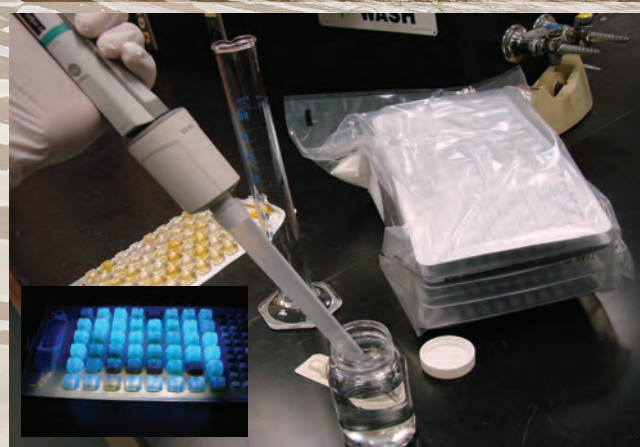
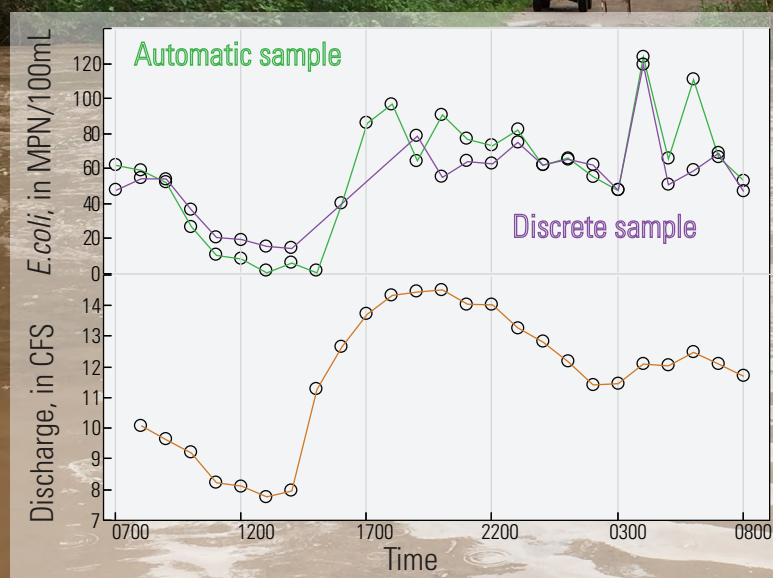


Prepared in cooperation with the National Park Service, Tumacácori National Historical Park

# Collection Methods and Quality Assessment for *Escherichia coli*, Water Quality, and Microbial Source Tracking Data within Tumacácori National Historical Park and the Upper Santa Cruz River, Arizona, 2015-16



Scientific Investigations Report 2017–5139



## COVER

Background photograph: Santa Cruz River at Santa Gertrudis Lane, Tumacácori National Historical Park southern boundary, Arizona. Photograph by Jay R. Cederberg, USGS.

Foreground graph, left: Time series plot of *Escherichia coli* concentrations, in most probable number per 100 milliliters and discharge, in cubic feet per second collected during a twenty-four hour sampling comparing collection methods.

Foreground photograph, right: Analytical preparation for the dilution of bacteria sample and analytical tray under fluorescent light showing cells positive for *Escherichia coli*. Photograph by Nicholas V. Paretti, USGS.

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By Nicholas V. Paretti, Alissa L. Coes, Christopher M. Kephart, and Justine P. Mayo

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Scientific Investigations Report 2017–5139

**U.S. Department of the Interior  
U.S. Geological Survey**

**U.S. Department of the Interior**

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## Contents

Abstract.....	1
Introduction.....	1
Purpose and Scope .....	3
Study Area.....	3
Methods .....	6
Friends of the Santa Cruz River and Tumacácori National Historical Park.....	6
International Boundary and Water Commission .....	6
Other Data Sources.....	7
U.S. Geological Survey.....	7
Field Methods.....	7
Analytical Methods .....	10
<i>Escherichia coli</i> .....	10
Microbial Source Tracking.....	13
Suspended Sediment .....	14
Data-Quality Assurance .....	14
Data-Quality Assessment.....	15
Bias.....	15
Variability.....	17
<i>Escherichia coli</i> .....	17
Additional Analytical Variability Analysis for <i>Escherichia coli</i> .....	20
Microbial Source Tracking.....	20
Suspended Sediment .....	20
Suspended-Sediment Sample Reference Samples.....	20
<i>Escherichia coli</i> Holding Time .....	23
Summary.....	27
References Cited.....	28

## Figures

1. Map of the study area including Tumacácori National Historical Park and the upper Santa Cruz River watershed.....	2
2. Photographs of sample collection using telescoping rod at Santa Cruz River at Tubac, AZ; automatic sampler at SC14; covered automatic sampler at SC14; and automatic sampler at Nogales Wash at Ruby Road .....	9
3. Chart showing relative standard deviation of <i>Escherichia coli</i> and suspended sediment irreplicate samples collected using a D-95, discrete, and automatic sampler. ....	10
4. Photographs of mini drive point transect along stream channel at Santa Cruz River at Santa Gertrudis Lane; peristaltic pump and mini drive point in wetted stream channel at SC10; mini drive point in dry stream channel at SC10; mini drive point on stream edge and within stream channel at Nogales Wash at Ruby Road; and pore water in dry channel bed at SC10.....	11
5. Photographs of pressure transducers deployed at Santa Cruz River near Nogales International Wastewater Treatment Plant; Nogales Wash at Ruby Road; Agua Fria Canyon; and, Sonoita Creek Canyon.....	12
6. Chart showing number of laboratory and field blank detections of <i>E. coli</i> and concentrations of field and environmental replicate paired concentrations from Tumacácori National Historical Park and the upper Santa Cruz River watershed.....	17
7. Discrete and automatic sample <i>Escherichia coli</i> concentrations from Tumacácori National Historical Park and the upper Santa Cruz River watershed plotted as boxplots; and as bivariate regression compared to a 1:1 linear fit.....	18
8. Discrete and automatic sample suspended-sediment concentrations from Tumacácori National Historical Park and the upper Santa Cruz River watershed plotted as A, box-plots; and as bivariate regression compared to a 1:1 linear fit.....	18
9. Plots of the logarithm of replicate standard deviation versus logarithm of mean replicate concentration for different replicate types: mean concentration of replicates versus standard deviation of replicate concentrations, in most probable number per 100 milliliters for samples from Tumacácori National Historical Park and the upper Santa Cruz River watershed .....	21
10. <i>Escherichia coli</i> concentrations plotted as a boxplot and as point data with confidence intervals from a discrete sampled multiple times from Santa Cruz River at Santa Gertrudis Lane; collection consisted of one clear surface water base flow; and one turbid pore water sample.....	22
11. Microbial source tracking replicate data from the Santa Cruz River at Tubac, Arizona, and the Santa Cruz River at Santa Gertrudis Lane presented as a bar graph with relative percent differences labeled for each source tracking marker analyzed.....	23
12. Cascade Volcano Observatory laboratory results from the branch of quality systems reference samples graphed as boxplots of the percent difference of the actual and reported substrate weights and concentration.....	24
13. Plots holding time quantile distribution plot colored by holding time category; boxplots of holding times grouped by the quantile distribution holding time quartile thresholds; <i>Escherichia coli</i> concentrations ranked and colored by holding time category; boxplots of <i>E. coli</i> concentrations grouped by holding time category. ....	25
14. <i>Escherichia coli</i> concentrations plotted as time series from base flow samples resampled over 5 days.....	26
15. <i>Escherichia coli</i> concentrations plotted as time series from flood samples resampled over 3 days.....	27

## Tables

1. Description of stations in the Tumacácori National Historical Park and the upper Santa Cruz River watershed study area.....	3
2. Standard curve characteristics and limits of detection and quantitation for microbial source tracking methods used in Tumacácori National Historical Park and the upper Santa Cruz River watershed.....	14
3. <i>Escherichia coli</i> by Colilert quality-control samples from IDEXX .....	16

## Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
Volume		
liter (L)	33.81402	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
cubic meter (m <sup>3</sup> )	264.2	gallon (gal)
liter (L)	61.02	cubic inch (in <sup>3</sup> )
cubic meter (m <sup>3</sup> )	35.31	cubic foot (ft <sup>3</sup> )
cubic meter (m <sup>3</sup> )	1.308	cubic yard (yd <sup>3</sup> )
Flow rate		
meter per second (m/s)	3.281	foot per second (ft/s)
cubic meter per second (m <sup>3</sup> /s)	35.31	cubic foot per second (ft <sup>3</sup> /s)
cubic meter per day (m <sup>3</sup> /d)	35.31	cubic foot per day (ft <sup>3</sup> /d)
liter per second (L/s)	15.85	gallon per minute (gal/min)
Mass		
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)



## International System of Units to U.S. customary units

<b>Multiply</b>	<b>By</b>	<b>To obtain</b>
<b>Length</b>		
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
<b>Volume</b>		
gallon (gal)	0.003785	cubic meter (m <sup>3</sup> )
gallon (gal)	3.785	cubic decimeter (dm <sup>3</sup> )
million gallons (Mgal)	3,785	cubic meter (m <sup>3</sup> )
cubic foot (ft <sup>3</sup> )	28.32	cubic decimeter (dm <sup>3</sup> )
cubic yard (yd <sup>3</sup> )	0.7646	cubic meter (m <sup>3</sup> )
cubic mile (mi <sup>3</sup> )	4.168	cubic kilometer (km <sup>3</sup> )
<b>Flow rate</b>		
foot per second (ft/s)	0.3048	meter per second (m/s)
cubic foot per second (ft <sup>3</sup> /s)	0.02832	cubic meter per second (m <sup>3</sup> /s)
gallon per minute (gal/min)	0.06309	liter per second (L/s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32.$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) / 1.8.$$

## Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

## Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter (µg/L). Escherichia coli concentrations are given in most probable number per 100 milliliters (MPN/100 mL)

## Abbreviations

ADEQ	Arizona Department of Environmental Quality
ALERT	Automated Local Evaluation in Real Time
AZPDES	Arizona Pollutant Discharge Elimination System
AZWSC	USGS Arizona Water Science Center
BQS	branch of quality systems
CI	confidence interval
CVO	USGS Cascades Volcano Observatory
DI	deionized
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EPA	U.S. Environmental Protection Agency
FBC	full body contact
FOSCR	Friends of the Santa Cruz River
IBWC	International Boundary and Water Commission
LoB	limit of blank
LoD	limit of detection
LoQ	limit of quantification
MF	membrane filtration
MPN	most probable number
MST	microbial source tracking
NIWTP	Nogales International Wastewater Treatment Plant
NIST	National Institute of Standards and Technology
NPS	National Park Service
NWIS	USGS National Water Information System
OWML	Ohio Water Microbiology Laboratory
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PD	percent difference
PMP	Polymethylpentene
psi	pounds per square inch
qPCR	quantitative polymerase chain reaction
SCAMA	Santa Cruz Active Management Area
SLEDS	Sediment Laboratory Environmental Data System
SRL	sample reporting limit
SSC	suspended sediment concentration
TUMA	Tumacácori National Historical Park
USGS	U.S. Geological Survey

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By Nicholas V. Paretti, Alissa L. Coes, Christopher M. Kephart, and Justine P. Mayo

## Abstract

Tumacácori National Historical Park protects the culturally important Mission, San José de Tumacácori, while also managing a portion of the ecologically diverse riparian corridor of the Santa Cruz River. This report describes the methods and quality assurance procedures used in the collection of water samples for the analysis of *Escherichia coli* (*E. coli*), microbial source tracking markers, suspended sediment, water-quality parameters, turbidity, and the data collection for discharge and stage; the process for data review and approval is also described. Finally, this report provides a quantitative assessment of the quality of the *E. coli*, microbial source tracking, and suspended sediment data.

The data-quality assessment revealed that bias attributed to field and laboratory contamination was minimal, with *E. coli* detections in only 3 out of 33 field blank samples analyzed. Concentrations in the field blanks were several orders of magnitude lower than environmental concentrations. The microbial source tracking (MST) field blank was below the detection limit for all MST markers analyzed. Laboratory blanks for *E. coli* at the USGS Arizona Water Science Center and laboratory blanks for MST markers at the USGS Ohio Water Microbiology Laboratory were all below the detection limit. Irreplicate data for *E. coli* and suspended sediment indicated that bias was not introduced to the data by combining samples collected using discrete sampling methods with samples collected using automatic sampling methods.

The split and sequential *E. coli* replicate data showed consistent analytical variability and a single equation was developed to explain the variability of *E. coli* concentrations. An additional analysis of analytical variability for *E. coli* indicated analytical variability around 18 percent relative standard deviation and no trend was observed in the concentration during the processing and analysis of multiple split-replicates. Two replicate samples were collected for MST and individual markers were compared for a base flow and flood sample. For the markers found in common between the two types

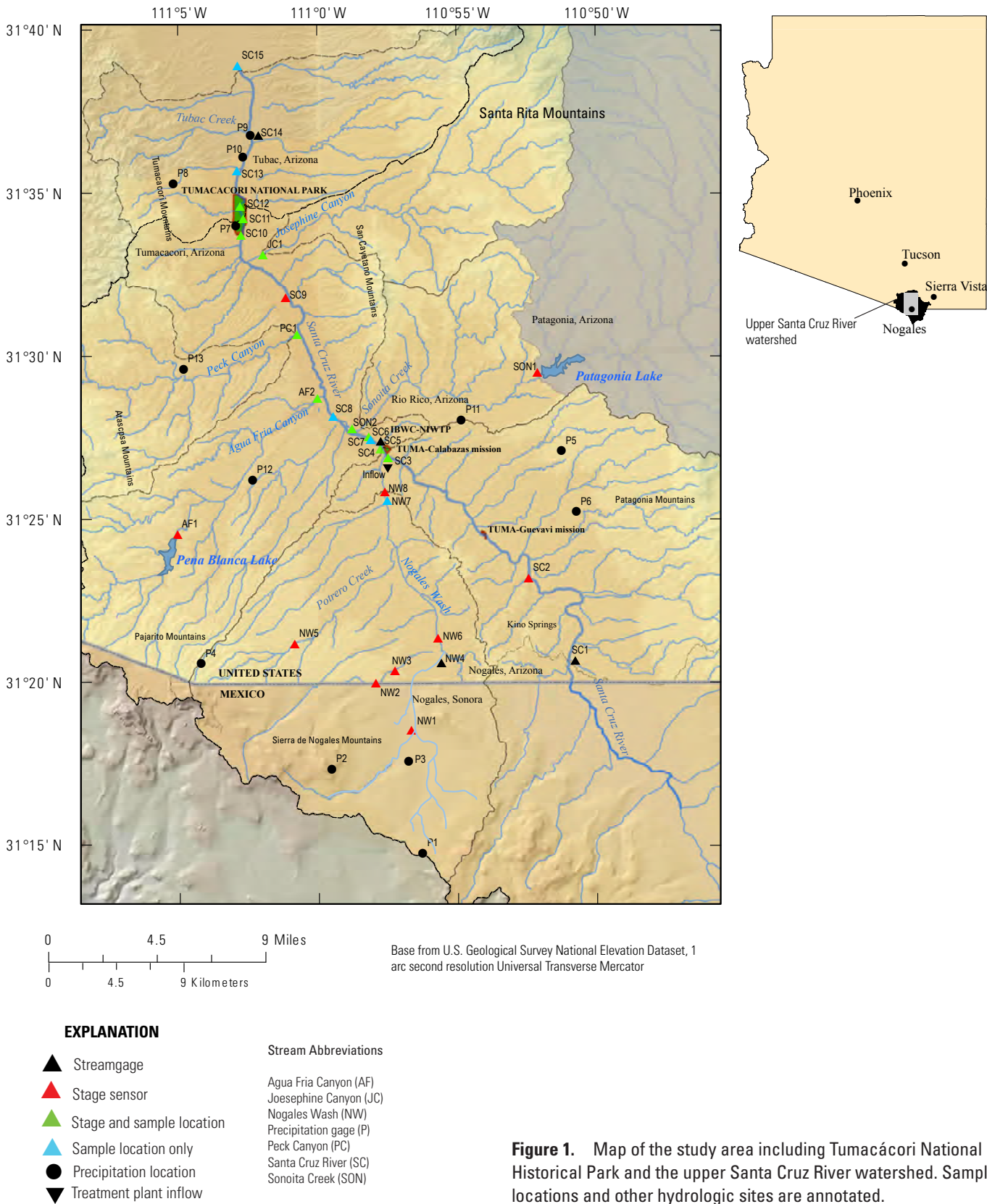
of samples, the relative standard deviation for the base flow sample was more than 3 times greater than the markers in the flood sample. Sequential suspended sediment replicates had a relative standard deviation of about 1.3 percent, indicating that environmental and analytical variability was minimal.

A holding time review and laboratory study analysis supported the extended holding times required for this investigation. Most concentrations for flood and base-flow samples were within the theoretical variability specified in the most probable number approach suggesting that extended hold times did not overly influence the final concentrations reported.

## Introduction

The region surrounding Tumacácori has a long and rich cultural history; it provided refuge and natural resources to the native O'odham culture and the Spanish missionaries exploring the Southwest in the seventeenth and eighteenth centuries. In 1691, Padre Eusebio Francisco Kino established the first missions in what is today Arizona, and later a National Monument was established in 1908 by President Theodore Roosevelt to protect Spanish and O'odham mission churches. In 1990, Congress created Tumacácori National Historical Park (TUMA), which included the original National Monument and the missions of Guevavi and Calabazas (fig. 1). In 2002, TUMA was expanded by 300 acres to include a 1-mile portion of the Santa Cruz River that protects a southwest cottonwood-willow riparian environment, which is one of the most endangered ecosystems in the United States (Noss and others, 1995; National Park Service, 2010, 2013). This riparian corridor provides an essential habitat for many plants, birds, and other animals that could not otherwise survive in the surrounding desert. TUMA views the Santa Cruz River as an important cultural and natural resource for the Park and as such is committed to understanding and maintaining a healthy river ecosystem.

2 Collection Methods and Quality Assessment within Tumacácori National Historical Park and the Upper Santa Cruz



TUMA is located approximately 10 miles downstream from the Nogales International Wastewater Treatment Plant (NIWTP), where perennial flow is established by the treated effluent discharged. The upstream hydrology presents challenges for TUMA in managing their water resources because the flow regime and water quality are determined by the NIWTP and upstream surrounding watershed activities. Several river reaches in the upper watershed, including the one that passes through TUMA, have been listed by the Arizona Department of Environmental Quality as impaired because of exceedances of *Escherichia coli* (*E. coli*) and other contaminants. As part of the U.S. Geological Survey (USGS)–National Park Service (NPS) Water-Quality Partnership, a three year investigation was conducted to provide TUMA with a better understanding of the spatial and temporal variability of *E. coli* concentrations and sources within and upstream of the TUMA. This investigation will provide much needed information to TUMA and the public, and support the development of best management practices in the region with the cooperation of local and state stakeholders.

## Purpose and Scope

The purpose of this report is to describe the field and laboratory methods used for the collection and processing of water samples during the 3-year investigation in TUMA and the upstream Santa Cruz River watershed. Specifically, the report describes:

1. Collection of water-quality parameters, stream discharge, water stage, and continuous turbidity,

2. Compilation and reviewing of furnished data,
3. Collection, processing, and analysis of suspended sediment and *E. coli*, and
4. Laboratory procedures and quantification of microbial source tracking markers used to characterize sources of fecal contamination,
5. Data quality assessment and a method variability analysis,
6. A review and assessment of *E. coli* holding time requirements.

## Study Area

The study area focuses on a portion of the upper Santa Cruz River beginning near Chavez Siding (1 mile south of Tubac, Arizona) and extends south to the United States-Mexico border about 3 miles southwest of Kino Springs, Arizona. Included in the study area are the major tributaries that drain mountainous areas to the east and west of Nogales, Arizona and Sonora, Mexico (fig. 1). Nogales Wash is a perennial flowing water source to the upper Santa Cruz River near Nogales. The Nogales Wash meets the Santa Cruz River adjacent to southeast side of the NIWTP and is dry most of the year. Most reaches of the main stream and tributaries from Sonora, Mexico and Nogales, Arizona are intermittent or ephemeral, and precipitation runoff events provide most surface water connectivity between the main stream and upstream reaches.

**Table 1.** Description of stations in the Tumacácori National Historical Park and the upper Santa Cruz River watershed study area.

[FCDSCC, Flood control district of Santa Cruz County; USGS, United States Geological Survey; NPS, National Park Service; FOSCR, Friends of the Santa Cruz; IBWC, International Boundary Water Commission; National Oceanic Atmospheric Administration; coordinate datum is North American Datum of 1983; NA, not applicable; ID, identification]

Station ID	Collecting agency	Station number	Station name	Instrumentation	Latitude, in decimal degrees	Longitude, in decimal degrees	Drainage area, in square miles
NW1	USGS/con-sultant	2524 and 2527	Chimineia Arroyo	Precipitation gage and stage sensor	31.30888889	-110.94333333	12.5
NW2	USGS/con-sultant	2540 and 2543	Las Canoas Wash	Precipitation gage and stage sensor	31.33305556	-110.96611111	4.79
NW3	USGS/con-sultant	2510 and 2513	Ephraim Wash	Precipitation gage and stage sensor	31.33944444	-110.95472222	5.23
NW4	USGS	09481000	Nogales Wash at Nogales, Arizona	Streamgage	31.3432722	-110.9316722	29.7
NW5	FCDSCC	2530 and 2533	Portrero Canyon	Precipitation gage and stage sensor	31.35305556	-111.0147222	5.36
NW6	FCDSCC	2550 and 2553	Nogales Wash	Precipitation gage and stage sensor	31.35583333	-110.9288889	44.1



#### 4 Collection Methods and Quality Assessment within Tumacácori National Historical Park and the Upper Santa Cruz

Table 1.—Continued

Station ID	Collecting agency	Station number	Station name	Instrumentation	Latitude, in decimal degrees	Longitude, in decimal degrees	Drainage area, in square miles
NW7	USGS	312536110573401	Nogales Wash Near Old Tucson Highway	Single discrete sample	31.426588	-110.95935	NA
NW8	USGS, FOSCR	312551110573901	Nogales Wash at Ruby Road	stage sensor, automatic sampler, turbidity sensor	31.43075	-110.9608333	94.5
INFLUENT	USGS	312640110575101	Nogales International Wastewater inflow	Treatment plant inflow (IBWC)	31.444571	-110.964106	NA
SC1	USGS, FOSCR	09480500	Santa Cruz River near Nogales, Arizona	Streamgage	31.3445443	-110.851474	532
SC2	FCDSCC	2549 and 2552	Santa Cruz River at State Route 82	Precipitation gage and radar	31.38694444	-110.8747222	573
SC3	USGS	312654110573201	Santa Cruz River above Nogales Wash	Stage sensor	31.4483611	-110.9589722	628
SC4	USGS	312710110574201	Santa Cruz River below confluence of Nogales Wash	Stage sensor	31.45275	-110.9616389	726
SC5	USGS	312733110581401	Santa Cruz River near Nogales International Wastewater Treatment Plant upstream of railroad trestle	Stage sensor	31.45913889	-110.9705278	728
SC6	USGS	312724110580501	Nogales International Wastewater Treatment Plant at outfall	Treatment plant effluent discharge (IBWC)	31.45669444	-110.9681111	NA
SC7	USGS	312730110581301	Nogales International Wastewater Treatment Plant downstream of outfall	None	31.45838056	-110.9701444	NA
SC8	USGS, FOSCR	312809110592801	Santa Cruz River near Rio Rico, Arizona	Stage sensor (temporary)	31.46926056	-110.9917549	1,000
SC8	USGS	09481710	Santa Cruz River at Rio Rico, Arizona	None	31.4703716	-110.9923104	1,000
SC9	FCDSCC	2541 and 2544	Santa Cruz River at Palo Parado bridge	Precipitation gage and radar	31.53	-111.0202778	1,116
SC9	USGS	313148111011101	Santa Cruz River at Palo Parado bridge	Stage sensor (temporary)	31.53002778	-111.0196944	1,116
SC10	USGS, FOSCR, NPS	313343110024701	Santa Cruz River at Santa Gertrudis Lane	Stage sensor	31.5620355	-111.0470346	1,180

Table 1.—Continued

Station ID	Collecting agency	Station number	Station name	Instrumentation	Latitude, in decimal degrees	Longitude, in decimal degrees	Drainage area, in square miles
SC11	USGS, NPS	313413111024400	Santa Cruz River at Tumacacori downstream site	Stage sensor (temporary)	31.5703611	-111.0456944	1,190
SC12	USGS, NPS	313436111025101	Santa Cruz River at Anza trail crossing	Stage sensor	31.57658333	-111.0475833	1,190
SC13	USGS, FOSCR	313542111025801	Santa Cruz River at Clarks crossing	None	31.595	-111.0494444	1,190
SC14	USGS	09481740	Santa Cruz River at Tubac, Arizona	Streamgage, automatic sampler, turbidity sensor, precipitation gage	31.61286778	-111.0414792	1,210
SC15	FOSCR	313854111025701	Santa Cruz River at Chavez Siding	None	31.64833333	-111.0491667	1,250
SON1	FCDS	2556 and 2557	Patagonia Lake	Precipitation gage and stage sensor	31.49194444	-110.8694444	NA
SON2	USGS	312750110582801	Sonoita creek canyon near Rio Rico, Arizona	Stage sensor	31.46380556	-110.9745278	259
AF1	FCDS	2502 and 2505	Pena Blanca	Precipitation gage and stage sensor	31.40916667	-111.085	13.8
AF2	USGS	312843111000201	Miscellaneous Agua Fria Canyon near Rio Rico, Arizona	Stage sensor	31.4787045	-111.0011995	40.2
PC1	USGS	313040111004601	Miscellaneous Peck Canyon near Nogales, Arizona	Stage sensor	31.5112033	-111.0134223	47.8
JC1	USGS	313307111020201	Josephine Canyon near Tumacacori, Arizona	Stage sensor	31.552	-111.0338333	48.6
P1	FCDS	2570	Cobach College	Precipitation gage	31.246	-110.938	NA
P2	FCDS	2560	San Fernando Hill	Precipitation gage	31.28888889	-110.9925	NA
P3	FCDS	2531	CILA Nogales, Sonora	Precipitation gage	31.29305556	-110.9463889	NA
P4	FCDS	2537	Calabasas Canyon	Precipitation gage	31.34305556	-111.0708333	NA
P5	NOAA	US1AZSC0003	Nogales 8.9 NNE	Precipitation gage	31.4519	-110.855	NA
P6	NOAA	USW00003196	Nogales International airport	Precipitation gage	31.4208	-110.846	NA
P7	NOAA	USC00028865	Tumacacori	Precipitation gage	31.56667	-111.05	NA
P8	NOAA	US1AZSC0010	Tubac 2.9 SW	Precipitation gage	31.5883	-111.0876	NA
P9	FCDS	6080	Santa Cruz River at Tubac	Precipitation gage	31.61286778	-111.0414792	NA
P10	NOAA	US1AZSC0011	Tubac 0.7 S	Precipitation gage	31.6019	-111.0458	NA
P11	NOAA	US1AZSC0007	Rio Rico 3.6 E	Precipitation gage	31.4677	-110.915	NA
P12	NOAA	US1AZSC0013	Rio Rico 4.4 WSW	Precipitation gage	31.437	-111.04	NA
P13	FCDS	2516	Peck Canyon	Precipitation gage	31.49361111	-111.0813889	NA

Perennial base flow in TUMA is maintained primarily by treated effluent discharged to the river channel at the NIWTP.

## Methods

*E. coli* and water-quality data collected from four sources were used in this study: (1) the Friends of the Santa Cruz River (FOSCR) between February, 2008 and January, 2017; (2) the NPS between June, 2007 and January, 2017; (3) the U.S. International Boundary and Water Commission (IBWC) between June, 2009 and January, 2017 and; (4) the USGS between and January, 2015 and January, 2017 (table 1). The types of data available, field and analytical methods, and quality assurance and quality control procedures for each data source are described below. Additional precipitation and water stage data were retrieved using the Water Resources Climate Assessment Tool developed for the Santa Cruz Active Management Area (SCAMA; Shamir, 2016). The datasets are assembled in real time from the Arizona Department of Water Resources, USGS, IBWC, National Weather Service, National Centers for Environmental Prediction, and the Santa Cruz County Flood Control District Automated Local Evaluation in Real Time (ALERT) system.

### Friends of the Santa Cruz River and Tumacácori National Historical Park

The FOSCR, a volunteer organization, began collecting discharge, field parameter, and *E. coli* data for the Santa Cruz River in 1986. In 1992, the Arizona Department of Environmental Quality (ADEQ) began working with FOSCR to provide guidance and support standardizing procedures for the collection of water quality samples as part of ADEQ's Clean Water Act requirements. In the mid-2000s, TUMA began working with FOSCR to aid in the collection of water-quality samples within TUMA. Prior to 2011, FOSCR sampled 6 locations on the Santa Cruz River at monthly intervals; in 2011 sampling was reduced to 4 locations at monthly intervals. Prior to 2013, TUMA sampled 2 to 3 sites on the Santa Cruz River within the TUMA boundary at a variable frequency; since 2013 TUMA sampling has focused on the Santa Gertrudis Lane site on an almost weekly basis.

A sampling-analysis and quality-assurance plan published by FOSCR and ADEQ outlines the sampling and analytical protocols followed by FOSCR and TUMA (Arizona Department of Environmental Quality, 2008; Friends of the Santa Cruz River, 2011). Discrete *E. coli* samples are collected by FOSCR and TUMA by dipping a 100 milliliter (mL) sterilized IDEXX bottle or an autoclaved 250 mL Polymethylpentene (PMP) bottle in the centroid of flow. One replicate sample is collected monthly during routine monitoring. Sequential replicates were collected prior to water year 2016; since 2016, split replicates are collected using the 250 mL PMP bottles. Samples are kept chilled after collection, and are analyzed

within 6 hours of collection to meet ADEQ holding time requirements for *E. coli*.

Sample processing and analysis is conducted at TUMA facilities. Water samples are analyzed for *E. coli* using the Colilert™-24 (Colilert) and Quanti-Tray®/2000 (Quanti-Tray) system manufactured by the IDEXX Corporation. Colilert methods are described below in the USGS—Analytical Methods section. When the U.S. Environmental Protection Agency (EPA) issued their 2007 Final Rule for the “Guidelines Establishing Test Procedures for the Analysis of Pollutants”, the Colilert most probable number (MPN) statistical approach became an approved method and was included in the Federal Register. In 2013 during “Revisions to the Total Coliform Rule” the National Primary Drinking Water Regulations included the Colilert method for quantifying total coliform and *E. coli* bacteria in drinking water and ambient waters (U.S. Environmental Protection Agency, 2013). ADEQ also uses this method in their ambient monitoring program. The Colilert method uses the MPN statistical approach in the determination of bacteria densities. One advantage of the Quanti-Tray is the 97-well design provides narrower confidence intervals in the MPN determination when compared to other density quantification methods. Densities of *E. coli* are expressed in MPN per 100 mL of water (MPN/100 mL) and confidence intervals are applied to concentration estimates using the tabulation of 95-percent confidence intervals provided by the manufacturer (IDEXX, 2013).

The data collected is entered in a Microsoft Excel® database maintained by FOSCR and reviewed by ADEQ. The database and scans of the field and analytical notes were provided to the USGS. The USGS assessed the quality of the discharge, field parameter, and *E. coli* data from June, 2007 to January, 2017 by reviewing the field notes for errors, and checking Quanti-Tray counts against the analytical notes and the MPN calculations to ensure that data were transferred and computed correctly. Additional logic and outlier checks were performed comparing *E. coli* outlier concentrations to river discharge, turbidity, and suspended sediment. The quality-assured environmental data were archived in the USGS National Water Information System (NWIS) database (U.S. Geological Survey, 2016). Analysis of the quality-control data is included in the Quality Assessment section of this report.

### International Boundary and Water Commission

The IBWC operates the NIWTP, which is located in Rio Rico, Arizona, and provides treatment of sewage for Nogales, Arizona, and Nogales, Sonora. The IBWC is required under their Arizona Pollutant Discharge Elimination System (AZPDES) permit to conduct regular sampling of various water-quality constituents, including *E. coli* analysis. IBWC sample collection, preservation and handling is described in “Standard Methods for the Examination of Water and Wastewater” (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1999), or

by procedures referenced in Arizona Revised Statutes (2016), Title 18, Chapter 9 of the Arizona Administrative Code.

Through the AZPDES permit, various sample frequencies are required of IBWC for the collection of *E. coli* samples. Discrete samples are collected at least weekly at the outflow of the NIWTP by dipping a 100 mL sterilized IDEXX bottle in the centroid of flow. Monthly 24-hour composite samples are collected at three locations: Santa Cruz River below the confluence of Nogales Wash (SC4), Santa Cruz River at Rio Rico (SC8), and Santa Cruz River at Santa Gertrudis Lane (SC10). Samples are collected using an automatic sampler and the carousel is filled with ice to maintain cool temperatures during sample collection. All sampling equipment is autoclaved before each use, and all samples are kept chilled until they arrive at the analytical laboratory. Turner Laboratories Inc. and Legend Technical Services Inc. complete sample analysis. Both laboratories use standard method 9223B (Environmental Protection Agency, 2007) for *E. coli* analysis, which uses the Colilert method. The holding time specified for wastewater is 30 hours.

The IBWC provided wastewater treatment plant unit-value flow-data to the USGS for the period of October, 2012 to January, 2017. Daily discharge data for the period was accessed on the Santa Cruz Active Management Area webpage (Shamir, 2016). The *E. coli* data was retrieved from ADEQ as a result of the AZPDES reporting, and USGS quality assured the April, 2009 to January, 2017 IBWC *E. coli* data. The IBWC *E. coli* data was not archived in the USGS NWIS database because the limited knowledge of collection methods and the quality-assurance procedures precluded a thorough data review. The data is available at <https://doi.org/10.5066/F73776ZN> (Mayo, 2017).

## Other Data Sources

Precipitation, stream-stage, and stream-volume data were retrieved from the Water Resources Climate Assessment Tool developed for the Santa Cruz Active Management Area (SCAMA; Shamir, 2016). The mean-daily datasets are assembled from sources provided by the IBWC, the National Weather Service, USGS, and the Santa Cruz County Flood Control District ALERT system. Higher resolution data for individual precipitation and flow events was retrieved from the Santa Cruz County Flood Control District ALERT system, which is maintained by a private Hydrological Consulting Firm in cooperation with the USGS Geology, Minerals, Energy, and Geophysics Science Center. They maintain and serve data for a series of precipitation gages, stream stage sensors (pressure transducer or radar), and weather stations spanning the upper Santa Cruz watershed (United States and Mexico) as part of a flood alert network for the Santa Cruz Flood Control District and Arizona Department of Water Resources. High Sierra Submersible Pressure Transducers (Model 6640-00) and Tipping Bucket Rain Gauges (2400 Series) were installed at several locations in the study area (fig. 1; table

1). Data are transmitted to a central server at high-resolution interval and accessible via the internet at <http://jefullerdata.com/Nogales/Nogales.html>. The Climate Assessment Tool for SCAMA is maintained by the Hydrologic Research Center and provides mean daily information.

## U.S. Geological Survey

The USGS collected stream discharge, field parameter data, and samples for analysis of suspended sediment, *E. coli*, and MST markers on the Santa Cruz River and its tributaries between January, 2015 and January, 2017. The USGS also collected subsurface shallow flow samples from the hyporheic zone using mini drivepoints (Duff and others, 1998) for analysis of *E. coli* concentrations. Samples were collected by the USGS to represent different hydrological and climatological conditions. These included 24-hour, stormflow runoff, and seasonal base flow sampling collected during most months of the year.

## Field Methods

Data collection methods followed protocols described in the USGS National Field Manual Collection of Water-Quality Data, Anderson (2005), Wilde (2005), U.S. Geological Survey (2006), Myers and others (2014), and water-quality parameters, including temperature, pH, specific conductance, turbidity, and dissolved oxygen were measured as conditions permitted for each water-sampling site using either a Yellow Springs Incorporated (YSI) EXO2 or an In-Situ Inc. smarTROLL multiparameter meter. Because the smarTROLL does not measure turbidity a HACH 2100P turbidimeter was used in conjunction with the smarTROLL. The multiparameter meter was calibrated daily before field measurements were collected using standards supplied by the USGS National Water Quality Laboratory, and YSI or HACH for turbidity. Discharge at locations without a gaging station was measured with a SonTek FlowTracker, using the midsection method of computing cross-section area for discharge measurements (Turnipseed and Sauer, 2010).

A Campbell Scientific OBS501 continuous turbidity sensor was deployed by the USGS Santa Cruz River at Tubac gaging station (SC14). The sensor is a dual probe that measures both 90-degree sidescatter (Formazin Nephelometric Units; FNUs) and backscatter (Formazin Backscatter Units; FBUs). The manufacture's reported range is from 0 to 4,000 FBUs and 0 to 1,000 FNUs. Four field calibration checks were conducted with the turbidity standards supplied by AMCO Clear®. Calibration standards included deionized water, 20 FNUs, 250 FBUs, and 1,000 FBUs. Check side-scatter measurements were also made on the OBS501 using a YSI EXO2, which was calibrated to 12.4 FNUs, 124 FNUs, and 1,010 FNUs. Two different sensors were used during the study, both were sent to Campbell Scientific for laboratory calibration



checks and each was verified to be within the specified  $\pm 10$  percent threshold.

Surface-water samples for *E. coli*, MST markers, and suspended sediment analysis were collected using two methods. The method selection was determined by the field conditions at the station, flow magnitude, season, and sample type. The first surface-water sample collection method was a discrete, or grab, sample approach from the centroid of the water column. Discrete samples were collected by either wading and submerging a widemouth 1 liter (L) polyethylene autoclaved (cleaning procedure in Myers and others, 2014) bottle by hand, or using a Nasco™ 12 to 24 feet (ft) telescoping swing sampler to lower a 1 L bottle into the centroid of flow (fig. 2a). The telescoping rod was used during nonwadeable or flood conditions. After samples were collected, they were kept chilled at 2–4°C until analysis.

The second surface-water sample collection method utilized Teledyne ISCO™ 6712 portable automatic samplers. Two automatic samplers were operated as permanent deployments for the duration of the study: one at SC14 (fig. 2b and 2c) and one at the Nogales wash at Ruby Road location (NW8; fig. 2d). Additional automatic samplers were operated as temporary deployments during runoff events or for the 24-hour collections. Each automatic sampler was configured with a carousel holding twenty-four 1 L polyethylene bottles. Upon deployment, the open center of the carousels was filled with ice. Automatic sampler carousels were periodically deployed with HOBO™ temperature loggers to ensure that cold temperatures were maintained throughout deployment. The automatic sampler intake tubing consisted of 3/8 inch (in.) inner diameter vinyl tubing inside conduit and affixed to a T-post. The height of the intake tubing for the permanent deployments was adjusted to approximately 6 to 12 in. above base flow or the dry channel to minimize the pumping of bed material and to sample a representative well-mixed water column during anticipated flooding events. During temporary deployments, the tubing was positioned at the centroid of flow above the streambed to avoid bed sediment or biological debris. Samplers were initiated at a specified time or using a liquid-level actuator.

The discrete and automatic sampler surface-water sampling methods were compared to an isokinetic depth-integrated sampling method to test reproducibility between sampling method results. The isokinetic depth-integrated sampling method has an advantage over discrete and automatic sampler sampling methods because it results in a composite sample that represents the discharge-weighted concentrations of a river cross section. This method is generally recommended for heterogeneous velocities and depths. However, in this study the intense localized precipitation events, and the rapid response of catchment to runoff resulted in steep hydrographs of stormwater flows in the Santa Cruz River; and the events did not allow for the time required to collect isokinetic depth-integrated samples. In order to compare the sampling methods used in this study to the isokinetic depth-integrated sampling method, samples were collected using a US D-95

depth-integrating sampler suspended by an A-reel during a high flow event on September 4<sup>th</sup>, 2016 at location SC14. Concurrent *E. coli* and suspended sediment samples were collected using discrete, automatic sampler, and isokinetic depth-integrated sampling methods to test reproducibility between the three sampling methods. The relative standard deviation between the *E. coli* and suspended-sediment concentrations collected using the different sampling methods ranged from 2.89 to 5.79 percent and 2.20 and 6.92 percent, respectively (fig. 3).

Groundwater samples were collected from the Santa Cruz River hyporheic zone. Clean stainless steel mini drivepoints were pushed into the streambed at various cross sectional locations (wetted channel, wetted edge, and dry-active channel) and various depths ranging from 1 to 3 ft (fig. 4). A peristaltic pump was used to develop or flush sediments until a visibly clear continuous flow was established. Field parameters were measured and *E. coli* samples were collected. As a potential maximum *E. coli* concentration, groundwater in the hyporheic zone was also sampled by digging into the sediments next to the river channel until groundwater was encountered and a sample of the turbid water was collected.

A total of 16 known-source fecal samples were collected with sterile equipment and placed in sterile 50 mL centrifuge tubes, individually sealed in bags, and immediately placed on ice. Samples were collected at several locations in the upper part of the watershed, mostly from cows and ducks on grazing lands. Efforts were made to collect fecal samples from fresh material and was only collected from sample types of known origin. One influent sample (untreated sewage from Nogales, United States and Nogales, Sonora) was collected from the NIWTP in a sterile 500 mL bottle and shipped on ice overnight to the Ohio Water Microbiology Laboratory (OWML).

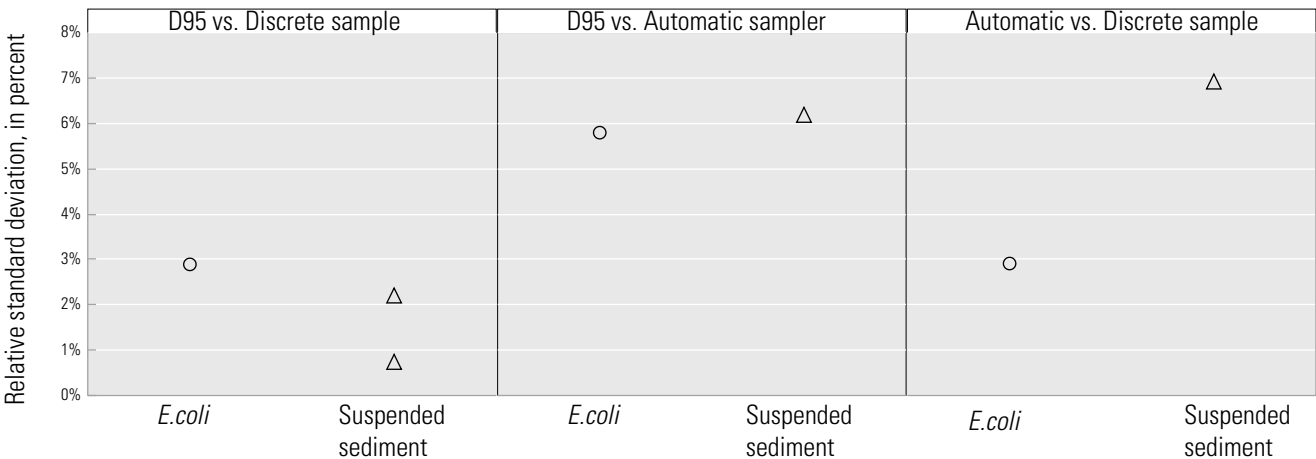
Stage was recorded using non-vented Solinst® 3001 Levellogger pressure transducers at 11 sites throughout the project area (fig. 1) for various time periods from February 12, 2015 to December 31, 2016. The data was logged at 15-minute intervals and manually downloaded. Non-vented pressure transducers do not take atmospheric pressure into account, therefore, the data collected via Levellogger were corrected for atmospheric pressure using a Solinst® Barologger located at SC14, mounted in the gage house. The Levelloggers were mounted on a metal stake below the water surface unless the site was ephemeral, then placement was generally 0.5 to 1.0 ft above the channel surface (fig. 5).

The data were processed and reviewed for erroneous data using Solinst Levellogger Software 4.2.0 and plotted to identify anomalous peaks. Erroneous data were deleted from the record. The pressure transducer that was deployed at the ephemeral location, Santa Cruz River above Nogales Wash (SC3), had a malfunctioning level sensor therefore the level data was not used. Instead, the temperature data collected from the pressure transducer were used to identify the presence of water through the rapid decrease in temperature indicating the shift from air temperature to water temperature. These data are





**Figure 2.** Photographs of A, sample collection using telescoping rod at Santa Cruz River at Tubac, AZ (SC14); B, automatic sampler at SC14; C, covered automatic sampler at SC14 (view towards river); and D, automatic sampler at Nogales Wash at Ruby Road (NW8).



EXPLANATION

Magnitude of concentrations used in relative percent difference

	Minimum concentration	Maximum concentration
○ <i>E.coli</i>	187,000 <i>Escherichia coli</i> , most probable number (MPN) per 100 milliliters	548,000 <i>Escherichia coli</i> MPN per 100 milliliters
△ Suspended sediment	3,052 milligrams per liter	5,410 milligrams per liter

**Figure 3.** Chart showing relative standard deviation of *Escherichia coli* (*E. coli*) and suspended sediment irrepsample samples collected using a D-95, discrete, and automatic sampler.

available in ScienceBase and can be retrieved at <https://doi.org/10.5066/F73776ZN> (Mayo, 2017).

Analytical Methods

The primary activity of the investigation was the collection and analysis of *E.coli*, MST, and suspended sediment. The analysis methods, quantification, and reported values can vary depending on the laboratory methods. Analysis and reporting methods for each laboratory are described in the following section.

*Escherichia coli*

Water samples were analyzed for *E. coli* at the USGS Arizona Water Science Center (AZWSC) laboratory using the Colilert system (Myers and others, 2014; IDEXX Laboratories, Inc., 2013). Samples collected in 1 L bottles are thoroughly shaken prior to analysis to fully suspend any sediment or fine particulate material and quickly poured (100 mL for undiluted) or pipetted (1 mL or 10 mL for dilution) into a sterilized IDEXX 100 mL bottle. Homogenization is one of most important steps in the analytical process of *E. coli* because particulates or sediments that are not adequately re-suspended during shaking can affect the analyzed concentration. A pre-measured dry medium containing two enzyme substrates—a chromogen that reacts with the enzyme found in total coliforms (galactosidase), and a fluorogen that reacts with

an enzyme found in *E. coli* (glucuronidase; IDEXX, 2013)—is added to each bottle. The samples are poured into a Quanti-Tray (49 large wells and 48 small wells per tray) and sealed using a Quanti-Tray Sealer PLUS and incubated at 35° C for 24 hours. While the Colilert tray is designed with an overflow well (the largest well at the top of the tray), inconsistent volumes can be a source of variability, and uneven pours or residual foam often result in empty or partially-filled wells. After incubation, an *E. coli*-positive reaction causes the medium to fluoresce under a long-wave ultraviolet light at 365 nanometers (nm). The proprietary medium used in the Colilert method suppresses other non-coliforms that may either interfere with *E. coli* bacteria growth or produce false positives. The density of *E. coli*, expressed as MPN/100 mL, is determined by counting the positive small and large tray wells and referencing the MPN table provided by IDEXX. The manufacturer suggests additional incubation upwards of 4 hours to determine if a lightly fluorescing well will intensify. In order to minimize potential variability introduced during these analytical steps, the USGS Arizona Water Science Laboratory developed and followed a consistent a laboratory protocol for processing and analyzing *E.coli* and trained staff in these protocols.

The Colilert method has a number of advantages over membrane filtration (MF), a common alternative used in many investigations and in routine monitoring. The time required with MF to process and plate several dilutions for each sample would have significantly reduced the number of samples that could have been analyzed in this study during high





**Figure 4.** Photographs of *A*, mini drive point transect along stream channel at Santa Cruz River at Santa Gertrudis Lane (SC10); *B*, peristaltic pump and mini drive point in wetted stream channel at SC10; *C*, mini drive point in dry stream channel at SC10; *D*, mini drive point on stream edge and within stream channel at Nogales Wash at Ruby Road (NW8); and *E*, pore water in dry channel bed at SC10.





**Figure 5.** Photographs of pressure transducers deployed at *A*, Santa Cruz River near Nogales International Wastewater Treatment Plant (SC5); *B*, Nogales Wash at Ruby Road (NW8); *C*, Agua Fria Canyon (AF2), outlined by white box; and *D*, Sonoita Creek Canyon (SON2).

sampling-frequency periods, such as runoff events. In addition, several researchers have shown that the Colilert method is a good alternative to MF methods because it has a slightly shorter incubation time, is more accurate (fewer false positives and false negatives), and is considered easier to use (Olson and others, 1991; Buckalew and others, 2006; Lawrence, 2012).

Dilutions were critical in the processing of samples using the Colilert method. Every sample collected during runoff events required a dilution in order to achieve quantifiable *E. coli* concentrations. Depending on the suspended-sediment concentration, turbidity, and location of collection, dilutions generally were 1:100 or 1:1,000. A dilution of 1:10,000 was used in a few situations such as when the samples had contributions of untreated sewage from upper Nogales Wash. For a 1:100 dilution, 1 mL of sample was pipetted using an Eppendorf™ Research Pipette into 99 mL of sterile deionized (DI) water. A 1:1,000 dilution was the most common dilution for flood samples and preparation consisted of pipetting 1 mL of sample into 99 mL of sterile DI water, then pipetting 10 mL from that dilution into 90 mL of sterile DI water. Samples affected by untreated effluent required a 1:10,000 dilution, which required pipetting 1 mL of sample into 99 mL of sterile DI water, then pipetting 1 mL of that dilution into 99 mL of sterile DI water. Periodic replication of 1:100 and 1:1,000 dilutions were conducted to ensure that dilution results were reproducible.

## Microbial Source Tracking

Microbial source tracking (MST) is a process of characterizing the sources of fecal contamination in the environment. MST using molecular markers is carried out by detecting genetic sequences in the deoxyribonucleic acid (DNA) of fecal-origin bacteria that are specific to the host species that produced the feces. Host-associated molecular markers have been identified based on the theory that the physiology in the digestive system of the host animal (for example, diet, temperature, or antibiotic treatment) is unique from one animal to another (Rivera, 2011). These unique conditions create unique subsets of microorganisms in the gut. Host-associated markers have been identified from different groups of fecal-origin bacteria, often from the genus *Bacteroides*, a bacterium abundant in the digestive system of warm-blooded animals (Dick and others, 2005).

Samples for MST analysis were kept below 2° C and shipped overnight to USGS Ohio Water Microbiology Laboratory. Upon receipt of samples, 2 aliquots of the water sample (up to 100 mL each), 10 to 20 mL of wastewater influent (one sample), or 0.1 to 1 mL of fecal slurry—consisting of 0.25 gram (g) wet-weight fecal material added to 10 mL of phosphate buffered saline (PBS)—was filtered through a 0.4 micrometer (μm), 47 millimeter (mm) polycarbonate filter (Bushon and others, 2017). Filters were aseptically folded and placed into a 2 mL screw-cap tube containing 0.3 g of acid-washed, 212 to 300 μm glass beads (Sigma-Aldrich Corp., St.

Louis, Missouri). Negative filtration controls consisting of 100 mL of PBS were filtered each day samples were processed. Filters were immediately frozen and stored at −70° C until further analysis.

DNA was extracted from the samples using a DNA-EZ extraction kit (GeneRite LLC., North Brunswick, NJ) according to manufacturer's instructions, except that no pre-filter was used. Known-source fecal samples were extracted in separate batches from the water samples in order to avoid cross contamination. A negative extraction control was included with each batch of extractions. Final DNA extracts were stored at 4°C until a quantitative polymerase chain reaction (qPCR) was run within 7 days. The qPCR assays allow for more rapid detection of markers by detecting a specific DNA sequence in a sample while simultaneously determining the actual copy number (relative concentration) of the sequence relative to a standard in real-time.

All samples were analyzed for six MST markers (table 2). Five out of the six MST markers used in this study originate from *Bacteroides* species bacteria, one of the prominent bacterial groups inhabiting the intestinal tracts of warm-blooded animals. Analysis included quantification of total *Bacteroides* providing a general indicator of fecal contamination (Gen-Bac; Siefring and others, 2008), a human-associated marker (HF183; Seurnick and others, 2005), a canine-associated marker (BacCan; Kildare and others, 2007), a ruminant-associated marker (Rum2Bac; Mieszkina and others, 2010), and a cattle-associated marker (CowM2; Shanks and others, 2008). Human polyomavirus was also analyzed as an additional human-specific marker (HPyV; McQuaig and others, 2009).

All qPCR analyses were performed using either the Applied Biosystems® StepOne Plus™ or Model 7500 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems®, Foster City, CA). All samples were analyzed in duplicate and a no template control was included on each qPCR plate. Standard curves were generated using plasmids containing the sequences for each of the targeted genes and were run on each qPCR plate. Sample MST marker concentrations were determined by use of standard curve equations characterized in table 2. Matrix inhibition was tested using matrix spikes and when inhibition was detected, data were generated using the results from diluted samples (Francy and others, 2017).

To aid in the interpretation of qPCR results, the limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) were determined for each assay to describe the lowest concentration of each MST marker that can be differentiated from blank sample results, and can be reliably detected and quantified (Francy and others, 2017). The LoB is the lowest concentration that can be reported with 95-percent confidence to be above the concentration of the blanks; it was determined for each qPCR run and included results from negative filtration controls, negative extraction controls, and no template controls. The LoB was not used for reporting results unless the concentration was greater than the LoD, in which case the LoB replaced the LoD. The LoD is the lowest



concentration of a marker that can be detected with 95-percent confidence that it is a true detection and distinguishable from the blank sample results; it was determined by analyzing at least ten replicates of each of several dilutions of a positive control. The LoQ is the lowest concentration of each MST marker that can be accurately quantified and is calculated from the standard deviation of the LoD replicates. If a sample result was below the LoQ, but above the LoD, then the result was considered an estimate. If the MST marker was not detected in a sample or if the concentration was less than the LoD, then results were reported as less than the sample reporting limit (SRL). The SRL is specific to each sample and is calculated using the LoD and taking into account the dilution analyzed and the initial sample volume filtered. The LoD and LoQ for each MST assay can be found in table 2.

Suspended Sediment

Suspended-sediment samples were analyzed by the USGS Cascades Volcano Observatory (CVO) using the filtration method (ASTM D3977-97, 2002) described in the CVO Quality Assurance plan (Cascades Volcano Observatory, 2014) with additional details from Shreve and others (2005). The advantage of this method is the dissolved solids present in the sample water will pass through the filter; therefore, mathematical adjustments for dissolved solids are not needed (Shreve and others, 2005). Samples were allowed to settle for a minimum of two weeks. Overlying sediment-free water in the sample bottle was decanted using a vacuum system to suction out the supernatant water. A filter (Whatman #934-AH, with a porosity of 1.5 µm) was inserted into the clean crucible, placed under vacuum, and a minimum of 50 mL of deionized water was flushed through the crucible and filter. The crucibles were then oven dried. All sediment was washed from the sample container with deionized water into the tared crucible on the vacuum manifold. Crucibles were dried in the oven for at least two hours at 103°C, and then transferred to desiccator enclosure and allowed to cool. Crucibles were weighed with an analytical balance to the ten-thousandth of a gram. Both tare and gross weights of individual crucibles were loaded into

Sediment Laboratory Environmental Data System (SLEDS) software program. The SLEDS program generates a sediment summary report, which was reviewed for accuracy. Data were then loaded into the USGS NWIS database. The standard scientific unit used for expressing sediment concentration in the laboratory is milligrams per liter (mg/L). The sediment concentration is calculated automatically by SLEDS as follows (Shreve and others, 2005):

mg/L = Concentration (ppm) = Concentration

$$\left[ \frac{\text{Weight of sediment} \times 10^6}{\text{Weight of water} - \text{sediment mixture}} \right]$$

Data-Quality Assurance

The temperature probe on each multiparameter meter was checked annually against a National Institute of Standards and Technology (NIST) certified thermometer and found to be within ± 0.1 ° C. Blind measurements of sample alkalinity, pH, and specific conductance were made by project staff annually as part of a USGS National Field Quality Assurance program and were within acceptable limits each year of sample collection. Microbiology quality-assurance practices as described in Myers (2014) and Franczy and others (2017) were followed throughout this study. All sample-collection and analytical information was recorded on USGS microbiology field forms. Before automatic sampler deployment, the sampler intake tubing was rinsed by pumping 2 to 5-percent soap water (Liquinox™ nonphosphate detergent), followed by a 5-percent bleach solution, tap water, deionized (DI) water, and autoclaved water through the tubing. Cleaning of all other sampling equipment and bottles followed Myers and others (2014). All sample collection bottles were autoclaved at 121°C at 15 pounds per square inch (psi) for specified time depending on the bottle or liquid volume (Myers and others, 2014). Each autoclave cleaning process was verified using heat-indicating autoclave tape.

**Table 2.** Standard curve characteristics and limits of detection and quantitation for microbial source tracking methods used in Tumacácori National Historical Park and the upper Santa Cruz River watershed.

[MST, microbial source tracking; R-squared, coefficient of determination; qPCR, quantitative polymerase chain reaction]

MST marker	Marker source identifier	Targeted bacterium	Number of standard curves	Average efficiency, in percent	Average R-squared	Average slope	Average intercept	Limit of detection (copies/qPCR)	Limit of quantification (copies/qPCR)
GenBac	General	<i>Bacteroides</i>	6	93	0.997	-3.50	40.1	3	11
RumBac	Ruminant	<i>Bacteroides</i>	6	102	0.996	-3.29	39.4	21	42
HF183	Human	<i>Bacteroides</i>	7	103	0.999	-3.25	33.8	11	28
BacCan	Canine	<i>Bacteroides</i>	6	93	0.997	-3.51	39.7	39	85
CowM2	Cattle	<i>Bacteroides</i>	6	95	0.998	-3.45	40.7	66	130
HPyV	Human	Human poly- omavirus	5	95	0.998	-3.45	39.3	21	43

Many of the *E. coli* water samples exceeded recommended hold time of 6 hours compliance for non-potable water and 24 hours for noncompliance purposes (Environmental Protection Agency, 2007; American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1999). An in-depth discussion of sample hold times for *E. coli* analysis is presented later in this report. Laboratory blanks for *E. coli* were analyzed periodically to estimate any bacteria contamination from the DI water autoclaving process, IDEXX Colilert supplies, and analytical procedures. The incubators used in the *E. coli* analysis were monitored with a glass or digital thermometer, which was checked against a NIST thermometer. Between water years 2015 and 2016 the USGS Arizona Water Science Center tested 5 sets of positive- and negative-control cultures for *E. coli*. The OWML tested 13 sets during the same period of time. These tests help verify that Colilert reagent, processing materials, and incubation procedure are all working to provide a known range of concentrations. Results from the control cultures were all within the acceptable range provided by IDEXX (table 3).

Five to ten percent of the samples analyzed by the CVO sediment laboratory were laboratory quality control samples inserted by the analyst or the laboratory chief (Cascades Volcano Observatory 2014). Balances were checked multiple times daily and recalibrated if differences in the standard weight measurements exceeded an established threshold. Ovens were checked twice daily during operation. All balance and oven data were logged electronically or recorded in the instrument logbook (Cascades Volcano Observatory, 2014). Laboratory blanks were prepared from DI water and processed identically to sediment samples. Blank samples were distributed throughout the set of concentration determination samples and (or) sand/fine separation samples. Laboratory replicates were analyzed from split samples. Proficiency testing by an external source was supplied through the U.S. Geological Survey Branch of Quality System and results can be retrieved at [https://bqs.usgs.gov/slqa/frontpage\\_study\\_results.htm](https://bqs.usgs.gov/slqa/frontpage_study_results.htm).

For the MST analyses, each qPCR run included a filtration blank (negative control at the filtration step), an extraction blank (negative control at the extraction step), and a no-template control (negative control at the qPCR step). The LoB concentration is the lowest concentration that can be reported with 95-percent confidence to be above the concentration of the blanks and it is determined from the results of each qPCR run that includes the negative filtration controls, negative extraction controls, and no template controls. These quality measures aid in the development and reporting of the MST results. Details of this process can be found in the previous “Microbial Source Tracking” section.

Field blank water for *E. coli* and microbial source tracking analysis was prepared at the AZWSC Laboratory and consisted of DI water autoclaved at 121 °C at 15 psi for 30 to 45 minutes depending on the volume (Meyers and others, 2014). Field blanks for *E. coli* were analyzed to estimate bacteria contamination from the cleaning procedures, sample collection

methods, environmental conditions, and laboratory processing and analysis. An onsite MST field blank was collected by pouring sterile DI water into a sterile 1-L wide-mouth bottle.

Field irreplicates, defined by Mueller and others (2015) were collected to estimate bias introduced to the analytical results from using two different sample collection methods. Irreplicates for *E. coli* were collected by collecting two independent *E. coli* samples simultaneously: one sample collected using the discrete sampling method, and one sample collected using the automatic sampler sampling method. Both *E. coli* samples were then prepared and analyzed identically. Irreplicates for suspended sediment samples were collected by using a discrete sediment sampler (bottle or extended rod) at the same time the automatic sampler collected a sample. Both sediment samples were then prepared and analyzed identically.

Two types of *E. coli* field replicate samples were collected by the USGS for this study. Split replicates were collected by generating two samples from one sample collection bottle; this type of replicate examines analytical variability. Sequential replicates were prepared by collecting two samples in the field, one right after the other, using the same sampling method; this type of replicate examines analytical variability in addition to variability of environmental conditions, particularly to determine how well mixed the water body is when it was sampled. In addition, MST sequential field replicates and six suspended sediment sequential field replicates were collected for this study.

## Data-Quality Assessment

The process of collecting and analyzing water samples for *E. coli*, MST, and suspended sediment includes a number of steps that can affect the quantification. Microorganisms responding to changes in environmental conditions are naturally variable and different collection methods, flow-conditions, and laboratory analysts can introduce additional error. Quality-control samples are collected to estimate the magnitude of error associated with the process of obtaining environmental data and these are analyzed as bias and variability. Bias is the systematic error inherent in a method or measurement system and variability is the random error that occurs in independent measurements (Mueller and others, 2015).

### Bias

Eight *E. coli* laboratory blanks and 33 *E. coli* field blanks were collected by the USGS during the course of the study. No detections (minimum detection limit is 1 MPN/100 mL) of *E. coli* were found in the laboratory blanks, and three of the field blanks had *E. coli* detections (fig. 6). The highest *E. coli* detection in the field blanks was 5.2 MPN/100 mL; the upper confidence limit is 97 percent that potential *E. coli* contamination is no greater than this concentration in at least 90 percent of the samples. With the exception of one sample,

**Table 3.** *Escherichia coli* (*E. coli*) by Colilert quality-control samples from IDEXX. *E. coli* is a positive control and *Pseudomonas aeruginosa* (*P. aeruginosa*) is a negative control.

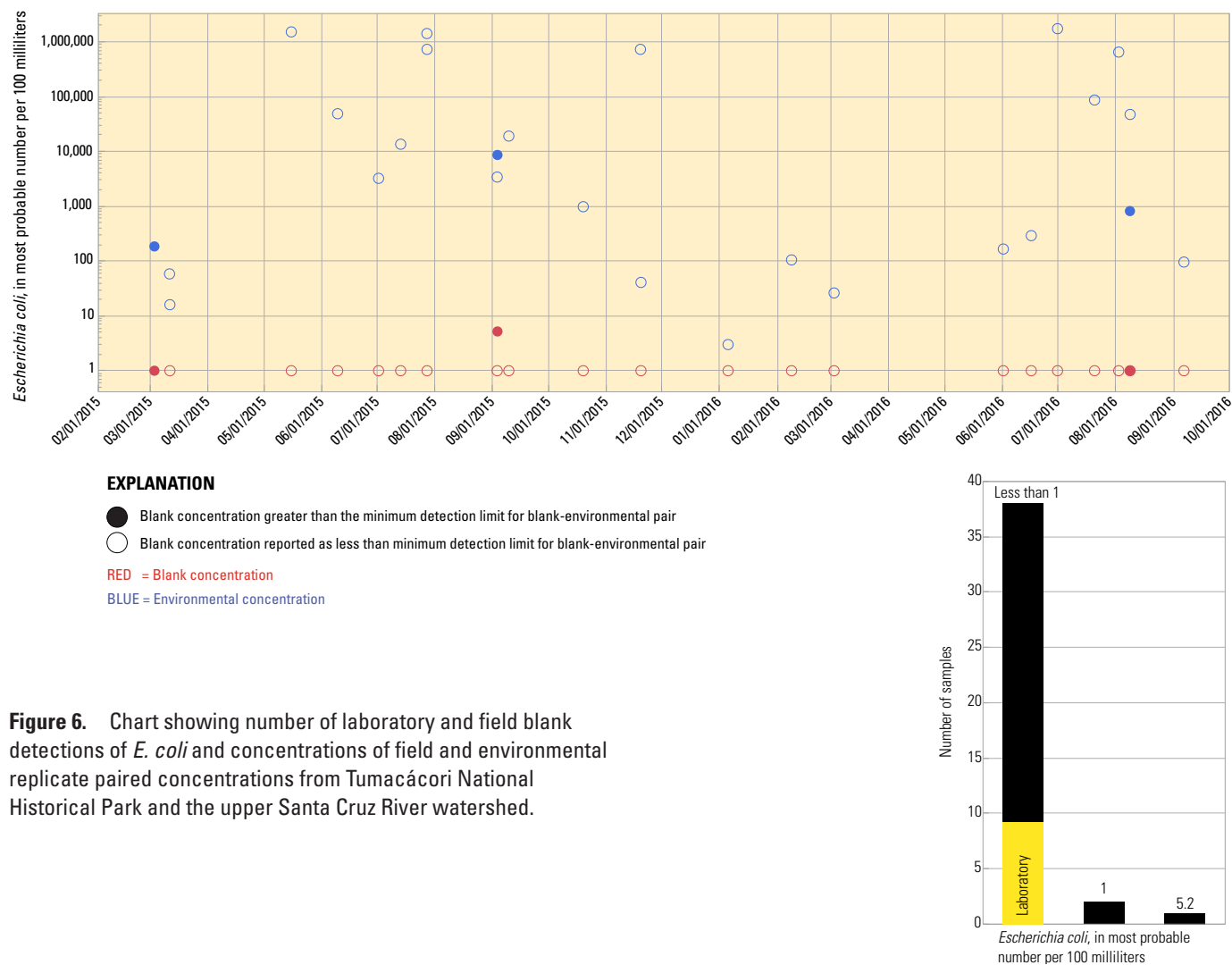
[MPN, most probable number; mL, milliliters; &lt;, less than]

Date of analysis run	Analyzing center	Colilert reagent lot number	Quality Control sample lot number	IDEXX <i>E. coli</i> control acceptable range (MPN/100 mL)	<i>E. coli</i> result (MPN/100 mL)	<i>P. aeruginosa</i> result (MPN/100 mL)
8/22/2015	Arizona Water Science Center	070314	082514	50-250	115.3	<1
9/8/2015	Arizona Water Science Center	070314	082514	50-250	86	<1
10/26/2015	Arizona Water Science Center	022015	042315	15-127	61.7	<1
12/8/2015	Ohio Water Microbiology Laboratory	CL326	21915	43-138	47	<1
1/13/2016	Arizona Water Science Center	022015	042315	15-127	51.2	<1
3/10/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	53	<1
5/11/2016	Arizona Water Science Center	022015	042315	15-127	77.1	<1
5/12/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	66	<1
6/7/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	96	<1
6/14/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	86	<1
6/21/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	88	<1
6/28/2016	Ohio Water Microbiology Laboratory	LL806	102015	30-138	76	<1
8/2/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	110	<1
8/9/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	120	<1
8/16/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	120	<1
8/23/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	130	<1
8/30/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	120	<1
9/13/2016	Ohio Water Microbiology Laboratory	EM522	52616	9-143	110	<1

the *E. coli* concentrations measured in this study are greater than 5 MPN/100 mL: the USGS *E. coli* data are considered to not be affected by high bias. The MST field blank had no detections above the detection limit for any of the markers analyzed. Likewise, all laboratory blanks for MST analysis had no detections above the detection limit for any of the markers analyzed. The CVO ran 441 laboratory blanks for suspended sediment concentrations between November, 2015 and January, 2017. The median concentration was 0 mg/L and the mean was 0.07 mg/L. Concentrations ranged from 1 to -1

mg/L because of the mass error associated with the balance tare and gross weights.

Thirty-four *E. coli* irreplicates and 6 suspended sediment irreplicates were collected to estimate bias introduced to the analytical results from using two different sample collection methods (discrete and automatic sampler). The sign test was used to determine whether one collection method was biased high or low compared to the other. The sign test was used instead of a rank-sum test because the variation among streams may obscure the difference between methods, which



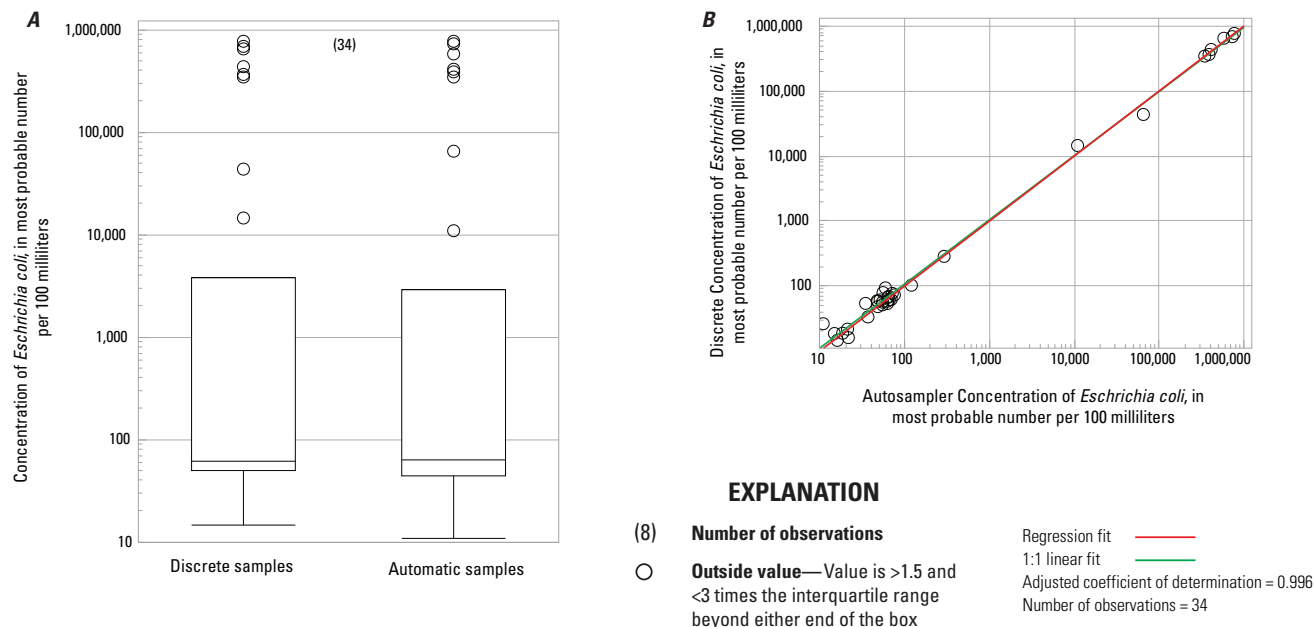
is the focus of the sign test (Helsel and Hirsh, 2002). The distributions of *E. coli* concentrations in the irreplicates were positively skewed because of the high concentrations collected during flooding (fig. 7A); the sign test, however, is a nonparametric test and no assumptions about the distribution of the data are necessary. The sign test indicated that neither collection method tended to produce *E. coli* concentrations higher than the other (p-value = 0.72, M-test statistic = -1.5). In addition, when *E. coli* irreplicate concentrations are plotted and fit with a linear regression, the residuals are random and the fitted line is very close to a 1:1 line (fig. 7B). Similarly, the sign test indicated that neither collection method tended to produce suspended sediment concentrations higher than the other (p-value = 0.62, M-test statistic = -1.0; fig. 8A). The linear regression fit of the suspended sediment irreplicate concentrations is also close to 1:1 (fig. 8B). The irreplicate data indicates that neither sampling method introduced bias relative to the other, and supports the compilation of autosampler results with discrete sample results for the purpose of the overall statistical analysis.

## Variability

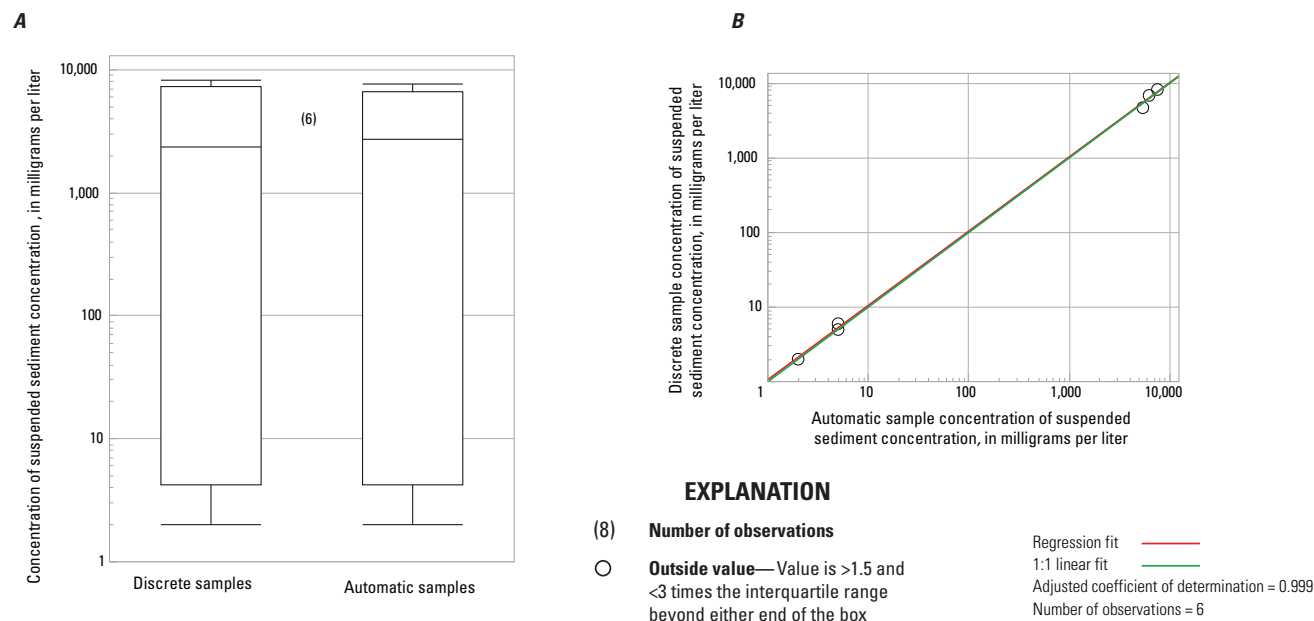
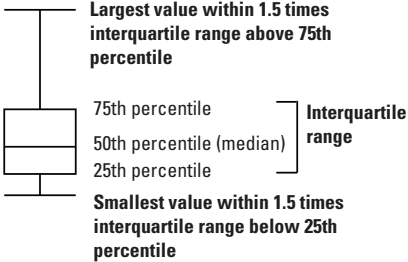
The variability of *E. coli*, MST, and suspended sediment was assessed using replication to measure the random error that occurs in independent measurements. For *E. coli*, both analytical and environmental variability was assessed. The analysis assesses the variability of different agencies collecting and analyzing *E. coli* samples, quantifies the error associated with method, and demonstrates an application of reporting uncertainty.

## *Escherichia coli*

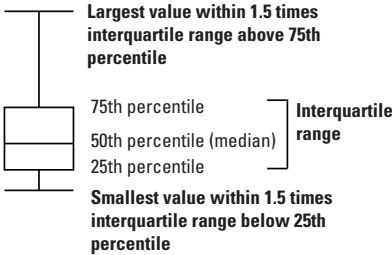
Two types of *E. coli* replicates were collected to examine different sources of variability. Eighty-nine split replicates were collected to examine analytical variability, and 109 sequential replicates were collected to examine the variability introduced by analytical approaches and environmental conditions. Twelve of the split replicates were collected by FOSCR or TUMA and 77 were collected by USGS, and 101 of



**Figure 7.** Discrete and automatic sample *Escherichia coli* (*E. coli*) concentrations from Tumacácori National Historical Park and the upper Santa Cruz River watershed plotted as *A*, boxplots; and *B*, as bivariate regression compared to a 1:1 linear fit. MPN/100 mL, most probable number per 100 milliliters.



**Figure 8.** Discrete and automatic sample suspended-sediment concentrations from Tumacácori National Historical Park and the upper Santa Cruz River watershed plotted as *A*, boxplots; and *B*, as bivariate regression compared to a 1:1 linear fit.





the sequential replicates were collected by FOSCR or TUMA and 8 were collected by USGS. For the purpose of assessing variability, the replicate samples collected by FOSCR, TUMA, and by USGS were combined within each replicate type.

A bias-corrected log-log regression model (Mueller and others, 2015) was used to model the variability, as standard deviation, for each type of replicate. This model is based on the approximately linear relation between the logarithms of replicate standard deviation and mean concentration, where:

$$\log(SD) = B_0 + B_1 \log(C) \quad (1)$$

where

- $(SD)$  is the replicate standard deviation
- $B_0$  is the intercept of the regression line, estimated by least-squares
- $B_1$  is the slope of the regression line, estimated by least-squares, and
- $C$  is the mean replicate concentration.

Standard deviation residuals from the log-log equation are then transformed back to their original units; the mean of the transformed standard deviation residuals is the bias-correction factor ( $b_{cf}$ ). The  $b_{cf}$  is multiplied by the estimated standard deviations of each replicate in order to express the modeled standard deviation ( $SD_M$ ):

$$SD_M = b_{cf} \left\{ 10^{[B_0 + B_1 \log(C)]} \right\} \quad (2)$$

The slopes and intercepts of the regression lines of the logarithms of standard deviation and mean concentration for the 2 replicate types are similar (slopes of 0.98 and 1.05; intercepts of -0.83 and -1.15; fig. 9A). This results in similar  $b_{cf}$  values (1.41 and 1.50) and similar modeled standard deviations for the 2 replicate types (fig. 9B). The analytical variability determined from the split replicates is  $1.50 \{10^{[-1.15 + 1.05 \log(C)]}\}$ , the analytical and environmental variability determined from the sequential replicates is  $1.41 \{10^{[-0.83 + 0.98 \log(C)]}\}$ . The models indicate that the changing stream conditions and analysis do not introduce more variability than the variability inherent within a sample. One model describing overall variability in *E. coli* concentrations was generated by combining the results from the 2 different types of replicates (fig. 9A and 9B). The resulting variability for this study's *E. coli* data:

$$1.47 \left\{ 10^{[-0.93 + 1.01 \log(C)]} \right\} \quad (3)$$

Variability determined from field replicates can be used to make an evaluation of the uncertainty in environmental *E. coli* sample data collected in the upper Santa Cruz watershed. Once the standard deviation is estimated by the above model of variability, it is assumed to represent the true standard deviation (Mueller and others, 2015) and this may provide a more meaningful measure of precision than the theoretical precision provided by the MPN confidence intervals. The replicate variability can be used to estimate the uncertainty of the concentration measured in a single environmental sample.

The uncertainty in the measured concentration ( $C$ ) can be determined by constructing the confidence interval for the true concentration:

$$[C_L, C_U] = C \pm Z_{(1-\alpha/2)} SD \quad (4)$$

where

- $C_L, C_U$  is the lower and upper limits of concentration for the  $100(1-\alpha/2)$  percent confidence interval,
- $Z$  is the percentage point of the standard normal curve that contains an area of  $100(1-\alpha/2)$  percent,
- $\alpha$  is the probability that the confidence interval does not include the true concentration, and
- $SD$  is standard deviation of the measured concentration, independently estimated from replicate variability (Mueller and others, 2015).

The second term,  $Z(1-\alpha/2) SD$ , in equation 4 represents the error inherent in a single measurement of concentration due to field variability. For example, if an *E. coli* concentration in a sample is determined to be 204.6 MPN/100 mL, and the standard deviation of this measurement can be estimated using the bias-corrected log-log model (eq. 3):

$$SD = 1.47 \{10^{[-0.93 + 1.01 \log(204.6)]}\} = 37.3 \text{ MPN/100 mL}$$

The Z-value for a 95-percent confidence interval ( $\alpha/2 = 0.025$ ) is 1.960. Thus the 95-percent confidence interval (from eq. 4) is:

$$[C_L, C_U] = 204.6 \pm 1.960 (37.3) = [132.56, 277.65]$$

The actual concentration of *E. coli* in this sample is estimated, with 95-percent confidence, to be in the range of 132 to 278 MPN/100 mL. The IDEXX Quanti-Tray® MPN table lists the 95-percent confidence interval to be within 138 and 307 MPN/100mL which is about a 14.5 percent wider confidence interval.

A measured concentration also could be compared to a water-quality standard such as the ADEQ water quality criteria standards in order to estimate the probability that the true concentration in the sample exceeded the standard (Mueller and others, 2015). In this example the full body contact (FBC) water quality criteria is set equal to the one-sided confidence limit in one of the following equations:

$$C_L = C - Z_{(1-\alpha)} SD \quad (5)$$

$$C_U = C + Z_{(1-\alpha)} SD \quad (6)$$

If  $C$  is greater than the criteria then use equation 5 and if  $C$  is less than the criteria use equation 6 (note that if  $C$  is equal to the criteria, the probability of exceedance is 50 percent.) The equation is solved for  $Z$ , and the associated  $\alpha$  value is determined from a table of  $Z$  (standard normal) scores. The probability that the standard has been exceeded is  $100(1-\alpha)$  percent for measured concentrations greater than the standard and  $100\alpha$  percent for measured concentrations less than the standard. For the example above, the measured concentration

was 204.6 MPN/100 mL and the FBC criteria is 235 MPN/100 mL. Because the measured concentration is less than the standard, equation 6 is used:  $235 = 204.6 + Z_{(1-\alpha)} (37.3)$ .

Solving for  $Z$  yields a standard-normal score of 0.8150, for which  $\alpha$  is 0.793 (from a standard normal probability table). The probability of exceedance is equal to  $100\alpha$  percent. For this example, the measured concentration of 204.6 MPN/100 mL indicates there is roughly 80 percent likelihood that the true concentration in the sample does not exceed the 235 MPN/100 mL FBC criteria.

### Additional Analytical Variability Analysis for *Escherichia coli*

There are multiple sources of variability associated with the process of analyzing an *E. coli* sample, including:

- Homogenization of the sample during shaking,
- Pouring or pipetting the correct sample volume,
- Adding reagent and properly dissolving the reagent,
- Pouring the solution into the tray,
- Incubating at a consistent temperature and length of time, and
- Properly counting the positive tray-wells.

While split replicates quantify analytical variability, we sought to further understand analytical variability with an additional analysis of multiple split replicates from one clear sample, and multiple split replicates from one turbid sample. This understanding is especially critical when suspended sediment is present in samples because of the affinity of *E. coli* to sorb to particulates.

To better understand the analytical variability of a concentration associated with a single sample, multiple split subsamples were sequentially poured (9 clear water samples) or pipetted (8 turbid water samples at a 1:10 dilution) from two samples and processed using the Colilert method. Results from the clear sample showed normally distributed concentrations with a mean and median near 25 MPN/100 mL and a standard deviation of about 5 MPN/100 mL (fig. 10A). The turbid sample concentration results were also normally distributed, with a mean and median near 300 MPN/100 mL and a standard deviation of about 56 MPN/100 mL (fig. 10B). The relative standard deviation for both sample types was around 18 percent suggesting that analytical variability is minimal and the results support a more precise confidence interval than the one reported with the MPN approach. Average 95-percent confidence intervals associated with MPN are  $\pm 11$  and 125 MPN/100 mL for the clear and turbid samples, respectively (figs 10A and 10B) whereas the 95-percent confidence intervals computed for this analysis are  $\pm 3$  and 40 MPN/100 mL for the clear and turbid samples, respectively. A final consideration in this analysis is the process of subsampling multiple times from the same bottle. There is a possibility that the prior

samples introduce bias to the subsequent sample concentration as the volume is reduced with each subsample. The order in which the samples were processed was considered and concentrations did not show a significant trend over the course of this analysis. These results lend confidence in obtaining a representative concentration from processing one or more subsamples from a 1 L sample bottle.

### Microbial Source Tracking

Two field replicates were collected for MST analysis at SC10, and another two at SC14. At both locations human markers HF183 and HPyV were below the reporting level for the environmental and replicate samples (fig. 11). The small samples sizes precluded the use of a bias-corrected log-log regression model; instead, the relative standard deviation (RSD) between each replicate pair was calculated using the following equation:

$$RSD(C_{primary}, C_{replicate}) = \left( \frac{\text{Standard Deviation}(C_{primary}, C_{replicate})}{\text{Mean}(C_{primary}, C_{replicate})} \right) * 100, (7)$$

where

$RSD(C_{primary}, C_{replicate})$  is the relative standard deviation between the concentration determined in the primary sample relative to the replicate sample concentration,

$C_{primary}$  is the concentration of the primary sample, and  $C_{replicate}$  is the concentration of the replicate sample.

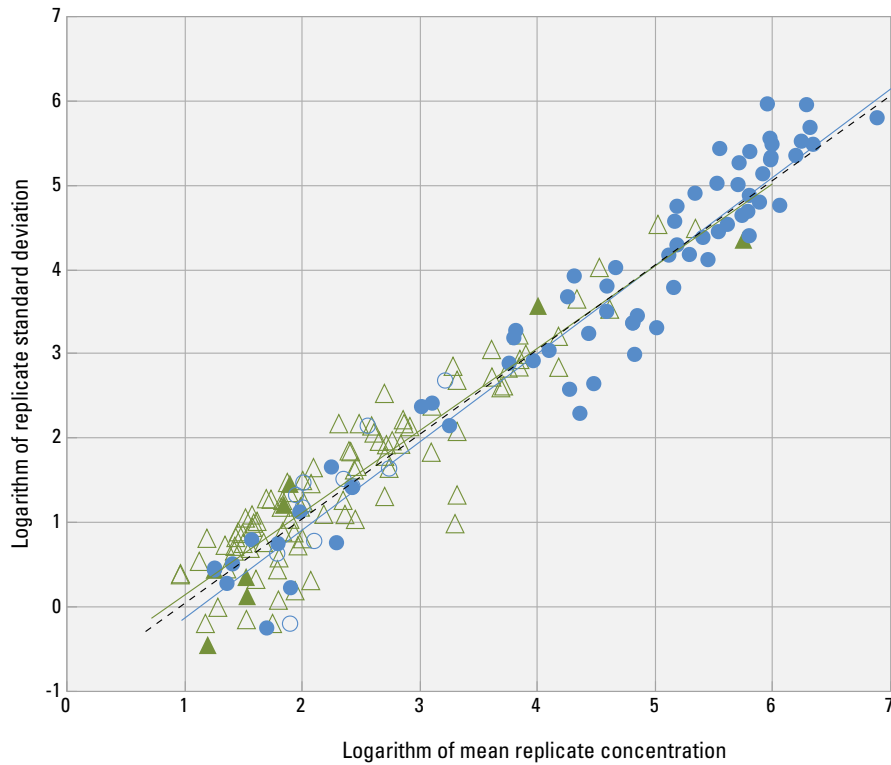
The general and ruminant markers had greater RSDs for the SC14 base flow sample (110 and 113 percent, respectively) than the flood sample at SC10 (33 and 34 percent, respectively), but the concentrations were roughly 1 to 2 orders of magnitude greater. Cattle and canine markers were present in the SC10 sample and had RSDs in concentrations of 55 and 27 percent, respectively.

### Suspended Sediment

Five sequential suspended sediment replicates were collected to examine environmental and analytical variability of suspended sediment concentrations. The median RSD of the sequential replicates was 1.3 percent (fig. 12). The low median sequential RSD supports representative and well-mixed water during flooding conditions and repeatability in the sediment analysis.

### Suspended-Sediment Sample Reference Samples

The CVO participates in the branch of quality systems (BQS) sediment laboratory quality assurance project. The BQS sends known quantities of fines, sand, and sediment in a solution to the participating laboratories. The CVO analyzes the samples and returns a report with water volume, weights, and suspended-sediment concentrations (SSC). The BQS

**A****EXPLANATION****Split Replicates**

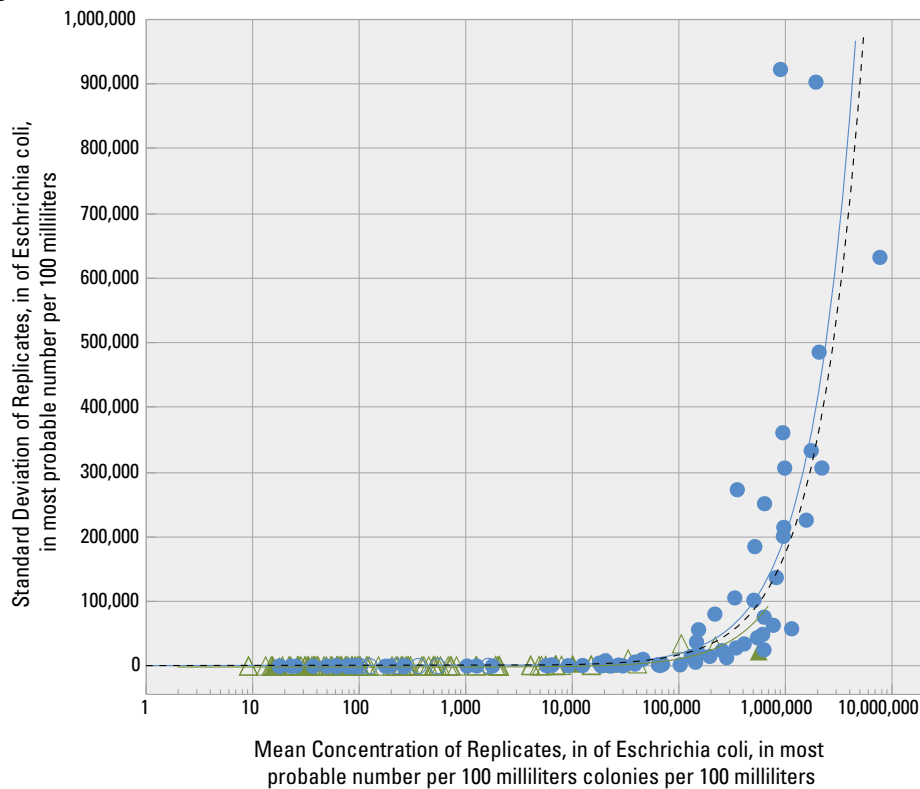
- USGS
- FOSCR/NPS
- $-1.15 + 1.05\log(\text{mean concentration})$

**Sequential Replicates**

- ▲ USGS
- △ FOSCR/NPS
- $-0.83 + 0.98\log(\text{mean concentration})$

**All Replicates**

- $-0.93 + 1.01\log(\text{mean concentration})$

**B****EXPLANATION****Split Replicates**

- USGS
- FOSCR/NPS
- $1.50\{10^{-1.15 + 1.05\log(\text{mean concentration})}\}$

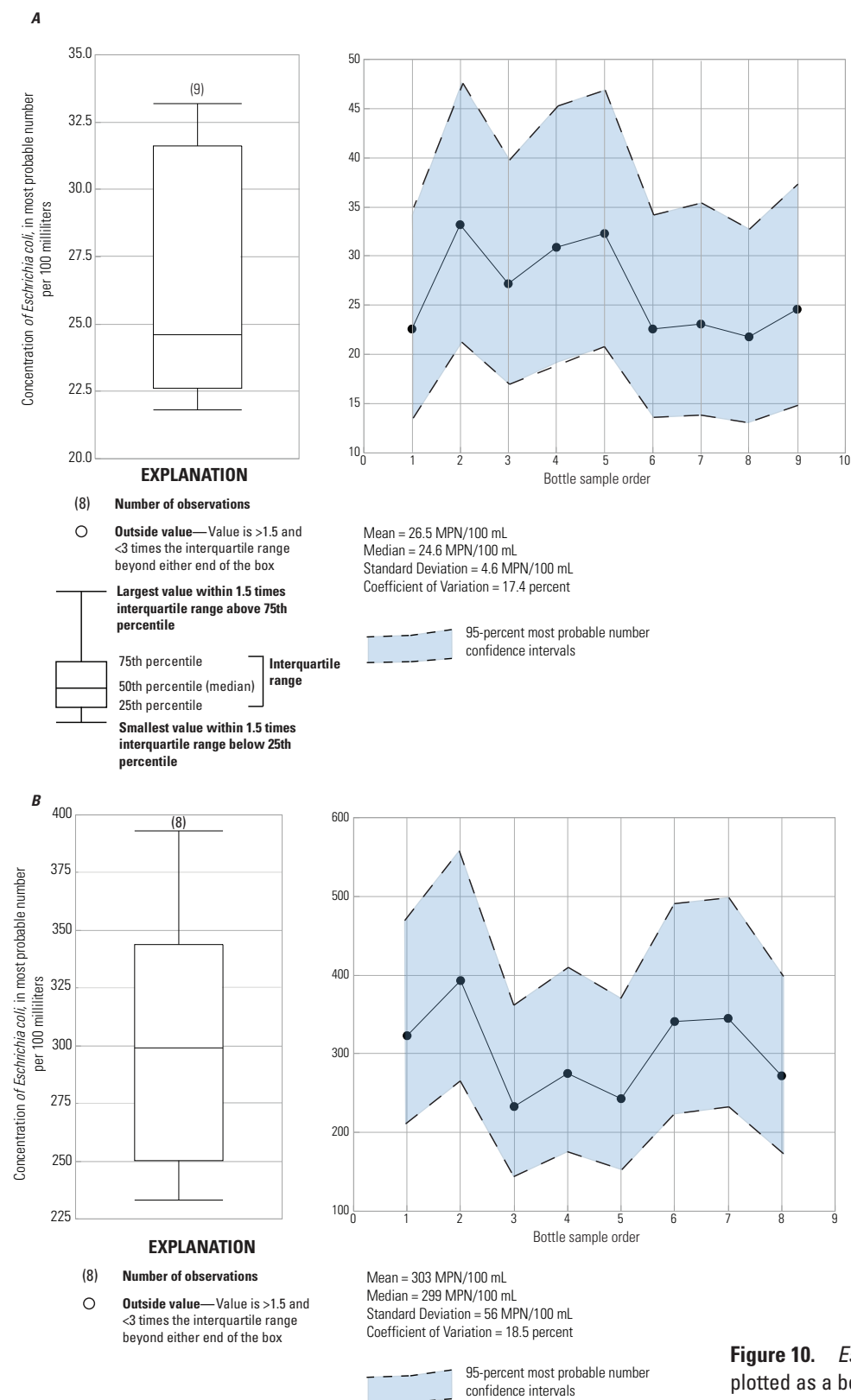
**Sequential Replicates**

- ▲ USGS
- △ FOSCR/NPS
- $1.41\{10^{-0.83 + 0.98\log(\text{mean concentration})}\}$

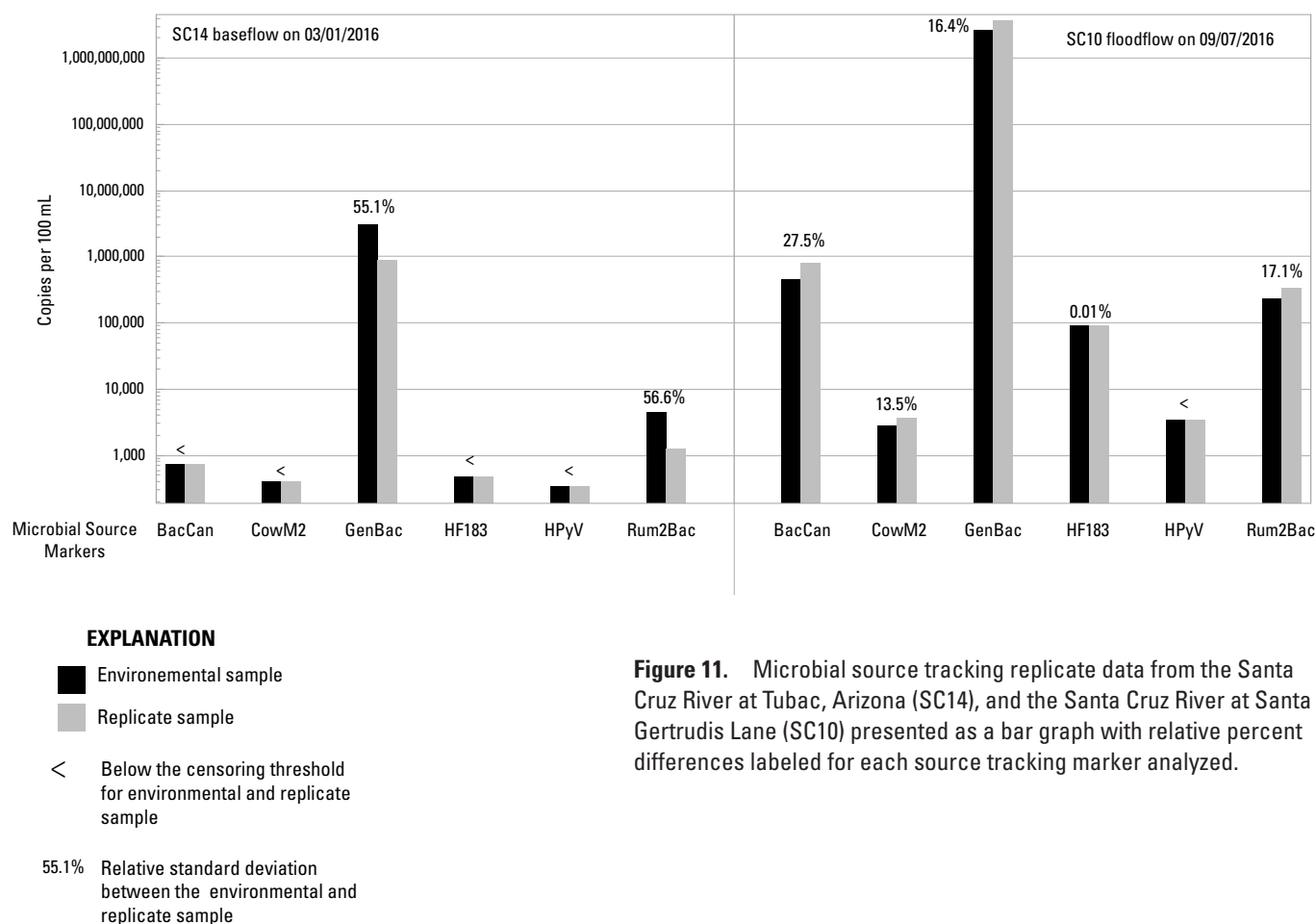
**All Replicates**

- $1.47\{10^{-0.93 + 1.01\log(\text{mean concentration})}\}$

**Figure 9.** Plots of *A*, the logarithm of replicate standard deviation versus logarithm of mean replicate concentration for different replicate types: *B*, mean concentration of replicates versus standard deviation of replicate concentrations, for samples from Tumacácori National Historical Park and the upper Santa Cruz River watershed. USGS, U.S. Geological Survey; FOSCR, Friends of the Santa Cruz River; NPS, National Park Service.



**Figure 10.** *Escherichia coli* (*E. coli*) concentrations plotted as a boxplot and as point data with confidence intervals from a discrete sampled multiple times from Santa Cruz River at Santa Gertrudis Lane (SC10); collection consisted of *A*, one clear surface water base flow; and *B*, one turbid pore water sample. MPN/100 mL, most probable number per 100 milliliters.



**Figure 11.** Microbial source tracking replicate data from the Santa Cruz River at Tubac, Arizona (SC14), and the Santa Cruz River at Santa Gertrudis Lane (SC10) presented as a bar graph with relative percent differences labeled for each source tracking marker analyzed.

2015–2016 results can be found at [https://bqs.usgs.gov/slqa/frontpage\\_study\\_results.htm](https://bqs.usgs.gov/slqa/frontpage_study_results.htm). Historical data (2008 to 2016) for the reference samples show median percent differences ( $PD = ((\text{actual-reported})/\text{actual}) \times 100$ ) near zero, but biased slightly negative for all measures except sand, indicating on average the CVO is reporting concentrations that are biased slightly high, although the mean PD is less than 1 percent for fines mass, total mass, and SSC (fig. 12). This investigation only analyzed for SSC and the historical data interquartile range for the percent differences is 2.73 percent, meaning that 50 percent of the 144 samples analyzed for SSC were within a 3 percent band between -1.86 and 0.87 PD. These results indicate that CVO is meeting the SSC quality standards of the project.

### *Escherichia coli* Holding Time

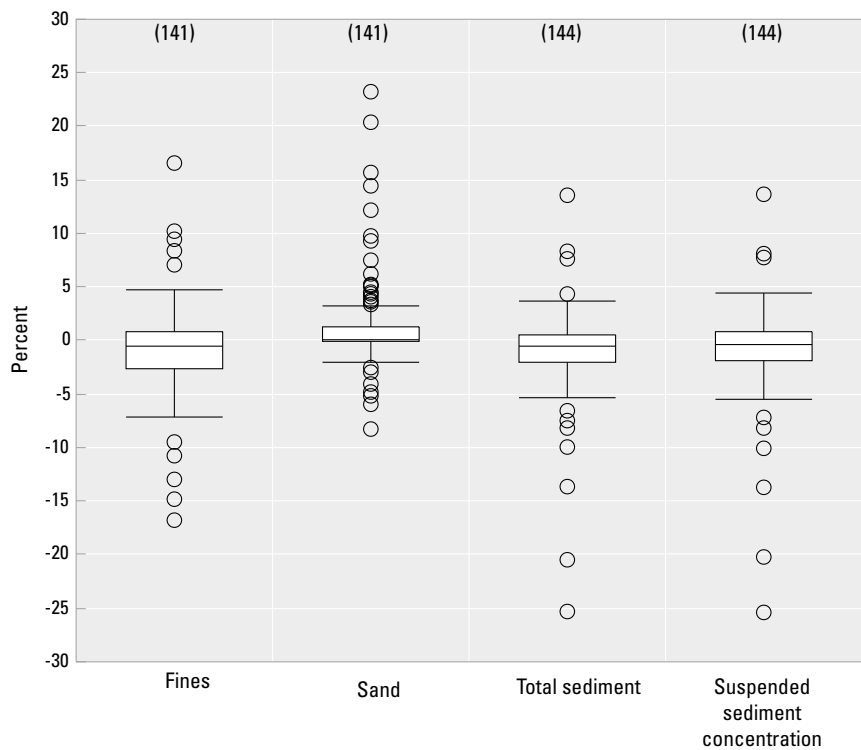
Hold times specified for *E. coli* are 6 hours in nonpotable water for compliance and 24 hours in water for noncompliance purposes (U.S. Environmental Protection Agency, 2007; American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1999). The EPA specifies 8 hours for source water compliance samples, and 30 hours for drinking water samples (U.S. Environmental Protection Agency, 2013). Multiple studies

have confirmed that hold times beyond 6–8 hours still produce quality data, though there is not agreement on the upper limit of an extended hold time. The conditions in which the sample was collected, the environmental matrix, magnitude of the concentration, and storage temperature are all factors in defining the hold time upper limit.

Pope and others (2003) investigated whether holding time and storage conditions affected *E. coli* densities in surface water using samples from 24 locations in the United States. Colilert was one of several methods used to compare storage-temperature and hold-time effects. For most of the locations where samples were stored at 4°C, the *E. coli* density showed no significant difference between time 0 and 48 hours. For locations where significant declines were observed, this occurred generally at 30 and 48 hour holding periods. Pope and others (2003) concluded that most surface water *E. coli* samples analyzed up to 48 hours after sample collection could generate *E. coli* data comparable to those generated within 8 hours of sample collection.

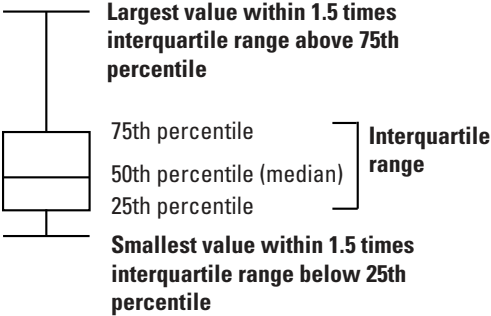
A study conducted by Aulenbach (2009) suggested that holding times greater than 18.5 hours might call results into question. However, the time series of *E. coli* concentrations presented from the 5 locations sampled in Aulenbach's study showed relatively no trend with time and is stated as such, and the inconsistent relation of the regular data with respect





EXPLANATION

- (8) Number of observations
- Outside value—Value is >1.5 and <3 times the interquartile range beyond either end of the box



**Figure 12.** Cascade Volcano Observatory laboratory results from the branch of quality systems reference samples graphed as boxplots of the percent difference of the actual and reported substrate weights and concentration. Samples were analyzed for the mass weight of fines, sand, total sediment, and the concentration of suspended sediment.

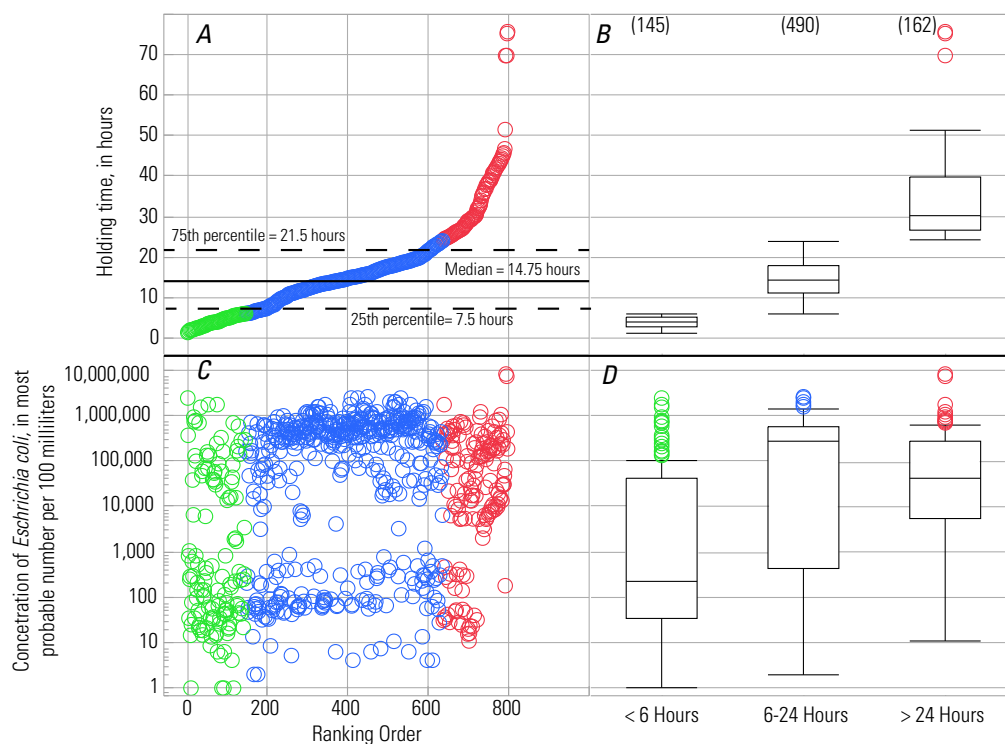
to the replicate data further suggest high variability that might confound a clear trend. Furthermore, after 27 hours (3 locations) and 48–60 hours (2 locations) the concentrations appear to be statistically indistinguishable from the initial concentrations (no significant differences in the nonparametric pair-wise tests). Statistical assumptions should be defined on the basis of sample population structure and precaution should be taken when conducting pair-wise statistical tests on small samples sizes (number of samples of 2 to 4). A visual assessment (considering the MPN confidence intervals) of the time series presented suggests that conclusions similar to Pope and others (2003) could be interpreted about *E. coli* concentrations observed in the Aulenbach study.

Bushon and others (2015) conducted a holding time comparison study for groundwater samples and showed that extending the holding time from within 8 hours to between 18 and 30 hours did not reduce the number of detections of fecal-indicator bacteria (total coliforms, *E. coli*, and enterococci). In addition, more detections were made in samples analyzed within 18 to 30 hours using quantitative methods than samples processed within 8 hours using presence/absence methods. A common observation in several holding time studies, including the one conducted in this investigation, is an increase in the concentration above the initial quantification at some point in the holding time-series analysis.

Other holding time studies using plating techniques have also shown that the 6 to 8 hour holding time could be extended

without comprising the results (Selvakumar and others, 2004; Pertunia, 2016). Completing the analysis within the recommended 6 to 8 hour holding time has the clear advantage of being within EPA compliance and should be followed when possible. However, completing analysis within the 6 to 8 hour holding time is not always realistic, especially in remote locations or streams that experience rapid water-stage changes and therefore require collection by automatic samplers. As demonstrated by the studies discussed, holding time extensions are supported by several studies, but it is also clear that bacterial indicator results are naturally variable for reasons that are likely site and study specific, such as temperature, particulates, sediment, concentration, and other chemical constituents (for example, chlorine). For every study, efforts should be taken to demonstrate holding time effects as they apply to delays in the analysis of bacteria indicators.

In this study, the driving distance to the study area and off-hour collection of samples (nights and weekends) by the automatic sampler resulted in many samples exceeding the 6 and 24 hour holding times (fig. 13). A majority of the new USGS samples collected for this study exceed the 6 hour holding time, and about 20 percent exceeded the 24 hour threshold. The overall median holding time was 14.75 hours. Generally, the concern with holding a sample past the recommended timeframe is the reduction in *E. coli* densities. This study attempted to quantify the effect from various holding times. Base flow samples were collected at two sites and a Colilert test was conducted every day for 4 days past the original analysis to understand how sample concentrations change with different holding times. In addition, flooding samples were collected at three sites and a Colilert test was conducted every day for 3 days past the original analysis. Samples were kept in

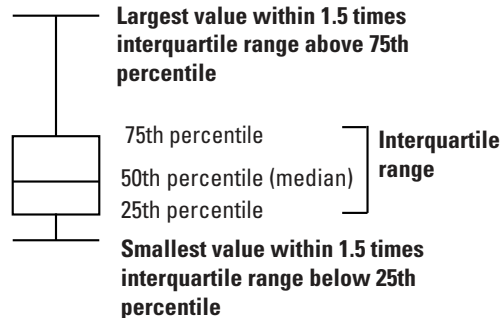


## EXPLANATION

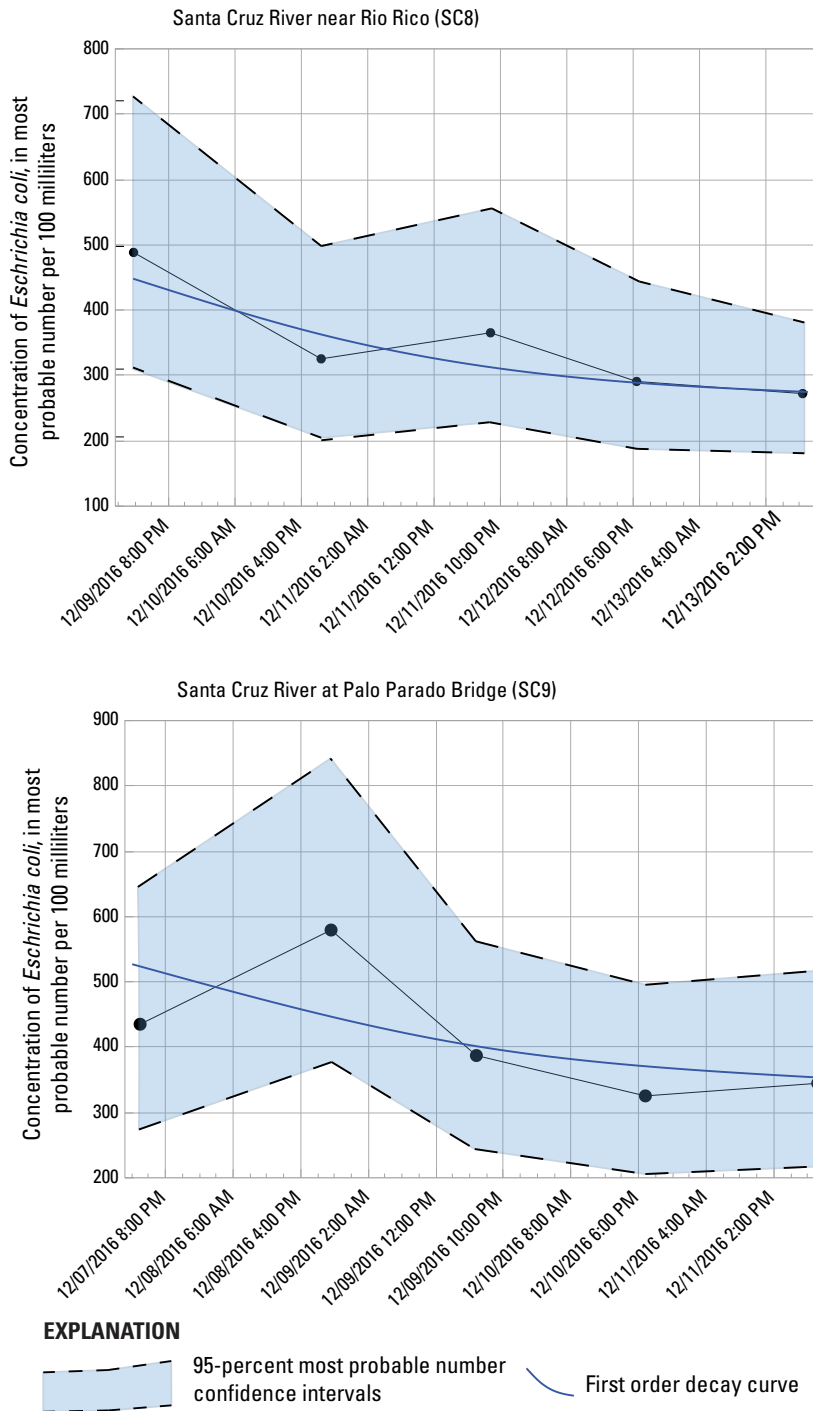
### (8) Number of observations

○ Outside value—Value is >1.5 and <3 times the interquartile range beyond either end of the box

Largest value within 1.5 times interquartile range above 75th percentile



**Figure 13.** Plots of A, holding time quantile distribution plot colored by holding time category; <6 hours (green); 6-24 hours (blue); and >24 hours (red); B, boxplots of holding times grouped by the quantile distribution holding time quartile thresholds; C, *Escherichia coli* (*E. coli*) concentrations ranked and colored by holding time category; and D, boxplots of *E. coli* concentrations grouped by holding time category.



**Figure 14.** *Escherichia coli* (*E. coli*) concentrations plotted as time series (with most probable number confidence intervals) from base flow samples resampled over 5 days; samples were collected from Santa Cruz River near Rio Rico (SC8) and the Santa Cruz River at Palo Parado (SC9).

a refrigerator maintained at 6–8°C. Confidence intervals (CI) were applied to concentration estimates by the MPN methods according to a tabulation of 95-percent CI provided by the manufacturer (IDEXX, 2013).

Base flow samples were collected at SC8 and SC9 and analyzed over four days. Both samples showed a decrease in *E. coli* concentrations with time and (fig. 14) at similar rates from the initial concentration to the last date concentration. The mean decrease (linear regression) is about 50 MPN/100 mL per day or approximately 8 to 10 percent decrease per day.

Using a first-order decay rate, which may better describe *E. coli* die-off over time shows about 80 to 90 MPN/100 mL on the first day, then the rate decreases slightly more than half of that each subsequent day or approximately 10 to 15 percent the first day and to 2 to 5 percent decrease by day 4 (fig. 14). While there is evidence between the two sites to suggest a similar holding time effect, this test needs to be repeated to reduce the uncertainty of the MPN CI. If the CI are taken into account, the magnitude of the decrease over time is less certain due to error associated with MPN calculation.

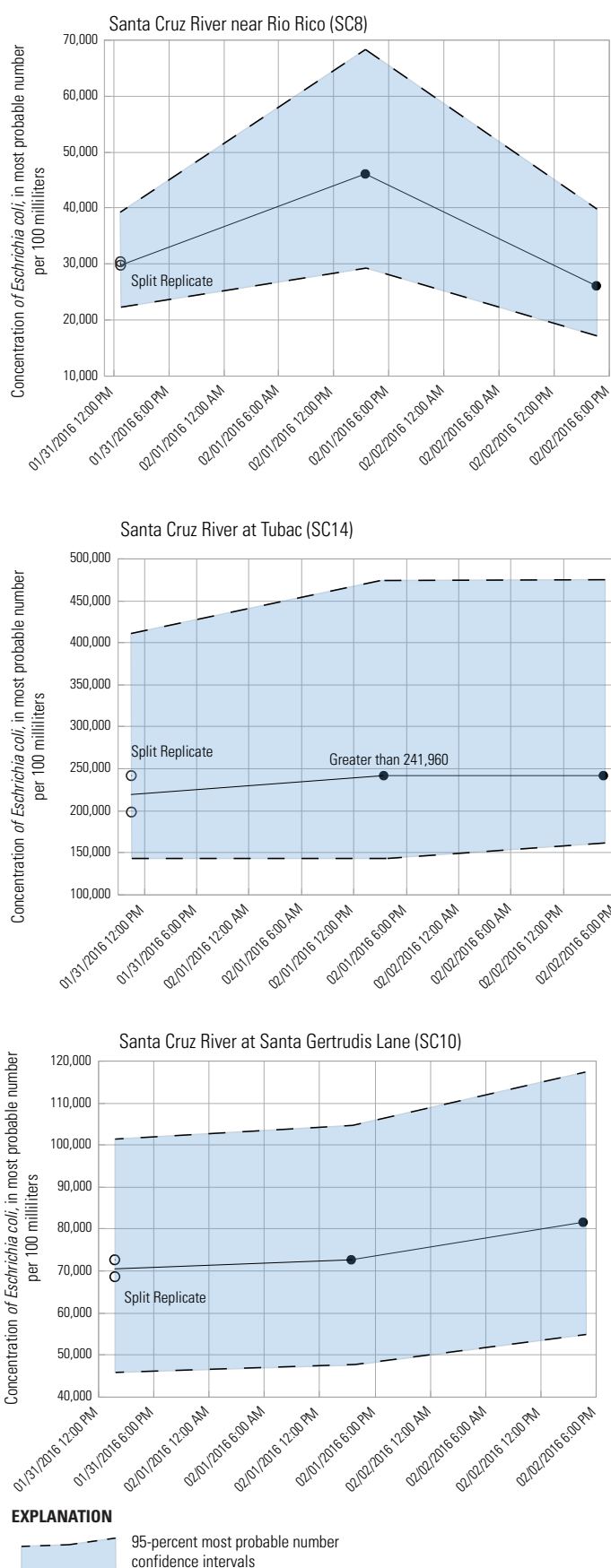
Flooding samples were collected from SC8, SC9, and SC14 during a late January, 2015 runoff event and analyzed over three days (fig. 15). The flooding samples required a 1:100 dilution for each Colilert test. All three samples were initially analyzed after a 24 hour hold time, then analyzed again on the second and third days after sample collection. Concentrations increased in all samples in the second day analysis, then decreased, increased, and remained the same in the third day analysis for each respective sample. If the CI are considered then there is no significant difference between the three sample concentrations for any of the sites. If concentrations are decreasing over time, the accurate quantification of that decrease remains challenging due to the variability associated with the method and MPN approach, especially for very turbid samples that needed to be diluted.

Based on previous studies of *E. coli* holding times and the limited holding time analysis completed as part of this study, the bacteria data analyzed from samples analyzed past the 6 to 24 hours holding times are considered acceptable. In addition, concentrations of *E. coli* during these extended holding times often exceeded concentrations where a decrease in concentration would be indecipherable from the inherent variability in the environmental data previously determined from the replicate quality-control data.

## Summary

Tumacácori National Historical Park is interested in protecting and promoting the integrity of the Santa Cruz River and its water quality because the River provides ecosystem services to visitors and the surrounding community. This report describes the methods and quality-assurance procedures used in the collection of water samples for the analysis of *E. coli*, microbial source tracking, suspended sediment, water-quality parameters, turbidity, and other flow-related data. Beyond describing methods and data integration approaches, this report also provides a quantitative assessment of the quality of the *E. coli*, microbial source tracking, and suspended sediment data.

Bias from contamination or using different sample collection methods was determined to have no consequential effects to the larger environmental dataset. Variability introduced through the analytical process and environmental conditions was quantified and determined to be more precise than the theoretical confidence intervals provided with the MPN method. A single bias-corrected equation was developed to quantify analytical variability and provide a means to estimate confidence intervals and the probability



**Figure 15.** *Escherichia coli* (*E. coli*) concentrations plotted as time series (with confidence intervals) from flood samples resampled over 3 days; samples were collected from the Santa Cruz River near Rio Rico (SC8), Santa Cruz River at Tubac (SC14), and Santa Cruz River at Santa Gertrudis Lane (SC10).

of exceeding water quality criteria. Results from the quality-assurance analysis support the use and integration of other agency data with USGS data. Lastly, the extended holding times were summarized and analyzed to determine a rate of decay. Findings from this analysis and a literature review suggest that extended holding times, especially for samples with elevated concentrations, have a minimal effect on the overall concentration or vary in the range of the MPN confidence intervals.

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