the primary drivers of FIB concentrations identified for the Mono-Inyo County study area are generally relevant across much of the Lahontan Region. MST results for the Mono-Inyo County study area showing that ruminants (including cattle) are a much more important contributor to fecal contamination than are humans was also true for the Lahontan-collected samples that represented a substantially larger portion of the Lahontan Region. As is the case for the impaired reaches that were the focus of the current contract, samples from other areas across the Lahontan Region should be evaluated using a wider diversity of source-specific MST assays to better describe the potential contributions of fecal sources in addition to those from cattle and humans.

The analysis of the Lahontan-collected FIB dataset was hindered by the inconsistent recording of livestock presence/absence in the vicinity of the sampling location, and the organization of the collected data. A field to indicate the presence/absence of cattle or other livestock is not included on the

Lahontan FIB datasheet, and as a result any information regarding livestock was recorded in field notebooks. Consequentially, the recorded information was difficult to retrieve (during dataset development) and inconsistent in what was recorded. More than a thousand FIB records had to be excluded from the analysis because of missing information on livestock presence/absence, and even when livestock-related information was available it often consisted of verbal descriptions that were time consuming to read and translate into a categorical variable. It is our understanding that this issue has since been corrected.

CONCLUSIONS

Analyses conducted under this contract or previous contracts indicate that streams in Bridgeport Valley, Long Valley, Round Valley, and the Bishop Creek watershed are characterized by high levels of fecal contamination; these levels commonly exceed the EPA criteria of 100 and 126 CFU per 100 mL. Results from membrane filtration and MST assays provide compelling evidence that cattle are a major contributor to fecal contamination of these streams and those located across a large portion of the Lahontan Region. In addition, results from MST assays also indicate that many of the samples collected below cattle-grazed areas showed substantial fecal contamination from ruminants, but that no sites showed any significant human-sourced contamination. As such, in the Mono-Inyo County study area ruminants (including cattle) are a much more important source of contamination than are humans. For waters that are exposed to a diversity of potential fecal sources, an important next step will be describing the relative contributions of as many of these sources as possible. Additional testing of assays that are more narrowly-targeted at cattle (instead of more broadly targeted at ruminants) would also be useful for distinguishing cattle-derived fecal contributions from contributions by other ruminants. However, even if more narrowly-targeted assays prove useful, because such assays usually have lower sensitivity than more broadly-targeted assays, it will likely be important to analyze samples with both types of assays.

ACKNOWLEDGEMENTS

Funding for this project was provided through Contract 13-054-160 from the California State Water Resources Control Board to R. Knapp and C. Nelson. The contents of this document do not necessarily reflect the views and policies of the California State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. We thank K. Rose and T. Bartley for their assistance in the field and laboratory, L. Petrusa for assembling the Lahontan FIB data, K. Remple for assistance running qPCR assays, and E. Hegeman for assistance with data analysis and figure preparation. We also thank T. Suk and M. Fiore-Wagner for their logistical support and insightful discussions that improved the design of the project.

LITERATURE CITED

American Public Health Association, American Water Works Association, Water Pollution Control Federation, and Water Environment Federation. 1998. Standard methods for the examination of water and wastewater. Vol. 20. American Public Health Association.

Boehm, A. B., L. C. Van De Werfhorst, J. F. Griffith, P. A. Holden, J. A. Jay, O. C. Shanks, D. Wang, and S. B. Weisberg. 2013. Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. Water Research 47:6812–6828.

Chern, E. C., S. Siefring, J. Paar, M. Doolittle, and R. A. Haugland. 2011. Comparison of quantitative PCR

assays for Escherichia coli targeting ribosomal RNA and single copy genes. Letters in Applied Microbiology 52:298–306.

Dick, L. K., and K. G. Field. 2004. Rapid estimation of numbers of fecal Bacteroidetes by use of a quantitative PCR assay for 16S rRNA genes. Applied and Environmental Microbiology 70:5695–5697.

Green, H. C., R. A. Haugland, M. Varma, H. T. Millen, M. A. Borchardt, K. G. Field, W. A. Walters, R. Knight, M. Sivaganesan, C. A. Kelty, and O. C. Shanks. 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Applied and Environmental Microbiology 80:3086–3094.

Haugland, R. A., M. Varma, M. Sivaganesan, C. Kelty, L. Peed, and O. C. Shanks. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. Systematic and Applied Microbiology 33:348–357.

Howell, J. M., M. S. Coyne, and P. L. Cornelius. 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio.

Kildare, B. J., C. M. Leutenegger, B. S. McSwain, D. G. Bambic, V. B. Rajal, and S. Wuertz. 2007. 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. Water Research 41:3701–3715.

Knapp, R. A., and C. E. Nelson. 2015. Assessment of bacterial water quality in the Lahontan Region. Report to the Lahontan Regional Water Quality Control Board, South Lake Tahoe, California. Unpublished final report.

Layton, B. A., Y. Cao, D. L. Ebentier, K. Hanley, E. Ballesté, J. Brandão, M. Byappanahalli, R. Converse, A. H. Farnleitner, J. Gentry-Shields, M. L. Gidley, M. Gourmelon, C. S. Lee, J. Lee, S. Lozach, T. Madi, W. G. Meijer, R. Noble, L. Peed, G. H. Reischer, R. Rodrigues, J. B. Rose, A. Schriewer, C. Sinigalliano, S. Srinivasan, J. Stewart, L. C. Van De Werfhorst, D. Wang, R. Whitman, S. Wuertz, J. Jay, P. A. Holden, A. B. Boehm, O. Shanks, and J. F. Griffith. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. Water Research 47:6897–6908.

R Development Core Team. 2015. R: a language and environment for statistical computing, v. 3.2.2.

Raith, M. R., C. A. Kelty, J. F. Griffith, A. Schriewer, S. Wuertz, S. Mieszkin, M. Gourmelon, G. H. Reischer, A. H. Farnleitner, J. S. Ervin, P. A. Holden, D. L. Ebentier, J. A. Jay, D. Wang, A. B. Boehm, T. G. Aw, J. B. Rose, E. Balleste, W. G. Meijer, M. Sivaganesan, and O. C. Shanks. 2013. Comparison of PCR and quantitative real-time PCR methods for the characterization of ruminant and cattle fecal pollution sources. Water Research 47:6921–6928.

Siefring, S., M. Varma, E. Atikovic, L. Wymer, and R. Haugland. 2008. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. Journal of Water and Health 6:225–237.

U.S. Environmental Protection Agency. 1976. Quality criteria for water. Washington, D.C.

U.S. Environmental Protection Agency. 1986. Ambient water quality criteria for bacteria - 1986. Washington, D.C.

U.S. Environmental Protection Agency. 2010. Method B: Bacteroidales in water by TaqMan® quantitative polymerase chain reaction (qPCR) assay. Washington, D.C.

U.S. Environmental Protection Agency. 2012. Recreational water quality criteria.

Whitman, R. L., M. B. Nevers, G. C. Korinek, and M. N. Byappanahalli. 2004. Solar and temporal effects on Escherichia coli concentration at a Lake Michigan swimming beach. Applied and Environmental Microbiology 70:4276–4285.

Willden, G. D. 2006. Loading and attentuation of fecal indicator bacteria in Emigration Creek. University of Utah.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Tables

Table 1. Description of five qPCR-based microbial source tracking assays used in the current study.

Assay Target	Assay Name	Gene Target	References	Nucleotide Sequences (Forward, Reverse, 5' Exonuclease Probe)
Escherichia	EC23S857	23S rRNA	Chern et al. (2011)	GGTAGAGCACTGTTTTGGCA TGTCTCCCGTGATAACTTTCTC 6-FAM™/TCATCCCGA/ZEN™/CTTACCAACCCG/IB®FQ/
Bacteroidales	GenBac3	16S rRNA	Dick and Field (2004), Siefring et al. (2008), U.S. Environmental Protection Agency (2010b)	GGGGTTCTGAGAGGAAGGT CCGTCATCCTTCACGCTACT 6-FAM™/CAATATTCC/ZEN™/TCACTGCTGCCTCCCGTA/IB®FQ/
Human Bacteroidales	HF183	16S rRNA	Haugland et al. (2010), Green et al. (2014)	ATCATGAGTTCACATGTCCG CTTCCTCTCAGAACCCCTATCC 6-FAM™/CTAATGGAA/ZEN™/CGCATCCCCAT/IB®FQ/
Human Bacteroidales	BacHum	16S rRNA	Kildare et al. (2007)	TGAGTTCACATGTCCGCATGA CGTTACCCCGCCTACTATCTAATG 6-FAM™/TCCGGTAGA/ZEN™/CGATGGGGATGCGTT/IB®FQ/
Ruminant Bacteroidales	BacCow	16S rRNA	Kildare et al. (2007)	CCAACYTTCCCGWTACTC GGACCGTGTCTCAGTTCCAGTG 6-FAM™/TAGGGGTTC/ZEN™/TGAGAGGAAGGTCCCCC/IB®FQ/

Table 2. Predictor variables used to identify the drivers of source-specific fecal bacteria concentrations in streams of the eastern Sierra Nevada, California.

Variable	Code	Description	Type	Model effect
Site identification number	SITEID	Unique five-digit alphanumeric code used to identify each sampling location	Categorical	Random
Upstream lakes	LAKE	Presence/absence of one or more water bodies (>1 ha in surface area, >3 m deep) within a 1.5 km radius upstream-oriented sector	Continuous	Fixed
High-intensity land use	LANDUSE23	Area of moderate and high-intensity land use within a 1.5 km radius upstream-oriented sector, including high density of buildings (in km^2)	Continuous	Fixed
Livestock grazing	COW	Presence/absence of livestock (usually cows) within a 1.5 km radius upstream-oriented sector	Categorical	Fixed
Sampling year	YEAR	Year during which sample was collected	Categorical	Random
Sampling day	DAY	Day of the year (since January 1) on which sample was collected.	Continuous	Fixed
Sampling time	TIME	Time of day when sample was collected	Continuous	Fixed

Table 3. Estimated parameters for the final generalized linear model used to identify significant predictors of BacCow concentrations across the study area

Variable name	**Estimate**	**Std. error**	***z***	***P***
Intercept	0.98	0.410	2.41	1.58×10^{-2}
COW(yes)	1.59	0.13	12.46	$< 1.00 \times 10^{-10}$
TIME	0.10	0.03	3.12	1.80×10^{-3}
DAY	0.00	0.00	2.65	8.00×10^{-3}
LAKE(yes)	-0.55	0.27	-2.02	4.31×10^{-2}

Table 4. For the dataset collected by Lahontan staff, predictor variables used to identify the drivers of fecal coliform bacteria concentrations in streams within the study area.

Variable	Code	Description	Type	Model Effect
Station Code	SITEID	Unique nine-digit alphanumeric code identifying each sampling location	Categorical	Random
Sampling year	YEAR	Year during which sample was collected	Categorical	Random
Elevation	ELEVATION	Height above sea level (in meters)	Continuous	Fixed
Upstream lakes	LAKE	Presence/absence of one or more water bodies (>1 ha in surface area) within a 1.5 km radius upstream-oriented sector	Categorical	Fixed
Road length	ROAD	Total length of all road segments (paved and unpaved) within a 1.5 km radius upstream-oriented sector	Continuous	Fixed
Developed land use	DEVELOPED	Cell (30m x 30m) count of developed open space, and low, medium and high intensity land use development within a 1.5 km radius upstream-oriented sector	Continuous	Fixed
High intensity developed land use	DEVELOPEDHIMED	Cell (30m x 30m) count of medium and high intensity land use development within a 1.5 km radius upstream-oriented sector	Continuous	Fixed
Meadow vegetation type	MEADOW	Cell (30m x 30m) count of vegetation classified as herbaceous, hay/pasture, cultivated crops, woody wetlands, and emergent herbaceous wetlands within a 1.5 km radius upstream-oriented sector	Continuous	Fixed
Livestock grazing	STOCK	Presence/absence of livestock (cows, sheep, horses, or goats) within a 1.5 km radius upstream-oriented sector at the time of sample collection	Categorical	Fixed
Sampling day	DATE	Day of the year (since January 1) on which sample was collected	Continuous	Fixed
Sampling time	TIME	Time of day when sample was collected	Continuous	Fixed

Table 5. Estimated parameters for the final GAM model used to identify significant predictors of fecal coliform bacteria concentrations for samples collected and analyzed by Lahontan personnel: (a) parametric coefficients, and (b) smooth terms.

a.

Variable name	Estimate	Std. error	*t*	*P*
Intercept	1.34×10^{0}	1.52×10^{-1}	*8.87*	$<2.00 \times 10^{-16}$
STOCK (yes)	4.11×10^{-1}	3.64×10^{-2}	11.29	$<2.00 \times 10^{-16}$
MEADOW	3.64×10^{-4}	5.37×10^{-5}	6.77	1.48×10^{-11}
DEVELOPED	4.52×10^{-4}	6.78×10^{-5}	6.68	2.89×10^{-11}
ELEVATION	-3.46×10^{-4}	7.82×10^{-5}	-4.43	9.81×10^{-6}

b.

Variable name	EDF[1]	*F*	*P*
s(DATE)	7.72	142.79	$<2.00 \times 10^{-16}$
s(TIME)	3.91	28.38	$<2.00 \times 10^{-16}$

[1]Effective degrees of freedom: 1 indicates a straight line, and higher values indicate an increasingly non-linear smoothing spline.

Figures

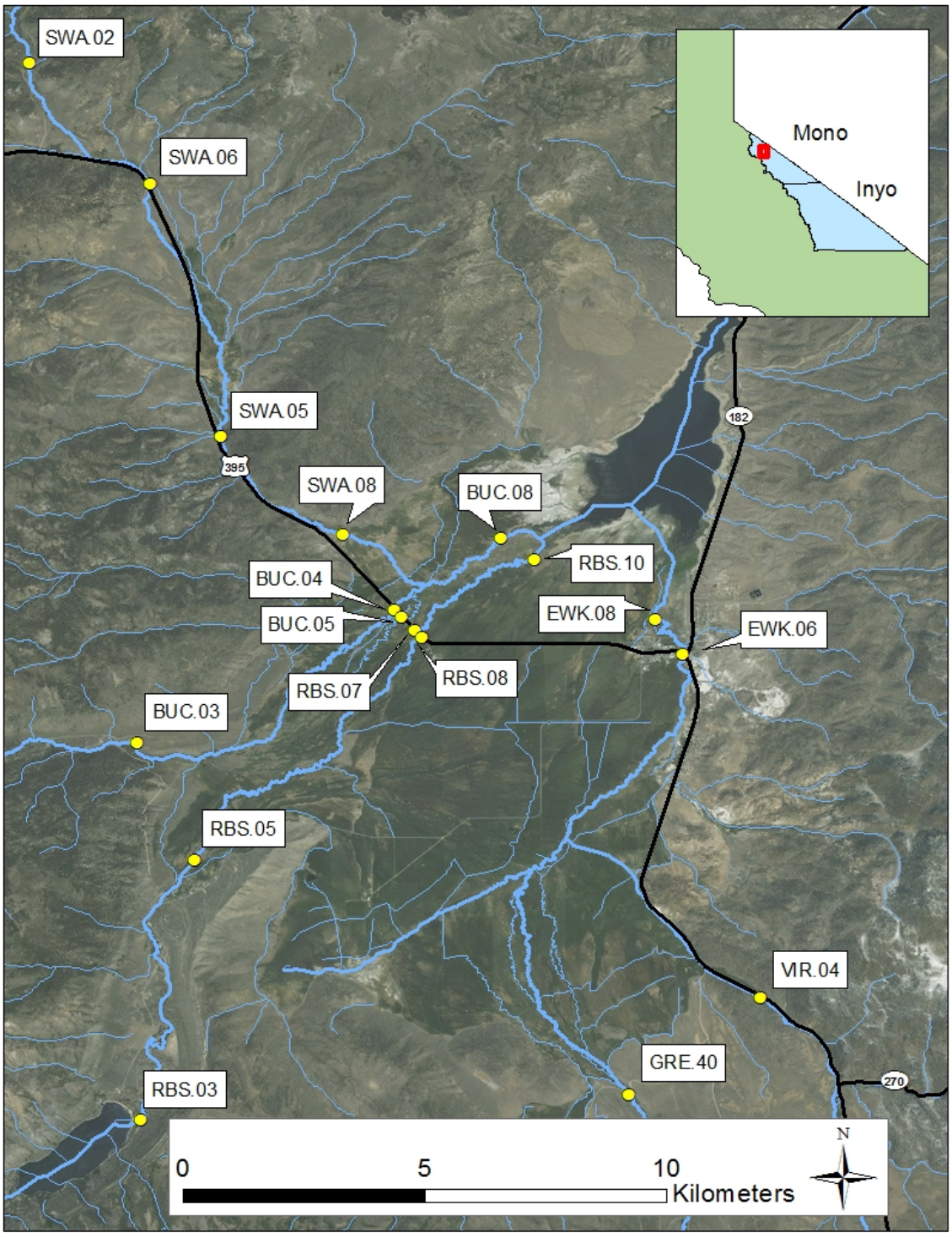

Figure 1. Map of the East Walker River headwaters in Bridgeport Valley, showing sampling locations on Swauger, Buckeye, Robinson, Green, and Virginia creeks (labeled yellow circles). The large water body in the upper-right is Bridgeport Reservoir. Major highways are shown as wide black lines. Information about each sampling location is provided in Appendix A. The inset map locates the sites in Mono and Inyo counties.

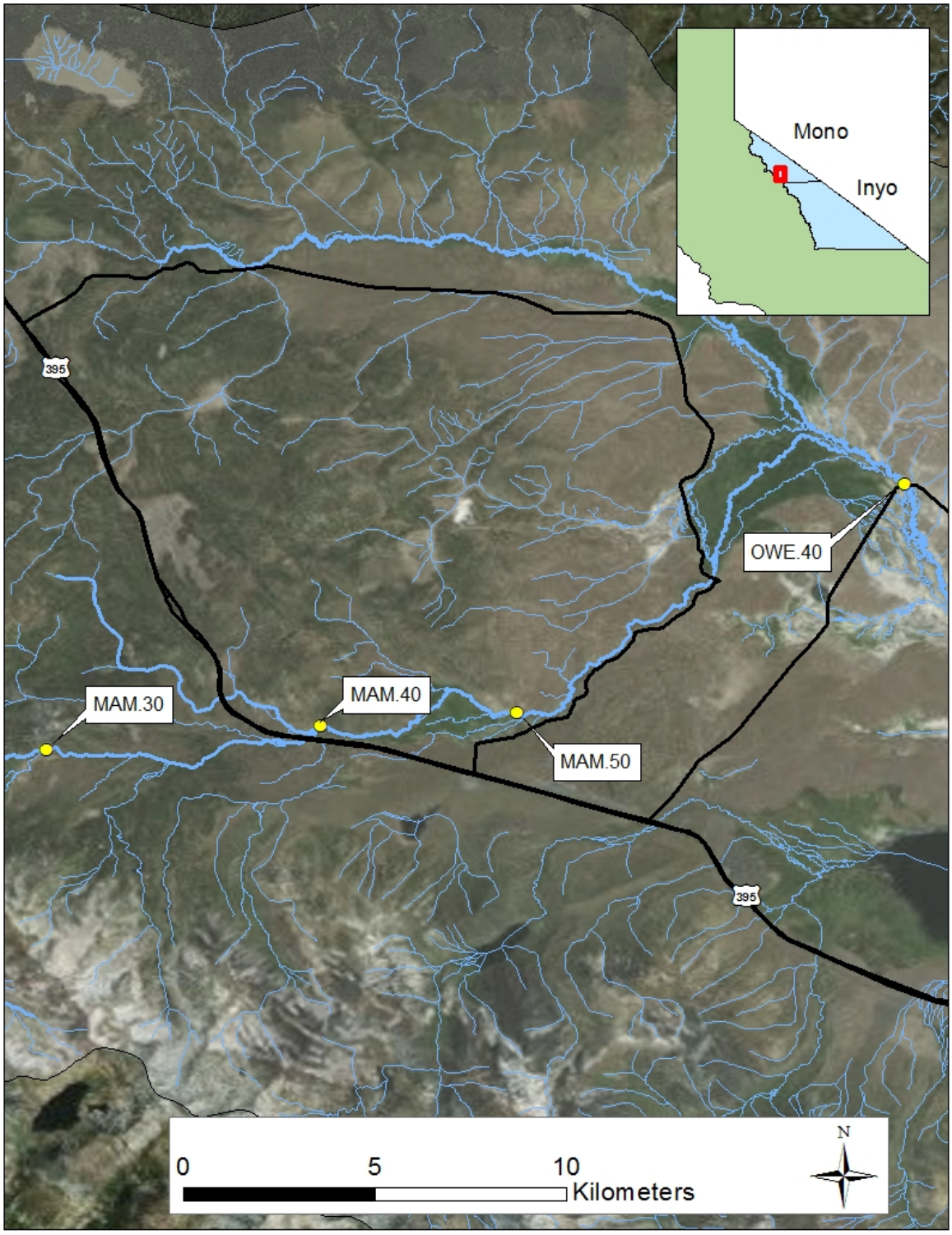

Figure 2. Map of lower Mammoth Creek and upper Owens River in Long Valley, showing sampling locations (labeled yellow circles). A portion of Crowley Reservoir is visible in the lower-right. Major highways and more minor roads are shown as thick and thin black lines, respectively. Information about each sampling location is provided in Appendix A. The inset map locates the sites in Mono and Inyo counties.

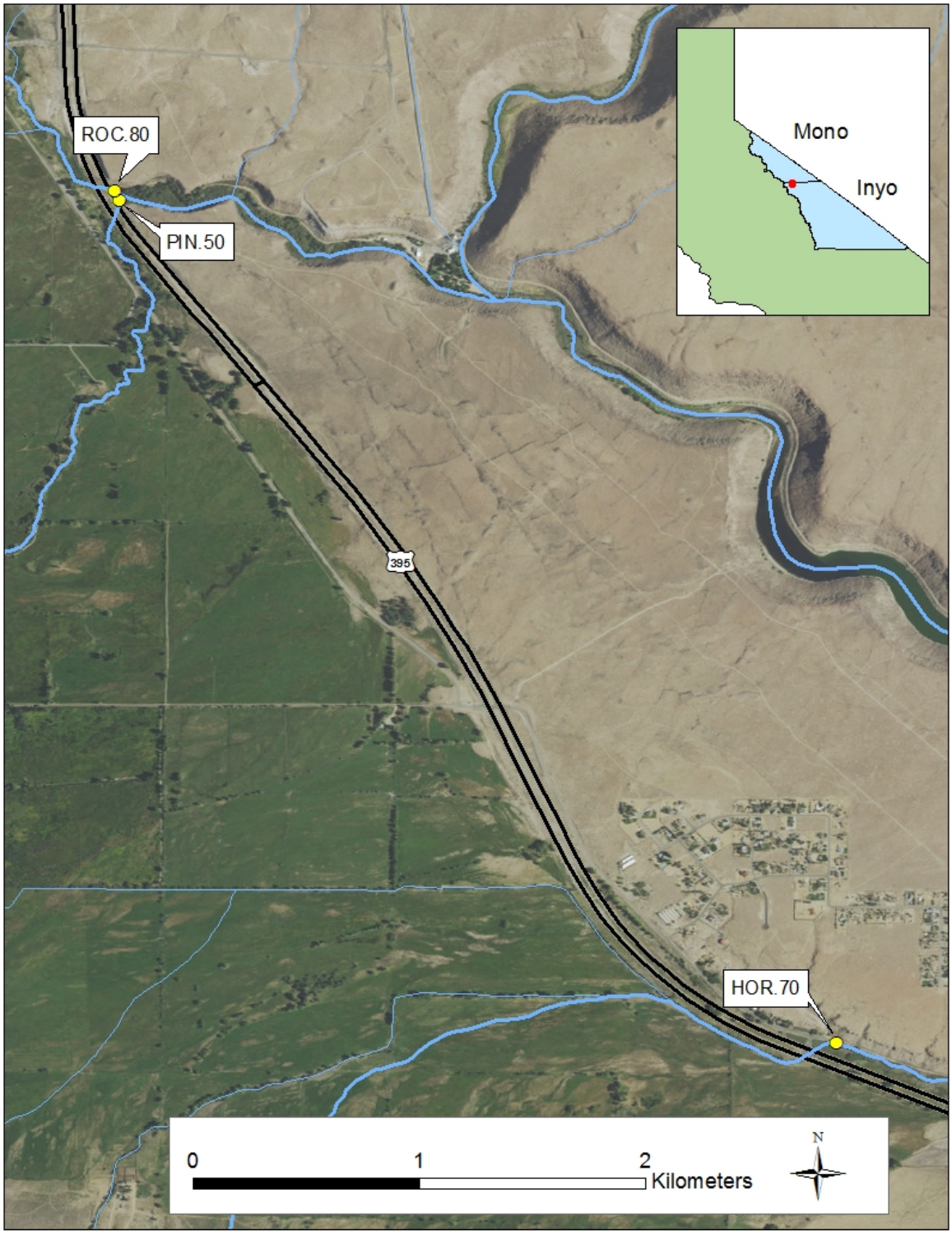

Figure 3. Map of eastern Round Valley, showing sampling locations on lower Rock, Pine, and Horton creeks (labeled yellow circles). The upper portion of Pleasant Valley Reservoir is shown in the center-right. Major highways are shown as a wide black lines. Information about each sampling location is provided in Appendix A. The inset map locates the sites in Mono and Inyo counties.

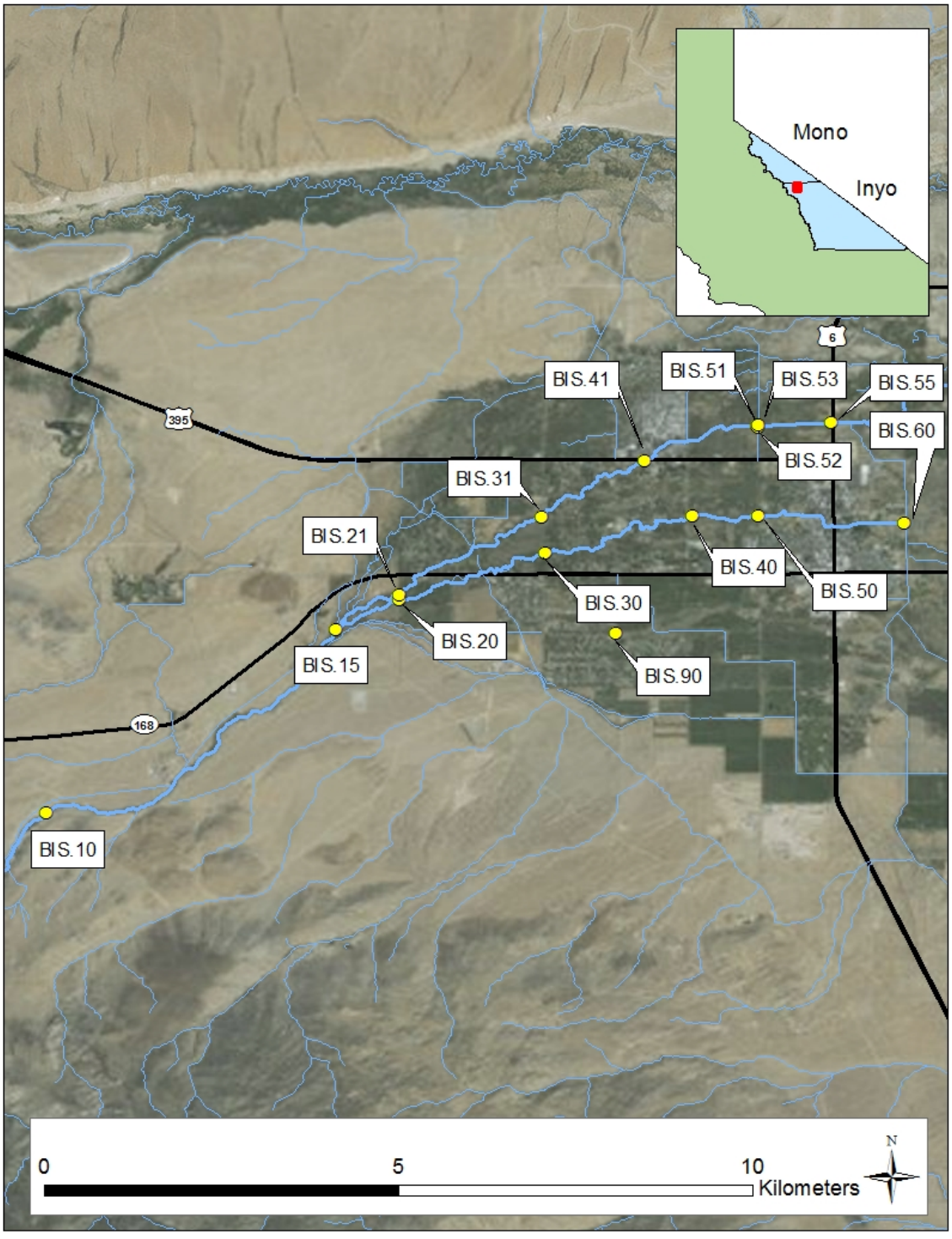

Figure 4. Map of the City of Bishop and outlying areas, showing sampling locations along Bishop Creek (labeled yellow circles). Major highways are shown as a wide black lines. Information about each sampling location is provided in Appendix A. The inset map locates the sites in Mono and Inyo counties.

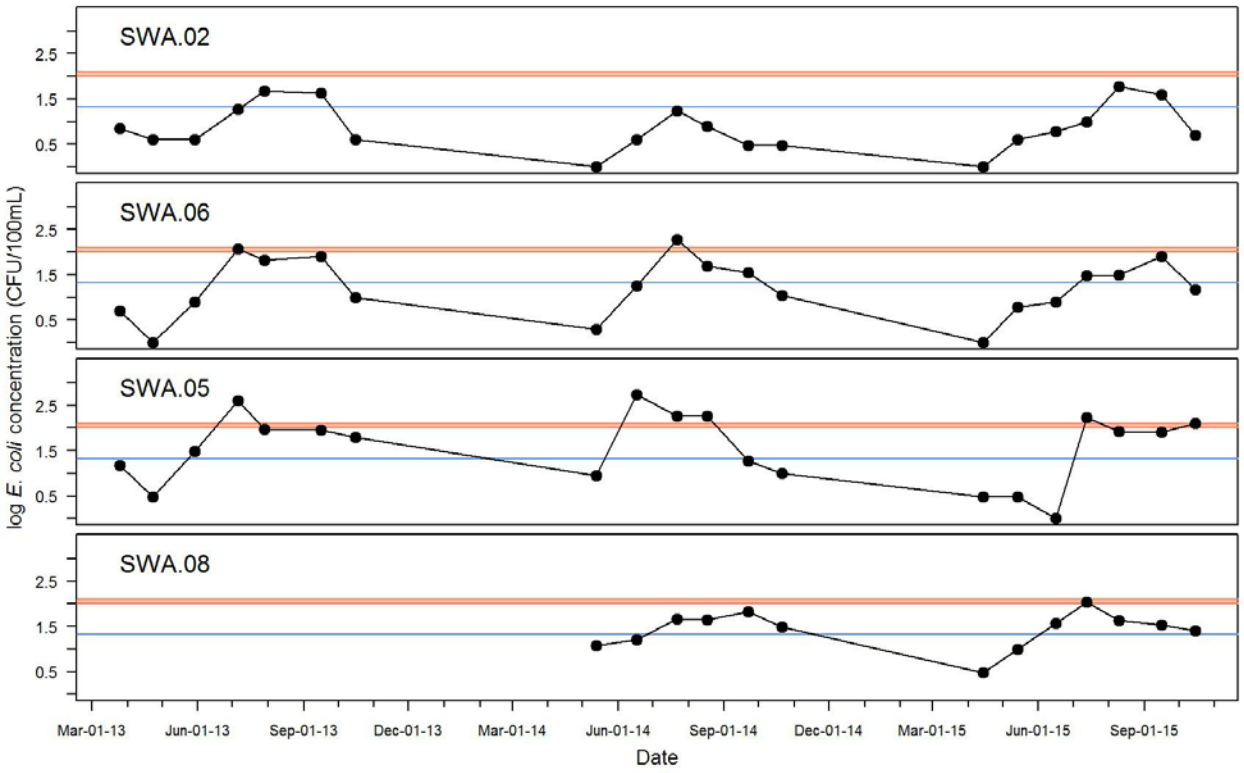

Figure 5. For Swauger Creek in Bridgeport Valley, temporal patterns of *E. coli* concentrations from upstream (SWA.02) to downstream reaches (SWA.08; Figure 2). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. Data collected from May-2014 to Oct-2015 were collected under the current contract, and those data from prior to May-2014 were collected under previous contracts.

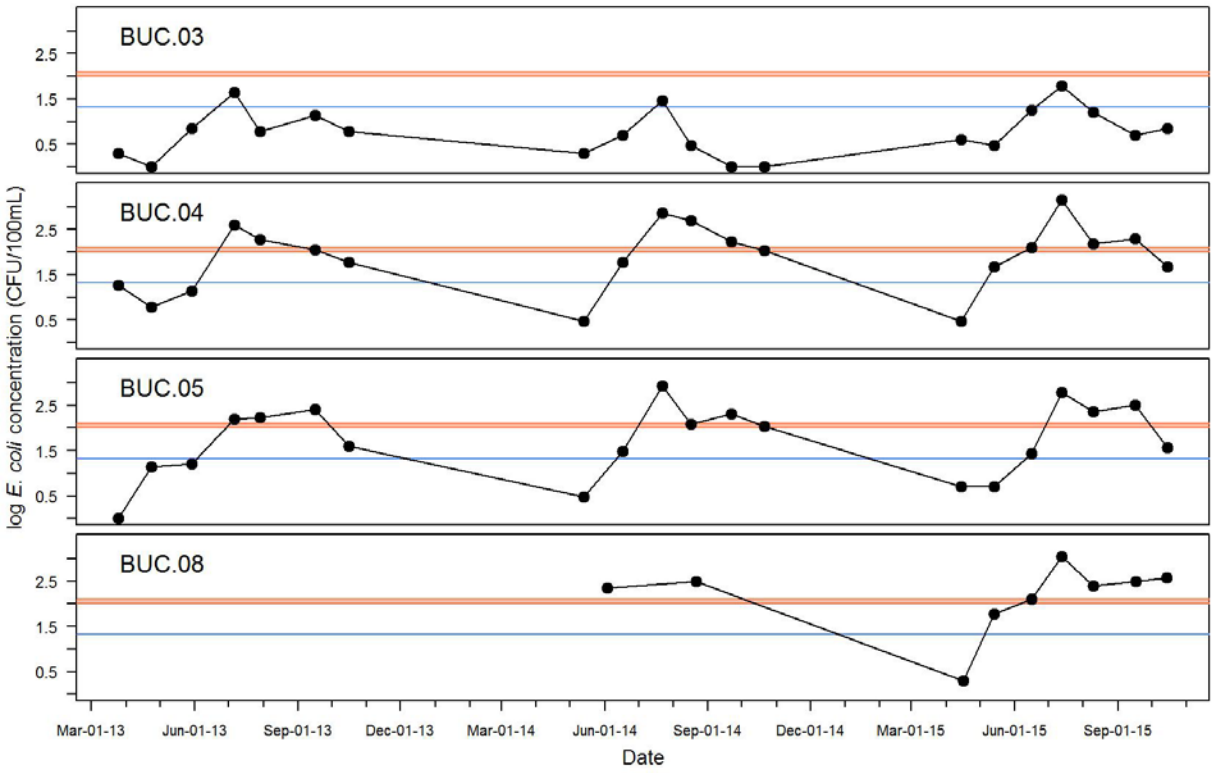

Figure 6. For Buckeye Creek in Bridgeport Valley, temporal patterns of *E. coli* concentrations from upstream (BUC.03) to downstream reaches (BUC.08; Figure 2). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. Data collected from May-2014 to Oct-2015 were collected under the current contract, and those data from prior to May-2014 were collected under previous contracts.

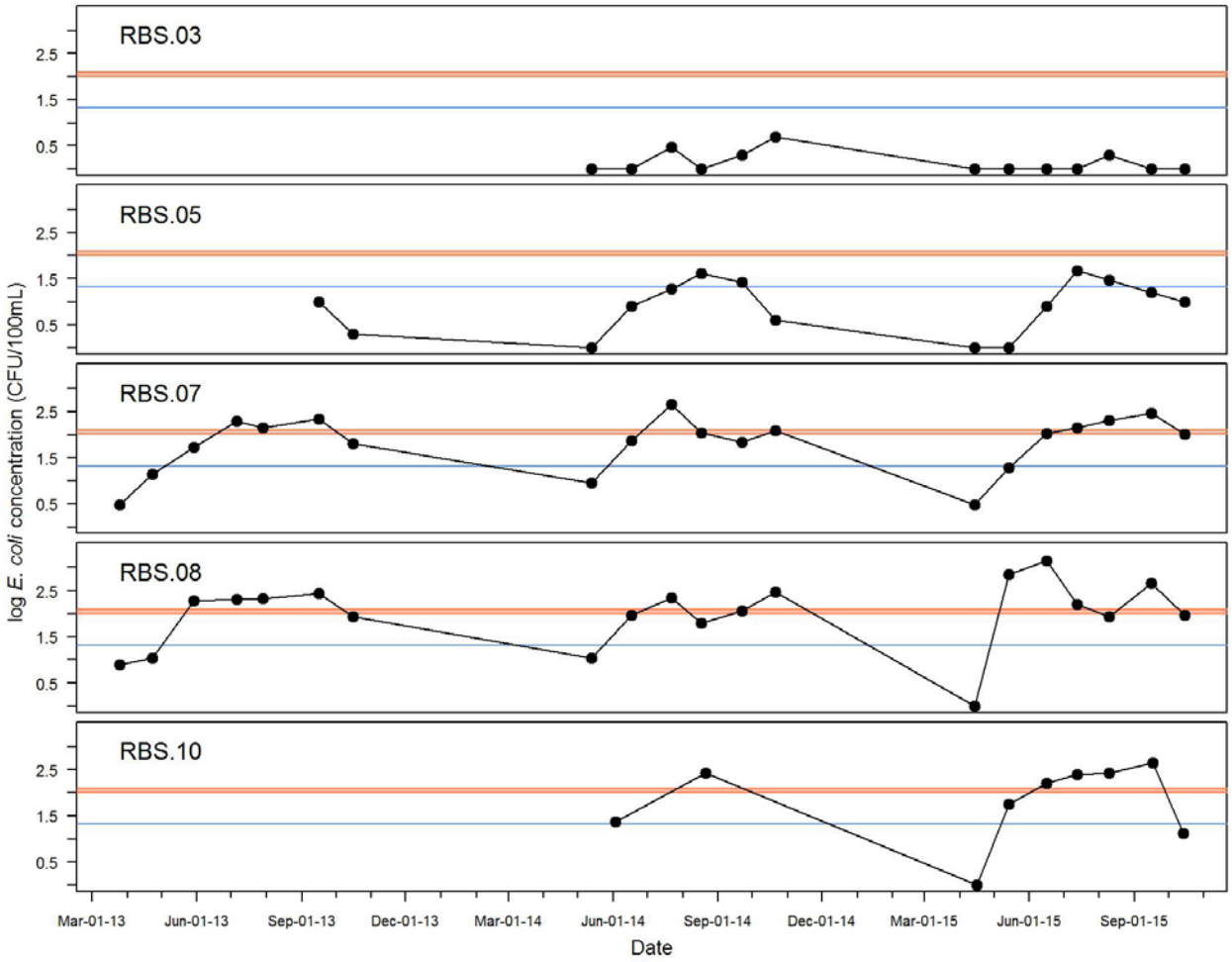

Figure 7. For Robinson Creek in Bridgeport Valley, temporal patterns of *E. coli* concentrations from upstream (RBS.03) to downstream reaches (RBS.10; Figure 2). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. Data collected from May-2014 to Oct-2015 were collected under the current contract, and those data from prior to May-2014 were collected under previous contracts.

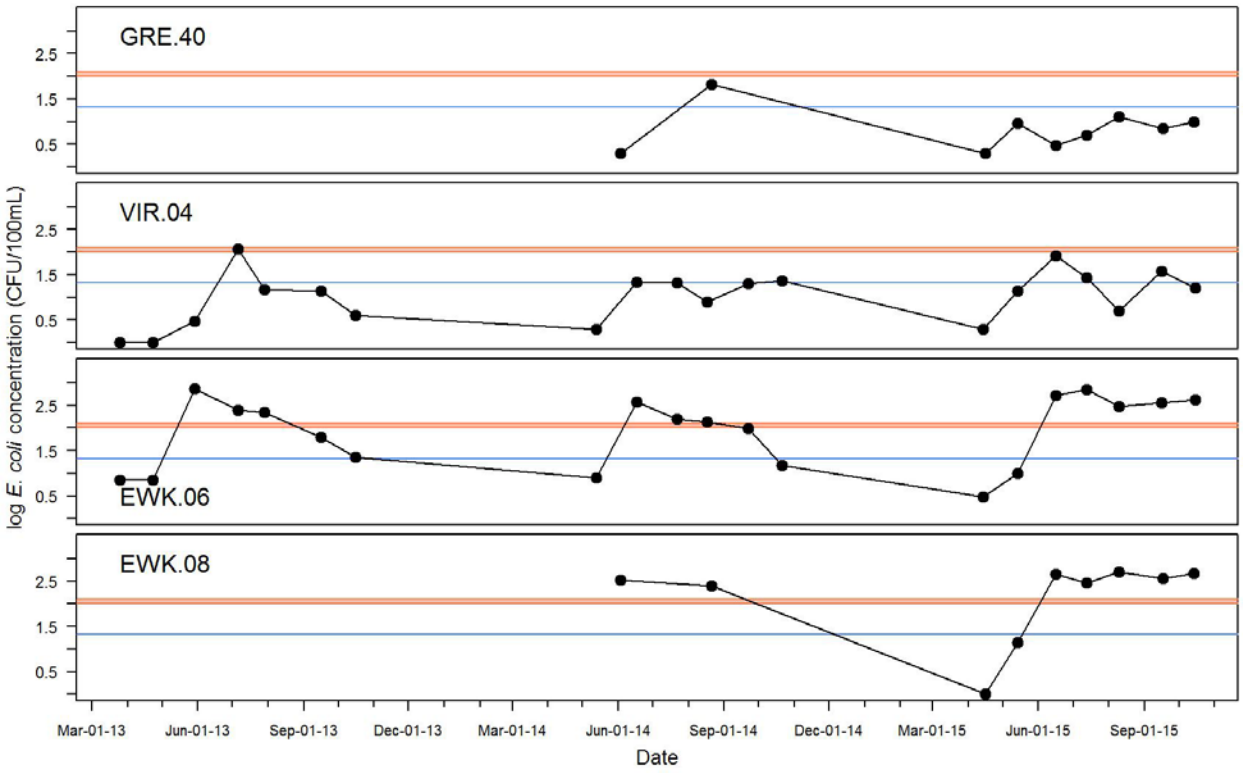

Figure 8. For the East Walker River and headwaters in Bridgeport Valley, temporal patterns of *E. coli* concentrations from upstream reaches on Green and Virginia creeks (GRE.40, VIR.04) to downstream reaches (EWK.06, EWK.08; Figure 2). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. Data collected from May-2014 to Oct-2015 were collected under the current contract, and those data from prior to May-2014 were collected under previous contracts.

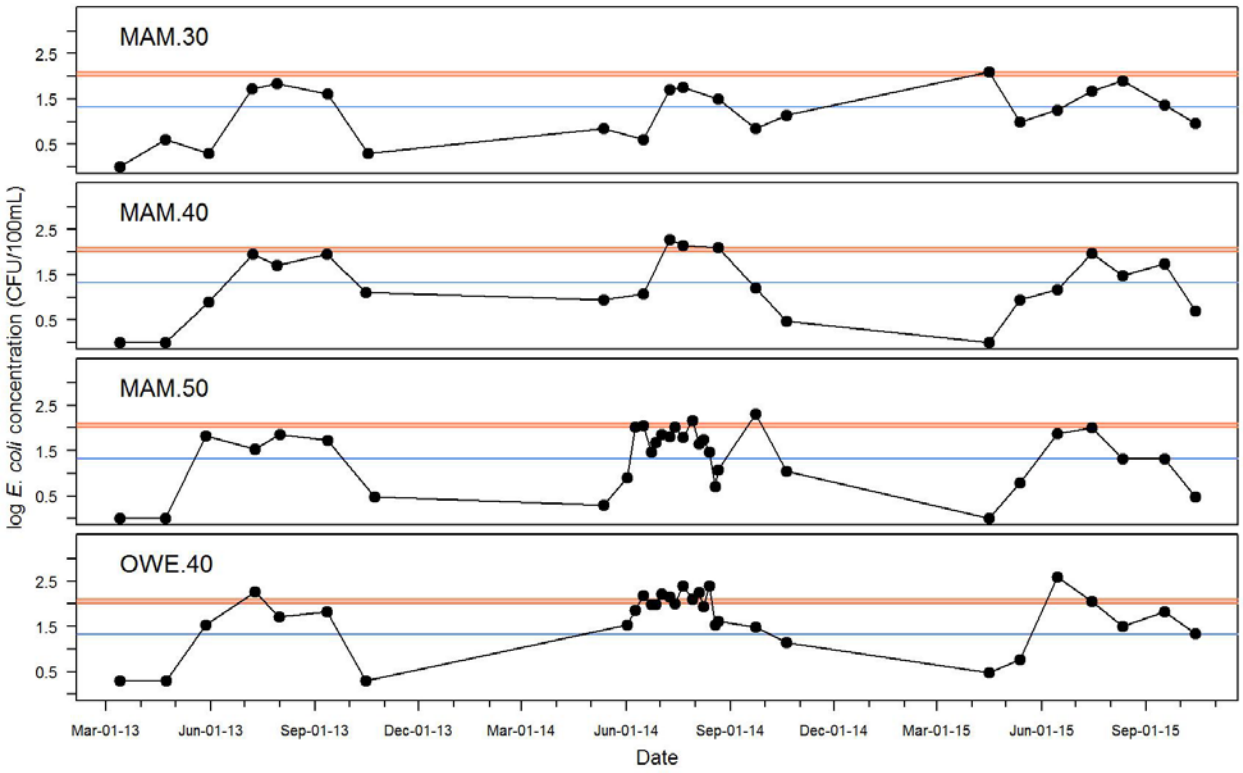

Figure 9. For Mammoth Creek and the Upper Owens River in Long Valley, temporal patterns of *E. coli* concentrations from upstream (MAM.30) to downstream reaches (OWE.40; Figure 3). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. Data collected from May-2014 to Oct-2015 were collected under the current contract, and those data from prior to May-2014 were collected under previous contracts.

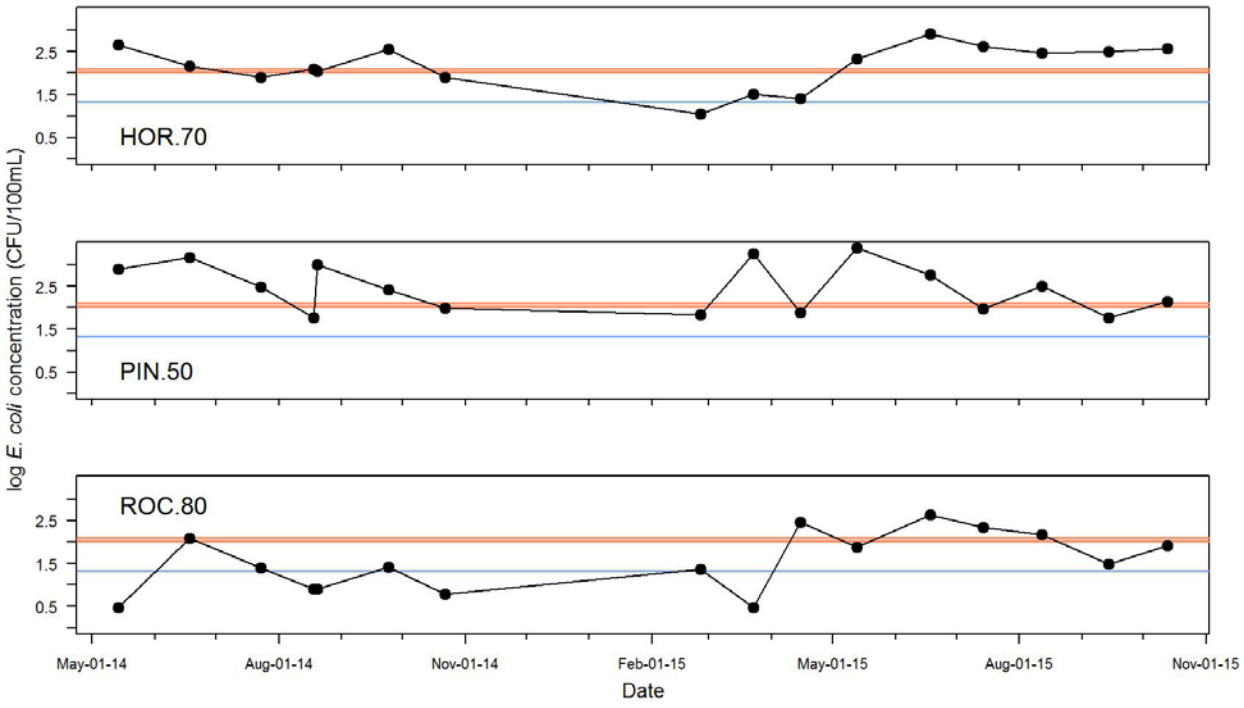

Figure 10. For Horton, Pine, and Rock creeks in Round Valley, temporal patterns of *E. coli* concentrations (Figure 4). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. All data were collected under the current contract.

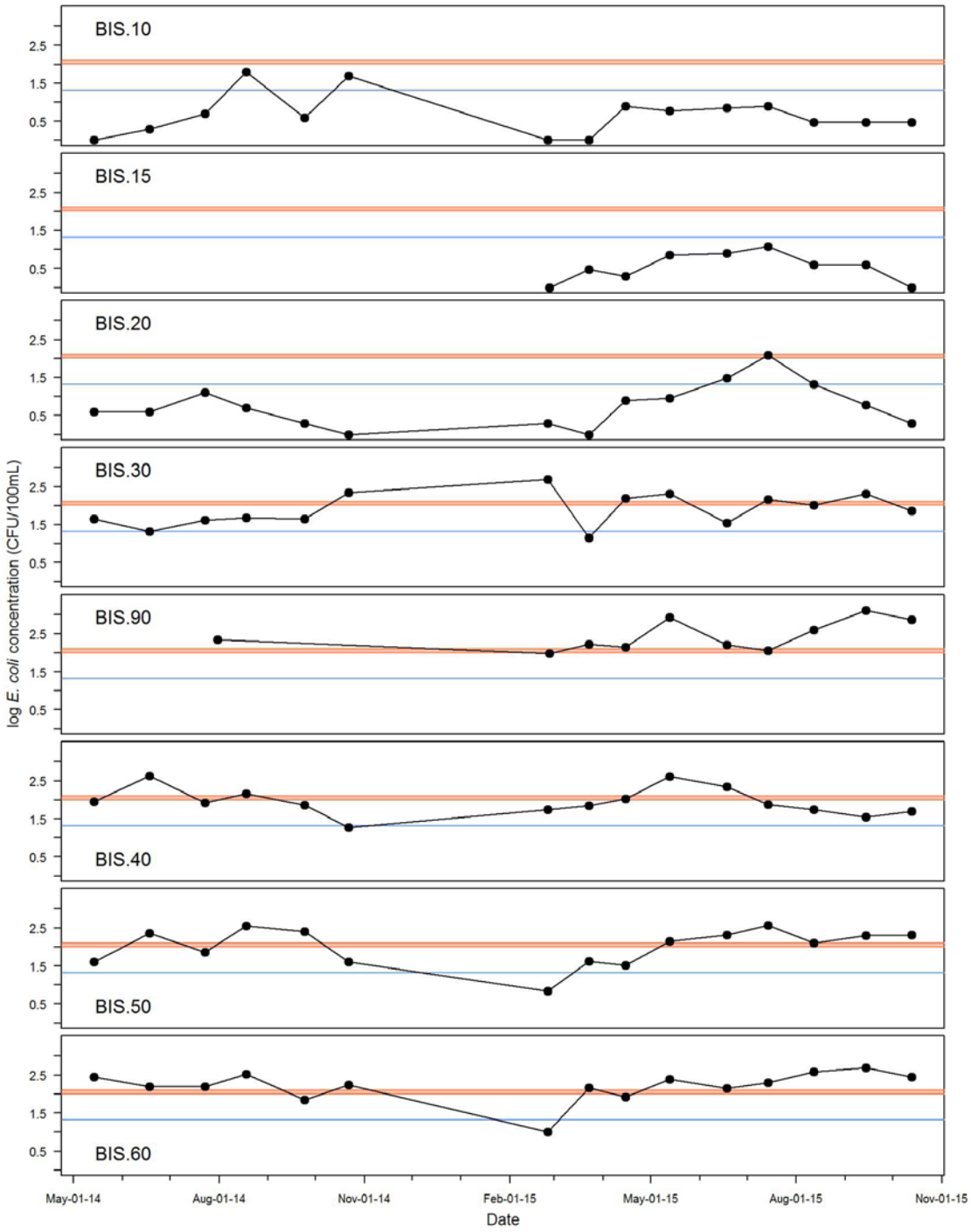

Figure 11. For Bishop Creek in the Owens Valley, temporal patterns of *E. coli* concentrations from upstream reaches on Bishop Creek (BIS.10, BIS.15) to downstream reaches on South Fork Bishop Creek (BIS.20-BIS.60; Figure 5). Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. All data were collected under the current contract.

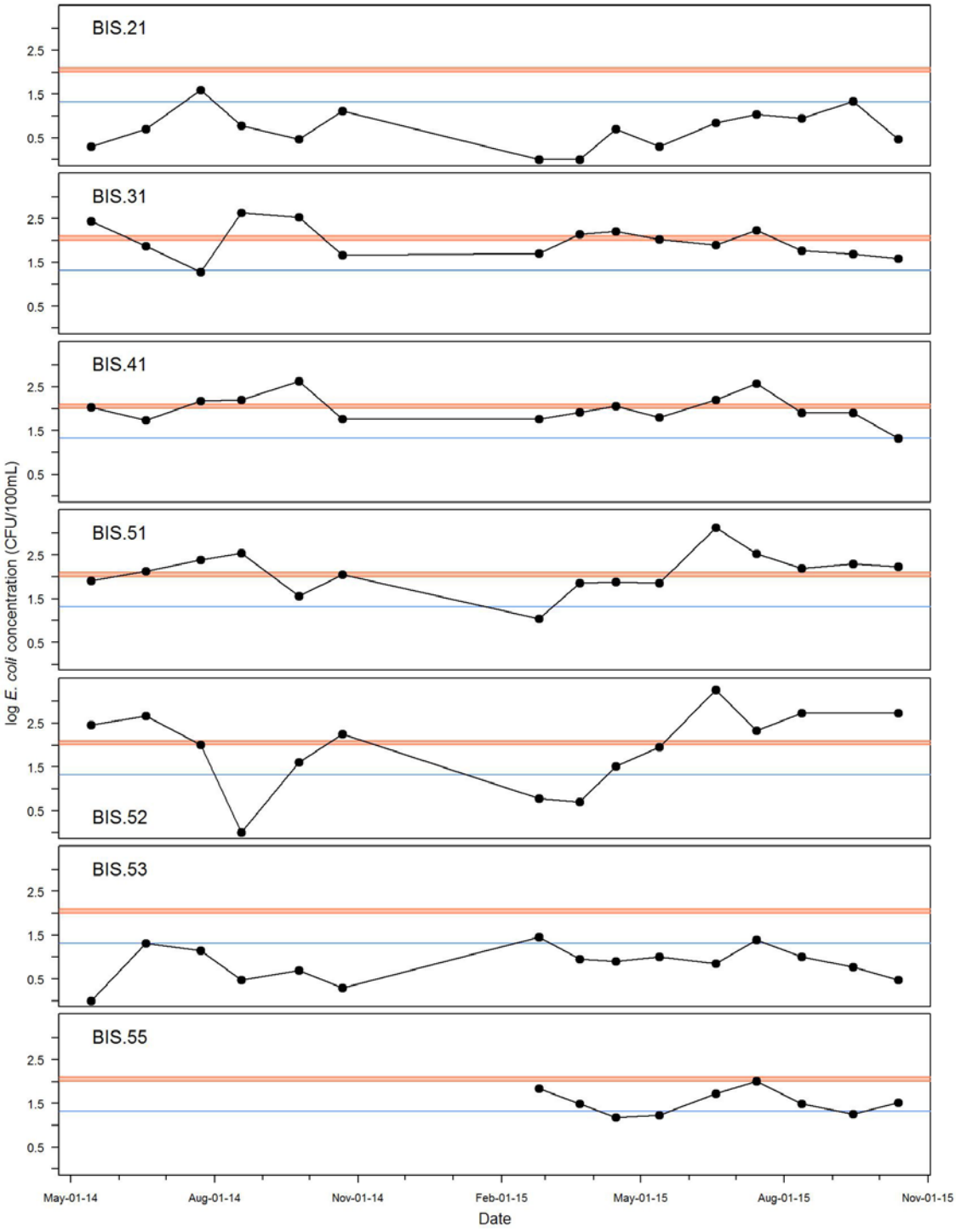

Figure 12. For North Fork Bishop Creek in the Owens Valley, temporal patterns of *E. coli* concentrations from upstream (BIS.21) to downstream reaches (BIS.55; Figure 5). Data for the main-stem Bishop Creek (BIS.10, BIS.15) upstream of BIS.21 are shown in Figure X. Note that the y-axis is on a $\log_{10}$ scale. The blue and red horizontal lines indicate the current Lahontan standard of 20 *E. coli* colonies per 100 mL (assuming equivalence of fecal coliform and *E. coli* concentrations) and a possible future *E. coli* standard of 100/126 *E. coli* colonies per 100 mL, respectively. All data were collected under the current contract.

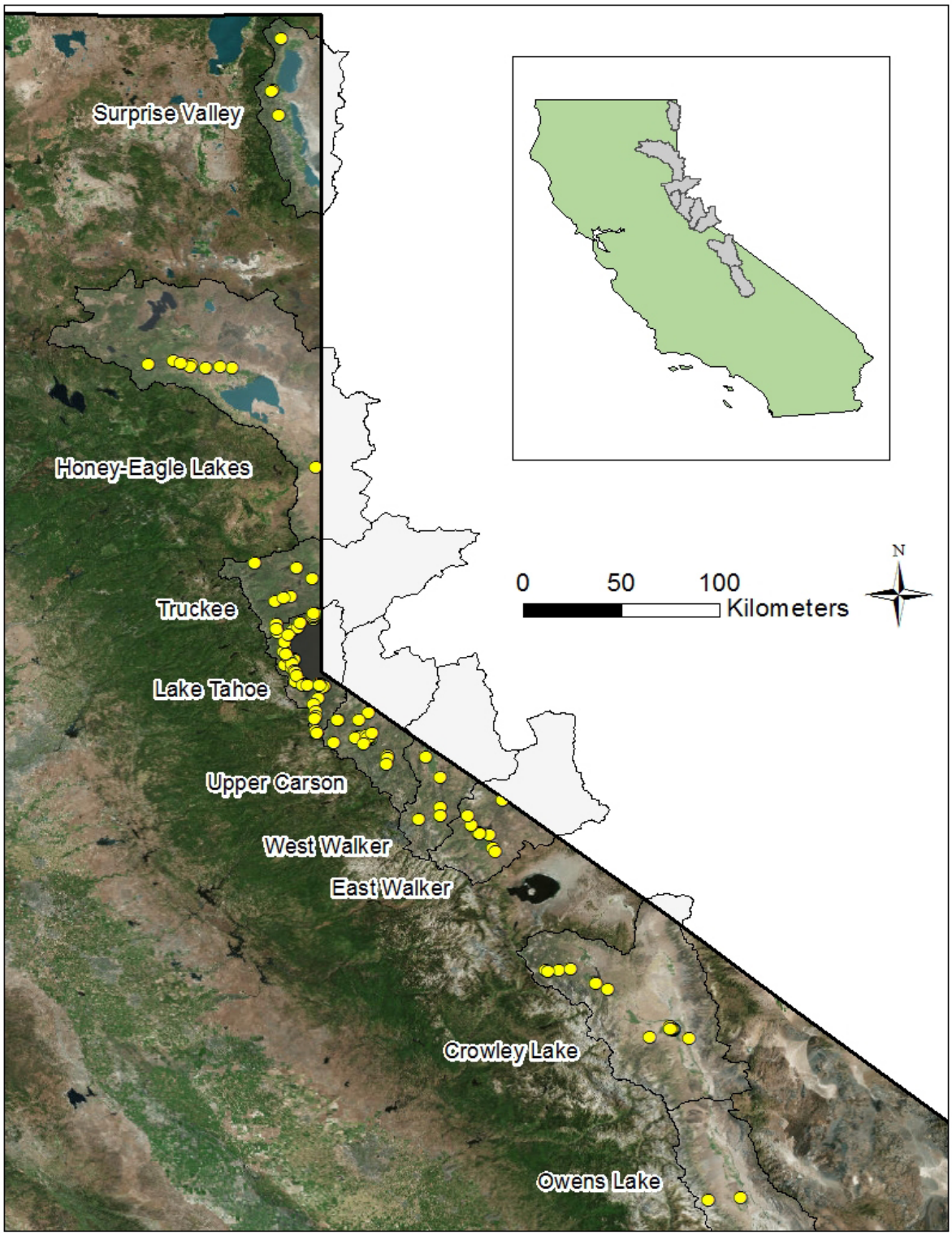

Figure 13. Map of the study area showing the sampling sites (yellow circles) within the HUC8 sub-basins in California at which Lahontan personnel collected one or more water samples for FIB analysis. The inset map shows the location of the study sub-basins within California.

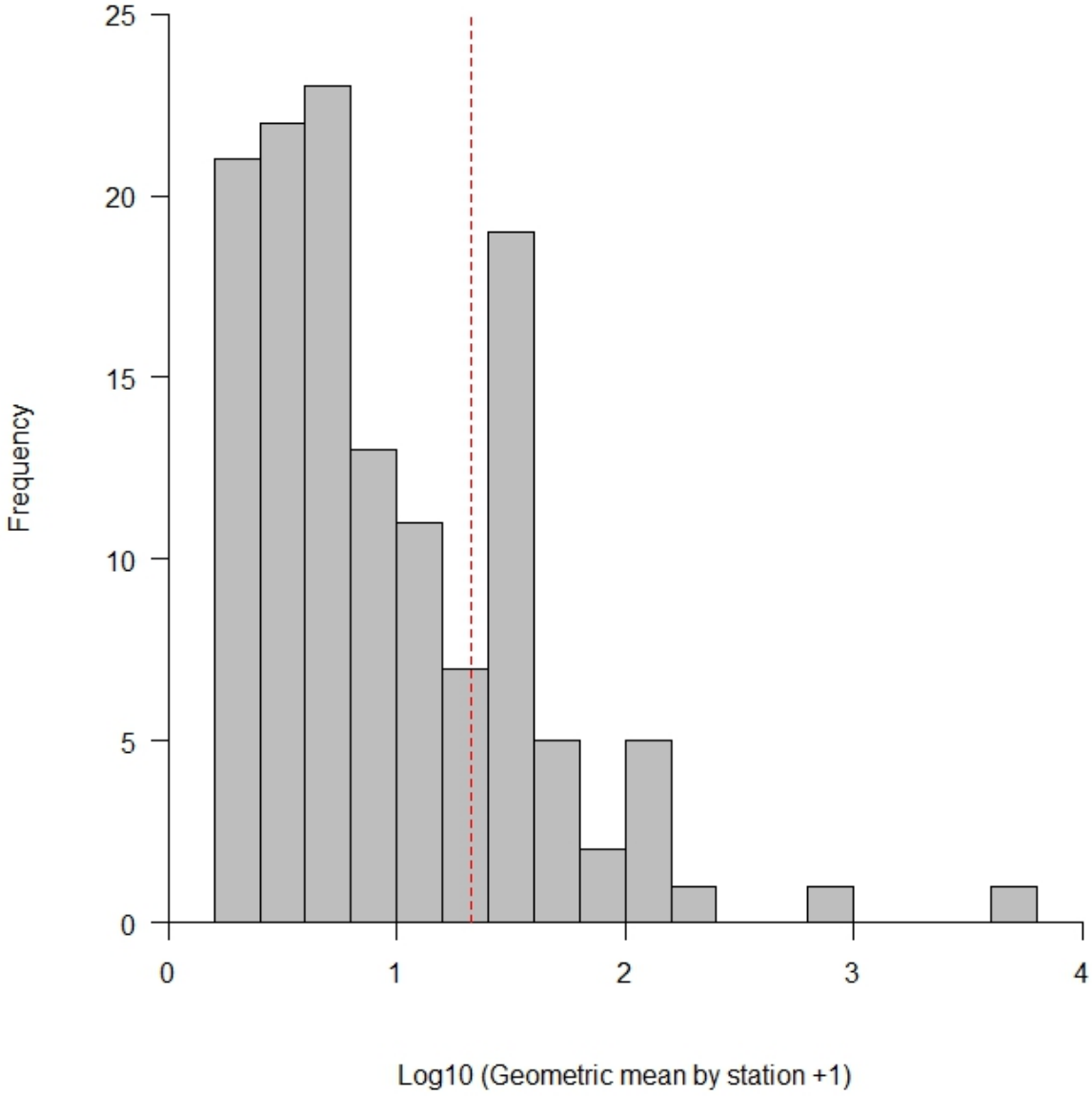

Figure 14. Histogram of fecal coliform bacteria CFU per 100 mL averaged by sampling site (geometric mean). The red dashed line shows the current FIB standard in the Lahontan region of 20 CFU per 100 mL ($\log_{10}(20 + 1) = 1.3$). For the majority of sites, the average fecal coliform concentration was less than the current standard.

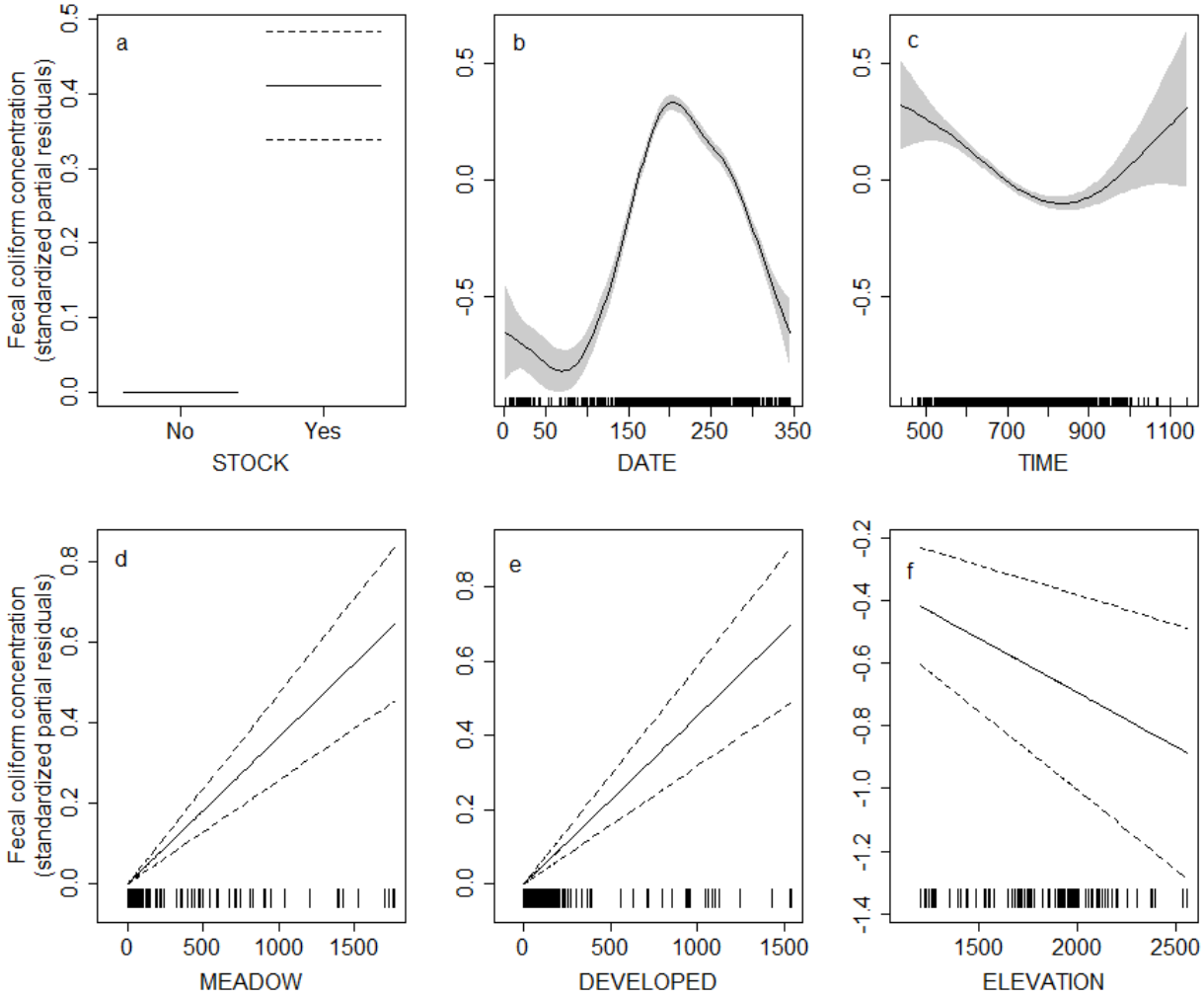

Figure 15. Plots showing the relationships (based on standardized partial residuals) between fecal coliform bacteria concentrations (log_{10}(CFU 100 per mL + 1) and all significant predictor variables ($P < 0.05$) in the final GAM model: (a) presence/absence of livestock, (b) day of year, (c) time of day, (d) area of meadow land cover, (e) area of developed land cover, and (f) elevation. Confidence intervals (95%) are shown as dashed lines. Plots are arranged in order of the strength of each predictor variable's effect, from strongest (a) to weakest (f). Hatch marks above the x-axis for the continuous variables indicate the observed values. In (b), x-axis values correspond to the following dates: 100 = 10 April, 200 = 19 July, 300 = 27 October.

Appendix A. Description of 39 sites sampled under the current contract by personnel from the Center for Eastern Sierra Aquatic Microbial Ecology, Sierra Nevada Aquatic Research Laboratory.

SNARL ID	SWAMP ID	Stream	Drainage	County	Elevation	Latitude	Longitude	Location Description
BIS.10	603BSP111	Bishop_Ck	Owens	Inyo	1527	37.3311	-118.4952	At USFS boundary (Inyo NF sign), above Plant 5 on E. Bishop Creek Road
BIS.15		Bishop_Ck	Owens	Inyo	1357	37.3543	-118.4584	At diversion structure at end of Otey Road, below SCE Plant 6
BIS.20	603BSPB55	Bishop_Ck_SF	Owens	Inyo	1342	37.3581	-118.4504	South Fork, immediately above Mumy Lane
BIS.21	603BSPB65	Bishop_Ck_NF	Owens	Inyo	1341	37.3587	-118.4504	North Fork, immediately above Mumy Lane
BIS.30	603BSPB50	Bishop_Ck_SF	Owens	Inyo	1305	37.3640	-118.4318	South Fork, immediately above Brockman Lane
BIS.31	603BSPB60	Bishop_Ck_NF	Owens	Inyo	1297	37.3686	-118.4323	North Fork, immediately above Brockman Lane
BIS.40		Bishop_Ck_SF	Owens	Inyo	1277	37.3687	-118.4132	South Fork, below See-Vee Lane
BIS.41	603BSPB20	Bishop_Ck_NF	Owens	Inyo	1282	37.3757	-118.4193	North Fork, immediately above Hwy 395
BIS.50	603BSP011	Bishop_Ck_SF	Owens	Inyo	1272	37.3687	-118.4048	South Fork, 190 m S of end of Sierra Street
BIS.51	603BSPB22	Bishop_Ck_NF	Owens	Inyo	1269	37.3801	-118.4050	North Fork, 35 m above confluence with Bishop Canal, just upstream of B-1 drain
BIS.52	603BSPB23	Bishop_Ck	Owens	Inyo	1269	37.3800	-118.4049	B-1 Drain, immediately before confluence with North Fork Bishop Creek
BIS.53		Bishop_Ck	Owens	Inyo	1269	37.3802	-118.4049	Bishop Canal, immediately upstream of confluence with North Fork Bishop Creek
BIS.55		Bishop_Ck_NF	Owens	Inyo	1263	37.3805	-118.3955	North Fork, immediately upstream of Hwy 6
BIS.60	603BSP004	Bishop_Ck_SF	Owens	Inyo	1259	37.3678	-118.3863	South Fork, immediately upstream of confluence with Bishop Canal, below Hanby Avenue
BIS.90		Bishop_Ck_SF	Owens	Inyo	1295	37.3539	-118.4229	South Fork Bishop Creek, A-1 Ditch, 53 m N of Highland Dr-Barlow Ln intersection
BUC.03		Buckeye_Ck	Walker	Mono	2105	38.2389	-119.3252	Immediately below Buckeye Hot Springs
BUC.04	630BUC004	Buckeye_Ck	Walker	Mono	1985	38.2637	-119.2773	North branch of Buckeye Creek @ Hwy 395, 860 m N of Centennial Ranch driveway
BUC.05	630BUC005	Buckeye_Ck	Walker	Mono	1985	38.2622	-119.2759	Middle branch of Buckeye Creek @ Hwy 395, 630 m N of Centennial Ranch drivewa
BUC.08		Buckeye_Ck	Walker	Mono	1972	38.2769	-119.2574	780 m W of Buckeye Creek-Robinson Creek confluence
EWK.06	630EWK006	East_Walker_Rvr	Walker	Mono	1976	38.2553	-119.2237	30 m upstream of Hwy 395 bridge
EWK.08		East_Walker_Rvr	Walker	Mono	1966	38.2619	-119.2288	400 m N of Stock Drive, just downstream of mid-channel island
GRE.40		Green_Ck	Walker	Mono	2096	38.1734	-119.2336	Immediately upstream of Upper Summers Meadow Road bridge over creek
HOR.70		Horton_Ck	Owens	Inyo	1364	37.4061	-118.5417	Horton Creek, immediately below Hwy 395 off of Mill Creek Road
MAM.30		Mammoth_Ck	Owens	Mono	2393	37.6352	-118.9648	S of Mammoth Creek Road, 185 m E of Old Mammoth Road, 75 m W of pedestrian bridge
MAM.40		Mammoth_Ck	Owens	Mono	2200	37.6407	-118.9004	Below bridge on Old Highway 395, immediately below USGS weir
MAM.50		Mammoth_Ck	Owens	Mono	2154	37.6438	-118.8540	160 m upstream of confluence with Hot Creek, 50 m below Chance Ranch fenceline
OWE.40		Owens_Rvr	Owens	Mono	2079	37.6977	-118.7629	Immediately upstream of Benton Crossing Road bridge over Owens River
PIN.50		Pine_Ck	Owens	Inyo	1363	37.4396	-118.5702	Pine Creek, immediately below Hwy 395 in Round Valley, 100 m S of Rock Ck site (ROC.80)
RBS.03		Robinson_Ck	Walker	Mono	2162	38.1686	-119.3245	120 m below outlet dam on Lower Twin Lake, access from S. Twin Road
RBS.05		Robinson_Ck	Walker	Mono	2063	38.2169	-119.3146	At NE end of Hackamore Place, immediately above Hunewill fenceline
RBS.07	630RBS007	Robinson_Ck	Walker	Mono	1986	38.2598	-119.2736	North branch of Robinson Creek @ Hwy 395, 290 m N of Centennial Ranch driveway
RBS.08	630RBS008	Robinson_Ck	Walker	Mono	1987	38.2584	-119.2723	South branch of Robinson Creek @ Hwy 395, 120 m N of Centennial Ranch driveway
RBS.10		Robinson_Ck	Walker	Mono	1971	38.2730	-119.2512	600 m SW of Buckeye Creek-Robinson Creek confluence
ROC.80		Rock_Ck	Owens	Inyo	1363	37.4400	-118.5704	Rock Creek, immediately below Hwy 395 in Round Valley
SWA.02		Swauger_Ck	Walker	Mono	2368	38.3654	-119.3452	Immediately downstream of Swauger Creek Road at first creek crossing, 2 km N of Hwy 395
SWA.05	630SWA005	Swauger_Ck	Walker	Mono	2059	38.2959	-119.3097	Below Huntoon Valley, 2.9 km N of Buckeye Road/Forest Service compound on Hwy 395
SWA.06	630SWA006	Swauger_Ck	Walker	Mono	2208	38.3429	-119.3229	Above Huntoon Valley, 2 km S of Swauger Ck Rd on Hwy 395 @ dirt road that crosses creek
SWA.08		Swauger_Ck	Walker	Mono	2002	38.2777	-119.2870	At USFS compound, immediately upstream of bridge over creek and private land boundary
VIR.04	630VIR004	Virginia_Ck	Walker	Mono	2045	38.1914	-119.2092	450 m N of Willow Springs Resort on Hwy 395, at USGS stream gage

Appendix B. Microbial source tracking results for 273 samples collected by personnel from the Center for Eastern Sierra Aquatic Microbial Ecology, Sierra Nevada Aquatic Research Laboratory and analyzed under the current contract. Membrane filtration results for fecal coliform (FC100) and *E. coli* (EC100) are also provided to allow direct comparison with microbial source tracking results. MST results are expressed as copies per 100 mL.

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
BIS.10	603BSP111	5/14/2014	0	0	800	6099	ND	ND	ND	ND
BIS.10	603BSP111	6/18/2014	1	1	800	8412	ND	ND	ND	ND
BIS.10	603BSP111	7/23/2014	10	4	800	8885	ND	ND	ND	ND
BIS.10	603BSP111	8/18/2014	66	61	800	4001	36	ND	ND	ND
BIS.10	603BSP111	9/24/2014	5	3	800	4422	76	ND	ND	ND
BIS.20	603BSPB55	5/14/2014	4	3	600	11606	ND	ND	ND	ND
BIS.20	603BSPB55	6/18/2014	7	3	800	14246	ND	ND	ND	ND
BIS.20	603BSPB55	7/23/2014	16	12	800	4670	ND	ND	ND	ND
BIS.20	603BSPB55	8/18/2014	4	4	800	9008	ND	ND	ND	ND
BIS.20	603BSPB55	9/24/2014	4	1	800	7386	ND	ND	ND	ND
BIS.21	603BSPB65	5/14/2014	1	1	600	16679	ND	ND	ND	ND
BIS.21	603BSPB65	6/18/2014	7	4	800	8298	ND	ND	ND	ND
BIS.21	603BSPB65	7/23/2014	86	38	800	12718	8	ND	ND	ND
BIS.21	603BSPB65	8/18/2014	5	5	800	8241	ND	ND	ND	ND
BIS.21	603BSPB65	9/24/2014	3	2	800	833	ND	ND	ND	ND
BIS.30	603BSPB50	5/14/2014	45	43	400	ND	51	11013	ND	ND
BIS.30	603BSPB50	6/18/2014	50	20	800	16191	138	197	ND	ND
BIS.30	603BSPB50	7/23/2014	43	40	800	8654	9	ND	ND	ND
BIS.30	603BSPB50	8/18/2014	54	47	800	10754	50	150	ND	ND
BIS.30	603BSPB50	9/24/2014	64	44	800	17121	5	1083	ND	ND
BIS.31	603BSPB60	5/14/2014	324	276	400	299	1991	1626	ND	ND
BIS.31	603BSPB60	6/18/2014	80	74	600	ND	67	437	ND	ND
BIS.31	603BSPB60	7/23/2014	24	18	800	7856	ND	ND	ND	ND
BIS.31	603BSPB60	8/18/2014	514	426	800	10031	1051	46	11	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
BIS.31	603BSPB60	9/24/2014	340	335	800	31519	480	ND	25	ND
BIS.40		5/14/2014	104	90	600	53825	120	4483	ND	ND
BIS.40		6/18/2014	1150	420	600	37747	1580	ND	ND	ND
BIS.40		7/23/2014	90	82	800	29932	66	1572	ND	ND
BIS.40		8/18/2014	172	144	800	20408	272	84	ND	ND
BIS.40		9/24/2014	78	72	800	69866	238	960	ND	ND
BIS.41	603BSPB20	5/14/2014	156	106	600	11614	212	733	ND	ND
BIS.41	603BSPB20	6/18/2014	102	54	700	53045	36	5420	ND	ND
BIS.41	603BSPB20	7/23/2014	195	150	800	23458	699	1543	12	ND
BIS.41	603BSPB20	8/18/2014	160	160	600	12475	313	ND	ND	ND
BIS.41	603BSPB20	9/24/2014	448	424	800	129473	2887	432	ND	ND
BIS.50	603BSP011	5/14/2014	40	40	600	53475	ND	9683	ND	ND
BIS.50	603BSP011	6/18/2014	240	230	800	30992	816	2705	ND	ND
BIS.50	603BSP011	7/23/2014	72	72	800	20985	59	2499	ND	ND
BIS.50	603BSP011	8/18/2014	1000	360	400	41969	1188	2732	ND	ND
BIS.50	603BSP011	9/24/2014	275	255	800	42482	63	1777	ND	ND
BIS.51	603BSPB22	5/14/2014	104	80	600	46932	496	2873	ND	ND
BIS.51	603BSPB22	6/18/2014	135	135	600	44303	650	2147	ND	ND
BIS.51	603BSPB22	7/23/2014	780	240	600	31937	838	5841	25	ND
BIS.51	603BSPB22	8/18/2014	400	350	600	54217	2532	4075	25	51
BIS.51	603BSPB22	9/24/2014	45	35	800	28158	10	1327	17	250
BIS.52	603BSPB23	5/14/2014	3190	290	400	52484	2278	3228	ND	ND
BIS.52	603BSPB23	6/18/2014	740	460	550	57521	1212	8752	ND	ND
BIS.52	603BSPB23	7/23/2014	110	100	800	79343	100	9744	ND	ND
BIS.52	603BSPB23	8/18/2014	3	0	800	68730	502	7088	ND	ND
BIS.52	603BSPB23	9/24/2014	46	40	800	254695	728	53894	ND	ND
BIS.53		5/14/2014	2	0	400	23047	ND	ND	ND	ND
BIS.53		6/18/2014	20	20	400	29837	108	ND	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
BIS.53		7/23/2014	26	13	600	77232	ND	ND	ND	ND
BIS.53		8/18/2014	5	2	400	29283	55	ND	ND	ND
BIS.53		9/24/2014	6	4	800	788	ND	ND	ND	ND
BIS.60	603BSP004	5/14/2014	573	285	400	134317	2606	1834	ND	98
BIS.60	603BSP004	6/18/2014	158	158	700	151298	1489	902	ND	ND
BIS.60	603BSP004	7/23/2014	470	155	800	84475	629	765	ND	ND
BIS.60	603BSP004	8/18/2014	345	335	600	101462	1871	1203	ND	ND
BIS.60	603BSP004	9/24/2014	88	70	800	137438	708	1097	ND	ND
BUC.02		7/6/2013	44	44	800	40633	198	4101	ND	ND
BUC.03		7/6/2013	43	43	800	8486	63	712	ND	ND
BUC.03		7/22/2014	58	28	800	5827	10	188	ND	ND
BUC.04	630BUC004	7/6/2013	440	400	800	45400	1670	2659	ND	ND
BUC.04	630BUC004	7/29/2013	187	187	400	13955	259	1023	ND	ND
BUC.04	630BUC004	9/16/2013	123	113	800	68486	628	10801	ND	ND
BUC.04	630BUC004	6/17/2014	62	59	800	22292	441	1557	ND	ND
BUC.04	630BUC004	7/22/2014	980	740	400	55065	3515	8870	ND	ND
BUC.04	630BUC004	8/17/2014	530	500	400	67857	1275	18223	ND	ND
BUC.04	630BUC004	9/22/2014	190	170	800	37664	531	6840	ND	ND
BUC.05	630BUC005	7/6/2013	156	156	400	144313	1239	30622	ND	ND
BUC.05	630BUC005	7/29/2013	193	167	400	45465	379	2811	ND	ND
BUC.05	630BUC005	9/16/2013	300	260	800	149861	1470	28968	ND	ND
BUC.05	630BUC005	6/17/2014	33	29	800	24802	4	12287	ND	ND
BUC.05	630BUC005	7/22/2014	1090	850	400	58642	3111	3730	ND	ND
BUC.05	630BUC005	8/17/2014	120	120	400	40533	419	8762	ND	ND
BUC.05	630BUC005	9/22/2014	210	203	800	56800	615	32820	ND	ND
BUC.08		6/3/2014	240	224	400	149047	1062	51424	ND	ND
BUC.08		8/21/2014	330	310	400	131489	1597	39315	24	ND
CON.15		7/7/2013	13	13	800	1538	ND	ND	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
CON.15		7/29/2013	32	24	800	1252	ND	ND	ND	ND
CON.15		9/11/2013	23	17	800	1020	603	ND	ND	ND
CON.15		10/17/2013	4	4	800	4181	ND	ND	ND	ND
CON.20		7/7/2013	39	38	800	1130	ND	ND	ND	ND
CON.20		7/29/2013	51	35	800	9808	198	ND	ND	ND
CON.20		9/11/2013	44	35	800	8463	1360	ND	ND	ND
CON.20		10/17/2013	7	7	800	6806	ND	ND	ND	ND
EWK.06	630EWK006	5/29/2013	830	740	400	138772	1740	19039	ND	ND
EWK.06	630EWK006	7/6/2013	287	247	400	108295	810	5514	ND	ND
EWK.06	630EWK006	7/29/2013	250	220	400	71323	980	11909	ND	ND
EWK.06	630EWK006	9/16/2013	67	60	600	109622	1839	25968	ND	ND
EWK.06	630EWK006	6/17/2014	395	380	400	87799	1987	14629	ND	ND
EWK.06	630EWK006	7/22/2014	160	160	400	140595	901	5717	ND	ND
EWK.06	630EWK006	8/17/2014	135	135	400	93477	ND	21487	ND	ND
EWK.06	630EWK006	9/22/2014	96	96	400	125233	1417	56698	ND	ND
EWK.08		6/3/2014	360	330	400	84206	479	6302	ND	ND
EWK.08		8/21/2014	280	250	400	201076	2690	35658	ND	ND
GRE.40		8/21/2014	73	66	800	89015	460	ND	ND	ND
HOR.70		5/14/2014	512	460	400	83011	888	499	ND	ND
HOR.70		6/18/2014	148	144	600	29904	ND	900	ND	ND
HOR.70		7/23/2014	92	78	800	131619	536	ND	ND	ND
HOR.70		8/18/2014	124	124	800	164731	896	652	ND	ND
HOR.70		9/24/2014	378	358	600	965911	2412	50194	ND	ND
LEE.30		7/7/2013	47	43	800	4908	ND	ND	ND	ND
LEE.30		7/30/2013	31	28	800	2065	24	ND	ND	ND
MAM.10		4/22/2013	0	0	400	7300	ND	ND	ND	ND
MAM.10		5/30/2013	0	0	400	15863	ND	ND	ND	ND
MAM.10		7/8/2013	0	0	400	11614	863	ND	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
MAM.10		8/1/2013	4	4	400	17292	ND	ND	ND	ND
MAM.10		9/11/2013	2	2	400	7749	ND	ND	ND	ND
MAM.10		10/15/2013	0	0	400	8011	ND	ND	ND	ND
MAM.10		5/12/2014	0	0	600	10163	ND	ND	ND	ND
MAM.20		4/22/2013	0	0	400	9646	ND	ND	ND	ND
MAM.20		5/30/2013	1	0	700	8371	22	ND	ND	ND
MAM.20		7/7/2013	1	1	600	4835	8	ND	ND	ND
MAM.20		7/29/2013	8	8	600	3019	4	ND	ND	ND
MAM.20		9/11/2013	18	21	500	2988	25	ND	ND	ND
MAM.20		10/15/2013	0	0	600	13076	ND	ND	ND	ND
MAM.20		5/12/2014	1	1	800	2219	ND	ND	ND	ND
MAM.30		4/22/2013	3	3	800	16673	ND	ND	ND	ND
MAM.30		5/30/2013	1	1	800	18663	ND	ND	ND	ND
MAM.30		7/7/2013	53	52	700	20143	134	160	35	ND
MAM.30		7/29/2013	78	69	700	10285	139	ND	ND	ND
MAM.30		9/12/2013	49	40	600	8772	89	121	ND	47
MAM.30		10/17/2013	1	1	700	ND	ND	ND	ND	ND
MAM.30		5/12/2014	6	6	800	14125	20	ND	ND	ND
MAM.30		6/16/2014	3	3	400	12622	ND	ND	ND	ND
MAM.30		7/9/2014	93	51	700	13177	187	243	ND	ND
MAM.30		7/21/2014	59	57	800	16446	68	ND	ND	ND
MAM.30		8/21/2014	32	31	800	24975	134	ND	ND	ND
MAM.30		9/23/2014	6	6	800	15894	ND	ND	ND	ND
MAM.40		4/22/2013	1	0	800	28724	ND	ND	ND	ND
MAM.40		5/30/2013	7	7	800	14796	ND	ND	ND	ND
MAM.40		7/8/2013	99	88	800	168540	95	39368	ND	ND
MAM.40		7/29/2013	56	51	800	13400	71	204	ND	ND
MAM.40		9/11/2013	123	88	800	42532	981	3322	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
MAM.40		10/15/2013	12	12	600	6871	55	ND	ND	ND
MAM.40		5/12/2014	8	8	700	9622	5	ND	ND	ND
MAM.40		6/16/2014	11	11	400	14082	657	ND	ND	ND
MAM.40		7/9/2014	192	192	800	156991	913	47044	ND	ND
MAM.40		7/21/2014	142	142	800	17388	509	907	ND	ND
MAM.40		8/21/2014	125	125	700	10545	65	644	ND	ND
MAM.40		9/23/2014	15	15	800	14344	182	2033	1253	21
MAM.50		4/22/2013	0	0	800	111893	3	134	ND	ND
MAM.50		5/28/2013	67	67	600	388777	2565	47674	ND	32
MAM.50		7/10/2013	33	33	800	17973	50	328	ND	ND
MAM.50		8/1/2013	80	70	800	ND	2232	9929	ND	ND
MAM.50		9/12/2013	53	53	700	ND	113	29709	ND	ND
MAM.50		5/12/2014	0	0	700	27199	ND	ND	ND	3
MAM.50		6/2/2014	7	7	600	49168	ND	ND	ND	ND
MAM.50		7/2/2014	84	70	800	65857	102	194	18	ND
MAM.50		8/4/2014	56	44	600	12048	16	3499	ND	ND
MAM.50		9/23/2014	206	206	600	2142248	1021	854517	ND	ND
MCG.30		7/10/2013	60	60	800	11294	ND	ND	ND	ND
MCG.30		8/1/2013	58	55	800	15266	ND	ND	ND	ND
MIL.80		7/7/2013	31	27	700	6417	ND	ND	ND	ND
MIL.80		7/30/2013	32	23	800	15168	5	ND	ND	ND
OWE.15		8/1/2013	33	25	800	8249	ND	ND	ND	ND
OWE.20		7/8/2013	60	56	800	166	13	ND	ND	ND
OWE.40		7/10/2013	180	180	600	63317	86	3612	ND	ND
OWE.40		7/31/2013	63	50	600	678874	1852	6943	ND	ND
OWE.40		9/11/2013	70	67	600	37427	225	ND	ND	ND
OWE.40		6/2/2014	32	32	600	40942	28	ND	ND	ND
OWE.40		7/2/2014	166	162	800	46883	22	ND	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
OWE.40		8/4/2014	186	176	600	19712	46	ND	ND	ND
OWE.40		8/8/2014	90	86	400	76864	311	ND	ND	ND
OWE.40		9/23/2014	32	30	800	121093	197	419	ND	ND
PIN.50		5/14/2014	976	796	400	10266	49	ND	ND	ND
PIN.50		6/18/2014	2340	1480	400	38955	50	ND	ND	ND
PIN.50		7/23/2014	310	310	800	69321	137	2394	ND	ND
PIN.50		8/18/2014	56	56	800	7837	5	ND	ND	ND
PIN.50		9/24/2014	354	260	800	7541	213	ND	ND	ND
RBS.02		4/23/2013	0	0	800	56880	ND	ND	ND	ND
RBS.02		5/29/2013	1	1	800	62831	ND	ND	ND	ND
RBS.02		7/6/2013	15	17	800	81923	ND	180	64	ND
RBS.02		7/29/2013	20	20	400	33023	8	ND	ND	ND
RBS.02		9/16/2013	13	9	800	11125	9	ND	ND	ND
RBS.02		10/16/2013	1	1	800	23385	ND	ND	ND	ND
RBS.03		5/13/2014	0	0	800	273	ND	ND	ND	ND
RBS.03		6/17/2014	0	0	700	214	ND	ND	ND	ND
RBS.03		7/22/2014	2	2	800	122	ND	ND	ND	ND
RBS.03		8/17/2014	0	0	800	294	ND	ND	ND	ND
RBS.03		9/22/2014	1	1	800	746	ND	ND	ND	ND
RBS.04		4/23/2013	1	1	800	3926	ND	ND	ND	ND
RBS.04		5/29/2013	2	2	800	3953	ND	ND	ND	ND
RBS.04		7/6/2013	4	3	800	7086	4	50	ND	ND
RBS.04		7/29/2013	15	13	800	2518	ND	ND	ND	ND
RBS.04		9/16/2013	7	6	800	19943	ND	3350	ND	ND
RBS.04		10/16/2013	1	1	800	6544	ND	ND	ND	ND
RBS.05		9/16/2013	9	9	800	6675	16	612	ND	ND
RBS.05		10/16/2013	7	1	800	8476	ND	ND	ND	ND
RBS.05		5/13/2014	0	0	800	15255	ND	ND	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
RBS.05		6/17/2014	7	7	700	2364	ND	ND	ND	ND
RBS.05		7/22/2014	24	18	800	5734	77	ND	ND	ND
RBS.05		8/17/2014	41	40	800	6247	74	770	ND	ND
RBS.05		9/22/2014	27	26	800	12106	56	1434	ND	ND
RBS.07	630RBS007	4/23/2013	15	13	800	107527	3	ND	ND	ND
RBS.07	630RBS007	5/29/2013	58	52	800	21020	122	383	ND	ND
RBS.07	630RBS007	7/6/2013	220	196	800	71687	1303	4329	ND	ND
RBS.07	630RBS007	7/29/2013	163	143	400	27869	396	2631	ND	ND
RBS.07	630RBS007	9/16/2013	220	220	800	81681	205	9406	ND	ND
RBS.07	630RBS007	10/16/2013	66	64	800	ND	321	7641	ND	ND
RBS.07	630RBS007	5/13/2014	8	8	800	52274	139	1578	ND	ND
RBS.07	630RBS007	6/17/2014	84	74	700	34447	458	7913	ND	ND
RBS.07	630RBS007	7/22/2014	480	460	400	39146	613	2317	ND	ND
RBS.07	630RBS007	8/17/2014	110	110	600	59353	512	7782	ND	ND
RBS.07	630RBS007	9/22/2014	76	68	400	22271	275	605	ND	ND
RBS.08	630RBS008	4/23/2013	11	10	750	125025	ND	ND	ND	ND
RBS.08	630RBS008	5/29/2013	328	192	600	148000	1193	873	336	106
RBS.08	630RBS008	7/6/2013	209	207	400	52143	507	2508	ND	ND
RBS.08	630RBS008	7/29/2013	230	213	400	65096	190	1023	ND	ND
RBS.08	630RBS008	9/16/2013	280	270	800	101972	674	16260	ND	ND
RBS.08	630RBS008	10/16/2013	87	84	800	ND	408	17651	ND	462
RBS.08	630RBS008	5/13/2014	10	10	800	29423	53	7511	ND	ND
RBS.08	630RBS008	6/17/2014	102	93	800	37403	546	8468	ND	ND
RBS.08	630RBS008	7/22/2014	288	220	400	54303	335	2013	ND	ND
RBS.08	630RBS008	8/17/2014	62	62	500	76360	68	2226	ND	ND
RBS.08	630RBS008	9/22/2014	122	114	800	89314	424	15877	ND	ND
RBS.10		6/3/2014	23	23	400	64252	11	3287	ND	ND
RBS.10		8/21/2014	280	270	400	41969	380	2233	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
ROC.30		9/12/2013	22	22	800	39684	176	ND	ND	ND
ROC.80		7/23/2014	24	24	800	102500	40	ND	ND	ND
ROC.80		9/24/2014	25	25	800	7397	8	ND	ND	ND
RUS.20		7/7/2013	43	43	800	2791	ND	ND	ND	ND
RUS.20		7/30/2013	176	168	800	4235	101	ND	ND	ND
RUS.20		9/17/2013	93	74	800	23609	733	ND	20	ND
RUS.80		7/30/2013	43	30	600	3338	ND	ND	ND	ND
SWA.02		4/23/2013	3	3	800	4513	ND	ND	ND	ND
SWA.02		5/29/2013	3	3	800	16022	ND	ND	ND	ND
SWA.02		7/6/2013	30	18	800	4920	43	ND	ND	ND
SWA.02		7/29/2013	46	46	800	5168	24	ND	ND	ND
SWA.02		9/16/2013	43	42	800	3981	65	106	ND	ND
SWA.02		10/16/2013	3	3	800	4032	ND	ND	ND	ND
SWA.02		5/13/2014	0	0	800	2815	ND	ND	ND	ND
SWA.02		6/17/2014	3	3	800	6141	ND	ND	ND	ND
SWA.02		7/22/2014	16	16	800	2973	14	ND	ND	ND
SWA.02		8/17/2014	7	7	600	5212	ND	ND	ND	ND
SWA.02		9/22/2014	5	2	800	4544	ND	ND	ND	ND
SWA.05	630SWA005	4/23/2013	2	2	800	19811	ND	ND	ND	ND
SWA.05	630SWA005	5/29/2013	30	29	600	14499	ND	ND	ND	ND
SWA.05	630SWA005	7/6/2013	460	400	800	524597	1718	82008	ND	ND
SWA.05	630SWA005	7/29/2013	93	93	700	84646	217	177	ND	ND
SWA.05	630SWA005	9/16/2013	107	90	800	110278	141	7175	ND	ND
SWA.05	630SWA005	10/16/2013	64	60	700	34616	29	510	ND	ND
SWA.05	630SWA005	5/13/2014	8	8	800	33279	26	668	ND	ND
SWA.05	630SWA005	6/17/2014	583	550	600	118259	5341	12298	ND	ND
SWA.05	630SWA005	7/22/2014	185	185	800	171865	566	5326	ND	ND
SWA.05	630SWA005	8/17/2014	184	184	800	133758	484	6594	ND	ND

			Membrane Filtration		Microbial Source Tracking					
SiteID	**SWAMPid**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
SWA.05	630SWA005	9/22/2014	18	18	800	50516	71	ND	ND	ND
SWA.06	630SWA006	4/23/2013	2	0	800	34357	ND	ND	ND	ND
SWA.06	630SWA006	5/29/2013	7	7	800	96699	22	73	ND	ND
SWA.06	630SWA006	7/6/2013	130	114	800	ND	ND	ND	ND	ND
SWA.06	630SWA006	7/29/2013	77	67	600	118551	418	ND	ND	ND
SWA.06	630SWA006	9/16/2013	80	80	800	94291	250	2474	ND	ND
SWA.06	630SWA006	10/16/2013	14	9	700	26199	ND	ND	ND	ND
SWA.06	630SWA006	5/13/2014	1	1	800	20195	5	ND	ND	ND
SWA.06	630SWA006	6/17/2014	18	17	700	8558	8	ND	ND	ND
SWA.06	630SWA006	7/22/2014	222	192	600	23023	1143	330	ND	ND
SWA.06	630SWA006	8/17/2014	104	48	600	16597	124	ND	ND	ND
SWA.06	630SWA006	9/22/2014	36	34	700	6522	ND	163	ND	ND
SWA.08		5/13/2014	11	11	400	487424	ND	591	ND	ND
SWA.08		6/17/2014	18	15	400	578355	ND	ND	ND	3
SWA.08		7/22/2014	54	46	400	490284	ND	ND	ND	ND
SWA.08		8/17/2014	192	44	400	500634	667	ND	ND	ND
SWA.08		9/22/2014	86	66	400	282841	ND	ND	ND	ND
VIR.03		7/29/2013	50	45	800	3404	166	287	261	299
VIR.03		9/16/2013	37	37	800	1727	91	ND	ND	ND
VIR.04	630VIR004	7/6/2013	142	118	800	37819	272	274	ND	ND
VIR.04	630VIR004	6/17/2014	27	21	800	28908	90	ND	ND	ND
VIR.04	630VIR004	7/22/2014	20	20	800	23295	26	ND	ND	ND
VIR.05	630VIR005	7/6/2013	122	45	600	25084	248	654	ND	ND
VIR.05	630VIR005	7/29/2013	38	38	800	18813	26	279	ND	ND
WAL.10		9/17/2013	52	32	600	2533	ND	ND	ND	ND

1. MST results are expressed as “copies per 100 mL”.

Appendix C. *Enterococcus*, *Escherichia, and Bacteroidales* qPCR assay Standard Operating Procedures.

Dr. Craig E. Nelson, January 2015
Standardized to USEPA document EPA-821-R-10-004: "Method A: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay" (April 2010)

Laboratory Details and Sample Handling

Sample collection and handling are described in the CESAME QA/QC Section E: Appendix. All equipment guidelines in EPA-821-R-10-004 are met. Reagent preparation is done in a bleach- and UV-sterilized laminar flow hood. Sample preparation (membrane filtration and subsequent DNA extraction) is done in separate laboratories, with DNA extraction done in benchtop area separated from reagent preparation that is bleach- and ethanol- cleaned after each use. Handling of amplified DNA is isolated to a separate room to avoid contamination of samples and reagents. All materials are disposed of according to institutional guidelines for biohazardous waste. Quantitative PCR is done on an Applied Biosystems StepOnePlus or ABI7300 or Eppendorf Mastercycler® ep realplex. All recommended safety guidelines are followed in accordance with EPA-821-R-10-004 and institutional recommendations.

Reagents and Standards

1) DNA Extraction Kits: MoBio PowerSoil® DNA Isolation Kit (12888)
2) qPCR Master Mix: 5Prime RealMasterMix Probe (2200710)
3) Primer and Probe Sets: We employ Integrated DNA Technologies PrimeTime® Assays
    a. Entero1a: (Ludwig & Schleifer 2000, Haugland et al. 2005, Method A EPA-821-R-10-004)
        i. Forward Primer - AGAAATTCCAAACGAACTTG
        ii. Reverse Primer - CAGTGCTCTACCTCCATCATT
        iii. Probe – 6-FAM™/TGGTTCTCT/ZEN™/CCGAAATAGCTTTAGGGCTA/IB®FQ/
    b. EC23S857: (Chern et al. 2011)
        i. Forward Primer - GGTAGAGCACTGTTTTGGCA
        ii. Reverse Primer - TGTCTCCCGTGATAACTTTCTC
        iii. Probe - 6-FAM™/TCATCCCGA/ZEN™/CTTACCAACCCG/IB®FQ/
    c. GenBac3: (Dick and Field 2004, Siefring et al. 2008, Method "B" EPA-822-R-10-003)
        i. GGGGTTCTGAGAGGAAGGT
        ii. CCGTCATCCTTCACGCTACT
        iii. 6-FAM™/CAATATTCC/ZEN™/TCACTGCTGCCTCCCGTA/IB®FQ/
    d. HF183: (Haugland et al. 2010, Green et al. 2014)
        i. ATCATGAGTTCACATGTCCG
        ii. CTTCCTCTCAGAACCCCTATCC
        iii. 6-FAM™/CTAATGGAA/ZEN™/CGCATCCCCAT/IB®FQ/ (add 3' CAT to avoid MGB)
    e. BacHum (Kildare et al. 2007)
        i. TGAGTTCACATGTCCGCATGA
        ii. CGTTACCCCGCCTACTATCTAATG
        iii. TCCGGTAGACGATGGGGATGCGTT
        iv. 6-FAM™/TCCGGTAGA /ZEN™/CGATGGGGATGCGTT /IB®FQ/

f. BacCow (Kildare et al. 2007)
    i. CCAACYTTCCCGWTACTC
    ii. GGACCGTGTCTCAGTTCCAGTG
    iii. 6-FAM™/TAGGGGTTC /ZEN™/TGAGAGGAAGGTCCCCC/IB®FQ/

4) Standards: Genomic DNA from the American Type Culture Collection or IDT gBlocks synthetics:
    a. Entero1a: *Enterococcus faecalis* strain V583 (ATCC® 700802D-5™)
    b. EC23S857: *Escherichia coli* strain Crooks (ATCC® 8739D-5™)
    c. GenBac3: *Bacteroides thetaiotamicron* Strain VPI 5482 [ATCC® 29148™]
    d. BacHum and HF183: IDT gBlocks dsDNA sequence AB242142.1 (Green et al. 2014). 16S rRNA gene sequence for the type strain of *Bacteroides dorei* Strain DSM 17855.
    e. BacCow: IDT gBlocks dsDNA sequence AF233400.1 (Bernhardt et al. 2000, Layton et al. 2009) – 16S rRNA gene sequence for uncultured clone CF123.

Quality Control

1) Method Blanks: A volume of 800 mL autoclaved deionized (Milli-Q) sterile water is filtered on every sampling date (4-8 samples) & filter and DNA extraction proceeds as for samples.
2) Positive and Negative Controls: Every day that samples are analyzed, or when reagents are changed,both control cultures are run for each assay (20,000 copies) to check for both positive and negative results for the target and non-target assay respectively.
3) No Template Controls (NTCs): Every day that samples are analyzed, on every plate, three wells are devoted to NTCs consisting of DNA elution buffer (Tris-EDTA).
4) DNA Standards and Standard Curves: Extracted genomic DNA or gBlocks synthetic DNA (see above) is quantitated (see below) and. Calculations are used to estimate copy number (see below). A composite standard dilution series is run in triplicate on each assay plate (see below) and analyzed using least squares log-linear regressions predicting Ct from Standard Quantity (Copies per Well). These regressions are standard curve equations to calculate Quantity from Ct for Samples and Controls.

Sample Analysis

1) DNA Extractions – Follow the MoBio Kit Directions with filter in bead tube: Elute 100 uL
2) Standard Dilution Series – See Below
3) qPCR Assays:
    a. Dilute working stocks of Standards and Control Samples to target correct copies per well in 5 uL volumes.
    b. Dilute Samples 1:5 to reduce inhibition; thus 5uL of Diluted = 1 uL sample per well
    c. Prepare qPCR Master Mix as follows for each sample (plus 10% extra for pipet error)
        i. 10uL of 5Prime RealMasterMix Probe (2.5X, without ROX), 0.25uL BSA 100X stock for 0.1 mg/mL final, 0.05uL Probe and 0.10 uL Primer (both 100 uM stock) for 200/400 nM final, 10uL Water. Multiply everything 100X for a full 96-well plate.
    d. Prepare assay plate 20 uL Master Mix per well for the following 96 well layout:
        i. Single wells for each of 64 samples or Triplicate wells for each of 21 samples (including method blanks) – 64 or 63 wells, respectively

ii. Triplicate wells for each 8-position standard dilution series – 24 wells
iii. Triplicate wells for NTCs, Positive Controls, Negative Controls – 8 wells

e. Aliquot Samples, Standards, and Controls 5 uL each to wells
f. Cap and centrifuge plate 1000 RPM for 1 min, check for bubbles
g. Set up Run Details with FAM Detection, ROX Background (depending on machine used), Auto Baseline, Ct Threshold = 0.03 or 300, depending on machine
h. Run Reactions 2 min 95°C followed by 45 cycles of 15s 95°C and 30s 60°C

4) Data analysis and calculation of sample copy numbers from standards.
   a. Standard curves yield gene copies per 1 uL of sample analyzed
   b. 1 uL sample analyzed is 1% of total sample collected if using a 100 uL elution.
   c. sample volume filtered (e.g. 800 mL) = 8 mL sample per 1 uL DNA analyzed
   d. Data are reported and calculated as Copies/100 mL = Quantity/8 mL

Standard Dilution and Preparation

1) Standards are purchased at a nominal amount of 5000 ng (typically more)
2) Genomic Standards are converted to gene copies using the following conversion factors:
   6.02E23 bp mol^{-1} / 660 g mol^{-1} = 9.12E11 bp ng^{-1} * ng purchased = total bp
   bp / bp $genome^{-1}$ = genomes * rRNA genes $genome^{-1}$ = total rRNA genes purchased
   Enterococcus faecalis V583: 3,359,974 bp genome with 4 copies of 23S gene
   Escherichia coli 8739: 4,746,218 bp genome with 7 copies of 23S gene
3) Standards are diluted with Tris-EDTA (TE) 750 uL – Primary Stock
4) Primary Stock is quantitated with PicoGreen on Invitrogen Qubit system
   a. Final concentrations typically 5-20 ng/uL, 10-20 million copies/uL
5) Standard Stock Solutions are aliquotted from the Primary Stock as follows:
   a. Master Stock is prepped at 1 million copies/uL (~5-10%) (1m storage)
   b. Working Stock is prepped at 10,000 copies/uL (1:100) (destroy after thaw)
   c. Dilution series are prepped by serial dilution planning for 15 uL per well. This is then aliquotted across three wells of the plate for a final of 5 uL per well in triplicate.
      i. 50,000 copies (15uL Working Stock - WS)
      ii. 10,000 copies (3uL WS + 12uL water)
      iii. 5,000 copies (1:10 of row A)
      iv. 1,000 copies (1:10 of row B)
      v. 500 copies (1:10 of row C)
      vi. 100 copies (1:10 of row D)
      vii. 50 copies (1:10 of row E)
      viii. 10 copies (1:10 of row F)
      ix. This series is best accomplished as follows according to Rows
         A. 17 uL of WS, remove 1.7uL for Row C
         B. 3.4 uL of WS, add 13.6 water, remove 1.7ul for Row D
         C. 15.3uL of water, add 1.7uL Row A, remove 1.7uL for Row E
         D. 15.3uL of water, add 1.7uL Row B, remove 1.7uL for Row F
         E. 15.3uL of water, add 1.7uL Row C, remove 1.7uL for Row G

F. 15.3uL of water, add 1.7uL Row D, remove 1.7uL for Row H
G. 15.3uL of water, add 1.7uL Row E
H. 15.3uL of water, add 1.7uL Row F

References

Bernhard, Anne E., and Katharine G. Field. "Identification of Nonpoint Sources of Fecal Pollution in Coastal Waters by Using Host-Specific 16S Ribosomal DNA Genetic Markers from Fecal Anaerobes." Applied and Environmental Microbiology 66, no. 4 (April 1, 2000): 1587–94. doi:10.1128/AEM.66.4.1587-1594.2000.

Chern, E. C., S. Siefring, J. Paar, M. Doolittle, and R. A. Haugland. 2011. Comparison of quantitative PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. Letters in Applied Microbiology 52:298–306.

Dick, L. K., and K. G. Field. 2004. Rapid Estimation of Numbers of Fecal Bacteroidetes by Use of a Quantitative PCR Assay for 16S rRNA Genes. Appl. and Environmental Microbiology 70:5695–5697.

Green, H. C., Haugland, R. A., Varma, M., Millen, H. T., Borchardt, M. A., Field, K. G., … Shanks, O. C. (2014). Improved HF183 Quantitative Real-Time PCR Assay for Characterization of Human Fecal Pollution in Ambient Surface Water Samples. Applied and Environmental Microbiology, 80(10), 3086–3094. doi:10.1128/AEM.04137-13

Haugland, R. A., S. C. Siefring, L. J. Wymer, K. P. Brenner, and A. P. Dufour. 2005. Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Research 39:559–568.

Haugland, Richard A., Manju Varma, Mano Sivaganesan, Catherine Kelty, Lindsay Peed, and Orin C. Shanks. "Evaluation of Genetic Markers from the 16S rRNA Gene V2 Region for Use in Quantitative Detection of Selected Bacteroidales Species and Human Fecal Waste by qPCR." *Systematic and Applied Microbiology* 33, no. 6 (October 2010): 348–57. doi:10.1016/j.syapm.2010.06.001.

Kildare, B. J., C. M. Leutenegger, B. S. McSwain, D. G. Bambic, V. B. Rajal, and S. Wuertz. 2007. 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. Water Research 41:3701–3715.

Layton, Alice, Larry McKay, Dan Williams, Victoria Garrett, Randall Gentry, and Gary Sayler. "Development of Bacteroides 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water." Applied and Environmental Microbiology 72, no. 6 (June 1, 2006): 4214–24. doi:10.1128/AEM.01036-05.

Ludwig, W., and K. H. Schleifer. 2000. How quantitative is quantitative PCR with respect to cell counts? Systematic and applied microbiology 23:556–562.

Seurinck, S., T. Defoirdt, W. Verstraete, and S. D. Siciliano. 2005. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environmental Microbiology 7:249–259.

Siefring, S., M. Varma, E. Atikovic, L. Wymer, and R. A. Haugland. 2008. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. Journal of Water and Health 06:225.

Appendix D.

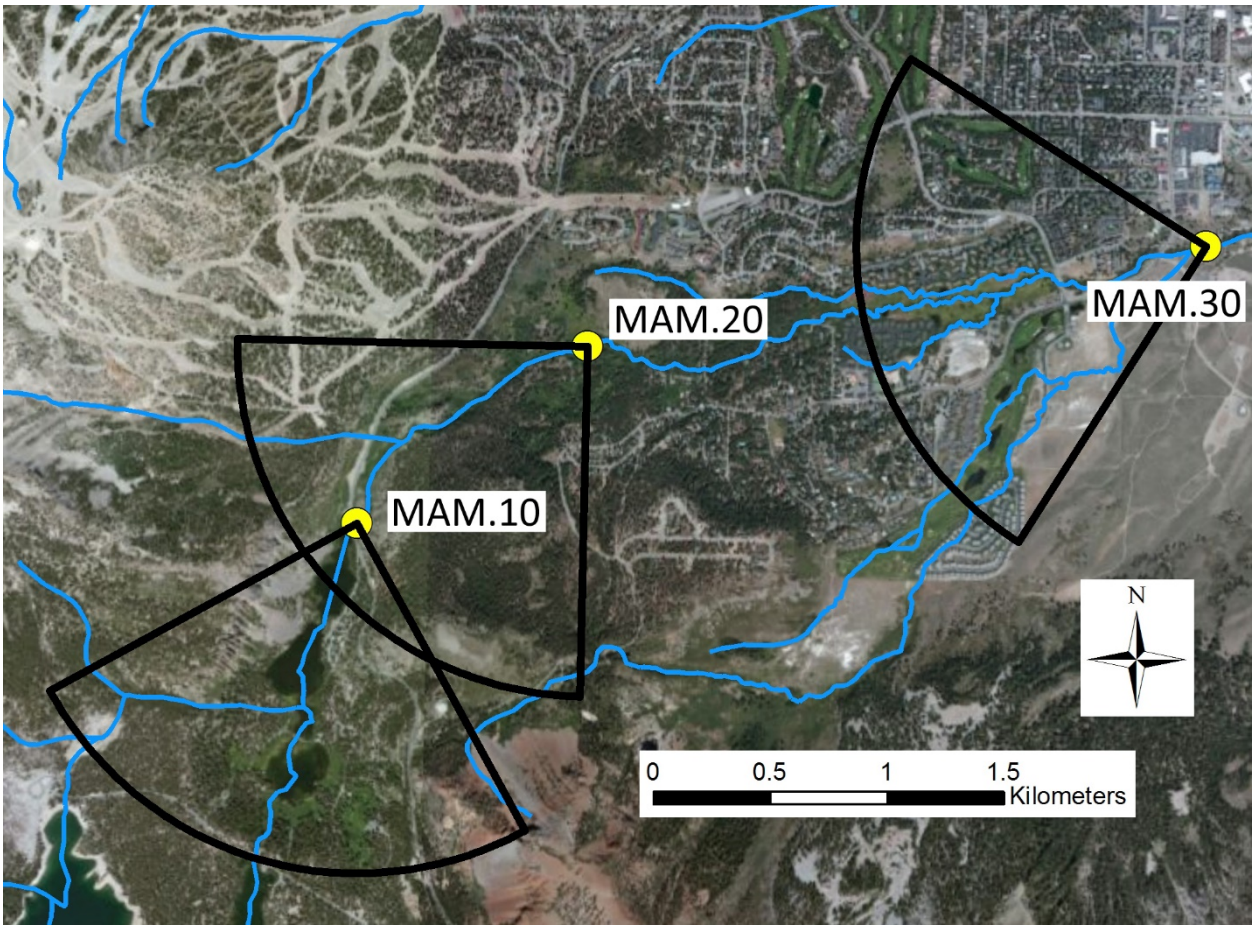

Image from the Geographic Information System used in this study, showing sectors associated with three sampling sites (yellow circles) on Mammoth Creek (MAM.10, MAM.20, MAM.30). Each sector has a 1.5 km radius, an angle of 90°, and is oriented upstream. The sectors for MAM.10 and MAM.20 include mostly undeveloped national forest lands, and the sector for MAM.30 includes the highly developed areas associated with the town of Mammoth Lakes. Streams are indicated with blue lines.

Appendix E. Details related to the dataset of samples collected and analyzed by Lahontan personnel and used by SNARL to identify significant predictors of fecal coliform bacteria concentrations.

Six Excel files were provided by Lahontan staff for use in this analysis. As shown in the table below, each workbook contained six to ten worksheets of FC results for each calendar year or quarter of a year. All workbooks contained FC data for the period 2009-2014 except R6Ecoli which contained FC data for 2009 only; the remaining worksheets contained only *E. coli* data. The Markleeville workbook also included worksheets that contained notes regarding the livestock data, and all workbooks included spatial data on the sampling locations (i.e., SITEID).

File name	Abbreviation	Total number of records
ESB_2011_2014_Final.xlsx	ESB	830
Markleeville_2009_2014_final.xlsx	Markleeville	2364
R6_E.coli_2009-2014_Roland.xlsx	R6Ecoli	321
R6_Fecal_2009-2014_Roland.xlsx	R6Fecal	2639
R6_SWAMP_Fecal_2009-2014.final.xlsx	R6Swamp	2352
Swamp_2009_2014-Final.xlsx	Swamp	420

Each individual worksheet of FC results (46 in total) was exported from Excel to a comma separated value (csv) file for import into R (version 3.2.2; R Core Development Team 2015), where all data manipulation took place. Each set of worksheets from the six workbooks was merged into a unique data frame and a field was added to identify the source workbook. In many cases, the field names were inconsistent within an individual worksheets and had to be renamed before merging the worksheets. For example, DilutionFactor was sometimes DilFactor, and UnitName was sometimes Unit.

Once all 46 worksheets were merged into a single data frame, numerous duplicate FC results were identified. The following process was used to remove duplicate records. First, all data with lab sample identification codes (i.e., unique codes that identified each unique sample) that ended in “D”, indicating duplicates, were removed. Next, all lab sample identification codes that were labeled “Lab Blank” were also removed. Finally, all results with identical lab sample identification codes and identical SITEID were removed. In situations where all data were identical except for the livestock variables, we manually selected the duplicate with more complete livestock data. Finally, data with station codes labeled as 000NONPJ were removed because these were non-project quality control samples.

After this process, several lab sample identification codes (13ESB378, 14ESB034, 14RB6179, 14RB6185, 14RB6191, 14RB6198, 001BT694, 090RB125, 090RB222) were found to be duplicates, but were associated with different SITEIDs. We found that despite having duplicate lab sample identification codes, these samples were unique records and the lab sample identification codes were modified (e.g. 13ESB378 became 13ESB378_1 or 13ESB378_2) to ensure that each sample in the data set had a unique lab sample identification code.

Livestock data was recorded using a different methodology in the ESB, Markleeville, R6Ecoli, and SWAMP workbooks versus the R6Fecal and R6Swamp workbooks. In the former four workbooks, there were two columns of livestock data recorded for each site. First, a “Livestock” variable recorded as Yes, No, or NR (not recorded) indicated whether livestock (cows, horse, goats, or sheep) were observed during sample collection. Second, an “Upstream” influence variable was used to describe the influence of livestock in the region upstream of the sampling site. In contrast, in the R6Ecoli and SWAMP workbooks, a single livestock variable (“Presence of upstream source”) was recorded and included a variety of data and notes regarding livestock presence. In order to combine the workbooks with different livestock data collection methodologies,

we created a new field named STOCK. STOCK was coded “Yes” whenever the “Livestock” variable was coded “Yes”, and the “Upstream” or “Presence of upstream source” field included the word cattle, horse, goats, or sheep.

Additional revisions were made to the STOCK variable after the workbooks were combined. Livestock results were reclassified based primarily on capitalization differences so there were only the three categories of Yes, No and NR. We also modified the STOCK variable from “Yes” to “No” in situations where the upstream variable noted 'manure' or 'poop' since old fecal matter is not expected to have an influence on FC results. Due to the high number of FC results without any associated livestock data recorded in the field, we improved the consistency of the STOCK variable by incorporating knowledge by Lahontan staff of sites that never contained livestock. These revisions resulted in a change in the value of the STOCK variable from “NA” to “No” at 70 stations.

Several other fields required revision before the data base was finalized. Missing and potentially incorrect spatial location data (latitude, longitude) were revised based on input from Lahontan staff. The date field had inconsistent formatting between worksheets and had to be revised to a single, consistent format. A DATE variable was created from this revised date field and represented the sample collection date as the number of days since January 1. From this revised field, we also created a continuous TIME variable to describe when the sample was collected (number of minutes instead of hours:minutes). Negative FC concentrations in the data base were all changed to zero except concentration = “-88” which indicates that the results were estimated. All such records were removed from the data base. We also used the comment field (“LabResultComments”) to flag other estimated FC results for removal. Finally, we removed all records for which any field were blank. This reduced the data set from 161 unique station codes and 4,404 unique lab samples to 130 unique station codes and 3,383 unique lab samples.

Appendix F. Sites from which samples were collected and analyzed for FIB by Lahontan personnel. Results were used by CESAME personnel to identify the significant predictors of fecal coliform bacteria concentrations[1].

Station Code	Station Name	Latitude	Longitude	HUC code[2]	HUC Name	FC100[3]	# of samples
603BSP006	South Fork Bishop Creek at Spruce St	37.3678	-118.3906	18090102	Crowley Lake	670	1
603BSP005	South Fork Bishop Creek at Hanby St	37.3678	-118.3885	18090102	Crowley Lake	122.7	29
603BSP008	South Fork Bishop Creek at Creekside Inn	37.3673	-118.3958	18090102	Crowley Lake	112.3	23
603BSP004	South Fork Bishop Creek above Bishop Creek Canal	37.3679	-118.3863	18090102	Crowley Lake	110.5	34
603BSP021	North Fork Bishop Creek above Bishop Creek Canal	37.3801	-118.4047	18090102	Crowley Lake	68.4	20
603BSP010	South Fork Bishop Creek at Home St	37.3689	-118.4022	18090102	Crowley Lake	51.5	46
603BSP002	Bishop Creek Canal at East Line St	37.3616	-118.3861	18090102	Crowley Lake	29.3	29
603BSP003	Bishop Creek Canal above South Fork Bishop Creek	37.3679	-118.3862	18090102	Crowley Lake	21.8	43
603LOW011	Lower Owens River at Warm Springs Rd	37.3253	-118.3137	18090102	Crowley Lake	9.9	4
603MAM006	Mammoth Creek, at Hwy 395	37.6380	-118.9077	18090102	Crowley Lake	6.3	24
603HIL001	Hilton Creek, at Lake Crowley	37.5795	-118.7415	18090102	Crowley Lake	5.9	21
603MAM013	Mammoth Creek above confluence with Hot Creek	37.6434	-118.8534	18090102	Crowley Lake	5.7	25
603MAM014	Mammoth Creek above Horsecamp	37.6348	-118.9676	18090102	Crowley Lake	3.5	34
603MAM003	Mammoth Creek, Horsecamp	37.6339	-118.9595	18090102	Crowley Lake	2.8	19
603BSP111	Bishop Creek at National Forest Boundary	37.3303	-118.4958	18090102	Crowley Lake	1.4	16
603RCK002	Rock Creek, above diversion	37.5498	-118.6867	18090102	Crowley Lake	1.2	29
630SWA005	Swauger Creek, below Huntoon Valley	38.2959	-119.3097	16050301	East Walker	62.3	67
630EWK006	East Walker River, at HWY 395	38.2553	-119.2238	16050301	East Walker	35.9	27
630RBS008	So. Branch Robinson Creek, upstream bridge	38.2585	-119.2723	16050301	East Walker	25.9	24
630BUC004	No. Branch Buckeye Creek, upstream bridge	38.2637	-119.2773	16050301	East Walker	15.7	26
630BUC005	Mid Branch Buckeye Creek, upstream bridge	38.2622	-119.2758	16050301	East Walker	13.7	24
630RBS007	No. Branch Robinson Creek, upstream bridge	38.2597	-119.2735	16050301	East Walker	12.1	27
630SWA006	Swauger Creek, above Huntoon Valley	38.3428	-119.3231	16050301	East Walker	11.6	46
630VIR005	Virginia Creek, above Willow Springs	38.1794	-119.1963	16050301	East Walker	10.6	34
630VIR004	Virginia Creek, below Willow Springs (at USGS gage)	38.1919	-119.2092	16050301	East Walker	10	41
630EWK001	East Walker River, at CA/NV state line	38.4140	-119.1657	16050301	East Walker	2.8	22
637SUSB01	Susan River @ Chappuis Lane	40.3857	-120.4519	18080003	Honey-Eagle Lakes	131.7	13

Station Code	Station Name	Latitude	Longitude	HUC code[2]	HUC Name	FC100[3]	# of samples
637SUSB04	Susan River @ Hwy 36	40.4028	-120.6312	18080003	Honey-Eagle Lakes	31.7	10
637SUS001	Susan River, nr Litchfield	40.3790	-120.3981	18080003	Honey-Eagle Lakes	29.9	32
637BRKB02	Brockman Slough @ Center Road	40.3966	-120.5863	18080003	Honey-Eagle Lakes	26.8	12
637LNG002	Long Valley Creek, upstream	39.9310	-120.0198	18080003	Honey-Eagle Lakes	23	14
637SUSB03	Susan River @ Johnsonville Road	40.3889	-120.5876	18080003	Honey-Eagle Lakes	18.4	7
637SUS002	Susan River at Lassen St	40.4137	-120.6648	18080003	Honey-Eagle Lakes	11.6	24
637SUSB02	Susan River @ Leavitt Lane	40.3793	-120.5214	18080003	Honey-Eagle Lakes	8.3	11
637SUS003	Susan River, above confluence w/ Willard Creek	40.3961	-120.7808	18080003	Honey-Eagle Lakes	3.5	4
634GETB01	East Tributary of Griff Creek above the confluence	39.2458	-120.0291	16050101	Lake Tahoe	5120	1
634GRFB10	Griff Creek above SR 28	39.2380	-120.0303	16050101	Lake Tahoe	159.1	9
634GRFB01	Griff Creek at Lake Tahoe	39.2370	-120.0306	16050101	Lake Tahoe	128.1	7
634GRFB80	Griff Creek above Gasline Road	39.2626	-120.0294	16050101	Lake Tahoe	43.2	6
634UTRB01	Upper Truckee River, at Lake Tahoe	38.9413	-120.0012	16050101	Lake Tahoe	42	1
634GETB80	East Tributary of Griff Creek above Beaver Street	39.2583	-120.0124	16050101	Lake Tahoe	41.2	5
634GRFB40	West Fork of Griff Creek above the confluence	39.2457	-120.0294	16050101	Lake Tahoe	37.4	6
634GRFB50	Griff Creek above Cambridge Street	39.2492	-120.0309	16050101	Lake Tahoe	28	5
634GRFB60	Griff Creek above Canterbury Street	39.2535	-120.0306	16050101	Lake Tahoe	26.6	5
634TRTB02	Trout Creek confluence South Upper Truckee	38.9416	-119.9960	16050101	Lake Tahoe	15.3	157
634TRTB03	Trout Creek at Highway 50	38.9320	-119.9792	16050101	Lake Tahoe	9.8	184
634TALB01	Tallac Creek at Baldwin Beach	38.9432	-120.0690	16050101	Lake Tahoe	7.3	177
634DOLB10	Dollar Creek above SR 28	39.1983	-120.0984	16050101	Lake Tahoe	7.2	2
634HWCB10	Homewood Canyon Creek above Hwy 89	39.0803	-120.1575	16050101	Lake Tahoe	6.8	6
634UTR009	Upper Truckee River, at Venice Dr	38.9348	-120.0004	16050101	Lake Tahoe	6.1	22
634UTRB40	Upper Truckee River, at Grass Lake Rd.	38.8138	-120.0172	16050101	Lake Tahoe	4.6	20
634UTRB30	Upper Truckee River, at Hwy 50 Meyers	38.8486	-120.0268	16050101	Lake Tahoe	4.5	22
634TAYB10	Taylor Creek above Hwy 89	38.9332	-120.0558	16050101	Lake Tahoe	4.4	2
634TALB10	Tallac Creek abov Hwy 89	38.9351	-120.0786	16050101	Lake Tahoe	4	2
634UTRB10	Upper Truckee River, at River Dr.	38.9223	-119.9905	16050101	Lake Tahoe	4	24
634UTRB50	Upper Truckee River, at bridge Hawley Grade	38.7963	-120.0192	16050101	Lake Tahoe	3.9	22

Station Code	Station Name	Latitude	Longitude	HUC code[2]	HUC Name	FC100[3]	# of samples
634MKNB20	McKinney Creek above McKinney Creek Road	39.0658	-120.1468	16050101	Lake Tahoe	3.5	2
634MKSB10	Meeks Creek above Hwy 89	39.0359	-120.1257	16050101	Lake Tahoe	3.5	3
634TALB03	Tallac Creek at Highway 89	38.9350	-120.0783	16050101	Lake Tahoe	3.5	182
634PRDB10	Paradise Flat above Hwy 89	39.0026	-120.1130	16050101	Lake Tahoe	3.4	3
634EAGB10	Eagle Creek above Hwy 89	38.9514	-120.1120	16050101	Lake Tahoe	3.3	2
634UTRB20	Upper Truckee River, at Elks Club Dr.	38.8751	-120.0056	16050101	Lake Tahoe	3.3	22
634WRDB10	Ward Creek above Hwy 89	39.1326	-120.1578	16050101	Lake Tahoe	3.1	3
634GENB01	General Creek, at Lake Tahoe	39.0551	-120.1132	16050101	Lake Tahoe	3	10
634GENB10	General Creek, above Hwy 89	39.0518	-120.1180	16050101	Lake Tahoe	2.8	12
634GENB20	General Creek, above campground	39.0499	-120.1356	16050101	Lake Tahoe	2.5	10
634UTRB60	Upper Truckee River, above swim hole	38.7854	-120.0247	16050101	Lake Tahoe	2.5	21
634UTRB80	Upper Truckee River, Meiss Meadow, upper	38.7192	-120.0114	16050101	Lake Tahoe	2.1	5
634BLKB05	Blackwood Creek below Hwy 89	39.1074	-120.1610	16050101	Lake Tahoe	2	2
634CASB10	Cascade Creek above Hwy 89	38.9495	-120.0845	16050101	Lake Tahoe	2	1
634MADB10	Madden Creek above Hwy 89	39.0907	-120.1628	16050101	Lake Tahoe	2	2
634TRTB10	Trout Creek above Hwy 50	38.9317	-119.9786	16050101	Lake Tahoe	1.7	2
634RCNB10	North Fork Rubicon Creek upstream above Hwy 89	38.9911	-120.1107	16050101	Lake Tahoe	1.4	2
635TRK099	Truckee River, below dam	39.1666	-120.1446	16050101	Lake Tahoe	1.4	10
634GENB40	General Creek, above Lily Pond	39.0308	-120.1597	16050101	Lake Tahoe	1.3	10
634BARB10	Barton Creek above SR 28	39.1858	-120.1211	16050101	Lake Tahoe	1	2
634BURB10	Burton Creek above SR 28	39.1853	-120.1216	16050101	Lake Tahoe	1	1
634GENB30	General Creek, above loop road	39.0430	-120.1491	16050101	Lake Tahoe	1	10
634LONB10	Lonely Gulch above Hwy 89	39.0147	-120.1237	16050101	Lake Tahoe	1	2
634QUAB10	Quail Creek above Hwy 89	39.0764	-120.1524	16050101	Lake Tahoe	1	1
634RCNB20	North Fork Rubicon Creek upstream above Hwy 89	38.9961	-120.1097	16050101	Lake Tahoe	1	2
634RCSB10	South fork Ribicon Creek above Hwy 89	38.9777	-120.1033	16050101	Lake Tahoe	1	1
634UTRB70	Upper Truckee River, Meiss Meadow, lower	38.7282	-120.0190	16050101	Lake Tahoe	1	3
634WATB10	Watson Creek above SR 28	39.2185	-120.0873	16050101	Lake Tahoe	1	1
603LPC001	Lone Pine Creek, at USGS gage	36.6012	-118.0823	18090103	Owens Lake	4	18

Station Code	Station Name	Latitude	Longitude	HUC code[2]	HUC Name	FC100[3]	# of samples
603LPC002	Lone Pine Creek, at Whitney Portal	36.5897	-118.2272	18090103	Owens Lake	2.7	9
641CDR002	Cedar Creek, above Cedarville	41.5303	-120.1875	18080001	Surprise Valley	7.5	10
641BID001	Bidwell Creek, below Mill Creek nr Fort Bidwell	41.8825	-120.1744	18080001	Surprise Valley	4	10
641MIL002	Mill Creek, above Lake City	41.6408	-120.2190	18080001	Surprise Valley	3.5	12
641MIL001	Mill Creek, below Lake City	41.6455	-120.2124	18080001	Surprise Valley	3	6
636LTRB30	Little Truckee Below Stampede Dam	39.4689	-120.1038	16050102	Truckee	35	1
635DONB01	Donner Creek, above Truckee River	39.3164	-120.2007	16050102	Truckee	5.7	10
636LTRB70	Little Truckee Above Independence Creek	39.4913	-120.2949	16050102	Truckee	3	1
635SQLB01	Squaw Creek, above Truckee River	39.2115	-120.1996	16050102	Truckee	2.1	15
635TRK002	Truckee River, above Farad	39.4226	-120.0339	16050102	Truckee	2	15
635TRKB50	Truckee River, above River Ranch	39.1730	-120.1891	16050102	Truckee	1.7	11
635TRKB30	Truckee River, above Squaw Creek	39.2119	-120.1990	16050102	Truckee	1.4	10
635TRKB10	Truckee River, above TTSA	39.3381	-120.1332	16050102	Truckee	1.3	3
635TRKB20	Truckee River, below Town of Truckee	39.3327	-120.1629	16050102	Truckee	1.3	8
635BER001	Bear Creek, lower (moraine)	39.1900	-120.1983	16050102	Truckee	1.2	13
635TRKB40	Truckee River, above Bear Creek	39.1900	-120.1975	16050102	Truckee	1.2	10
632WLFB10	Wolf Creek, Below Ranch	38.6007	-119.6889	16050201	Upper Carson	38.2	10
632MRKB03	Markleeville Creek at Swim Hole	38.6938	-119.7795	16050201	Upper Carson	35.4	61
632MLBB01	Confluence Millberry Creek with Markleeville Creek	38.6950	-119.7785	16050201	Upper Carson	34	86
632MLBB02	Millberry Creek behind Post Office	38.6954	-119.7795	16050201	Upper Carson	32.2	45
632MRKB02	Markeeville Creek at USFS Campground	38.6965	-119.7740	16050201	Upper Carson	30.3	95
633WFCB02	West Fork Carson River at Paynesville Bridge	38.8089	-119.7771	16050201	Upper Carson	30	113
632MRKB04	Markleeville Creek at Library Bridge	38.6933	-119.7818	16050201	Upper Carson	28.9	90
632MLBB03	Millberry Creek at 30 mph Sign	38.6969	-119.7818	16050201	Upper Carson	28.7	5
633WFCB30	West Fork Carson River, above Forestdale Creek	38.6743	-119.9379	16050201	Upper Carson	27	1
632WLFB01	Wolf Creek, above East Fork Carson River	38.6137	-119.6924	16050201	Upper Carson	16.5	5
632PLVB04	Pleasant Valley Creek	38.6698	-119.8013	16050201	Upper Carson	11.6	20
632ECR005	East Fork Carson River, at USGS gage below Markleeville	38.7157	-119.7631	16050201	Upper Carson	10.9	20
632WLFB20	Wolf Creek above the ranch	38.5773	-119.6962	16050201	Upper Carson	6.3	5

Station Code	Station Name	Latitude	Longitude	HUC code[2]	HUC Name	FC100[3]	# of samples
632DTCB01	The town ditch above Millberry Creek	38.6994	-119.7843	16050201	Upper Carson	6	1
632HSPB05	Hotsprings Creek at Hotsprings Creek Road	38.6985	-119.8258	16050201	Upper Carson	3.9	144
632ECRB10	East Fork Carson River, above Hangman's bridge	38.6896	-119.7639	16050201	Upper Carson	3.8	6
633WFCB03	West Fork Carson River at Woodford's Bridge	38.7750	-119.8230	16050201	Upper Carson	2.8	85
632HSPB06	Hotsprings Creek above Grover Hotsprings Campground	38.6978	-119.8380	16050201	Upper Carson	2.7	146
632MLBB04	Millberry Creek above house	38.7066	-119.7907	16050201	Upper Carson	2.3	48
633WCR004	West Fork Carson River, at HWY 89 (Hope Valley)	38.7782	-119.9169	16050201	Upper Carson	2	1
633WFCB04	West Fork Carson River at Pickett's Bridge	38.7783	-119.9188	16050201	Upper Carson	2	53
633WCR002	West Fork Carson River, below Willow Creek	38.7781	-119.9161	16050201	Upper Carson	1.8	38
632ECRB40	East Fork Carson River, above Wolf Creek	38.6140	-119.6921	16050201	Upper Carson	1.7	3
631HOT001	Hot Creek above confluence with Little Walker River	38.3421	-119.4507	16050302	West Walker	42.8	51
631LWK004	Little Walker River above confluence with Hot Creek	38.3417	-119.4509	16050302	West Walker	17.6	53
631LWK003	Little Walker River above confluence with West Walker R.	38.3793	-119.4507	16050302	West Walker	9.4	8
631WWK008	West Walker River at Topaz	38.6105	-119.5176	16050302	West Walker	8.9	62
631WWK007	West Walker River above confluence with Little Walker R.	38.3793	-119.4511	16050302	West Walker	1.9	19
631WWK001	West Walker River, at Coleville	38.5134	-119.4488	16050302	West Walker	1.3	53
631WWK010	West Walker River above Pack Station	38.3232	-119.5487	16050302	West Walker	1.1	20

[1] Records are sorted by the hydrologic unit code (HUC8 sub-basin), then by geometric mean from highest to lowest within each hydrologic unit.

[2] HUC 8 sub-basins (1:250,000-scale hydrologic units of the United States)

[3] Average fecal coliform concentration was calculated as geometric means across all samples from each site (cfu or mfu per 100 mL).

Appendix G. Microbial source tracking results obtained from 63 samples collected by Lahontan personnel. MST results are expressed as "copies per 100 mL".

			Membrane Filtration		Microbial Source Tracking					
StationCode	**SampleID**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
603BSP004	15RB6189	4/7/2015	250	150	600	318	50	ND	ND	ND
603BSP004	15RB6174	5/20/2015	330	330	300	3089	112	506	ND	ND
631WWK001	15ESB091	8/19/2015	200	200	300	ND	ND	418	ND	ND
632MLBB01	14BAC205	6/26/2014	35	30	750	50	ND	ND	ND	ND
632MLBB01	14BAC232	7/7/2014	106	106	550	650	ND	ND	1049	ND
632MLBB01	14BAC244	7/23/2014	48	44	600	32	ND	ND	ND	ND
632MLBB01	15BAC006	2/17/2015	159	159	600	13128	1584	ND	ND	ND
632MLBB01	15BAC025	2/25/2015	66	66	800	88	61	ND	ND	ND
632MLBB01	15BAC068	6/8/2015	5	5	450	44	ND	ND	ND	ND
632MLBB01	15BAC089	7/28/2015	284	284	400	19	ND	ND	ND	ND
632MLBB01	15BAC100	7/30/2015	200	180	600	298	231	ND	ND	ND
632MLBB01	15BAC112	8/11/2015	84	84	400	2172	318	ND	ND	ND
632MLBB01	15BAC120	8/18/2015	100	100	400	17	ND	ND	ND	ND
632MLBB03	15BAC072	6/8/2015	6	6	600	97	140	ND	ND	ND
632MLBB03	15BAC093	7/28/2015	TNC	TNC	400	ND	ND	ND	ND	ND
632MLBB03	15BAC105	7/30/2015	600	540	500	34	ND	ND	ND	ND
632MRKB02	14BAC204	6/26/2014	29	23	800	3413	19	153	ND	ND
632MRKB02	14BAC234	7/7/2014	46	46	800	3066	90	2450	ND	ND
632MRKB02	15BAC066	6/8/2015	82	82	600	1005	99	1383	ND	ND
632MRKB02	15BAC087	7/28/2015	188	152	600	46600	3395	18733	ND	ND
632MRKB03	14BAC233	7/7/2014	58	58	700	1038	34	948	ND	ND
632MRKB03	14BAC245	7/23/2014	1730	1730	600	30330	1398	15924	ND	ND
632MRKB03	15BAC067	6/8/2015	100	100	600	27635	2033	22188	ND	ND
632MRKB03	15BAC088	7/28/2015	65	65	450	53846	1342	16641	ND	ND
632MRKB04	14BAC235	7/7/2014	34	28	700	21738	346	12248	ND	ND
632MRKB04	15BAC069	6/8/2015	TNC	TNC	500	30216	2783	22028	ND	ND

			Membrane Filtration		Microbial Source Tracking					
StationCode	**SampleID**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
632MRKB04	15BAC090	7/28/2015	840	840	600	36274	3242	13721	ND	ND
632MRKB04	15BAC102	7/30/2015	470	380	600	9178	ND	14485	ND	ND
632MRKB04	15BAC121	8/18/2015	1800	136	400	83597	1520	144590	ND	ND
633WFCB02	14BAC203	6/26/2014	138	138	800	5674	437	5090	ND	ND
633WFCB02	14BAC231	7/7/2014	45	40	700	10326	77	1956	ND	ND
633WFCB02	15ESB064	6/2/2015	6	6	600	228	29	ND	ND	ND
633WFCB02	15RB6314	7/21/2015	21	21	400	2506	33	225	ND	ND
633WFCB02	15BAC086	7/28/2015	46	41	600	10218	ND	4521	ND	ND
633WFCB02	15BAC109	8/11/2015	94	76	500	ND	ND	ND	ND	ND
633WFCB03	15BAC094	7/28/2015	7	7	450	1514	33	ND	ND	ND
634GETB80	14ESB084	7/14/2014	45	45	800	295	ND	ND	ND	ND
634GRFB01	14ESB034	6/23/2014	100	100	750	16251	2543	1048	ND	ND
634GRFB01	14ESB055	7/1/2014	120	120	650	546	8	ND	ND	ND
634GRFB01	14ESB083	7/14/2014	270	270	650	7034	98	66	ND	ND
634GRFB10	14ESB035	6/23/2014	142	142	600	26664	4415	1284	ND	ND
634GRFB10	14ESB056	7/1/2014	150	138	700	1402	107	50	ND	ND
634GRFB10	14ESB085	7/14/2014	230	230	700	16094	189	70	ND	ND
634GRFB40	14ESB037	6/23/2014	96	96	800	1055	44	ND	ND	ND
634GRFB80	14ESB061	7/1/2014	25	25	400	2384	181	ND	ND	ND
634GRFB80	14ESB079	7/14/2014	52	52	750	3488	1034	ND	ND	ND
634MKSB10	14ESB231	10/3/2014	14	6	600	51882	69	269	ND	ND
634TALB01	15BAC076	6/23/2015	82	82	200	2951	208	ND	ND	ND
634TALB01	15BAC083	6/29/2015	6	6	200	1665	ND	ND	ND	ND
634TRTB02	15BAC075	6/23/2015	106	100	55	93209	552	7362	ND	ND
634TRTB02	15BAC078	6/24/2015	232	196	200	8039	299	607	ND	ND
634TRTB02	15BAC081	6/29/2015	140	124	300	2114	48	ND	ND	ND
634TRTB02	15BAC085	7/28/2015	24	15	200	2670	ND	ND	ND	ND
634TRTB02	15BAC095	7/30/2015	17	17	500	8331	126	849	ND	ND

			Membrane Filtration		Microbial Source Tracking					
StationCode	**SampleID**	**CollectDate**	**FC100**	**EC100**	**mLqPCR**	**GenBac100mL**	**Ecoli100mL**	**BacCow100mL**	**BacHum100mL**	**HF183100mL**
634TRTB02	15BAC107	8/11/2015	35	35	300	7888	24	186	ND	ND
634TRTB03	15BAC080	6/29/2015	144	122	300	10503	ND	ND	ND	ND
634TRTB03	15BAC084	7/28/2015	10	10	300	82	ND	ND	ND	ND
634TRTB10	15BAC073	6/17/2015	8	6	300	2238	ND	ND	ND	ND
634TRTB10	15BAC074	6/23/2015	19	17	300	1912	57	ND	ND	ND
634TRTB10	15BAC079	6/24/2015	27	25	200	4977	ND	ND	ND	ND
634TRTB10	15BAC082	6/29/2015	89	84	200	78293	679	234	ND	ND
637SUS001	15ESB071	7/27/2015	19	19	150	586	ND	ND	ND	ND
637SUS004	15ESB072	7/27/2015	12	12	300	2010	162	ND	ND	ND

Appendix H. Description of deliverables that were required under Contract 13-054-160.

3.1. List of water body segments and sample sites with GPS location coordinates

See Appendix A.

3.2. Log of qPCR samples received from Lahontan staff

See Appendix G.

3.3. List of 250 samples for qPCR analysis

See Appendix B and Appendix G. 273 CESAME-collected and 63 Lahontan-collected samples were selected for qPCR analysis (total = 336).

3.4. Submit membrane filtration data for not fewer than 400 samples and source tracking data for 250 samples

Data were submitted from 539 membrane filtration samples (collected from 43 sites on 12 streams), and 336 source tracking samples. Data were submitted to CEDEN on March 4, 2016. Digital files containing MST data were submitted to Contract Manager Mary Fiore-Wagner on March 5, 2016.

4.1. Submit draft Final Report.

Submitted to Contact Manager Mary Fiore-Wagner on March 24, 2016.

4.2. Submit draft Final Report.

Submitted to Contact Manager Mary Fiore-Wagner on April 25, 2016.