MONITORING REPORT

Upper Willapa River Microbial Source Tracking Study

Prepared for

Cosmopolitan Engineering Group

and

Pacific County Department of Community Development

June 2005

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Prepared for

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June 24, 2005

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Introduction

The Willapa River, located in Pacific County, Washington is listed on the Washington Department of Ecology's (Ecology's) 303(d) list of threatened and impaired water bodies for fecal coliform bacteria, dissolved oxygen, and temperature (Ecology 2005a online). Studies of total maximum daily loads (TMDLs) are currently being conducted for these parameters to identify sources of pollution and recommend pollutant controls in the Willapa River watershed.

Conducted here is a microbial source tracking (MST) study that uses a genetic fingerprinting (molecular ribotyping) technique to identify sources of fecal coliform bacteria in the upper Willapa River. The results of this MST study will be used by the North Pacific County Infrastructure Action Team (NPCIAT) to establish source control goals and best management practices targeting specific sources of bacteria in the watershed.

The molecular ribotyping technique has been successfully used for many other studies to identify fecal sources in watersheds throughout western Washington (Herrera 1993, 1999, 2001, 2004) and is considered by Ecology to be an effective investigative technique (Ecology 1999). This MST method isolates pure cultures of *Escherichia coli (E. coli)*, (the most common member of the fecal coliform bacteria group) from samples of receiving waters and suspected sources (i.e., human sewage, industrial effluent, and fecal matter from warm-blooded animals). The *E. coli* cultures obtained are then genetically typed and compared to a library of unique *E. coli* genotypes to determine the presence of specific sources. Sources of other bacteria in the fecal coliform group (e.g., *Klebsiella*, *Enterobacter*, and *Serratia*) or other enteric bacteria (e.g., *Enterococcus*) are not determined with this method.

This document summarizes the findings of the upper Willapa River Microbial Source Tracking Study that was conducted from November 2003 through November 2004. The report that follows is organized into separate sections including: background information; project description; methods; results and discussion; and conclusions.

Background Information

Background information for the upper Willapa River MST study is presented here that includes a description of the Willapa River watershed and a summary of previous monitoring results for fecal coliform bacteria. The Willapa River watershed is briefly described in terms of land use and topography, climate and hydrology, and habitat and water quality. Fecal coliform bacteria data from Ecology's ambient monitoring program and recent fecal coliform TMDL survey are also presented.

Watershed Description

Land Use and Topography

The Willapa River is located in water resource inventory area (WRIA) 24 in Pacific County, Washington (Figures 1 and 2) and drains an area of approximately 260 square miles. The Willapa River basin is largely rural with the exception of the cities of Raymond and South Bend on the lower river, both of which operate municipal wastewater treatment facilities. There are several small towns, including Lebam, along the upper river. Residences in the upper river are not connected to municipal treatment facilities and rely on the use of onsite wastewater treatment facilities for the disposal of household sanitary waste. Principal industries in the cities are timber and seafood (mostly oysters). Agriculture, including dairy operations, is the primary land use in the upper river valley, and silviculture is the main use throughout the rest of the basin (Ecology 1998) (Figure 2).

The landscape in the Willapa River basin ranges from marshes, grasslands, farmlands, and low hills along the lower river to farmland, mixed deciduous forests, and low to moderate elevation hills along the upper river. At its confluence with Mill Creek at River Mile (RM) 18.1, the main stem of the Willapa River changes from a moderate gradient in the lower reaches to a high gradient in the upper reaches (WDF 1975). Elevations in the Willapa River basin range from sea level near the mouth, to 40 feet above sea level near the city of Raymond (RM 7), to 200 feet above sea level near the town of Lebam (RM 34), to greater than 2,000 feet above sea level near the headwaters (RM 43). Ecology distinguishes between the lower reach, below RM 17.7, and the upper reach of the Willapa River, near its confluence with Mill Creek. The upper Willapa River basin comprises approximately 60 percent of the entire river basin.

Climate and Hydrology

The climate of the basin is heavily influenced by its proximity to the ocean. The growing season lasts 180 days on average, with cool, wet winters and mild summers. Annual precipitation ranges from 80 inches in the lower river valley to 120 inches in the higher elevations. The heaviest rainfall occurs between October and June, but the lower watershed receives an average of 3 inches of rain per month during the summer (Ecology 2001b).

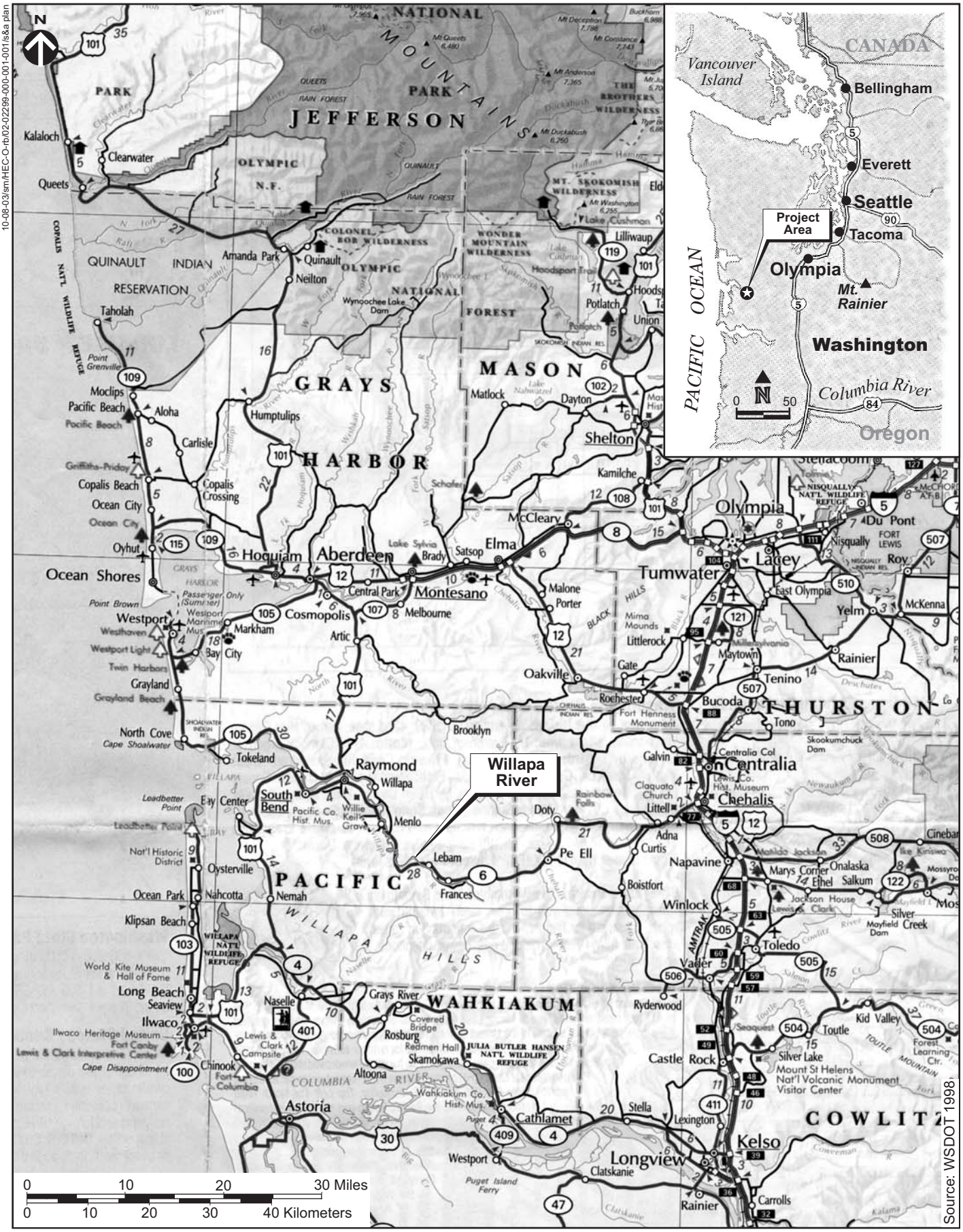

Figure 1. Vicinity map of the Willapa River Microbial Source Tracking Study, Pacific County, Washington.

10-08-03/sm/HEC-O-fb/02-02299-000-001-001/s&a plan

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Figure 2. Land use map of water resource inventory area (WRIA) 24 and the Willapa River.

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11 X 17 color

2nd page for color Figure 2

The hydrologic sources for freshwater streams in the Willapa River basin are precipitation, runoff, and ground water inputs. Because there are no glaciers in the headwater regions of this basin, precipitation plays an important role in water levels during the summer months. The lower Willapa River is a tidal estuary, characterized by mixing of marine water from Willapa Bay with freshwater from the river and other tributaries. Saline marine water moves 10 miles or more up the river, and the tidal effects on water levels in the river can be observed near Camp One Road at RM 14.5 (Ecology 1998). Numerous sloughs and streams contribute flows to the Willapa River, including (from downstream to upstream) Johnson Slough, the South Fork Willapa River, Elk Creek, and Wilson Creek in the lower Willapa River, along with Mill Creek, Trap Creek, Fork Creek, and Fern Creek in the upper Willapa River (WDF 1975).

The U.S. Geological Survey operates a continuous streamflow gauging station on the Willapa River at Camp One Road at RM 17.5 (USGS Station 12013500, Willapa River near Willapa, Washington). The Willapa River experiences a wide range of flows, ranging from less than 20 cubic feet per second (cfs) to more than 10,000 cfs (Ecology 1998). Low flows typically occur in August, and high flows occur between November and March. Average summer flows are typically less than 100 cfs, and average winter flows exceed 400 cfs.

Habitat and Water Quality

The Willapa River and its tributaries support fall chinook salmon, coho salmon, fall chum salmon, and winter steelhead trout. Major tributaries known to support salmon in the lower river include the South Fork Willapa River, Wilson Creek, and Ellis Slough. The lower Willapa River is tidally influenced, with marsh grass habitat existing in the side sloughs that provides important rearing and transitional habitat for chinook and chum salmon (WSCC and NWIFC 1999). Very little spawning habitat is present in the main stem below RM 7.0 (WDF 1975).

The upper Willapa main-stem serves as spawning habitat for chinook, chum, coho, and steelhead. In the upper river, important salmon-producing tributaries include Mill Creek, Stringer Creek, Trap Creek, and Forks Creek. A Washington Department of Fish and Wildlife salmon hatchery is located on Forks Creek, rearing and releasing fall chinook and coho salmon. Habitat-limiting factors for salmon and trout in the Willapa River basin include lack of large woody debris, sedimentation from bank instability and road runoff, and poor riparian conditions (WSCC and NWIFC 1999).

Based on data collected by Ecology, water quality in the Willapa River does not meet state standards for fecal coliform bacteria, dissolved oxygen, and temperature (Ecology 2000). The Willapa River is on the Ecology 1998 303(d) list and 2004 candidate 303(d) list of threatened and impaired water bodies in terms of fecal coliform bacteria, dissolved oxygen, and temperature (Ecology 2005a online). The Willapa River is designated as primary contact recreation by Washington State water quality standards (WAC 173-201A). The fecal coliform bacteria standard for primary contact recreation is that the geometric mean must not exceed 100 colony forming units (CFU)/100 mL with not more than 10 percent of the samples (i.e., 90th percentile) exceeding 200 CFU/100 mL.

The 2004 candidate 303(d) list specifies 10 segments of the main stem that are listed for fecal coliform bacteria, including sections near South Bend and Raymond, below the confluence with Mill Creek, near Lebam, below Falls Creek, and at the confluence with Fern Creek. Willapa Bay near the mouth of the Willapa River is also listed for fecal coliform bacteria. Two tributaries to the Willapa River, Half Moon Creek and Falls Creek, are also listed for fecal coliform bacteria. Ecology has initiated TMDL studies of fecal coliform bacteria, dissolved oxygen, and temperature for the Willapa River basin.

Fecal Coliform Bacteria Monitoring

Ecology has intermittently collected ambient water quality data at two upstream (freshwater) locations on the upper Willapa River since 1959 (Ecology 2005 online). Table 1 shows this station identification and period of record for these two stations (which also were used for the MST study). These two stations include the Willapa River near Willapa and the Willapa River at Lebam. Ecology is currently monitoring the station near Willapa but has not monitored the station at Lebam since 1992.

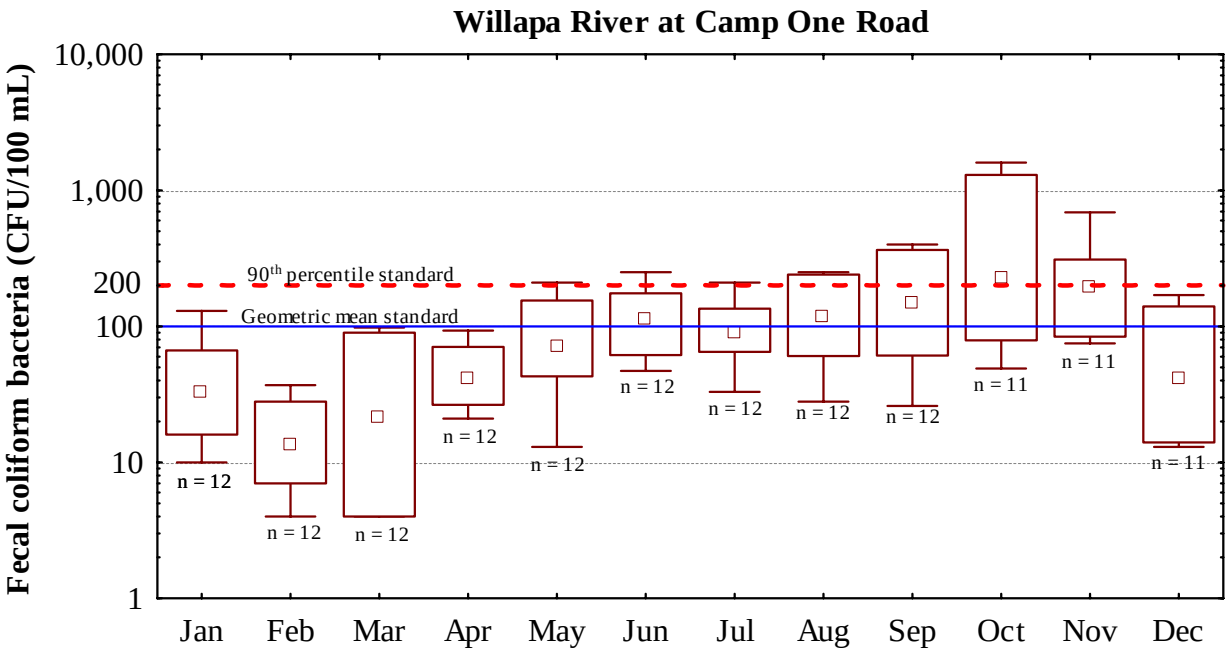
Table 1. Ecology ambient monitoring (freshwater) stations on the upper Willapa River.

Station Name	Station ID	Location	Period of Record
Willapa River near Willapa [a]	24B090	RM 17.5	1966-1967; 1969-1974;1976-1992; 1995-present
Willapa River at Lebam	24B130	RM 33.2	1959-1961; 1969, 1979-1992

[a] Also known as Willapa River at Camp One Road.
RM = river mile.

Fecal coliform bacteria data for monthly grab samples collected from the Ecology ambient monitoring station on the Willapa River near Willapa (also known as Willapa River at Camp One Road) were compared to the state water quality standard for fecal coliform bacteria (i.e., geometric mean must not exceed 100 colonies/100 mL and 90th percentile must not exceed 200 colonies/100mL). Based on monthly data collected during 12 years since 1990 (i.e., water years 1991, 1992, and 1995 through 2004 because no data were collected in 1993 and 1994), the levels of fecal coliform bacteria exceeded the water quality standard during the months of May through November (Figure 3). These results suggest that fecal sources to the river are greatest during the summer and fall.

Ecology also collected water quality data from the Willapa River and major tributaries during 12 surveys in 1998 as part of the Willapa River TMDL study of dissolved oxygen and fecal coliform bacteria (Ecology 2000). The entire main stem of the Willapa River was studied, from the mouth near Johnson Slough upstream to the headwaters below Patton Creek. Major tributaries were also included in the surveys. Water quality data were collected for 19 parameters, and flow measurements were recorded. Figure 4 shows the locations of over 40 sampling stations that have been monitored by Ecology. Table 2 summarizes the fecal coliform bacteria results for the Ecology study.



Source: Ambient (monthly) monitoring conducted by the Washington State Department of Ecology for water years 1991, 1992, and 1995 through 2004 (Ecology 2005 online).

Legend: Symbol = geometric mean; Box = 25th and 75th percentile; Whisker = 10th and 90th percentile

Figure 3. Fecal coliform bacteria concentrations by month from the Willapa River at Camp One Road for water years 1991, 1992, and 1995 through 2004.

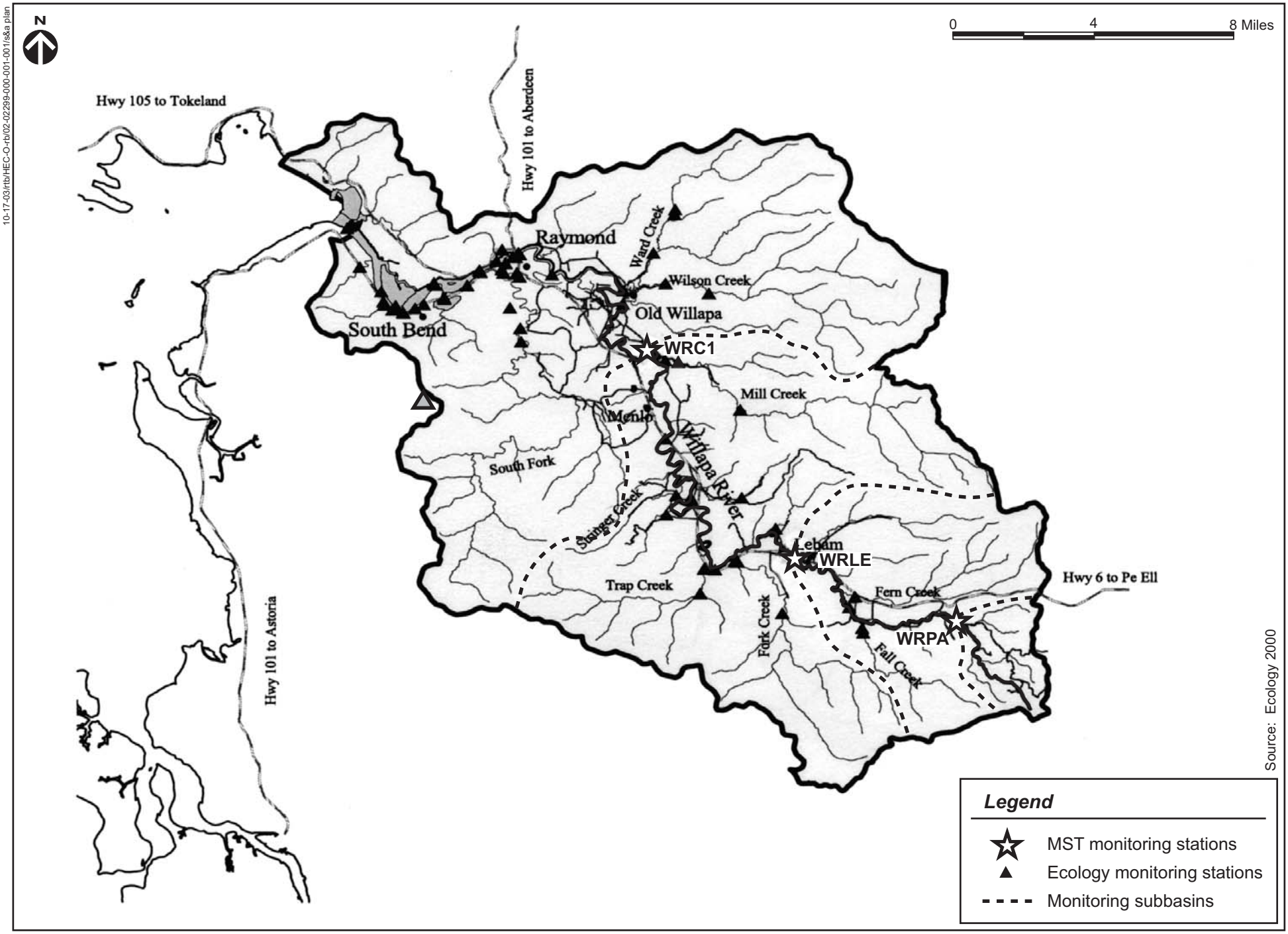


Figure 4. Sampling stations on the Willapa River, Pacific County, Washington.

Table 2. Compliance with fecal coliform bacteria standards at Ecology monitoring sites during the Willapa River TMDL study in 1998.

Main Stem River Mile	Tributary River Mile	Sampling Station	Site Code	Highest 60-Day 90th Percentile			Highest 60-Day Geometric Mean			Meets Standard?
				Value [a]	Date	Standard [a]	Value [a]	Date	Standard [a]	
Upper Willapa River										
41.20		Willapa River below Patton Creek	WRPA	64	July	200	39	May-Jul	100	yes
37.50	0.30	Falls Creek above Retreat Center	FALLS	**294**	November	200	58	Sep-Nov	100	**NO**
37.10		Willapa River at Swiss Picknik Rd	WRSW	**790**	July	200	**522**	Jul-Sep	100	**NO**
36.20	0.40	Fern Creek at Elk Prairie Rd	FERN	**720**	Early May	200	**446**	May-Jul	100	**NO**
33.20		Willapa River at Lebam	WRLE	**540**	Late May	200	**344**	May-Jul	100	**NO**
30.50	0.25	Fork Creek at State Hatchery	FORK	**730**	October	200	**166**	Sep-Oct	100	**NO**
30.00		Willapa River above Trap Creek	WRTR	160	August	200	**104**	Jun-Aug	100	**NO**
29.90	0.15	Trap Creek above Hwy 6	TRAP	63	August	200	45	Aug-Sep	100	yes
25.20		Willapa River at Oxbow Rd	WROX	**365**	Late May	200	**219**	May-Jul	100	**NO**
24.20	0.50	Stringer Creek at Highland-Stringer Rd	STRINGER	69	October	200	42	Aug-Sep	100	yes
21.40		Willapa River at SR 6 near Menlo	WRMN	**580**	Late May	200	**397**	May-Jul	100	**NO**
17.90	0.30	Mill Creek at first Mill Creek Rd bridge	MILLCK	194	June	200	**152**	Jun-Aug	100	**NO**
17.50		Willapa River at Camp One Rd	WRC1	**330**	July	200	**141**	Jul-Sep	100	**NO**
Lower Willapa River										
13.70		Willapa River at Willapa Rd	WRWI	**389**	Late May	200	94	Oct-Dec	100	**NO**
12.00	0.9/1.2	Ward Creek below Fairchild Creek	WARD	85	Late Sept	200	51	Sep-Oct	100	yes
12.00	1.45	Wilson Creek at first Weyco Bridge	WILSUP	140	Late Sept	200	51	Sep-Oct	100	yes
12.00	0.10	Wilson Creek near Willapa	WILSON	**240**	Late May	200	**127**	May-Jul	100	**NO**
7.70		Willapa River at Hwy 101 Bridge	WRHY	**417**	Late May	200	60	Oct-Dec	100	**NO**
7.20	0.40	Riverdale Creek at Lions Club Park	RAYSW-3	**300,000**	July	200	**35,088**	Jun-Aug	100	**NO**
7.10	4.20	South Fork Willapa River at Golf Course Rd	SFRK-F	185	August	200	**130**	Jul-Sep	100	**NO**
7.10	0.80	Drain at Bruckenhaus	RAYSW-5	**82,000**	June	200	**11,615**	May-Jul	100	**NO**
7.10	0.30	Raymond SW drain north of PHW	RAYSW-6	**302**	November	200	94	Oct-Dec	100	**NO**
6.40		Willapa River at Raymond (near Port)	WRRA	**464**	Late May	200	62	May-Jul	100	**NO**
5.90	0.10	Raymond SW at Delaware St.	RAYSW-2	**1,300**	Late May	200	**312**	Apr-Jun	100	**NO**
5.30	0.60	Skidmore Slough above tide gate	RAYSW-1	170	June	200	**105**	Jul-Sep	100	**NO**
5.00		Willapa River at the Narrows	WRNA	**529**	Late May	200	55	May-Jul	100	**NO**
3.75	0.00	South Bend SW pipe at SB Packers	SBSW-3	**10,000**	August	200	**1889**	Jul-Sep	100	**NO**
3.40	0.00	South Bend SW pipe near Gardner's	SBSW-1	**410**	November	200	**371**	Oct-Dec	100	**NO**
3.10	0.00	Creek at Coast Seafoods	SBSW-2	**3,800**	December	200	**1,755**	May-Jul	100	**NO**
0.40		Willapa River at Johnson Slough	WRJS	**94**	November	43	**16**	May-Jul	14	**NO**

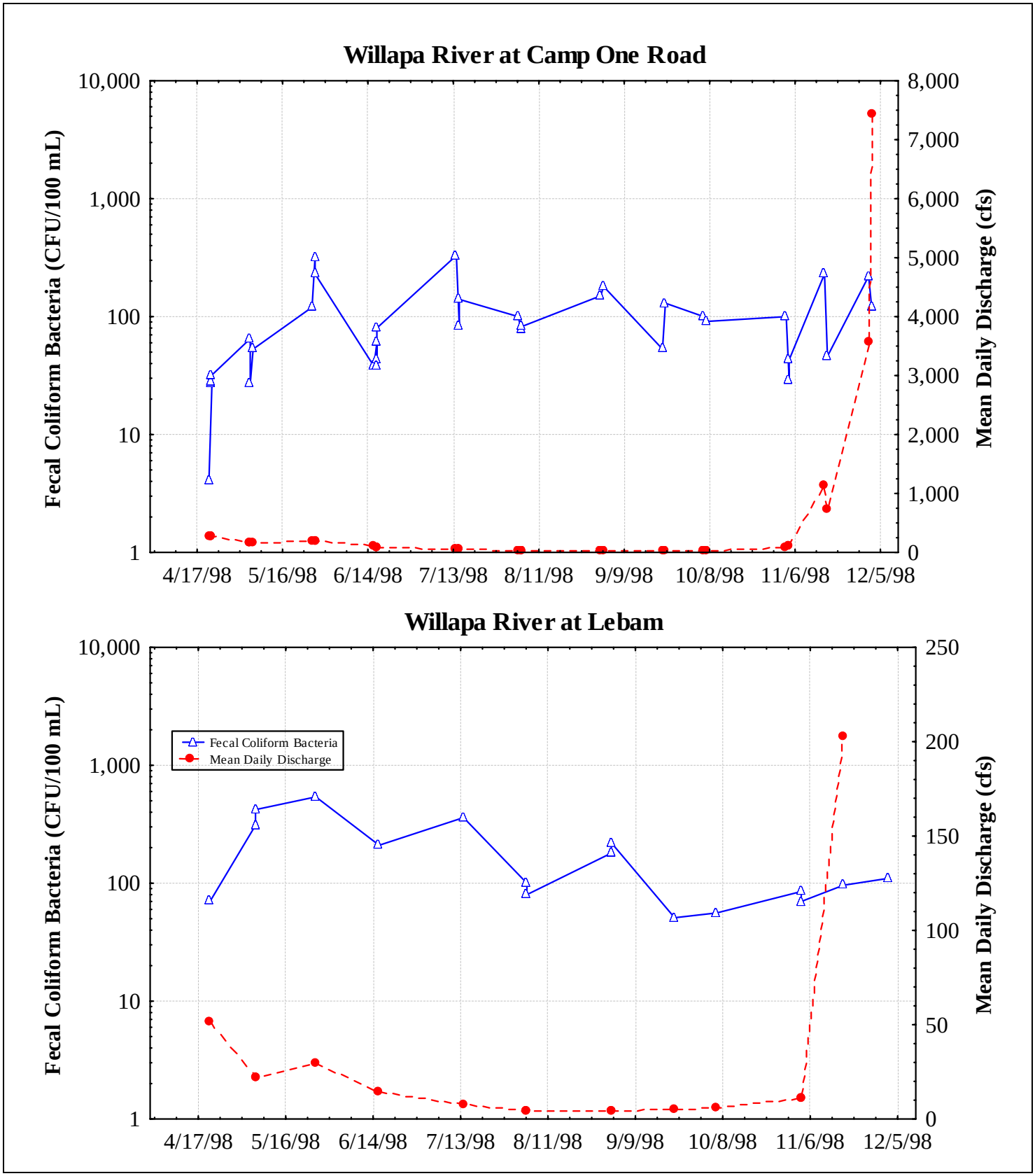
Shaded rows indicate stations located on the main stem. **Bold** values exceed the fecal coliform bacteria standard.

[a] Colony forming units (CFU)/100 milliliters (mL). The fecal coliform bacteria standard for the Willapa River upstream of Mailboat Slough (RM 1.8) is a geometric mean of 100 CFU/100 mL, with no more than 10% of the samples greater than 200 CFU/100 mL. Downstream of Mailboat Slough, the standard is a geometric mean of 14 CFU/100 mL, with no more than 10% of the samples greater than 43 CFU/100 mL. The standards were applied to a 60-day period to provide a minimum of three values per period without bridging different seasonal conditions.

Source: Ecology 2000.

Overall, temperature, fecal coliform bacteria, and dissolved oxygen levels did not meet water quality standards at the main stem and tributary stations throughout the basin. In terms of fecal coliform bacteria concentrations, 25 of 30 monitoring stations did not meet the water quality standards (see Table 2). Of the 12 stations along the main stem, only the uppermost station at RM 41.2 met the standards for fecal coliform bacteria. Ecology (2000) used a 60-day period to assess compliance with fecal coliform bacteria standards because a shorter period did not include enough data (i.e., less than three values) and a longer period bridged different seasonal conditions, which could have hidden non-compliance periods.

Figure 5 presents the data for fecal coliform bacteria and daily discharge collected during the TMDL study at two of the stations selected for this MST study (i.e., at Camp One Road and at Lebam). Both stations exhibited fecal coliform bacteria concentrations that exceeded the water quality standards. The upstream station, Willapa River at Lebam (WRLE), exhibited the highest fecal coliform concentrations during the early summer months. The downstream station, Willapa River at Camp One Road (WRC1), exhibited the highest fecal coliform concentrations during the middle to late summer. These findings generally agree with results of Ecology's ambient monitoring program (see Figure 3).



(data source: Ecology 2005 online)

Figure 5. Fecal coliform bacteria and mean daily discharge at two locations on the Willapa River from the Willapa River TMDL study in 1998.

Methods

The primary objective of the upper Willapa River MST study was to identify the sources of fecal coliform bacteria in the upper Willapa River using a genetic fingerprinting technique. A secondary objective was to collect sufficient data to evaluate how fecal bacteria sources vary with hydrologic conditions, seasons, and bacteria concentrations. The study design is summarized below, followed by sample collection and analysis methods. Quality assurance objectives and procedures are described in the sampling and analysis plan (Herrera 2003).

Study Design

To meet the study objectives, *E. coli* colonies were isolated from water samples and genetically typed using a molecular ribotyping technique to identify animal and human sources of fecal coliform bacteria. The genotype database developed was supplemented with a regional database containing genotypic *E. coli* data obtained from a wide variety of human sewage and animal feces. This combined database was used to determine the number of genetic matches between those bacteria ribotypes recovered from known sources and the ribotypes of those bacteria recovered from the water quality monitoring stations.

A total of 400 bacterial isolates were to be collected from the following three locations shown in Figure 4 in the upper Willapa River: 1) a background station located upstream of potential agricultural sources, 2) a midstream station located near potential human sources at the town of Lebam, and 3) a downstream station located near the lower boundary of the upper Willapa River. Fecal coliform bacteria were to be analyzed from each water sample collected during 20 sampling events in a one-year period from November 2003 through October 2004. During each sampling event, one water sample was to be collected from the background and midstream sampling stations, and two water samples were to be collected from the downstream station, for a total of 80 samples. The ribotyping technique was to be applied to approximately five *E. coli* isolates from each of the 80 collected samples.

The monitoring study was conducted in accordance with the sampling and analysis plan (Herrera 2003). The study components are summarized in Table 3 and the monitoring procedures are described below.

The water samples were collected and analyzed as designed, with 40 samples collected from the downstream station, and 20 samples each from the midstream and upstream stations. A total of 10 storm flow events and 10 base flow events were sampled from November 2003 through November 2004. A greater number of *E. coli* isolates were obtained from the collected samples than was designed (i.e., 552 isolates were obtained at an average of 6.9 isolates per sample versus the design of five isolates per sample for a total of 400 isolates).

Table 3. Sampling and analysis summary for the upper Willapa River Microbial Source Tracking Study.

Station	Location	River Mile	Fecal coliform bacteria analyses [a]	*Escherichia coli* isolates [b]
WRC1	Downstream at Camp One Road	17.5	40	275
WRLE	Midstream at Lebam	33.2	20	150
WRPA	Upstream below Patton Creek	41.2	20	127
Total			80	552

[a] Analysis of 2 grab samples at Station WRC1 and 1 grab sample at Stations WRLE and WRPA per each of 20 events (10 base flow and 10 storm flow events), conducted by the Pacific County Environmental Health Laboratory.
[b] Analysis of up to 18 isolates from membrane filter cultures of each fecal coliform bacteria analysis, conducted by the Institute of Environmental Health.

Water Sample Collection and Analysis

Water samples were collected at three locations used by Ecology in previous water quality monitoring projects in the Willapa River basin (Ecology 2000; 2005 online). These sampling stations were selected because they represent different areas and conditions within the upper Willapa River watershed that include various potential sources of fecal coliform bacteria. In addition, the selected stations have historically exhibited variable concentrations of fecal coliform bacteria and the stations are relatively easy to access.

Water samples were collected at the following three stations as shown in Figure 4 (presented in the order sampled moving downstream:

- WRPA: Willapa River below Patton Creek (Ecology TMDL study site WRPA at RM 41.2) is located at a bridge on a private road off of Breen Road, about 1 mile south of State Route 6 (via Elk Prairie Road). Station WRPA is located upstream of populated areas and commercial agricultural activities.

- WRLE: Willapa River at Lebam (Ecology TMDL study site WRLE and ambient station 24B130 at RM 33.2) is located at the bridge on State Route 6, 0.4 miles west of Lebam. Station WRLE is located immediately downstream of Lebam, which is the most populated area of the upper watershed and where the use of onsite wastewater treatment facilities is common. Commercial agricultural activities are present upstream of this location.

- WRC1: Willapa River at Camp One Road (Ecology TMDL study site WRC1 and ambient station 24B090 at RM 17.5) is located at the bridge on

Camp One Road (also known as Bullard Road), about 1 mile north of State Route 6. Station WRC1 is located near the lower boundary of the upper watershed (RM 17.7).

Pacific County field personnel collected water samples during 10 base flow events and 10 storm events from November 2003 through November 2004. At the two uppermost stations (WRPA and WRLE), one water sample was collected at each station during each of the 20 sampling events. At the lowermost station (WRC1), field personnel collected two water samples at least 10 minutes apart during each event. All samples were collected using aseptic techniques, placed on ice, and delivered to the Pacific County laboratory within 24 hours of sample collection. The samples were analyzed for the presence of fecal coliform bacteria using the membrane filter method (Method 9222D) as outlined in *Standard Methods for the Examination of Water and Wastewater* (APHA et al. 1995). Generally, sample volumes of 0.5 milliliters (mL), 5 mL, and 50 mL were filtered for the analysis. The Pacific County laboratory is accredited by Ecology to perform the required analyses. After enumeration, fecal coliform bacteria culture plates were delivered to the Institute for Environmental Health (IEH) laboratory for *E. coli* isolation and DNA analysis.

The Herrera quality assurance officer determined fecal coliform bacteria concentrations for each sample from the raw bacteria culture plate results received from the laboratory. Bacteria concentrations were calculated as the culture plate count divided by the milliliters (mL) of volume filtered, and multiplied by 100 to obtain units of colony forming units per 100 mL (CFU/100 mL). Data were validated as described in the sampling and analysis plan (Herrera 2003).

Fecal Source Sample Collection

Fecal source samples of wastewater influent were collected by staff from the City of South Bend Wastewater Treatment Plant (WWTP) and the City of Raymond WWTP. Samples were collected at the South Bend WWTP on February 17, 2004; April 6, 2004; August 10, 2004; and November 9, 2004. Samples were collected at the Raymond WWTP on January 28, 2004; March 23, 2004; June 14, 2004; and September 15, 2004. Culture plates containing at least 5 distinct colonies were sent to the IEH laboratory for bacteriological typing and to update the ribotype database for human fecal sources.

Pacific Conservation District staff collected samples of septic tank wastewater on April 14, 2004 and September 27, 2004 from a local septic tank maintenance company to further update the ribotype database for human fecal sources. The wastewater samples were collected using sterile techniques, immediately placed on ice, and shipped overnight to the Institute for Environmental Health.

Pacific Conservation District staff collected fecal samples deposited by warm-blooded animals in the Willapa River basin on several occasions during the monitoring period. All fecal samples

were collected using sterile techniques, immediately placed on ice, and shipped overnight to the Institute for Environmental Health. Fecal samples were collected from the following animal sources:

- Beef cow
- Dairy cow
- Horse
- Black Tailed Deer
- Roosevelt Elk
- Black Bear
- Wild Turkey
- Roughed Grouse
- Canada Goose
- Llama
- Coyote
- Sheep
- Goat
- Emu
- Seagull
- Crow
- Raccoon
- Bobcat.

Ribotyping

Fecal coliform bacteria culture plates were shipped overnight by Pacific County to the Institute for Environmental Health within 3 days of enumeration. Pure cultures of *E. coli* were obtained from the plate cultures by the Institute for Environmental Health. The laboratory employed the ribotyping technique described below.

The ribotyping technique involves several steps, including the following:

- Isolation and labeling of *E. coli* ribosomal ribonucleic acid (RNA) to create the genetic probe
- Isolation of genomic deoxyribonucleic acid (DNA) from the source samples and the receiving water samples
- Hybridization of the DNA with the genetic probe
- Analysis of the ribotype information to compare the characteristic patterns for the *E. coli* isolated from receiving waters with the *E. coli* isolated from source samples.

In addition, two blind source samples were prepared by Pacific County to check the accuracy of ribotyping method. Cultures from one cow sample and one deer sample were prepared and shipped to the Institute for Environmental Health for matching to the ribotype library.

Results and Discussion

Results of the microbial source tracking study are discussed separately below for precipitation and discharge, data validation, bacteria enumeration, and ribotyping analysis. A quality assurance report for the study is included in Appendix A. Laboratory analysis reports are presented in Appendix B. The project database is presented in Appendix C.

Precipitation and Discharge

Table 4 presents the schedule for storm and base flow sampling, rainfall totals, and daily mean river discharge for each sampling event. Storm events required at least 0.20 inches of rain in a 12-hour period preceding the sampling as measured at the King 5 precipitation gauge at the Chauncey Davis Elementary School located in South Bend, Washington (King 5 2004 online). During rain events, the real-time precipitation data obtained from the King 5 internet web site was used to determine if staff from Pacific County should mobilize for storm sampling. Daily mean river discharge was measured at the U.S. Geological Survey (USGS) gauging station located at the Camp One Road monitoring station (USGS 2005 online).

Table 4. Daily precipitation totals and daily mean river discharge recorded during sampling events for the upper Willapa River Microbial Source Tracking Study.

Sample Date	Event Type	Daily Precipitation (inches)	Daily Mean River Discharge (cfs)
11/18/2003	storm	1.78	3,520
12/2/2003	base	0	1,290
12/15/2003	base	0	1,790
1/21/2004	base	0	462
1/28/2004	storm	4.24	1,270
2/9/2004	base	0	867
2/25/2004	base	0	756
3/15/2004	base	0	403
3/24/2004	storm	1.26	447
4/14/2004	storm	0.23	308
5/17/2004	base	0	119
5/26/2004	storm	0.73	102
6/7/2004	base	0.14	211
7/12/2004	base	0	55
8/11/2004	base	0	32
8/25/2004	storm	0.71	421
9/1/2004	storm	0.46	83
10/19/2004	storm	0.36	950
10/25/2004	storm	0.41	385
11/1/2004	storm	0.58	491

Precipitation data source: King 5 (2004 online)
Discharge data source: USGS (2005 online)

The data presented in Table 4 indicate that all storm flow sampling events met the total rainfall goal of at least 0.20 inches of rain. Daily precipitation totals ranged from 0.23 to 4.24 inches for the storm flow sampling events. No rainfall occurred during the base flow sampling events with the exception that there was 0.14 inches of rain on one base flow sampling date (June 7, 2004). Daily discharge values for sampling events ranged from a low of 32 cubic feet per second (cfs) during summer base flow to a high of 3,520 cfs during winter storm flow. The maximum daily discharge rate during this study period was 5,590 cfs on the day after the November 18, 2003 sampling event.

Data Validation

The quality assurance report (Appendix A) presents the results of the quality assurance review. A description of the quality assurance objectives can be found in the sampling and analysis plan (Herrera 2003). The overall data quality objective of the study was to ensure that data of known and acceptable quality were obtained. Field notes and laboratory results were reviewed for precision, accuracy, representativeness, completeness, and comparability. Although quality control problems were identified, no data were rejected (flagged with an R) during the data review. Therefore, all collected data are considered to be of acceptable quality and are used here for evaluation purposes. Data validation results are summarized below for bacteria enumeration and ribotyping. A complete discussion of the qualified data can be found in the quality assurance report (Appendix A).

Bacteria Enumeration

The quality assurance review identified a source of uncertainty that is common in enumeration studies of bacteria. The method used to measure bacteria concentrations requires that between 20 and 60 colonies are present on the culture plate to achieve the most accurate count. In this study, 39 of 80 bacteria results (49 percent) were reported as estimates (flagged with a J) by the quality assurance officer because fewer than 20 colonies were present on the culture plate. Five (6 percent) of the bacteria results were reported as “greater than” the measured value (flagged with a G) because more than 60 colonies were present on the culture plate.

Bacteriological results were assessed for analytical precision using laboratory duplicates. The precision criteria consisted of two levels: the relative percent difference (RPD) of laboratory duplicates shall be less than or equal to 25 percent for values that are greater than 5 times the detection limit, and the difference between duplicates shall be within 2 times the detection limit for values that are less than or equal to 5 times the detection limit. These quality assurance objectives were not met for eight of the 20 laboratory duplicates analyzed. However, results for four of those eight duplicates had been qualified as estimates (flagged with a J) due to low colony counts. Therefore, results of four samples were qualified as estimates (flagged with a J) because the quality assurance objectives were not met.

Bacteria results were assessed for environmental variability by comparing results of the consecutive grab samples collected from station WRC1 to the precision criteria for laboratory duplicates. These criteria were not met for five of the 10 base flow samples and for five of the 10 storm flow samples. These results suggest that environmental variability is similar for base and storm flow samples, and that environmental variability (50 percent of consecutive grabs met criteria) is similar to the measured analytical precision (40 percent of laboratory duplicates met criteria).

Ribotyping

Blind water samples were prepared and sent to the Institute for Environmental Health to evaluate the accuracy of ribotyping fecal sources. One sample each containing bacteria from cow feces and from deer feces was prepared. Five isolates from the blind cow and six isolates from the blind deer sample were matched known sources in the ribotype library. All 11 isolates obtained from the blind samples were correctly identified.

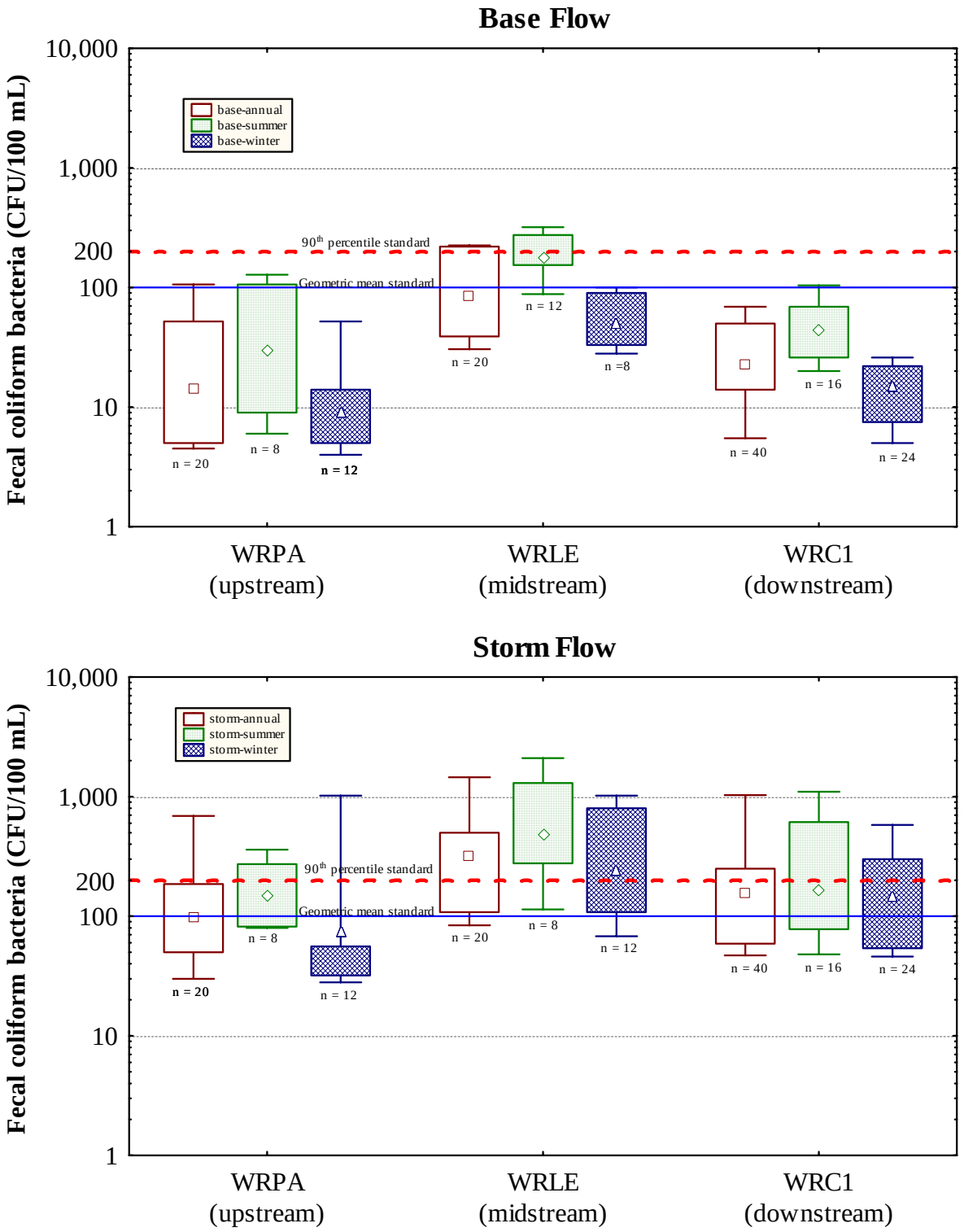
A study of microbial source tracking methods found that the ribotyping method used for this study performed well in several of the evaluation criteria (Myoda et al. 2003). The ribotyping method exhibited high sensitivity rates (i.e., the percentage of time that the source was correctly identified as present in the sample) for human and sewage sources (88 percent) and for all sources (81 percent). The false positive rates for this method were low, at 17 percent for human and sewage sources and 23 percent for all sources. The ribotyping method correctly identified the dominant source of contamination in 75 percent of all samples. A more detailed summary of the review can be found in the quality assurance report (Appendix A).

Bacteria Enumeration

In the following sections, fecal coliform bacteria data are summarized and discussed in terms of comparison to water quality standards, station location, hydrologic condition, season of the year, and historical data. The project database is presented in Appendix C.

Fecal coliform bacteria concentrations for the Willapa River MST Study are summarized as box and whisker plots in Figure 6. Box and whisker plots show the geometric mean as a point, 25th and 75th percentiles of the data as a box, and the 10th and 90th percentiles of the data as whiskers.

Nonparametric statistical procedures were used to test for significant differences among bacteria data sets because these data do not exhibit a normal distribution. A two-tailed Mann-Whitney U test was used to test for differences between hydrologic conditions (i.e., base versus storm flow), season (i.e., spring/summer versus fall/winter), and different studies (i.e., MST study versus Ecology data). The Mann-Whitney U test is a nonparametric analogue to the paired t-test. Differences between monitoring stations were tested using a Friedman's ANOVA. The Friedman's test is a nonparametric randomized block analysis-of-variance used to compare observations on the same subject. All tests were conducted at a significance level (α) of 0.05.



Legend: Symbol = geometric mean; Box = 25th and 75th percentile; Whisker = 10th and 90th percentile
Summer = April through September; Winter = October through March

Figure 6. Annual and seasonal fecal coliform bacteria concentrations at three monitoring stations in the Upper Willapa River during base flow and storm flow from November 2003 through November 2004.

Water Quality Standard Comparison

The upper Willapa River is designated as primary contact recreation by Washington State water quality standards (WAC 173-201A). The fecal coliform bacteria water quality standard for primary contact recreation is that the geometric mean must not exceed 100 colony forming units (CFU)/100 mL with not more than 10 percent of the samples (i.e., 90th percentile) exceeding 200 CFU/100 mL. As shown in Figure 6, one or both of these criteria were exceeded at all three monitoring stations during storm flow conditions in both spring/summer and fall/winter seasons. During base flow, the fecal coliform bacteria standard was exceeded at the midstream station (WRLE) in the spring/summer season (April through September) but not in the fall/winter season, and was not exceeded at the other stations in either season.

Spatial, Hydrologic, and Seasonal Comparisons

Bacteria data for samples collected at the upstream station (WRPA), midstream station (WRLE), and downstream station (WRC1) were compared to evaluate spatial, hydrologic, and seasonal differences in fecal coliform bacteria concentrations in the upper Willapa River. As shown in Figure 6, geometric means of these bacteria increase from the upstream station (WRPA) to the midstream station (WRLE), and decrease from the midstream station (WRLE) to the downstream station (WRC1). Results from the Friedman's ANOVA show that fecal coliform bacteria concentrations at the midstream station are significantly different from both the upstream and downstream stations, and that data for the upstream station are not significantly different from those for the downstream station ($p < 0.00001$). These results indicate that the primary sources of fecal coliform bacteria in the upper Willapa River watershed are located between the upstream station (below Patton Creek at RM 41.2) and the midstream station (below Lebam at RM 33.2).

A comparison of bacteria concentrations during base flow and storm flow conditions show that geometric means are substantially higher during storm flow than during base flow at all three stations (see Figure 6). Results from the Mann-Whitney U test indicate that fecal coliform bacteria concentrations during base and storm flow conditions are significantly different at all three stations ($p = 0.0113$ at WRPA, $p = 0.0113$ at WRLE, and $p = 0.0007$ at WRC1). These results indicate that stormwater runoff is a significant factor affecting bacterial contamination in the upper Willapa River.

Fecal bacteria concentration data were assigned to one of two seasons to compare data collected during the manure application season with data collected when manure is not typically applied. Because the application of manure typically occurs in the spring and summer growing season, this was defined as the spring/summer season (April through September), and the fall/winter season was defined as October through March. A comparison of spring/summer and fall/winter geometric means indicates that fecal coliform bacteria concentrations are higher in the spring and summer than the fall and winter at all stations (see Figure 6). Results from the Mann-Whitney U test indicate that fecal coliform bacteria concentrations for all flow conditions are significantly different in the spring/summer than fall/winter at the upstream station ($p = 0.0409$ at WRPA) and the midstream station ($p = 0.0491$ at WRLE). These results indicate that sources of fecal

coliform bacteria in the upper Willapa River are most prevalent during the spring and summer seasons.

Historical Data Comparison

As summarized in the Background Information section, Ecology has intermittently collected water quality data in the Willapa River since 1966, including intensive monitoring in 1998 in support of the development of a TMDL for fecal coliform bacteria. The fecal coliform bacteria enumeration data collected for this MST study were compared to fecal coliform bacteria concentrations measured by Ecology during the 1995 and 1996 water years (a water year extends from October through September). This time period was selected for a comparison to a continuous data set of similar size that was collected approximately 10 years prior to this MST study. Data collected at the downstream station (station WRC1) were used for the comparison because data from the midstream and upstream stations are not available for this time period.

Results from the Mann-Whitney U test indicate that there is not a statistically significant difference ($p = 0.2116$) between the historical and MST study data. Thus, fecal coliform bacteria concentrations measured at station WRC1 for the MST study are not significantly different than those measured at the same location during the 1995 and 1996 water years.

Ribotyping

A total of 552 *E. coli* isolates were obtained from a total of 80 water samples collected for this MST study. These isolates were then matched to the ribotype database (library) maintained by the Institute for Environmental Health. Approximately 95 percent of all isolates obtained were matched to a known fecal source. This high matching rate provides a high level of confidence indicating that major sources were correctly identified. Of those 552 isolates obtained from the water samples, 83 isolates (15 percent) were matched to new sources that were added to the library from source samples collected in the Willapa River watershed.

The ribotyping matching results are presented in Table 5. The number and percentage of isolates are presented for each station and for all stations combined. Several identified sources were combined into one category for ease of interpretation. The human category includes sources originating from human waste or sewage samples. The bovine category includes sources identified as cow or bovine. Bovine sources include bacteria strains that may be common to both cows and sheep. The deer/elk category includes sources identified as deer, elk, or deer/elk (i.e., bacteria strains common to both deer and elk). The canine category includes sources identified as dog, coyote, or canine (i.e., bacteria strains common to both domestic dog and coyote). The feline category includes sources identified as feline, which may have originated from domestic cats, bobcat, or cougar. The rodent/beaver category includes sources identified as rodent or rodent/beaver (i.e., bacteria strains common to both rodent and beaver). Rodents include rats, mice, voles, moles, and muskrats. The avian category includes sources identified as avian, which may have originated from a large number of species of birds or waterfowl.

Table 5. Number of *E. coli* isolates by fecal source category observed at the upstream, midstream, and downstream stations for the upper Willapa River Microbial Source Tracking Study.

	Upstream Station (WRPA)		Midstream Station (WRLE)		Downstream Station (WRC1)		Sum of All Stations	
Fecal Source	Number of Isolates	Percent of Total	Number of Isolates	Percent of Total	Number of Isolates	Percent of Total	Number of Isolates	Percent of Total
Human	0	0.0	17	11.3	24	8.7	41	7.4
Bovine	6	4.7	64	42.7	95	34.5	165	29.9
Horse	0	0.0	2	1.3	4	1.5	6	1.1
Bear	3	2.4	2	1.3	4	1.5	9	1.6
Deer/elk	44	34.6	14	9.3	39	14.2	97	17.6
Canine	12	9.4	12	8.0	17	6.2	41	7.4
Feline	1	0.8	0	0.0	4	1.5	5	0.9
Raccoon	6	4.7	2	1.3	7	2.5	15	2.7
Rodent/beaver	17	13.4	8	5.3	23	8.4	48	8.7
Avian	36	28.3	26	17.3	35	12.7	97	17.6
Unknown	2	1.6	3	2.0	23	8.4	28	5.1
Total	127	100.0	150	100.0	275	100.0	552	100.0

Figure 7 presents the percent matching results for each monitoring station in a series of stacked bars. The first bar in the series represents the percentage of sources observed in the entire data set. Three pairs of bars are then presented for comparison of matching percentages for: 1) samples that meet or exceed the fecal coliform bacteria standard of 100 CFU/100 mL, 2) samples collected during base or storm flow conditions, and 3) samples collected during the spring/summer (April through September) or fall/winter (October through March). The number of isolates (n) associated with each bar is included in parenthesis.

The overall matching results are described below for each station using the entire data set. Comparisons of matching results by environmental variable are then summarized in a separate section.

Overall Matching Results

Human sources of fecal coliform bacteria represented 7.4 percent of the total isolates obtained for the MST study. Human sources were not present at the upstream station (WRPA), but represented 11 percent of the isolates at the midstream station (WRLE) and 8 percent of the isolates at the downstream station (WRC1).

Bovine sources comprised the largest percentage (30 percent) of all ribotypes isolated from the upper Willapa River, and were the predominant sources observed at the midstream (43 percent) and downstream (35 percent) stations. Bovine fecal sources represented a relatively low percent of the isolates at the upstream station (4.7 percent). Horses were the other livestock source

observed in the MST study, but represented only 1 percent at the midstream and downstream stations and were not observed at the upstream station.

The percentages of wildlife fecal sources generally decreased from the upstream station to the downstream station. Deer/elk sources were the predominant ribotypes observed at the upstream station (35 percent), and were found in lower numbers at the midstream station (9 percent) and downstream stations (14 percent). Rodent/beaver sources were also observed in a higher percentage at the upstream station (13 percent) compared to the midstream (5 percent) and downstream (8 percent) stations. Avian ribotypes were relatively abundant at the upstream (28 percent) station, but less so at the midstream (17 percent) and downstream (13 percent) stations. Canine sources represented between 6 and 9 percent of all sources at each of the monitoring stations. Bear and raccoon sources were observed at a low frequency (less than 5 percent) at all of the monitoring stations. Feline sources were rarely observed (less than 1 percent) at the upstream and downstream stations, and were not observed at the midstream station.

Concentration, Hydrologic, and Seasonal Comparisons

Generally, percentages of fecal coliform bacteria sources did not vary substantially with fecal coliform bacteria concentration, hydrologic condition, or season (see Figure 7). However, small differences are apparent from comparisons of these environmental variables.

At the upstream (WRPA) and midstream (WRLE) stations, the percentage of avian sources increased (and cow sources decreased) when bacteria concentrations increased (i.e., when the fecal coliform bacteria concentration increased above 100 CFU/100 mL). Deer source percentages increased slightly with bacteria concentration at the downstream (WRC1) station. Thus, these wildlife sources were somewhat more abundant when the fecal coliform bacteria standard was exceeded.

Bovine and deer/elk sources increased in percentage from summer to winter at the midstream station (i.e., bovine increased from 29 to 53 percent and deer/elk increased from 5 to 13 percent). Avian sources decreased from 27 percent in summer to 10 percent in winter at the midstream station. Mergansers and other waterfowl have been observed foraging for fish and raising their young in the river during the spring and early summer (Johnson 2005). Forested bird populations also typically increase in the spring and summer in response to increased food availability. Thus, the seasonal differences in avian source percentages may be explained in part by the migration of bird populations.

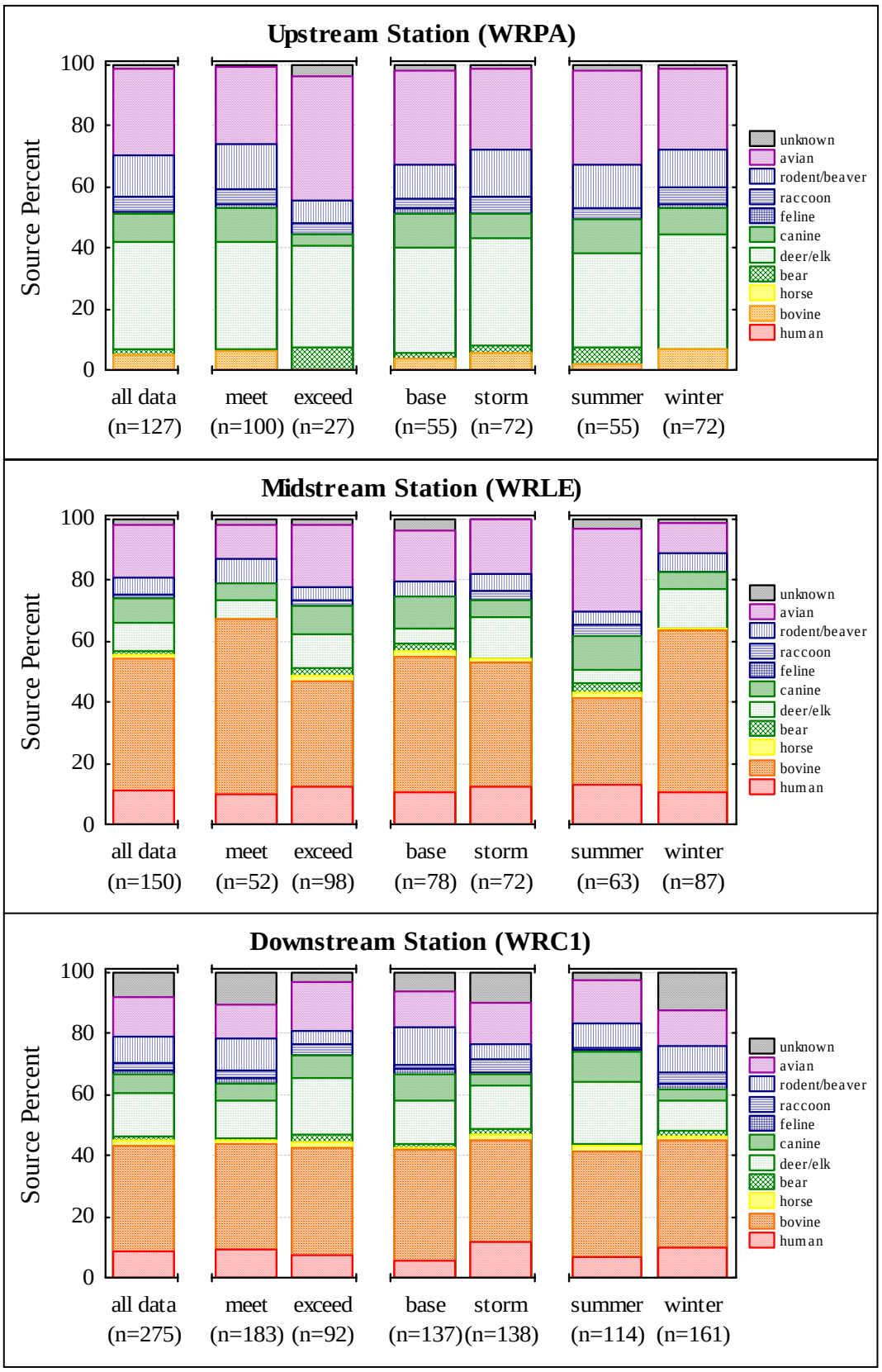


Figure 7. Fecal coliform bacteria sources observed at three stations on the Upper Willapa River using all ribotyping data and including comparisons of data for samples meeting or exceeding the water quality standard (100 CFU/100 mL), collected during base or storm flow conditions, and collected during summer (April through September) or winter (October through March) seasons.

Conclusions

Fecal Coliform Bacteria

A total of 80 fecal coliform bacteria enumeration analyses were performed on water samples collected from three monitoring stations on the upper Willapa River over a one-year period during base and storm flow conditions. Conclusions reached from the fecal coliform bacteria analysis performed for this study include the following:

- The Washington State water quality standard for fecal coliform bacteria in surface waters designated for primary contact recreation was exceeded at all three monitoring stations located on the upper Willapa River during storm flow conditions in both spring/summer and fall/winter seasons. During base flow conditions, the standard was only exceeded at the midstream station (below Lebam) in the spring/summer season.

- Fecal coliform bacteria concentrations were significantly higher at the midstream station than at the upstream or downstream station.

- Fecal coliform bacteria concentrations were significantly higher during storm flow than base flow at all three monitoring stations.

- Geometric mean concentrations of fecal coliform bacteria were higher during the spring/summer season than fall/winter season at all three monitoring stations, and the differences between seasons were statistically significant at the upstream and midstream stations.

- Fecal coliform bacteria concentrations measured at the downstream station (at Camp One Road) were not significantly different than historical data collected by Ecology in 1995 and 1996.

Ribotyping

The ribotyping analysis successfully matched 95 percent of the 552 *E. coli* isolates obtained from the bacteria analyses to known human or other animal fecal sources. Conclusions reached from the ribotyping analysis performed for this study include:

- Human fecal sources were not present at the upstream station, but represented approximately 10 percent of all sources observed at the midstream and downstream stations.

- Bovine fecal sources were not abundant at the upstream station (5 percent), but were the predominant source observed at the midstream station (43 percent) and the downstream station (35 percent). Horse fecal sources were not observed at the upstream station, and represented only 1 percent of all sources at the midstream and downstream stations.

- Wildlife fecal sources generally decreased in percentage from upstream to downstream. Deer/elk were the predominant fecal sources observed at the upstream station (35 percent), but represented only 9 percent at the midstream station and 14 percent at the downstream station. Avian fecal sources were also abundant at the upstream station (28 percent), and decreased to 17 percent at the midstream station and 13 percent at the downstream station.

- Additional fecal sources commonly observed at all three monitoring stations include rodent/beaver (ranging from 5 to 13 percent) and canine (ranging from 6 to 9 percent, and included both coyote and domestic dog). Bear, raccoon, and feline each represented less than 5 percent of the observed fecal sources at all stations.

- With few exceptions, fecal source matching percentages did not change substantially in relation to bacteria concentration (i.e., meet or exceed the 100 CFU/100 mL standard), hydrologic condition (i.e., base or storm flow) or season of the year (i.e., spring/ summer or fall/winter). Percentages of some wildlife sources were observed to be higher at some stations when fecal coliform bacteria concentrations exceeded the standard during storm flow and during the spring/summer season.

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