*Final Summary Report*

# Lower Boise River Coliform Bacteria DNA Testing

Prepared for

**Lower Boise River Water Quality Plan**

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CH2MHILL

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# Final Summary Report: Coliform Bacteria DNA Testing

## Watershed Setting

The lower Boise River watershed begins at Lucky Peak Dam and continues approximately 40 river miles to the confluence with the Snake River near Parma, Idaho. This watershed is approximately 1,300 square miles and contains about one-third of Idaho's population. The land use varies from urban and suburban uses to agricultural farmland. Approximately 163,270 acres of irrigated farmland are contained in the watershed. The irrigation water is diverted from the lower Boise River and distributed through a series of canals and ditches to individual farms. The return water from the agricultural fields, as well as storm runoff, is collected through privately owned drains that discharge to the lower Boise River.

According to numbers used in the 1998 lower Boise River Total Maximum Daily Load (TMDL), approximately 260,000 people living within the watershed are served by publicly owned wastewater treatment plants (WWTPs) that use secondary treatment technology prior to discharging to the lower Boise River. Approximately 100,000 people within the watershed are unsewered. These numbers are currently higher due to population growth that has occurred since the 1998 TMDL was developed.

In 1992, the Idaho Department of Environmental Quality (DEQ) placed the lower Boise River on the State 303(d) list as an impaired waterbody. The designated uses for the lower Boise River are cold water biota, salmonid spawning (upper reaches only), primary and secondary contact recreation, potable water (in upper reaches only), and agricultural water. Nutrients, dissolved oxygen (DO), grease and oils, temperature, sediment, and bacteria were identified at that time as impairing the designated uses.

A formal total maximum daily limit (TMDL) document was submitted to the U.S. Environmental Protection Agency (EPA) in December 1998 and approved by EPA in January 2000. During the Problem Assessment phase of this TMDL, several pollutants were eliminated from further consideration and only sediment and bacteria were addressed. The next phase of the TMDL process includes preparing an Implementation Plan for sediment and bacteria. This overall plan is being developed from source-specific implementation plans that are being prepared by source groups representing point source municipal and industrial stakeholders, urban and suburban storm drainage interests, and non-point agricultural interests.

Since the TMDL was approved, Idaho has changed its bacteria criteria. These criteria were originally expressed in terms of fecal coliform levels (30-day geometric mean concentrations of 50 and 200 per 100 mL [/100 mL] for primary and secondary contact recreation,

respectively)[1]. The current criteria is expressed in terms of *E. coli* concentrations (geometric mean concentrations of 126/100 mL for both recreational uses).

To address this discrepancy, both fecal coliform and *E. coli* results will be discussed in this report. The TMDL Implementation Plan will address how the previous wasteload and load allocations for bacteria will be addressed using the new criteria.

# Goals of DNA Testing Program

The lower Boise River TMDL Implementation Plan defines the program within the watershed for reduction of both sediment and bacteria. In some locations, more than 90 percent reductions are required for bacteria. To better focus the efforts for bacteria reduction through Best Management Practices (BMPs), the Lower Boise River Water Quality Plan (LBRWQP), the state-designated Watershed Advisory Group (WAG), received a 319 grant to conduct this bacteria DNA testing program. The DNA testing helps to define the actual sources of bacteria at sampling locations in the Boise River and major tributaries. A secondary purpose for this study is to show the applicability of this testing technology for use in other watersheds throughout Idaho that require bacteria TMDLs.

# Bacteria DNA Testing Procedures

## Bacteria Source Fingerprinting

Dr. Mansour Samadpour (Institute for Environmental Health [IEH], Seattle, Washington) has developed the DNA fingerprinting methodology used in this study. This methodology is based on using a library of DNA fingerprints of *E. coli* strains isolated from various sources, to identify the sources of *E. coli* strains isolated from water samples. This library was used to identify bacteria sources in the water quality samples taken from the lower Boise River, two of its tributaries, and a few stormwater discharges. In addition, stakeholders in the lower Boise River watershed collected scat samples of animals and wildlife within the watershed for IEH to develop a local bacteria DNA fingerprint, and the cities of Boise and Nampa provided several fecal coliform cultures developed from their WWTP influent to develop local human bacteria DNA fingerprints.

## Sampling Schedule and Locations

Sampling sites are generally classified into three areas: samples identified in the 319 grant; samples to characterize riparian area contributions; and samples to characterize urban stormwater contributions. The locations of these stations are presented in Figure 1.

In general, the eastern stations (Walnut Street, Ann Morrison, Americana, and Glenwood Bridge) are located within an urban environment, Eagle Island and Indian Creek are located in a portion of the watershed that is in transition between the urban and rural portion of the watershed, and the downstream two stations (Dixie Slough, and Parma Bridge) represent primarily a rural environment. Sampling site descriptions and numbers of samples taken at

[1] Fecal coliform criteria were also expressed in terms of instantaneous concentrations of 500/ and 800/100 mL colonies at any time for primary and secondary contact recreation, respectively. Current *E. coli* criteria include instantaneous concentrations of 406/100 mL and 576/100 mL at any time for primary and secondary contact recreation, respectively.

each location are described in this section (sampling plans for each of the types of samples are contained in Appendix A).

### 319 Grant Sampling Locations

These samples were collected to provide a prioritization of BMP locations to reduce bacteria. The sampling locations defined in the 319 grant include mainstem stations: Glenwood Bridge and the bridge over the Boise River near Parma; as well as two tributaries: Indian Creek (just downstream of the Riverside canal diversion) and Dixie Slough. The LBRWQP collaborated with the Boise State University engineering school to collect these water quality samples.

Water quality samples were taken during two periods to capture high runoff conditions and irrigation conditions. The first sampling period was targeted for just after the water was released to the irrigation canal and during the high water runoff period in the river (April 19–May 16, 2000). Samples were taken at the same sites again late in the irrigation season, between September 5–27, 2000.

The sampling trips to the LBRWQP 319 grant sample sites extended over two 4-week periods and resulted in approximately 12 sample visits per site for each season. This resulted in a total of 379 water quality samples delivered to the City of Boise Water Quality Laboratory (Boise WQ Laboratory) during both the spring and late summer sampling events.

The same sampling protocol was used in the spring and the late summer sampling periods. Three to four grab samples were taken at the Glenwood Bridge on the Boise River during each sampling trip to get a representative sampling of the river at this location. Although the study plan indicated that these samples were to be combined in a single sample container before delivering the water samples to the Boise WQ Laboratory, each discrete grab sample remained separate and individual fecal coliform counts were conducted on these grab samples.

At the Indian Creek and Dixie Slough sampling sites, only two grab samples were needed to get a representative sampling because these tributaries are smaller than the mainstem Boise River. At the Parma Bridge site on the Boise River (upstream of the confluence with the Snake River), four grab samples were taken longitudinally across the river in order to get a representative sampling of the mainstem.

### Riparian Sampling Locations

EPA expressed concern with the original 1998 lower Boise River TMDL submission and the lack of a bacteria load reduction target for the riparian corridor. The riparian area water samples collected at the Ann Morrison Park site and the Eagle Island site address these concerns by delineating riparian corridor bacteria sources and providing coliform counts needed to determine riparian corridor reduction targets.

DEQ personnel collected a total of 57 water quality samples from the Ann Morrison Park and Eagle Island sites. Samples were generally collected from each site once per week for 8 weeks during the spring (April 11–May 26, 2000) and summer (July 3–August 21, 2000) sampling periods. Additional samples were collected on June 12, September 19, September

22, and October 10, 2000, to provide an indication of monthly trends throughout the irrigation season.

### Ann Morrison Park Site.

The sample site is located in Ann Morrison Park between Americana Boulevard and Capital Boulevard approximately 100 meters upstream from the footbridge that crosses the river in the middle of the park. The specific sampling site is at the outfall of a small wetland, which serves as the final destination of the Bubb canal system. The Bubb Canal meanders throughout the riparian corridor, providing water for the park and collecting stormwater and park runoff. The outfall is approximately 20 meters above the wetlands confluence with the Boise River.

### Eagle Island Sampling Site.

The sample site is the Mason-Catlin Canal just before it discharges into the North Channel of the Boise River. It is located in the floodplain between two channels of the Boise River southwest of the City of Eagle. The drainage area includes a mix of farmland and residential. A 160-acre farm and a subdivision are located upstream in the same floodplain.

### Stormwater Sampling Locations

Ada County Highway District (ACHD) collected water quality samples from two separate storm drain pipes that discharge into the Boise River at Americana Boulevard and Walnut Street. The Walnut Street site currently serves as a National Pollutant Discharge Elimination System (NPDES) stormwater monitoring site. These outfalls discharge storm water and dry weather flows to the Boise River via complex subsurface storm drain networks. ACHD collected approximately 50 samples from each site for a total of 100 samples. Half of the samples (25) were collected from base flow in the pipe during dry weather periods, while the remaining samples were collected during storm events. For the purpose of this study, storm events were defined as precipitation events creating stormwater runoff. Samples were collected throughout the irrigation season from April 25, 2000 - September 22, 2000. Because measurable storm events are rare in the Boise area during the summer months , most of the 100 samples were collected during the spring. To obtain 50 samples from each site during this short time period, a minimum of five samples were targeted for the first flush of each storm.

### Americana Boulevard Site.

The Americana Boulevard storm drain outfall is located on the north bank of the Boise River, west of the Americana Bridge. There are two outfalls at this location, a 42-inch pipe and a 48-inch pipe. Samples were collected directly from the 48-inch pipe. The site drains approximately 150 acres of land classified as mixed use. The mixed use classification consists primarily of residential and commercial uses. Base flow is present at this site year round. Base flow contributions include irrigation return flows from the Boise City Canal and foothills drainage piped from Hulls Gulch.

### Walnut Site.

The Walnut site is located on Walnut Street on the north side of the Boise River. The site drains an area approximately 536 acres in size. Land use is comprised of the following: 309 acres (58 percent) residential, 54 acres (10 percent) high density residential, 172 acres (32 percent) recreation, and 0.2 acres (<1 percent) commercial and industrial.

An alternate sampling site was determined in an upstream storm drain manhole at the intersection of Strawberry Lane and Walnut Street. Samples were collected from the alternate site from April – June when Spring river flows caused the storm drain pipe to become surcharged. Sampling from the alternate site during high river flows eliminated the possibility of back flow from the Boise River influencing sample collection. Base flow is present at this site year round. Base flow contributions include natural foothill drainage and groundwater.

## Analytical Procedures

Fecal coliform cultures were prepared using the membrane filtration technique with m-FC agar (APHA 1992). For each sample the Boise WQ Laboratory developed a minimum of three fecal coliform petri dishes containing the water sample membrane filter and specific growth media. Dilutions were made to ensure between 20–80 bacteria colonies on each culture plate were available for IEH to sample and run the DNA analysis. Each of the isolated petri dish samples were then shipped to IEH for DNA fingerprinting analysis.

Upon sample receipt, IEH choose up to five colonies with *E. coli*-like morphologies from each petri dish. Following isolation, microbiochemical analyses were performed to positively identify the *E. coli* colony. DNA from each of the isolates was then isolated and extracted, and molecular characterization was performed on individual *E. coli* strains using ribotyping, which focuses on characteristics in the *E. coli* gene that codes for ribosomal RNA. A complete description of the ribotyping DNA sourcing methods is provided in Appendix B.

The field of bacteria source tracking continues to evolve rapidly and there are numerous methods available. Each of these methods has its limitations and benefits. Recently, the U.S. Department of Agriculture, the U.S. Geological Survey, the EPA, and the Southern California Coastal Waters Research Project are in the process of researching the various methods. Despite the rapid and intensive research in existing methods, EPA recommends that bacteria source tracking "should be used by federal and state agencies to address sources of fecal pollution in water… [because it] represents the best tools available to determine pathogen TMDL load allocations and TMDL implementation plan development" (EPA 2002a).

In comparison with other DNA fingerprinting methodologies, the ribotyping methodology appears to compare well (Werblow 1997). In a study of Deep Creek Lake, Philadelphia, Werblow indicates that the IEH ribotyping methodology showed consistent results with the repetitive DNA/PCR (Rep-PCR) methodology because both methods "concluded that it looked like resident geese and duck were to blame for 70 percent of the *E. coli* samples found." Dombeck and colleagues (2000) suggest that large-scale use of ribotyping methods are limited because they tend to require extensive manipulation of DNA. However, the IEH methodology has been used successfully in other water quality studies, such as Little Soos Creek, Washington, and Grand Teton National Park, Wyoming. Additional studies by IEH are in progress and are anticipated to be published in 2004. In addition, at other laboratories employing ribotyping techniques, studies have found that human and non-specific animal sources were correctly identified an average of 82 percent of the time (Perveen et al., 1999).

# Results

## DNA Source Fingerprinting Overview

Overall, a collection of 1564 *E. coli* strains was established from water and storm drain samples taken from the main stem of the Boise River (Glenwood Bridge and Parma Bridge), the two tributaries (Indian Creek and Dixie Slough), two riparian stations (Ann Morrison and Eagle Island), and two urban stormwater stations (Walnut and Americana). Of the total number of isolated *E. coli* colonies (1,564), a bacteria source for 1,079 (69 percent) was able to be positively identified (that is, attributed to a particular source). *A more detailed discussion of the implications of these unidentified sources is provided in the Discussion section of this report.*

Table 1 presents a summary of sample sizes and positively identified isolates for each station.

**TABLE 1. SUMMARY OF SAMPLES COLLECTED AND ISOLATES IDENTIFIED BY MONITORING LOCATION**

| Location [1] | Samples Collected | Total *E. coli* Isolates | Positively Identified *E. coli* Isolates | Unidentified *E. coli* Isolates | % Unidentified *E. coli* Isolates |
|---|---|---|---|---|---|
| *Mainstem Stations* | | | | | |
| Glenwood Bridge | 93 | 263 | 181 | 82 | 31% |
| Parma Bridge | 95 | 276 | 195 | 81 | 29% |
| *Outfalls and Tributaries* | | | | | |
| Walnut Street | 52 | 146 | 121 | 25 | 17% |
| Ann Morrison | 30 | 86 | 57 | 29 | 34% |
| Americana | 48 | 142 | 102 | 40 | 28% |
| Eagle Island | 29 | 93 | 72 | 21 | 23% |
| Indian Creek | 95 | 280 | 172 | 109 | 39% |
| Dixie Slough | 96 | 278 | 179 | 99 | 36% |
| SUM / AVERAGE | 538 | 1,564 | 1,079 | 486 | 31% |

[1] Monitoring locations are generally presented in downstream order (see Figure 1).

On average, 31 percent of the E. coli sources were unable to be positively identified. Although the absolute numbers of unidentified sources vary by location depending on how many total samples were collected, unidentifiable isolates consistently occur throughout the watershed.

The results for those *E. coli* isolates that were able to be positively identified for the watershed as a whole are presented in Table 2. The percent match column in Table 2 represents the total number of positively identified *E. coli* colonies (1,079).

**TABLE 2. SUMMARY OF DNA RESULTS FOR LOWER BOISE RIVER WATERSHED**

| Source | Number of Matches | Percent Match | Controllable Source | Uncontrollable Source |
|---|---|---|---|---|
| **Total Human** | 185 | 17.1% | X | |
| **Total Pet** | 233 | 21.6% | | |
| Cat | 29 | 2.7% | X | |
| Dog | 197 | 18.3% | X | |
| Dog-Cat (Pet) | 7 | 0.6% | X | |
| **Total Livestock** | 118 | 10.9% | | |
| Cow | 75 | 6.9% | X | |
| Goat | 2 | 0.2% | X | |
| Horse | 25 | 2.3% | X | |
| Pig | 5 | 0.5% | X | |
| Poultry | 1 | 0.1% | X | |
| Sheep | 10 | 0.9% | X | |
| **Total Avian / Waterfowl** | 377 | 34.9% | | |
| Avian | 319 | 29.6% | | X |
| Duck and Goose | 58 | 5.4% | | X |
| **Total Wildlife** | 166 | 15.4% | | |
| Deer and Elk | 34 | 3.2% | | X |
| Feline | 36 | 3.3% | | X |
| Canine | 23 | 2.1% | | X |
| Fox | 2 | 0.2% | | X |
| Opossum | 4 | 0.4% | | X |
| Rabbit | 4 | 0.4% | | X |
| Raccoon | 11 | 1.0% | | X |
| Rodent | 51 | 4.7% | | X |
| Squirrel | 1 | 0.1% | | X |

NOTE: The Cat, Dog, and Dog-Cat (Pet) categories only contain domesticated strains of these animals. In contrast, the Feline and Canine categories could include both wild and domestic strains. Although we elected to place these in the Wildlife group, if all of the Feline and Canine isolates actually represented domesticated strains, it would add an additional contribution of 5.4 percent to the Pet group).

The results indicate that human sources comprise 17 percent of total identifiable bacteria throughout the watershed. The other 83 percent is made up of avian and waterfowl (35 percent), pets (22 percent), wildlife (15 percent), and livestock (11 percent).

Table 3 provides a summary of results for each specific sampling station. These results have been grouped together by major classification, as specified in Table 2.

**TABLE 3. SUMMARY OF GROUPED DNA RESULTS BY SAMPLING LOCATION**

| Location | Human | Pets | Livestock | Avian / Waterfowl | Wildlife | Unknown |
|---|---|---|---|---|---|---|
| *Mainstem Stations* | | | | | | |
| Glenwood Bridge | 18% | 17% | 0% | 27% | 8% | 31% |
| Parma Bridge | 13% | 6% | 18% | 25% | 9% | 29% |
| *Drains and Tributaries* | | | | | | |
| Walnut Street | 10% | 29% | 0% | 29% | 15% | 17% |
| Ann Morrison | 13% | 14% | 0% | 26% | 14% | 34% |
| Americana | 21% | 35% | 0% | 13% | 4% | 28% |
| Eagle Island | 13% | 11% | 2% | 39% | 13% | 23% |
| Indian Creek | 9% | 13% | 8% | 20% | 11% | 39% |
| Dixie Slough | 3% | 8% | 16% | 23% | 14% | 36% |

In general, these data show that human influences (including pets) decrease and livestock influences increase throughout the watershed in the downstream direction (Figure 2). Avian and waterfowl sources are the largest contributors (between 13 and 39 percent), with no large differences between the upstream urban and downstream rural areas. These two sources have been lumped relative to the other sources, even though they each have somewhat different indicators of habitat use. Avian sources make up 84 percent of this lumped category (and are the largest singular contributor overall), while duck/goose wastes make up 16 percent in this category. That is, for every duck/goose source identified throughout the watershed, more than five avian sources were identified. Wildlife sources consistently range from 4 to 15 percent throughout the watershed.

Upstream from Eagle Island, the Boise River and its tributaries flow through a relatively urbanized area. At these stations (Walnut Street, Ann Morrison, Americana, Glenwood Bridge, and Eagle Island), the contribution from humans ranges from 10 to 21 percent, while the combined influence from humans and pets ranges from 27 to 56 percent in this reach. The only agricultural sources in this reach were observed at Eagle Island (2 percent).

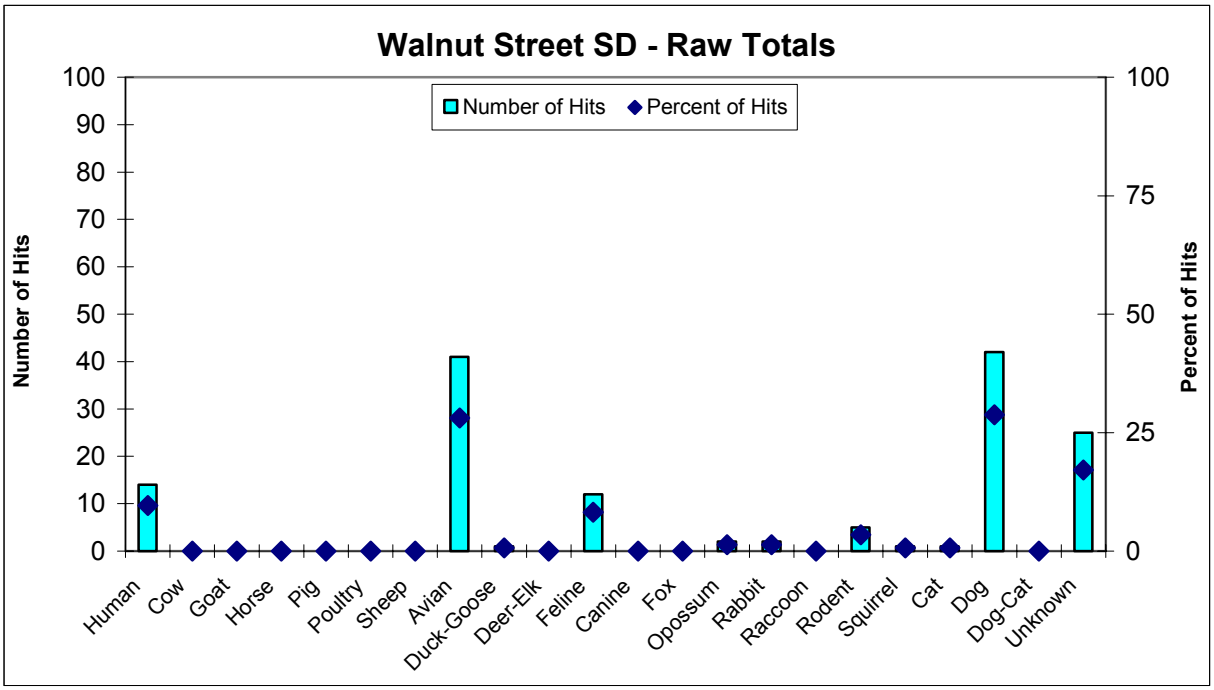
Downstream from Eagle Island, as the Boise River leaves the relatively urbanized area, the mainstem flows through a more predominantly agricultural area in the lower reaches of the watershed. At these stations (Indian Creek, Dixie Slough, and Parma Bridge), human sources decrease to between 3 and 13 percent, the combined contribution from humans and pets decreases to between 11 and 19 percent, and influences from livestock sources increase to between 9 and 14 percent. Avian/waterfowl and wildlife contributions remain relatively constant at 20 to 25 percent. The relationship between these general trends and actual fecal coliform concentrations will be presented following the station-specific summary of DNA sources below.

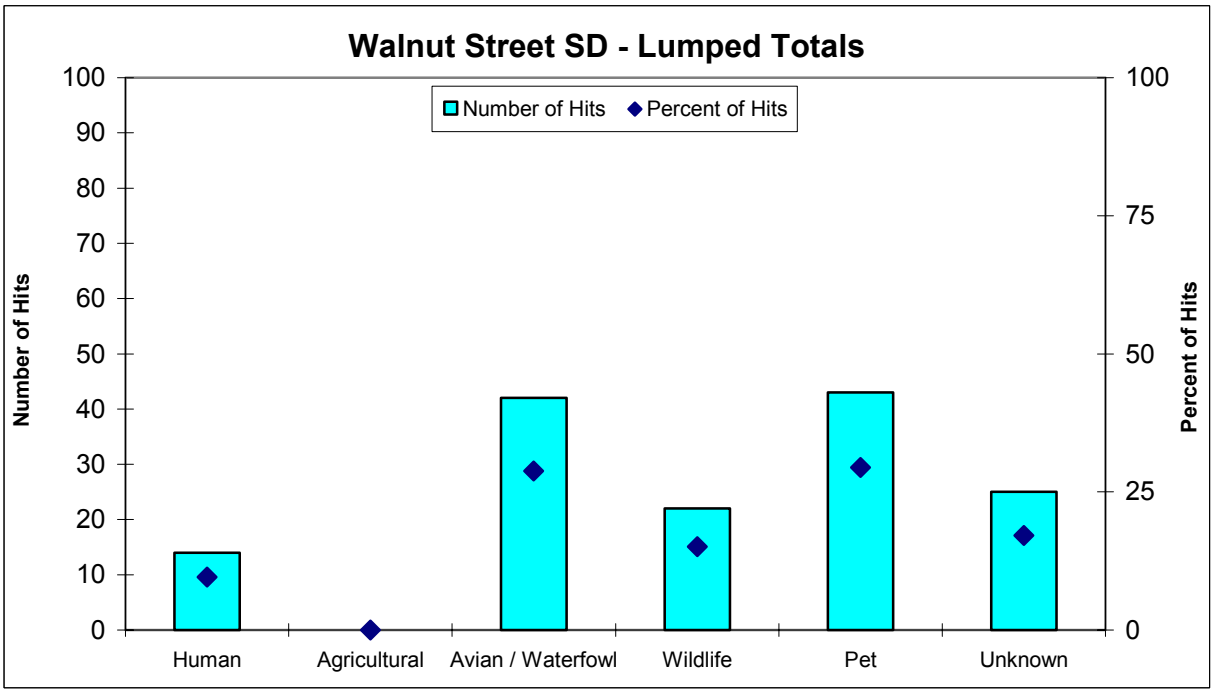
All data for each station are presented in downstream order in the remainder of this section. For each station, the total number of identifiable sources is presented both in raw form and in lumped categories (e.g., pets, livestock, avian/waterfowl, and wildlife; see Table 2). The results are also expressed as percentages to provide a relative measure of sources by location.

## Walnut Street SD

Of the 146 *E. coli* sampled, 121 sources were positively identified. The highest percentage of coliform DNA was seen in the Avian and Dog with 29 percent each, followed by Human sources with 9.6 percent. The rest of the percentages are as follows: Feline 8.2 percent, Rodent 3.4 percent, Opossum and Rabbit 1.4 percent, and Duck-Goose, Squirrel and Cat with 0.7 percent.

Of the 121 *E. coli* identified with lumped source categories (see Table 2), the highest impact can be seen in Avian and Pet with 29 percent each. The rest of the percentages are as follows: Wildlife 15 percent, and Human 9.6 percent.

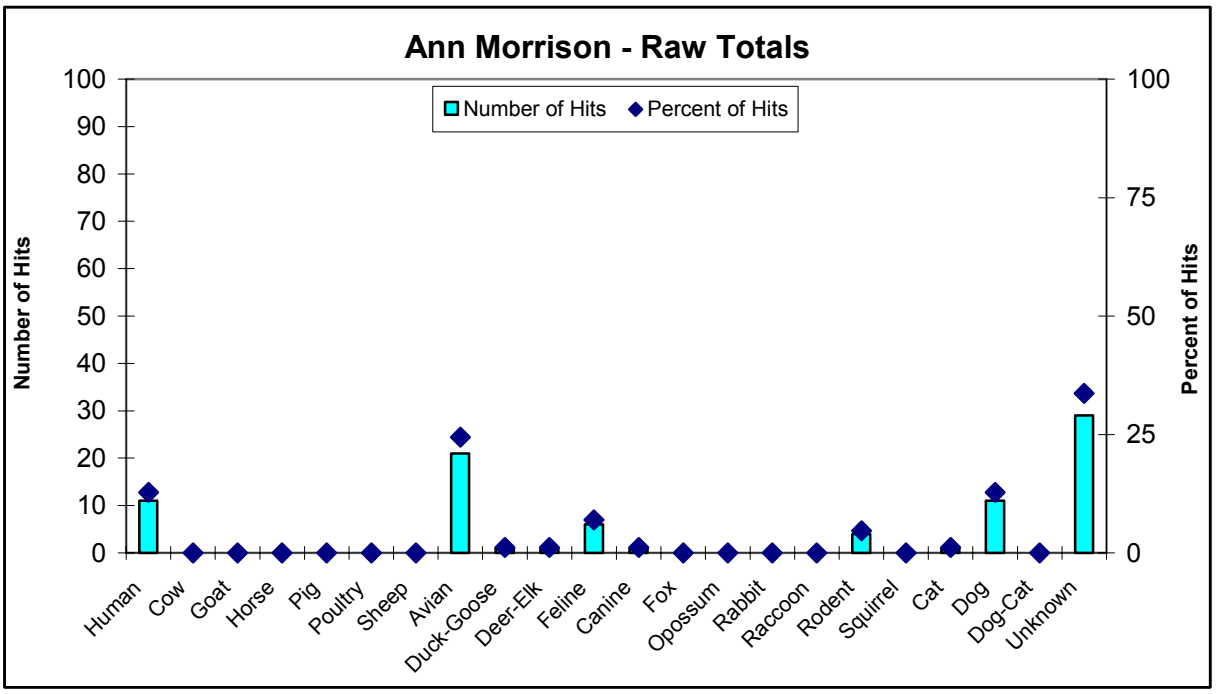


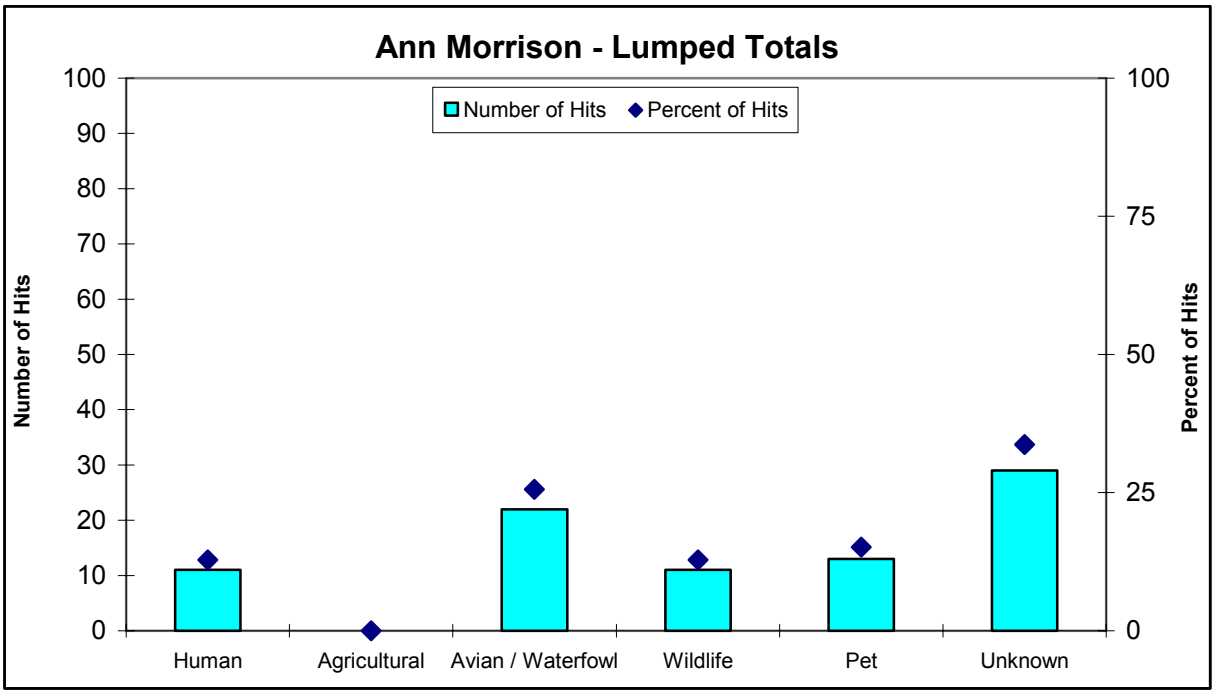



## Ann Morrison Site

Of the 86 *E. coli* sampled, 57 sources were positively identified. The highest percentage in coliform DNA was in the Avian category with 24 percent followed by Dog with 13 percent. The rest of the percentages are as follows: Human 13 percent, Feline 7.8 percent, Rodent 4.7 percent, and Duck-Goose, Deer-Elk, Canine and Cat 1.2 percent.

Of the 57 *E. coli* identified with lumped source categories (see Table 2), the highest area of impact was seen in the Avian / Waterfowl category with 26 percent followed by Pet with 15 percent. The rest of the percentages are as follows: Human and Wildlife 13 percent.

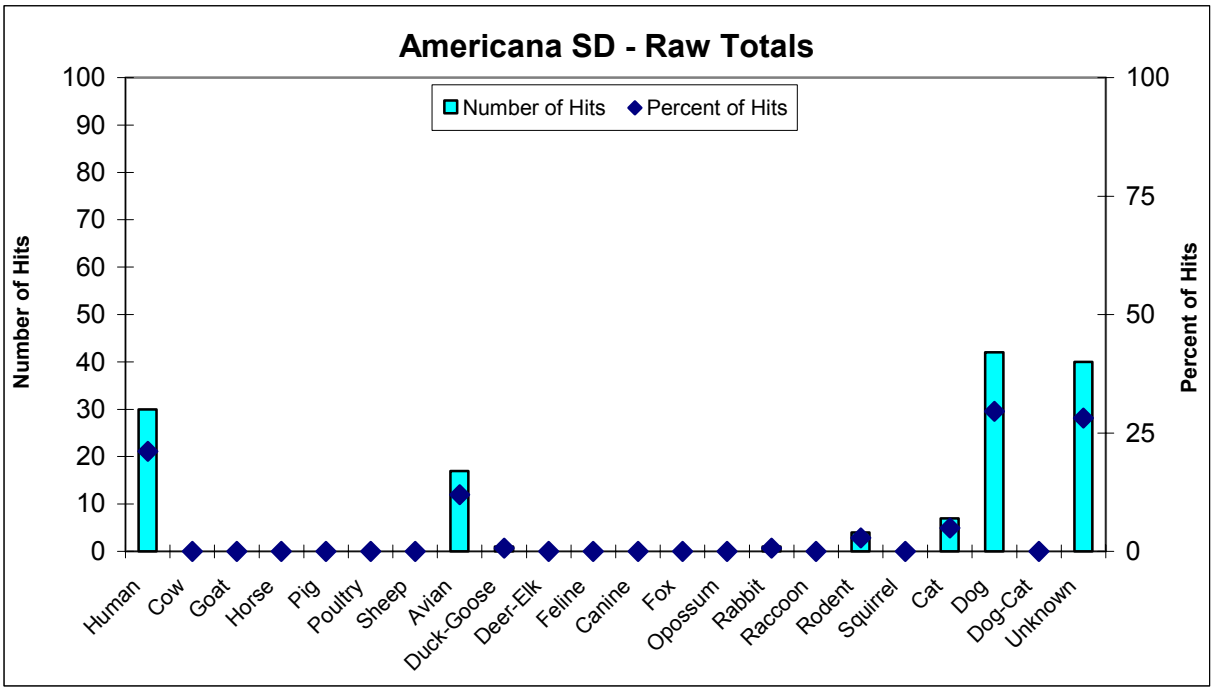


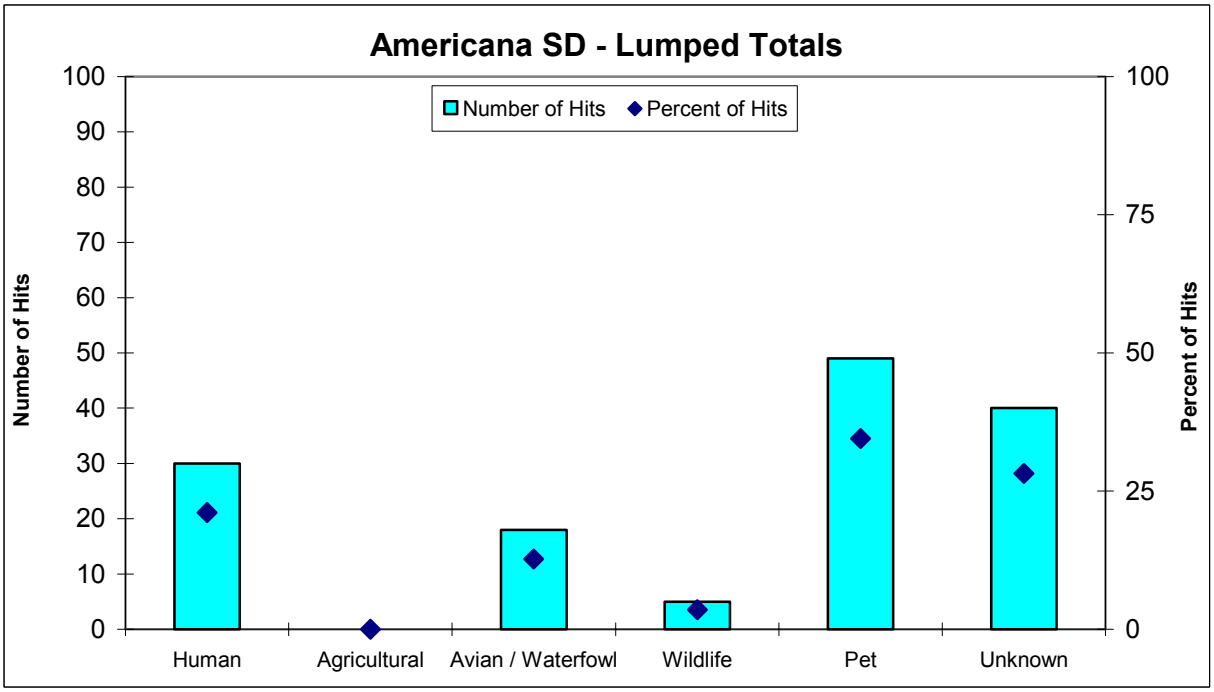



### Americana Boulevard Storm Drain

Of the 142 E. coli sampled, 102 sources were positively identified. The highest percentage of coliform DNA was seen in the Dog with 30 percent, followed by the Human 21 percent and Avian 12 percent each. The rest of the percentages are as follows: Cat 4.9 percent, Rodent 2.8 percent, and Duck-Goose and Rabbit 0.7 percent.

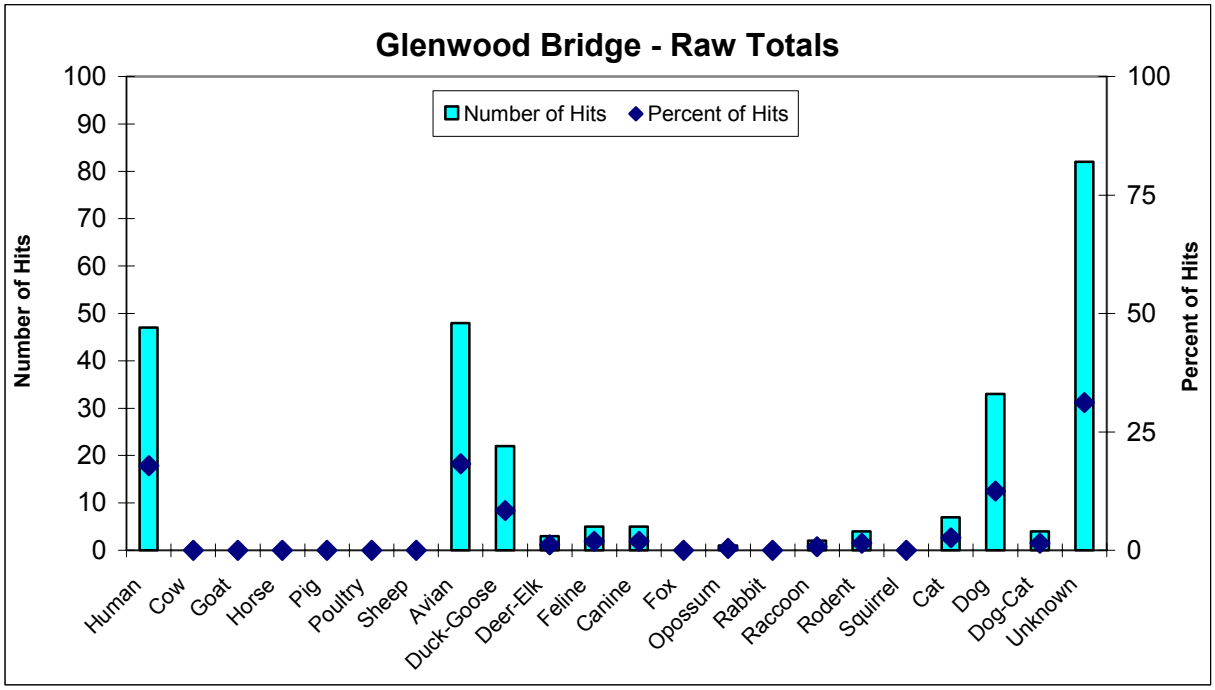
Of the 102 *E. coli* identified with lumped source categories (see Table 2), the largest impact by far was seen in the Pet category with 34 percent followed by Human with 21 percent. The rest of the percentages are as follows: Avian / Waterfowl 13 percent, Wildlife 3.5 percent.

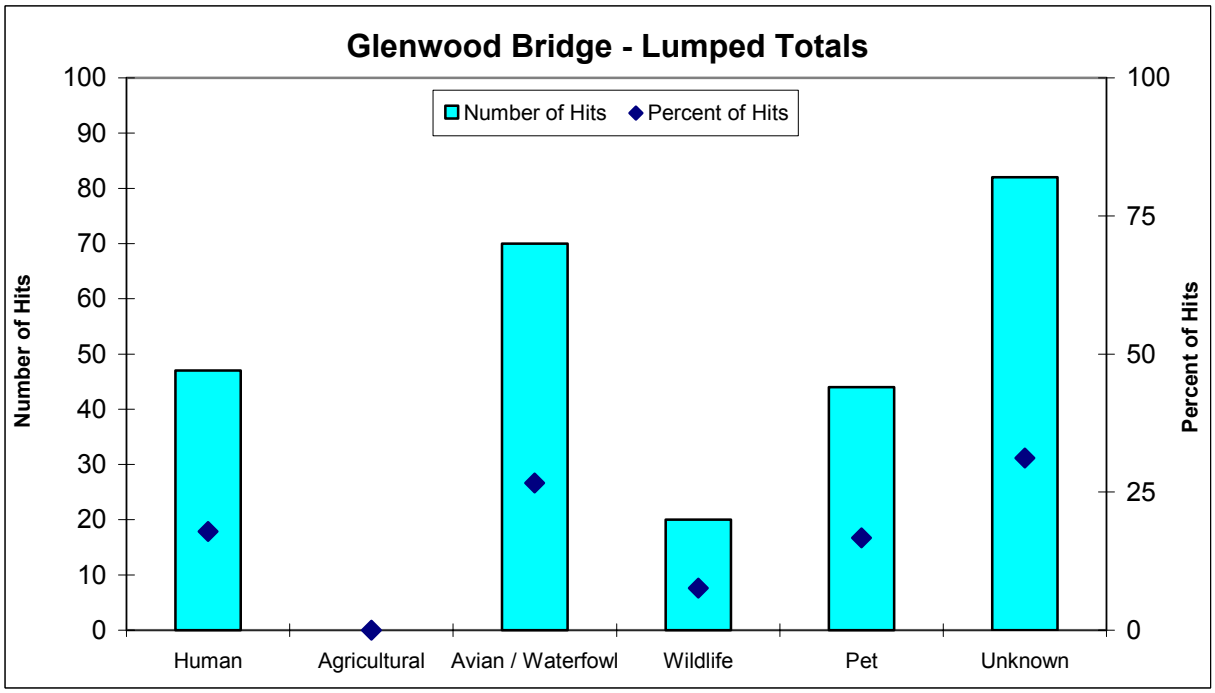


## Glenwood Bridge

Of the 263 *E. coli* sampled, 181 sources were positively identified. The highest percentage of coliform DNA was seen both in the Avian and Human with 18 percent. The rest of the percentages are as follows: Dog 13 percent, Duck-Goose 8.4 percent, Cat 2.7 percent, Feline and Canine 1.9 percent, Rodent and Dog-Cat 1.5 percent, Deer-Elk 1.1 percent, Raccoon 0.8 percent, Opossum 0.4 percent. Of the 181 *E. coli* identified with lumped source categories (see Table 2), the highest area of impact was seen in the Avian/Waterfowl with 27 percent followed by the Human category with 18 percent. The rest of the percentages are as follows: Pet 17 percent, and Wildlife 7.6 percent.

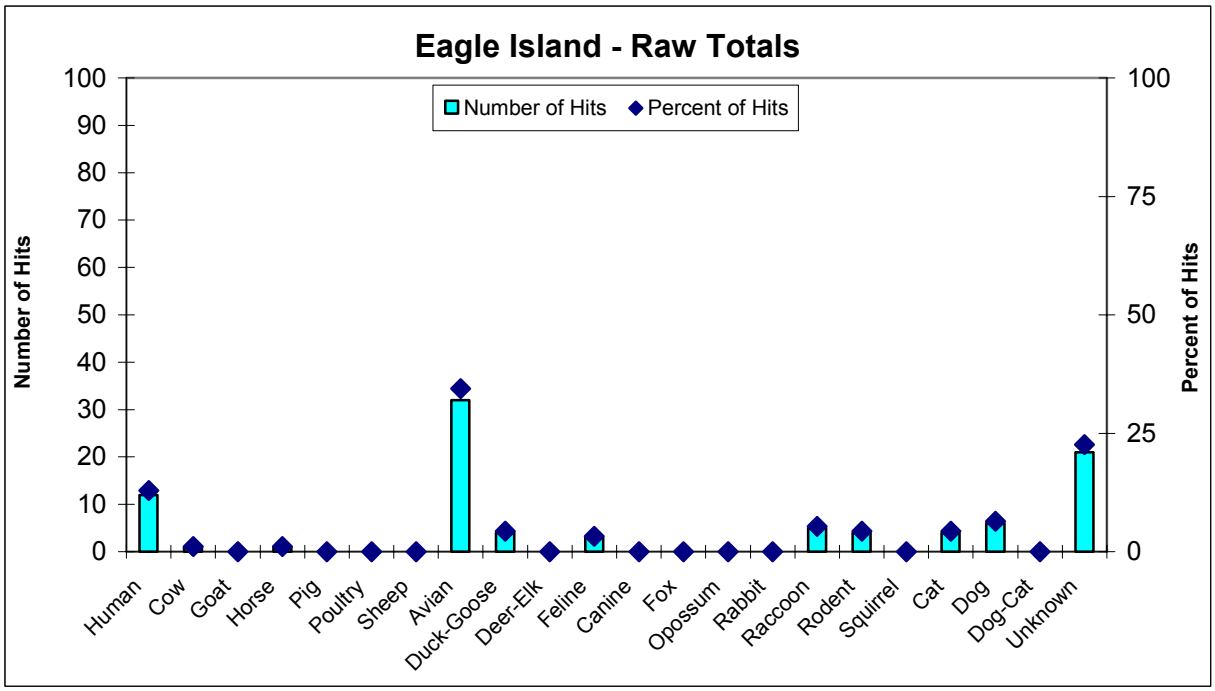


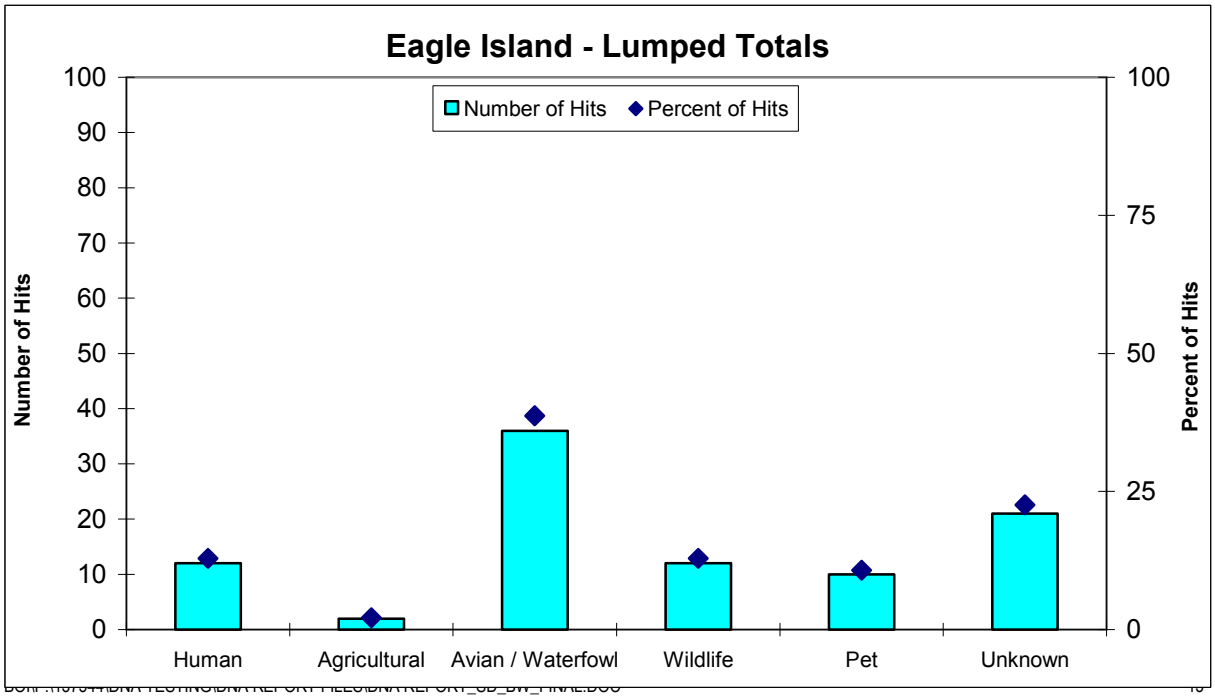



## Eagle Island

Of the 93 *E. coli* sampled 72 total sources were positively identified, the highest percentage in coliform DNA was seen in the Avian source with 34 percent followed by the Human with 13 percent. The rest of the percentages are as follows: Dog 6.5 percent, Raccoon 5.4 percent, Rodent and Cat 4.3 percent, Duck-Goose and Feline 3.2 percent, and Cow and Horse 1.1 percent.

Of the 72 *E. coli* identified with lumped source categories (see Table 2), the highest impact was seen in the Avian / Waterfowl category with 39 percent followed by Human and Wildlife with 13 percent. The other percentages are as follows: Pet 11 percent and Livestock with 2.2 percent.

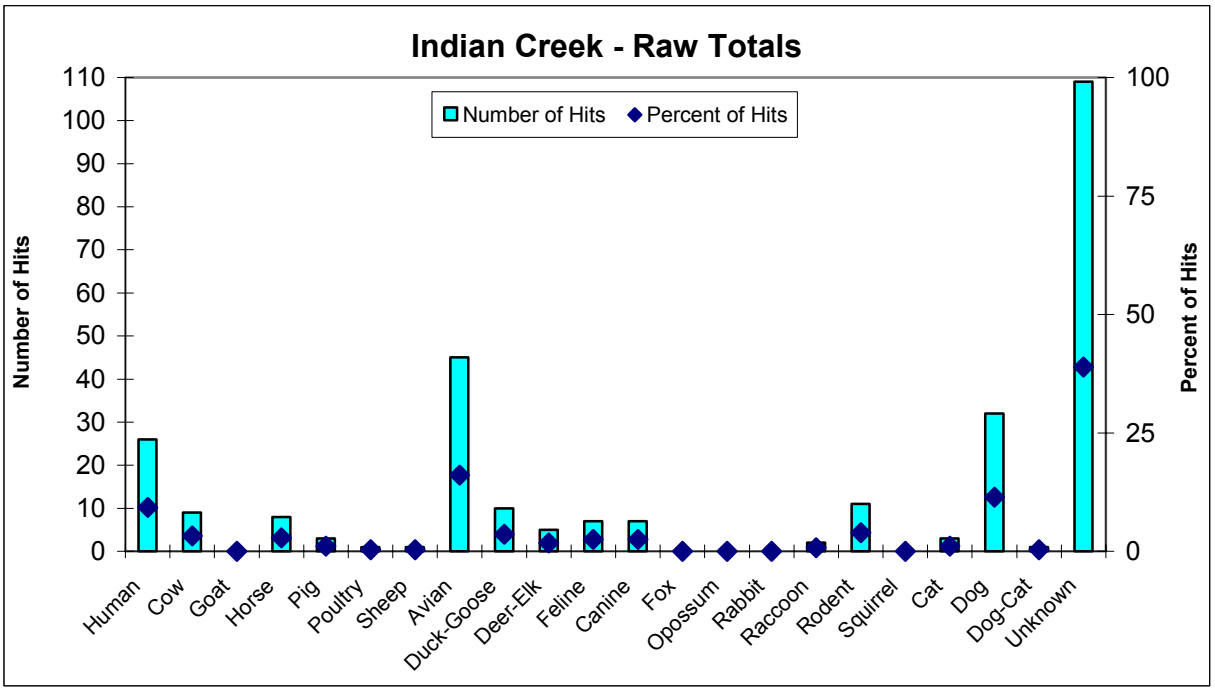


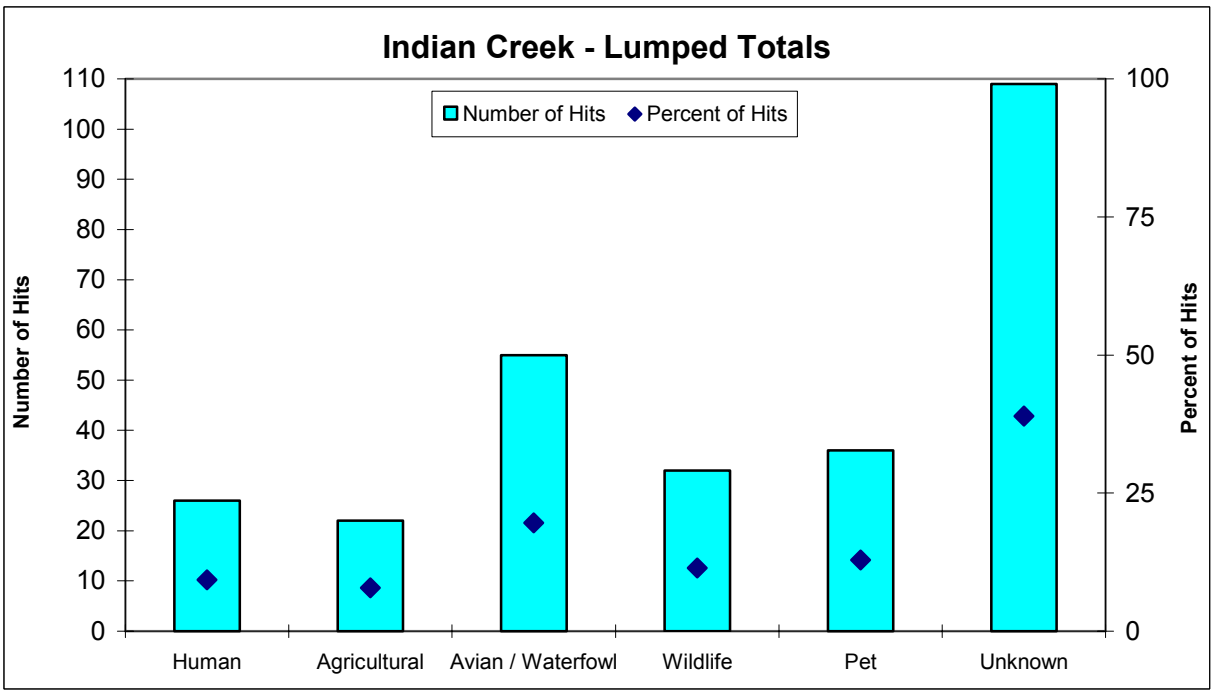



## Indian Creek

Of the 280 *E. coli* sampled, 172 sources were positively identified. The highest percentage was Avian with 16 percent followed by Dog with 11 percent. The rest of the percentages are as follows: Human 9.3 percent, Rodent 3.9 percent, Duck-Goose 3.6 percent, Cow 3.2 percent, Horse 2.9 percent, Feline and Canine 2.5 percent, Deer-Elk 1.8 percent, Pig and Cat 1.1 percent, Raccoon 0.7 percent, and Poultry, Sheep, and Dog-Cat 0.4 percent.

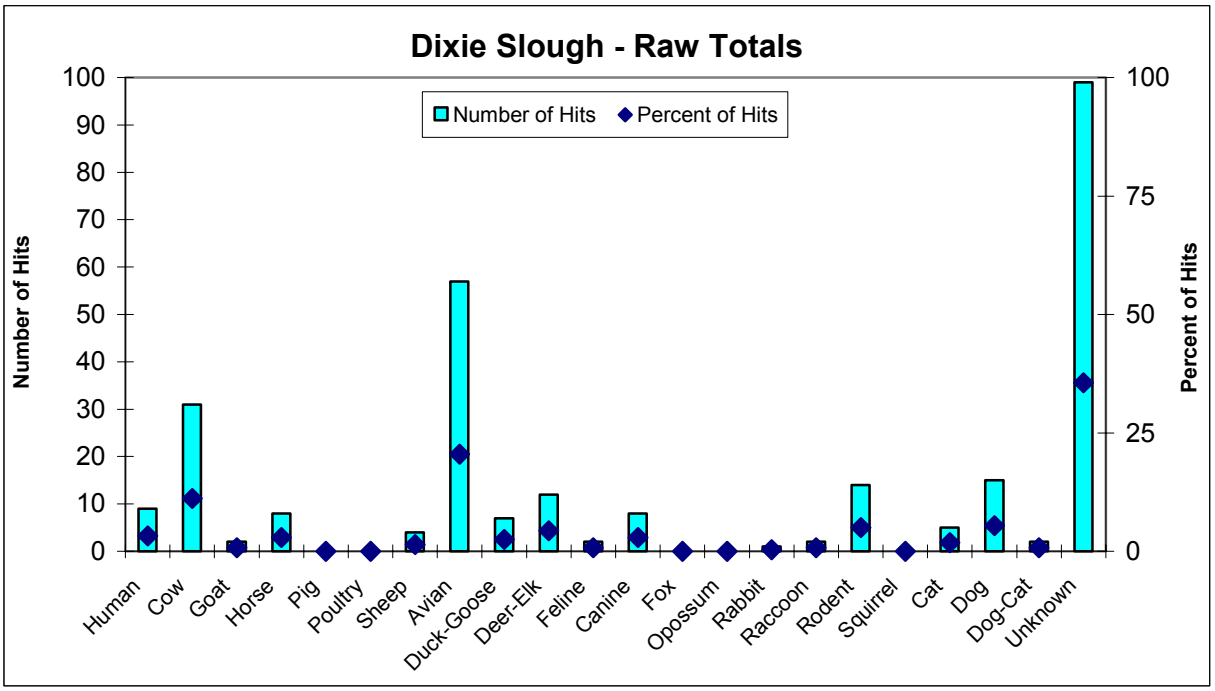
Of the 172 *E. coli* identified with lumped source categories (see Table 2), the greatest impact was seen as Avian / Waterfowl with 20 percent. The rest of the percentages are as follows: Pet 13 percent, Wildlife 11 percent, Human 9.3 percent, and Livestock 7.9 percent.

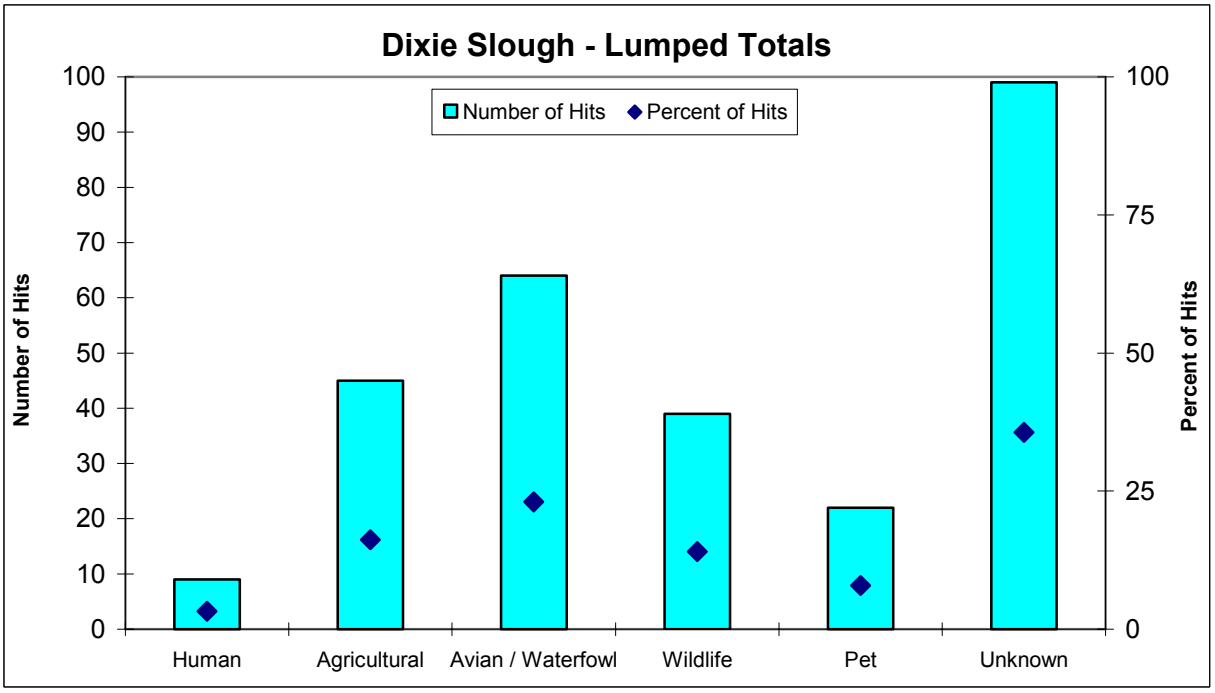


## Dixie Slough

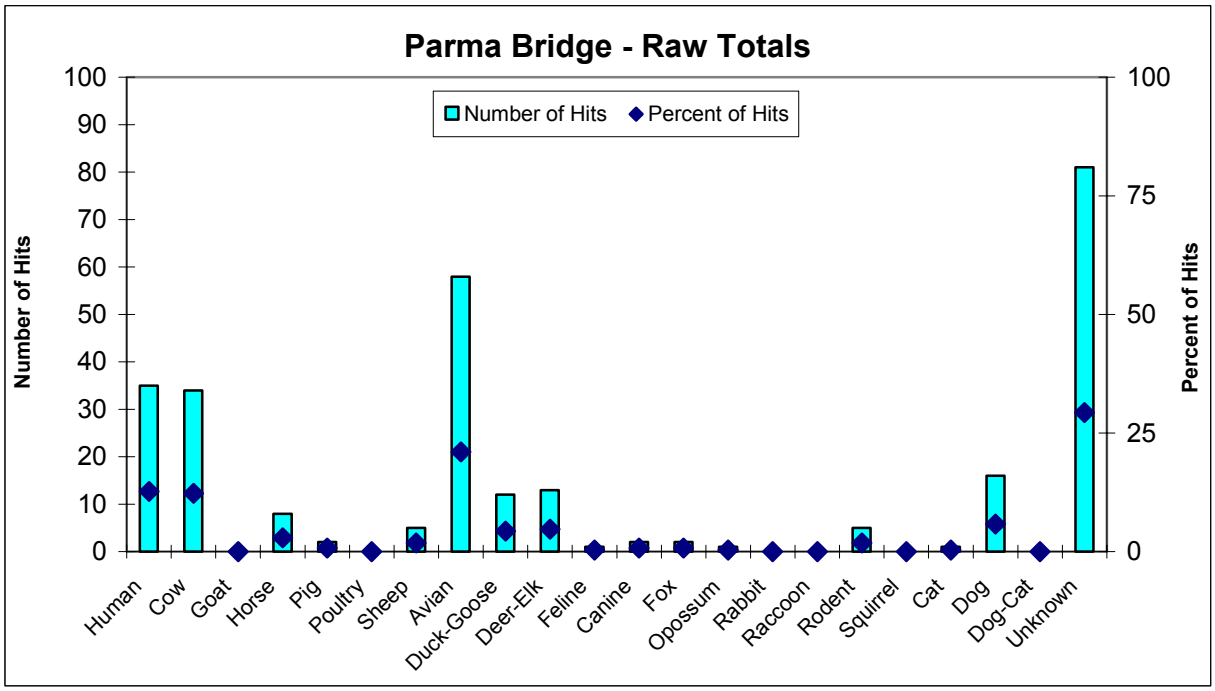
Of the 278 *E. coli* sampled, 179 sources were positively identified. The source which received the highest percentage in coliform DNA was Avian with 20 percent of the hits, then Cow with 11 percent. The rest of the percentages are as follows: Dog 5.4 percent, Rodent 5.0 percent, Deer-Elk 4.3 percent, Human 3.2 percent, Horse and Canine 2.9 percent, Duck-Goose 2.5 percent, Cat 1.8 percent, Sheep 1.4 percent, Goat, Feline, Raccoon and Dog-Cat 0.7 percent, and Rabbit 0.4 percent. Of the 179 *E. coli* identified with lumped source categories (see Table 2), the greatest impact was seen in the Avian / Waterfowl category with 23 percent, followed by Livestock with 16 percent. The other percentages are as follows: Wildlife 11 percent, Pet 7.9 percent, and Human 3.2 percent.

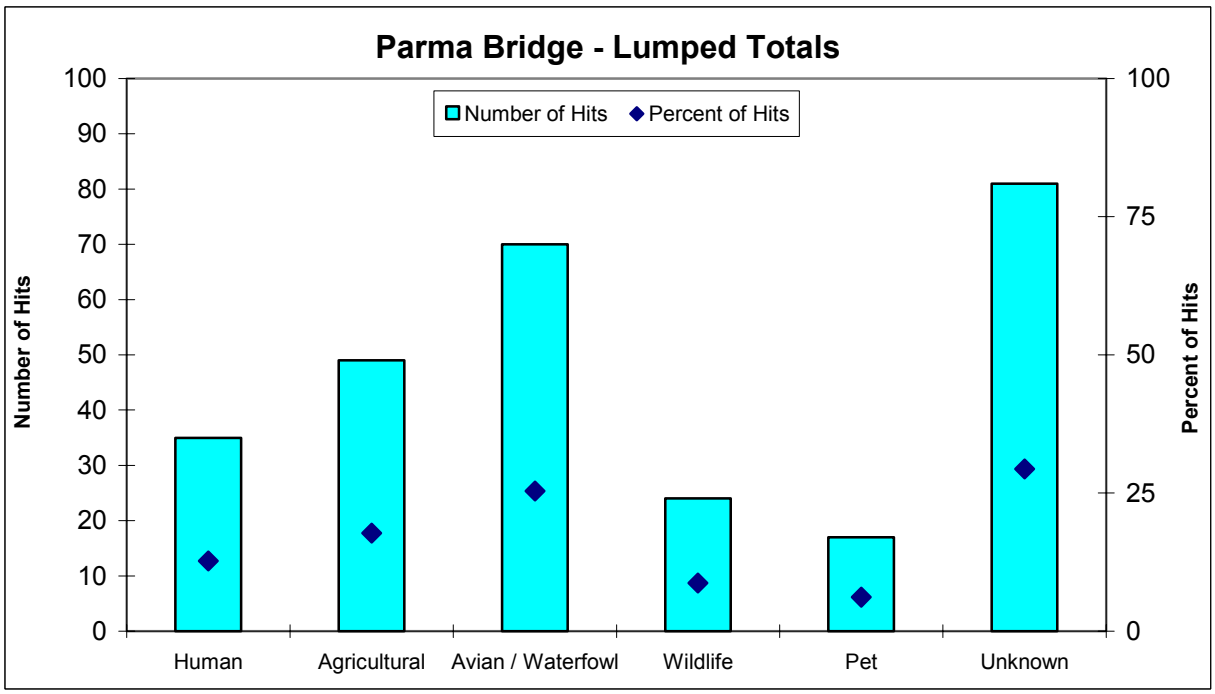


## Parma Bridge

Of the 276 *E. coli* sampled, 195 sources were positively identified. The highest percentage in coliform DNA was seen in the Avian with 21 percent, followed by the Human and Cow with 13 percent and 12 percent, respectively. The other percentages are as follows: Dog 5.8 percent, Deer-Elk 4.7 percent, Duck-Goose 4.3 percent, Horse 2.9 percent, Sheep and Rodent 1.8 percent, Pig and Fox 0.7 percent, and Feline, Opossum and Cat 0.4 percent. Of the 195 *E. coli* identified with lumped source categories (see Table 2), the highest impact was seen in the Avian / Waterfowl category with 25 percent followed by Livestock with 18 percent. The rest of the percentages are as follows: Human 13 percent, Wildlife 8.7 percent, and Pet 6.2 percent.



# Fecal Coliform Analyses Overview

## Fecal Coliform Results

A summary of fecal coliform concentrations are presented for each station in Figure 3.[2] To calculate geometric mean concentrations, all fecal coliform colonies expressed as greater than (>) values were taken to be the concentration. For example, if a fecal coliform count was reported as >1400/100 mL, then the concentration of 1400/100 mL was used in the calculation of the geometric mean.

Also, the criteria specify a geometric mean based on a minimum of five samples taken over a 30-day period. DEQ's criteria require this approach so that the numbers are both conservative and representative of the 30-day period. To compare the study data against this criteria, samples taken daily at each station were first averaged (using geometric mean) to find the fecal coliform level for that day.[3] This first step ensured that the final value represents changing concentrations over a 30-day period, not within a 24-hour period. The next step was to calculate 30-day geometric mean values using the available daily geometric mean concentrations. The final step was to select the highest 30-day geometric mean that was calculated from a minimum of five daily geometric mean values. The resulting number is the geometric mean concentration at each site provided in this section.

Within the urban areas of the watershed upstream from Eagle Island, geometric mean fecal coliform concentrations ranged between 77/100 mL in the mainstem at Glenwood Bridge to 1643/100 mL in the Walnut Street storm drain, as shown in Figure 3. (A more detailed discussion of concentrations within the storm drains during storms versus dry conditions is presented in the urban stormwater discussion section of this report.) Geometric mean concentrations from the riparian stations indicate that the riparian contribution of fecal coliform is 384/100 mL at Ann Morrison and 397/100 mL at the Eagle Island site.

Within the rural portion of the watershed, the geometric mean concentrations for each station are similar to trends in the data available for, and used in, the TMDL (which were collected generally between 1970 and 1998). Within the mainstem, concentrations of fecal coliform generally increase in the downstream direction from Glenwood Bridge (77/100 mL) toward Parma Bridge (803/100 mL). As shown in Figure 3, these values are somewhat higher than the TMDL concentrations (50 and 703/100 mL, respectively) but follow the same increasing trend in the downstream direction. These trends are also consistent with the most recent USGS database that contains fecal coliform concentrations for Glenwood Bridge and Parma Bridge. This database includes data used in development of the TMDL (data collected through 1998), as well as more recent data collected through September 1999. Fecal coliform geometric mean concentrations from the USGS database at Glenwood Bridge are 40/100 mL, and increase to 450/100 mL at Parma Bridge (Figure 3; USGS 2001).

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[2] To put the results of these results into context, the previous water quality criterion for fecal coliform (primary contact recreation between May 1 and September 30) was maximum of 500/100 ml at any time; 200/100 ml in more than 10 percent of the total samples taken over a 30-day period; and a geometric mean of 50/100 ml based on a minimum of five samples taken over a 30-day period. Current water quality criteria are expressed in *E. coli* concentrations, as discussed in the following section.

[3] Although taking the geometric mean of a geometric mean may result in skewing the data, this approach was taken in order to provide a meaningful representation of each day as an individual sample, in order to give each day of sampling equal weight within the 30-day period. Because the number of samples collected for each day was not equal and bacteria concentrations are generally lognormally distributed, we elected to use geometric mean values to represent daily average concentrations.

Geometric mean concentrations of fecal coliform in Indian Creek and Dixie Slough, tributaries that drain primarily sub-watersheds in the lower part of the watershed, range between 603 and 2101/100 mL, respectively. These values are slightly lower than the TMDL concentrations for these two tributaries (770 and 2987/100 mL, respectively).

The daily geometric mean concentration of fecal coliform for each station is shown in Figure 4. Stations are displayed by category (e.g., 319 grant stations) and this figure also shows the general sampling schedule for each category of station. It is important to note that the concentration scale for the ACHD stormwater stations is larger than the 319 grant stations and the riparian stations; these larger concentrations are due to the nature of the sampling program and will be discussed in further detail later in the report.

### *E. Coli* Estimates

Although the new bacteria criteria are expressed in terms of *E. coli*, data from this study were directly measured as fecal coliform colonies. In the absence of actual *E. coli* concentrations, information from IEH's fingerprinting method was used to estimate the associated *E. coli* concentrations for each station. An alternative correlation/regression method was also evaluated and rejected in favor of the IEH methodology. To see if there was a regional relationship between *E. coli* concentrations and fecal coliform concentrations, *E. coli* concentrations were plotted against fecal coliform concentrations measured in over 145 samples collected from various locations within the lower Boise River watershed (Figure 5; USGS 2001). The preliminary indication is that these concentrations appear to be correlated ($R^2 = 0.88$). However, further analysis suggests that if the two highest data points (concentrations of both fecal coliform and *E. coli* above 15000/100 mL, which represent less than 2 percent of the dataset) are removed, the correlation drops to below random chance ($R^2 = 0.37$). In addition, the data indicate that at very low fecal coliform concentrations (<100/100 mL), corresponding *E. coli* concentrations may range between 0 and 140/100 mL. Thus, this method was rejected.

To estimate *E. coli* concentrations using the IEH data, the total number of *E. coli* colonies per plate was divided by the total number of fecal coliform colonies per plate. This number was used as a surrogate to estimate the approximate *E. coli* concentration associated with a given sample for which fecal coliform concentrations were measured. For example, if a plate collected from the Glenwood Bridge station had a total of 10 fecal coliform colonies, and 8 of those colonies were positively identified as *E. coli*, the *E. coli* concentration was estimated to be 80 percent of the total fecal coliform concentration. The IEH colony count dataset is presented in Appendix C.

Again, this calculation is only an estimate because actual *E. coli* concentrations are not available for this study.[4] However, these estimates provide the best available data to determine the potential for meeting the state *E. coli* criteria on a site by site and watershed-wide basis. Table 4 presents a station-specific summary of the estimated *E. coli* concentrations, including the associated variability and number of samples for each station.

[4] In addition, no statistical metrics could be calculated because no actual E. coli concentrations were measured.

**TABLE 4. SUMMARY OF E. COLI CONCENTRATION ESTIMATIONS**

| Station | Geomean Fecal Coliform Concentration (#/100 mL) | n = | % *E. coli* Colonies of Total Fecal Colonies | Estimated Geomean *E. coli* Concentration (#/100 mL) |
|---|---|---|---|---|
| Walnut Street | 1,643 | 15 | 78.3% | 1,287 |
| Ann Morrison | 384 | 20 | 78.9% | 303 |
| Americana | 1,099 | 12 | 61.1% | 672 |
| GLENWOOD BRIDGE | 77 | 27 | 78.9% | 61 |
| Eagle Island | 397 | 20 | 93.8% | 372 |
| Indian Creek | 603 | 24 | 88.4% | 533 |
| Dixie Slough | 2,101 | 25 | 89.2% | 1,873 |
| PARMA BRIDGE | 803 | 24 | 86.3% | 693 |

NOTE: Mainstem stations are capitalized.

The percentage of *E. coli* colonies to total fecal coliform colonies ranges from 61 to 94 percent. The number of samples from each station appear to be sufficient (between 12 and 27) to be able to estimate a reasonable *E. coli* percentage to use in the context of interpreting the results of this study.

# Discussion

## Watershed Overview

To identify sources of microbial pollution that are impacting a waterbody, a collection of representative bacterial isolates in the watershed must be established. To effectively use this technique, this identification method has to have enough sensitivity to group the bacterial isolates on the basis of their species of origin. An epidemiological approach (which is used in this study) relies on a 100 percent match between *E. coli* strains isolated from water samples and strains from the source library. Analysis of *E. coli* strains of known origin using the IEH source tracking method has shown 96 percent source specificity (Buck 1997). The accuracy of the method is further increased by eliminating the 4 percent of *E. coli* that do not show host specificity from the library.

The margin of error in experimental execution and data analysis is another important factor in determination of the confidence in the results. In order to calculate the margin of error, in two separate studies, blind sets of E. coli were included in the study design. The first study (TMDL study of three watersheds in Virginia) was conducted in collaboration with the U.S. Geological Survey in Richmond, Virginia (Heyer, in press). In the second study in Morrow Bay, California, the study was conducted in collaboration with Dr. C. Kitts at the California Polytechnic University. Over 64 blind *E. coli* strains from both of these projects were identified with 100 percent accuracy using the IEH source tracking methodology.

Thus, the advantage of the method is the relatively high level of accuracy and certainty because if a 100 percent match is not possible, then no source is identified. Disadvantages of

the method are that it requires a large library of source isolates and that there is always a percentage of sample isolates for which matches are unable to be identified. Thus, at the beginning of each study a larger number of samples are collected for analysis under the presumption that a certain number will not yield source information. This allows the results of the study to remain significant because of the sufficiently large number of sample isolates that are initially collected.

## Unidentifiable Sources

Prior to discussing the sources that were able to be positively identified, it is important to note that 486 *E. coli* isolates were not able to be positively identified and that these unidentified isolates were distributed throughout the watershed. The fact that these unidentifiable isolates occur throughout the watershed suggests that there is no bias in overlooking a particular type of source. For example, if cow sources were underrepresented, a larger percentage of unidentifiable sources in the agricultural reaches would be expected. Because this is not the case, the unidentifiable sources likely represent a variety of bacteria wastes.

The exact distribution of unidentifiable sources cannot be determined because the DNA ribotyping method is not predictive. That is, unidentified isolates could either have the same distribution as the known isolates in the study, or they could represent source groups that are underrepresented (mostly wildlife) in the source library. While the reality is probably somewhere in between the two scenarios, speculative and predictive analysis of individual unknowns was not considered an appropriate use of the dataset.

However, since sources for approximately 30 percent of the overall dataset could not be identified, the treatment of these sources as a group warrants additional discussion to provide a preliminary indication of what these data mean within the context of the lower Boise River TMDL. For the purposes of this analysis, the group of unknown was classified either as controllable (i.e., anthropogenic) or uncontrollable (i.e., non-anthropogenic). Again, it is important to note that discussing these results in terms of controllable and uncontrollable terms is completely subjective because the sources of the unknowns cannot be predicted.

Treating the unidentified sources as controllable provides an indication of whether the criteria could be met if all controllable sources were controlled and/or eliminated. From a different perspective, treating the unidentified sources as uncontrollable provides a more environmentally protective perspective because then more resources would be applied toward those sources that are controllable and will have the largest impact on bacteria reductions. Table 5 illustrates these ranges for each of the sampling stations.

**TABLE 5. ESTIMATE OF POTENTIAL CONTROLLABLE VS. UNCONTROLLABLE E. COLI CONCENTRATIONS (#/100 ML)**

| | | Unknowns = Uncontrollable: Minimum Controllable Sources[2] | | | Unknowns = Controllable Maximum Controllable Sources[3] | | |
|---|---|---|---|---|---|---|---|
| Station[1] | Current Estimated *E. coli* Concentration | % | Controllable *E. coli* Concentration | Uncontrollable *E. coli* Concentration | % | Controllable *E. coli* Concentration | Uncontrollable *E. coli* Concentration |
| Walnut Street | 1287 | 39% | 502 | 784 | 56% | 723 | 564 |
| Ann Morrison | 303 | 27% | 81 | 222 | 60% | 183 | 120 |
| Americana | 672 | 56% | 374 | 298 | 84% | 563 | 109 |
| GLENWOOD | 61 | 35% | 21 | 40 | 66% | 40 | 21 |
| Eagle Island | 372 | 26% | 96 | 276 | 48% | 180 | 192 |
| Indian Creek | 533 | 30% | 160 | 373 | 69% | 368 | 166 |
| Dixie Slough | 1873 | 27% | 512 | 1361 | 63% | 1179 | 694 |
| PARMA | 693 | 37% | 254 | 440 | 66% | 457 | 236 |

1- Mainstem stations are capitalized.
2- Percent based on all bacteria sources. Minimum feasible controllable sources upstream from Glenwood Bridge include humans and pets. Minimum feasible controllable sources downstream from Glenwood Bridge include humans, pets, and livestock.
3- Percent based on all bacteria sources. Maximum feasible controllable sources upstream from Glenwood Bridge include humans, pets, and unidentified sources. Maximum feasible controllable sources downstream from Glenwood Bridge include humans, pets, livestock, and unidentified sources.

For example, at Parma Bridge if the unidentified sources are considered uncontrollable, then the controllable concentrations of *E. coli* are lower (254/100 mL) than if the unidentified sources are considered controllable (457/100 mL). From a watershed protectiveness perspective, these lower concentrations mean that additional resources could be spent mitigating the controllable sources, instead of assuming that a greater proportion of the *E. coli* bacteria cannot be controlled.

Instead of assuming that the unidentified sources are either completely controllable or completely uncontrollable, it is more realistic to presume that some of the unidentified sources fall into both categories. Because we cannot predict the actual split, the results of those positively identified sources should be used to begin to decide how to best allocate existing resources. Therefore, the remaining discussion focuses primarily on what is known from the results of the DNA fingerprinting analyses.

## Identifiable Sources

The total human contribution to bacteria appears to decrease somewhat as the river and its tributaries flow from predominantly urban areas to more rural areas in the downstream direction. If pets are included in this category, the combined contribution of humans and pets follows the same decreasing trend, while the influence of livestock increases. In urban areas, pet waste contributions are higher than human waste. In rural areas associated with agricultural sources, cow wastes contribute the highest percentage (in Dixie Slough), perhaps from livestock grazing along banks or an increase in density of concentrated animal feeding operations (CAFOs) and animal feeding operations (AFOs). This suggests that available resources should be spent controlling human and pet sources in the upper reaches

and human, pet , and agricultural sources in the lower reaches. Specific mechanisms for such controls should be included in the TMDL Implementation Plan.

Throughout the watershed, the avian/waterfowl/wildlife contribution to bacteria is consistently large (average of 50 percent of the total bacteria waste that was able to be identified). Deer/elk, felines (wild cats), and rodents are the greatest wildlife contributors. Controlling these sources in an urban or rural environment above and beyond existing controls may not be the best use of resources in terms of meeting the TMDL goals for bacteria reduction in the short-term.

It is important to note that in pristine environments with wildlife populations (e.g., headwater areas), bacteria concentrations are not typically as high as what was observed in this study and few of these areas are subject to the TMDL process for bacteria. Within the urban and rural areas of the lower Boise River watershed, bacteria concentrations associated with wildlife are relatively higher probably due to a number of factors:

1) The extensive irrigation canal and drainage system provides more opportunity for wildlife to be direct contact with water.

2) The riparian buffer zones that have been established within the urban areas concentrate wildlife directly along the river corridor, and the wildlife population is relatively dense in these riparian buffer zones.

3) The urban area has more impervious area and fewer wetlands, which decreases the assimilative capacity of the watershed to mitigate bacteria waste.

Additional factors that probably contribute to elevated wildlife concentrations should be explored in more detail in the long-term.

Duck/goose wastes contribute consistently throughout the system (5 percent), while avian wastes consistently average almost 30 percent. These non-anthropogenic sources are relatively more difficult to control than anthropogenic sources. For example, if Boise City developed ordinances limiting the population of ducks and geese, this might result in objections from wildlife groups. A surging geese population in Anchorage contributes to bacteria levels in an urban environment (EPA 1997). To address the goose population, the Anchorage Waterfowl Working Group initially recommended partially killing the adult population and gathering eggs, which drew protests from wildlife groups. This resulted in different recommendations such as prohibiting feeding of waterfowl and replacing short grass with tall grass (EPA 1997). If ordinances were passed to prevent the waterfowl population from reaching the river, it would likely be cost-prohibitive and infeasible, as well as inappropriate because it may adversely affect the current ecosystem. Finally, controlling the waterfowl population (average of 5 percent), would likely not be as effective as determining a long-term acceptable strategy for controlling the avian population (average of 30 percent).

If it is assumed that the wildlife/avian/waterfowl sources are essentially uncontrollable in the short-term, it appears that the current bacteria TMDL target (based on *E. coli* concentrations) cannot be met. Stated another way, if resources were targeted in the short-term toward controlling those sources that were feasible (i.e., humans and pets upstream from Glenwood Bridge and humans, pets, and livestock downstream from Glenwood

Bridge) the resulting *E. coli* concentrations would still be above the criteria for primary recreational contact. To illustrate this, Table 6 estimates how much the estimated mean *E. coli* concentration could be decreased by completely controlling and eliminating all of the feasible controllable sources for each of the sampling stations. The geometric mean component of the current *E. coli* recreational criteria (126/100 mL) is used for comparison purposes in this analysis.

**TABLE 6. ESTIMATE OF POTENTIAL E. COLI CONCENTRATIONS ((#/100 ML)**

| Station[1] | Current Estimated *E. coli* Concentration | Percent of Controllable Sources[2] | Controllable *E. coli* Concentration | Uncontrollable *E. coli* Concentration | *E. coli* Target Concentration | Percent Reduction Required |
|---|---|---|---|---|---|---|
| Walnut Street | 1287 | 47% | 606 | 681 | - -[3] | - -[3] |
| Ann Morrison | 303 | 40% | 122 | 181 | 126 | 0% |
| Americana | 672 | 77% | 520 | 151 | - -[3] | - -[3] |
| GLENWOOD | 61 | 50% | 31 | 30 | 126 | 0% |
| Eagle Island | 372 | 33% | 124 | 248 | 126 | 0% |
| Indian Creek | 533 | 49% | 262 | 271 | 126 | 52% |
| Dixie Slough | 1873 | 42% | 795 | 1078 | 126 | 84% |
| PARMA | 693 | 52% | 359 | 334 | 126 | 65% |

1- Mainstem stations are capitalized.
2- Percent based on positively identified bacteria sources only. Feasible controllable sources upstream from Glenwood Bridge include humans and pets. Feasible controllable sources downstream from Glenwood Bridge include humans, pets, and livestock.
3- Walnut and Americana storm drains do not have a load allocation for bacteria. Although the concentrations from these storm drains are relatively higher than the other urban stations, the loading for these sites is relatively low because the flows are small in relation to the other drainages and mainstem. Additional discussion is provided on these two stations later in the report.

This analysis is also shown graphically in Figure 6. These calculations show that even if all of the controllable sources were essentially eliminated (which is unrealistic), the uncontrollable sources are still higher than the new *E. coli* standards (thus, the standard still would not be met) at Ann Morrison, Eagle Island, Indian Creek, Dixie Slough, and Parma Bridge.

A more detailed discussion of specific station results is provided in the following sections.

## 319 Grant Stations

If the Glenwood Bridge station is used as a measure of bacteria sources in the upper reaches of the mainstem, the data suggest that humans and pets contribute a relatively higher percentage of waste in the urban versus rural areas. Compared to Parma Bridge, which represents an area of the mainstem that is affected more by agricultural inputs, Glenwood Bridge has approximately the same percentage of controllable sources (Figure 2). However, bacteria levels at Glenwood Bridge are already below the criteria. Because the relative level of *E. coli* concentrations is much higher at Parma Bridge, more resources should be spent on reducing bacteria levels to achieve the new *E. coli* standards. Specifically, humans and pets (for a total of 19 percent) appear to be largest human contributors, and cow and horse wastes (for a total of 15 percent) appear to be the largest livestock contributors to sources at Parma Bridge.

The results for Indian Creek and Dixie Slough support the same conclusions, although Dixie appears to have a more serious bacteria problem. At Indian Creek, horses and cows appear to contribute to the livestock sources, although human hits outnumbered livestock hits in this sub-watershed that contains the cities of Kuna, Nampa, and Caldwell. During development of the TMDL, the Nampa WWTP geometric mean fecal coliform concentration was 65/100 mL, a value that is one of the higher values for WWTPs in the basin. However, this point source is currently required to limit fecal coliform discharges to 50/100 mL (the previous standard) and to monitor for *E. coli* on a monthly basis; thus, the source of elevated human sources in Indian Creek remains somewhat unclear. At Dixie Slough cows and horses are again the largest contributors to livestock bacteria.

## Riparian Stations

Similar to the other sites described in this study, the DNA data from the riparian sampling locations suggest a majority contribution from avian sources. Of the identifiable isolates in the riparian area, avians compose 26 percent at Ann Morrison Park and 39 percent at Eagle Island. Given the proximity of the riparian sampling locations to the river and the readily available living space for birds within the riparian area, it is not surprising that a majority of identifiable isolates are from avian sources. Riparian areas provide a dense area of food, water, shade and cover for all species of wildlife, particularly birds. Cottonwood and willow (*Salix* spp.) by their very nature attract a large diversity of insectivorous birds (Manci 1989), and both tree species are found in the lower Boise River riparian corridor.

Another large source in the riparian area is humans. The data show that 13 percent of the identifiable isolates from both Ann Morrison Park and Eagle Island are from human sources. The origin of the human bacteria may include leaking septic systems, leaking sewer lines, illicit discharge to the water, and direct contamination by humans. Pets are also a large source at Ann Morrison Park and Eagle Island, with 14 and 11 percent, respectively. Other minor sources include wildlife species such as rodents and raccoons.

Table 6 shows the estimated geometric mean and the reduction necessary to meet the *E. coli* standard for the riparian sampling sites. When the percentage of uncontrollable sources (as described in this paper) are considered, the percent reduction necessary to meet 126 organisms/100 ml is 0 percent at both Ann Morrison Park and Eagle Island.

The aforementioned analysis implies that the uncontrollable number of *E. coli* in the riparian corridor is greater than the standard itself. However, there are controllable sources that must be addressed, particularly below Glenwood Bridge where the lower Boise River is no longer in compliance with the bacteria standard. Activities that control the movement of bacteria generated by pets, humans and livestock should be implemented and managed appropriately. This is discussed further in the Recommendations section of this report.

## Stormwater Stations

Sources identified at the Walnut and Americana outfalls are consistent with those identified downstream at the Glenwood Bridge with avian, pets (primarily dogs), and human comprising the largest percentages of the known sources. The percentage of avian sources identified at the Walnut station was larger than at the Americana station (28 and 12 percent, respectively). This is likely due to the bird populations in the ponds that contribute flow to

the Walnut storm drain system. Human sources are among the leading sources identified with 9.6 and 30 percent at Walnut and Americana, respectively. Further investigation is needed to determine the cause and control of human sources.

It is important to note that data collected during storm events and dry weather events (background flow) were combined to determine the estimated concentrations. Bacterial concentrations in the storm drains varied considerably among dry weather and storm flows and among samples collected consecutively during the same sampling period. For example, during the April 25, 2001, storm event, fecal coliform concentrations ranged from 90/100mL to 47,000/100mL at the Americana site for 5 samples collected during a 9-minute interval. Samples collected at the Walnut site for the same storm event during a 10-minute interval ranged from a fecal coliform concentration of 50/100mL to 230/100mL.

The in-pipe concentrations exceed the *E. coli* in-stream standards at both sampling locations. However, river samples collected downstream at the Glenwood Bridge were below the *E. coli* criteria. These results suggest that while in-pipe bacterial concentrations are high, the bacterial loads from the drains and downstream in the river remain relatively low. Contributing factors are likely the assimilative capacity of the river, the duration and intensity of storm events, dilution, and the variability in concentration between dry weather and storm flows.

# Recommendations

Complete elimination of controllable sources represents a best-case scenario that is unlikely given the available resources, particularly in the rural areas of the watershed where bacteria concentrations are high. This suggests that a more realistic short-term approach will entail enforcing existing ordinances and permit limits that control human and pet waste upstream from Glenwood Bridge and human, pet, and livestock waste downstream from Glenwood Bridge. In the urban portion of Ada County, these activities are currently underway as part of a joint NPDES stormwater permit held by Boise, Garden City, Ada County Highway District, Idaho Transportation Department District 3, Boise State University and Drainage District #3. ACHD is also currently working with Boise City to determine the condition of storm drain and sewer systems to identify and mitigate possible sources of human contamination.

In Canyon County and its associated municipalities located in the riparian corridor, there is less documented stormwater management. As stormwater management programs move to the forefront in Canyon County as part of the Phase II NPDES stormwater permit program, activities that control riparian-borne and other bacteria sources are anticipated to be included. Existing ordinances and state and federal regulations (for example, those pertaining to migratory birds) should be used in the rural areas of the watershed to control waterfowl sources to the extent practicable, in order to continue the reduction of bacteria levels. This meets the intent of the TMDL, and specific strategies for such controls will be outlined in the lower Boise River TMDL Implementation Plan.

Concurrently, the LBRWQP, DEQ and USGS are implementing an iterative process by which *E. coli* data will continue to be collected and bacteria levels will continue to be monitored. Also, as NPDES effluent permits are re-issued, additional *E. coli* monitoring is

being required and data are being collected. As the management practices that limit the controllable sources are implemented and become effective in the short-term, the number of anthropogenic *E. coli* organisms in the water should decrease, leaving the uncontrollable levels as background.

These monitoring data are expected to help long-term management decisions. In the long-term, the responsible entities should evaluate how to further control less feasible sources, such as the avian/waterfowl populations and wildlife populations. In addition, more DNA testing in the watershed does not appear to be warranted at this time; however, follow-up DNA source testing will be useful after the TMDL Implementation Plan has been in effect for a number of years. This testing will be useful to evaluate the program effectiveness and to better refine adaptive management strategies and effective long-term controllable actions.

Although the bacteria criteria is not expected to change as a result of this study and the goals of the TMDL will remain the same (protection of human health), in the long-term, it may be appropriate to re-visit this issue. It is interesting to note that in Florida, TMDL regulations have shifted such that only verified human sources will remain on the 303(d) impaired waters list. Thus, data values that are elevated solely due to wildlife are not used to determine impairment. Although Florida operates under a very different climate regime and bacteria growing conditions, it might be appropriate to evaluate a similar creative approach in the future as additional monitoring data become available in the lower Boise River watershed.

In the interim, additional pathogen testing in the subbasin could be conducted to provide more insight into the actual risk of disease because bacteria indicators may be misleading. That is to say that the correlation between the indicator organisms and presence of pathogens may be weak. Despite examples where *E. coli* outbreaks occurred (for example, in a reservoir near Vancouver, Washington, even though bacterial indicators suggested low risk), ongoing research is needed because in EPA's Draft Implementation Guidance for Ambient Water Quality Criteria for Bacteria, EPA states that "it is inappropriate to conclude that wildlife sources present no risk to human health from waterborne pathogens" (EPA 2002b).

Pathogen testing relies on advances in microbiological methods to conduct broad surveys for human pathogens in water, wastewater, and environmental samples. Water samples would be collected so that microbial cells could be concentrated, bacteria pathogens could be enriched, and DNA could be extracted and identified via PCR analysis for a broad group of human pathogens. These data would be used to conduct a risk assessment to determine the actual human health risk linked to recreational contact with water in the lower Boise River basin.

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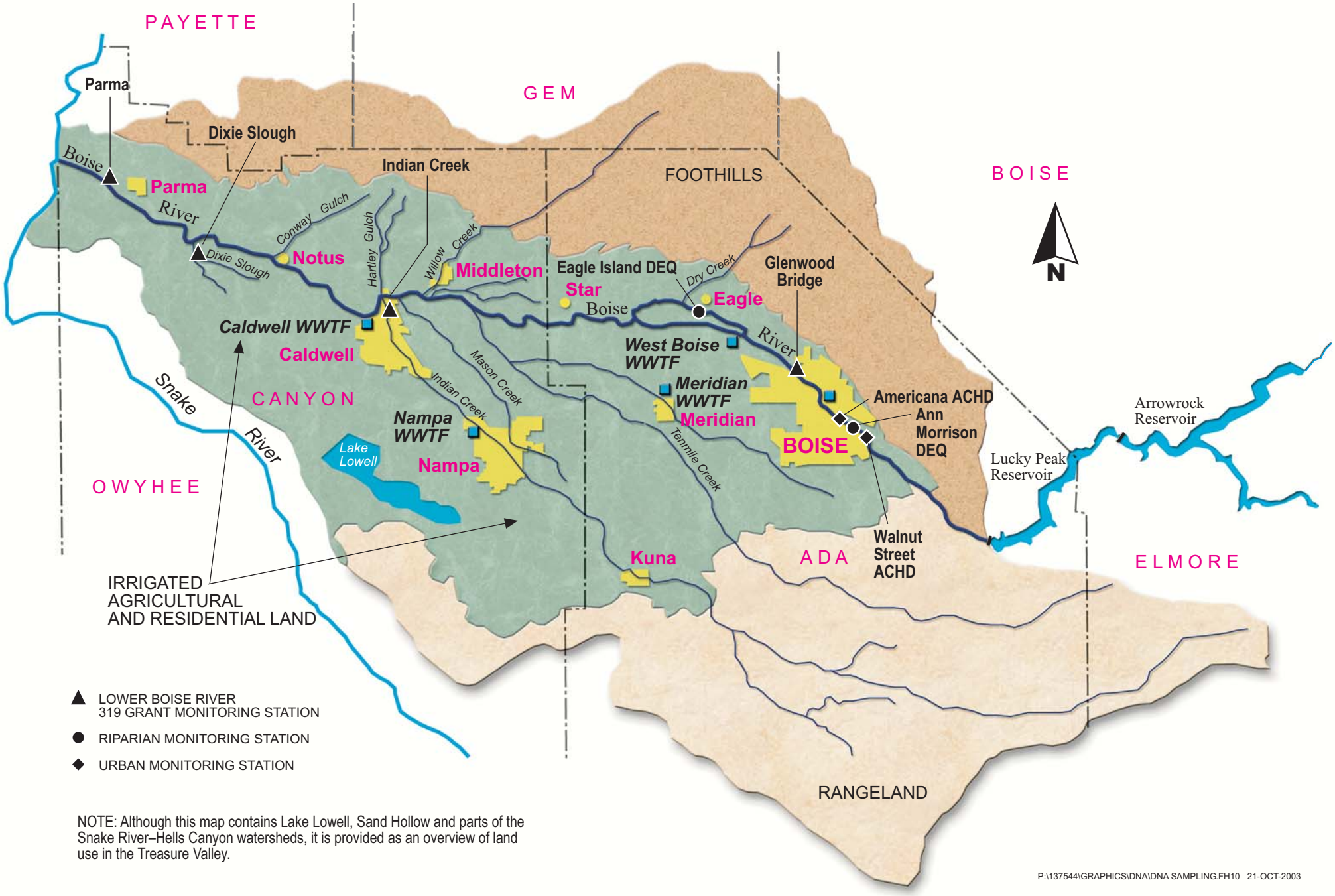
FIGURE 1

# Lower Boise River DNA Sampling Locations

## LOWER BOISE RIVER WATER QUALITY PLAN




NOTE: Although this map contains Lake Lowell, Sand Hollow and parts of the Snake River–Hells Canyon watersheds, it is provided as an overview of land use in the Treasure Valley.

P:\137544\GRAPHICS\DNA\DNA SAMPLING.FH10 21-OCT-2003

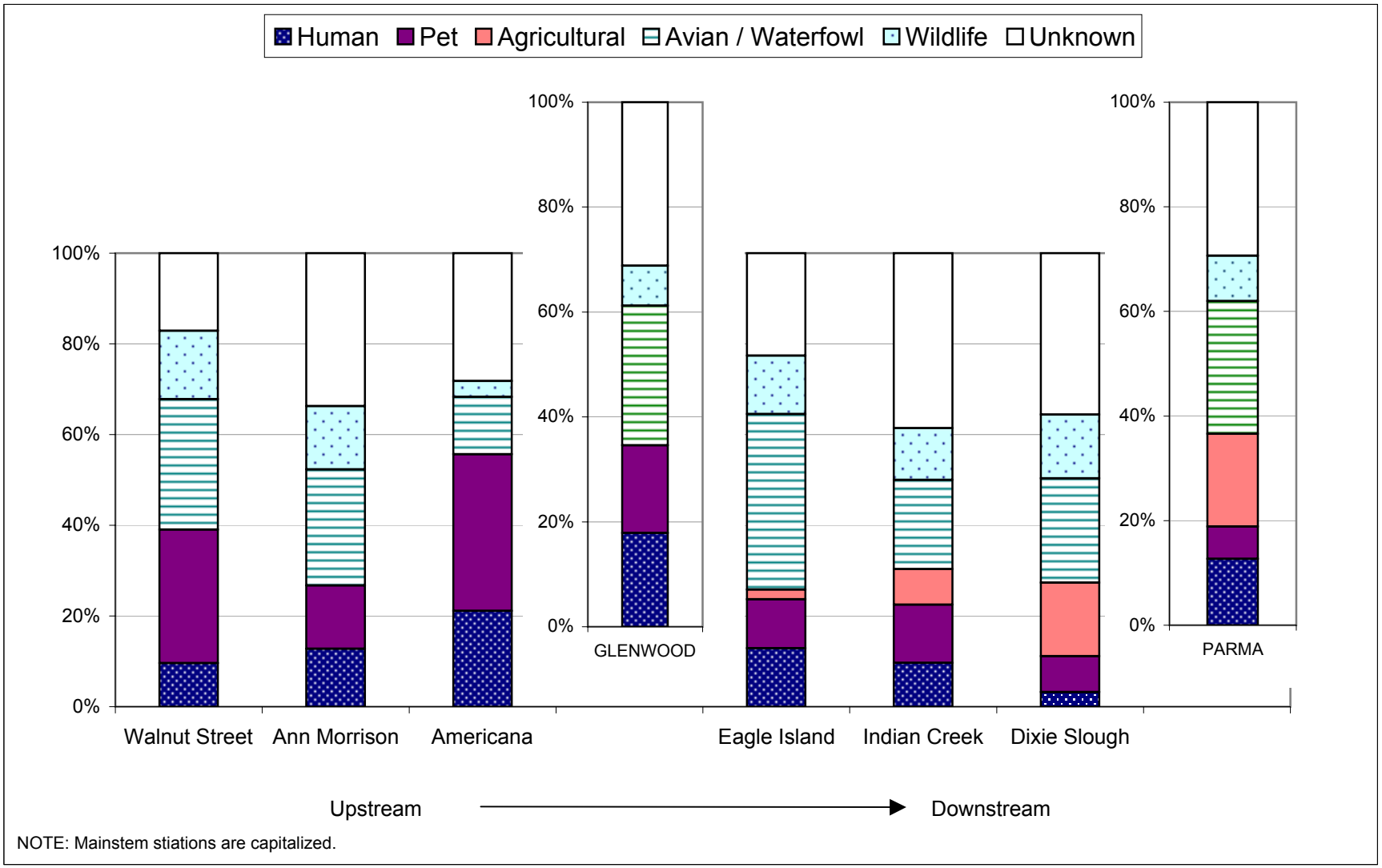



Figure 2. Summary of DNA hits by percent by location throughout the Lower Boise River basin.

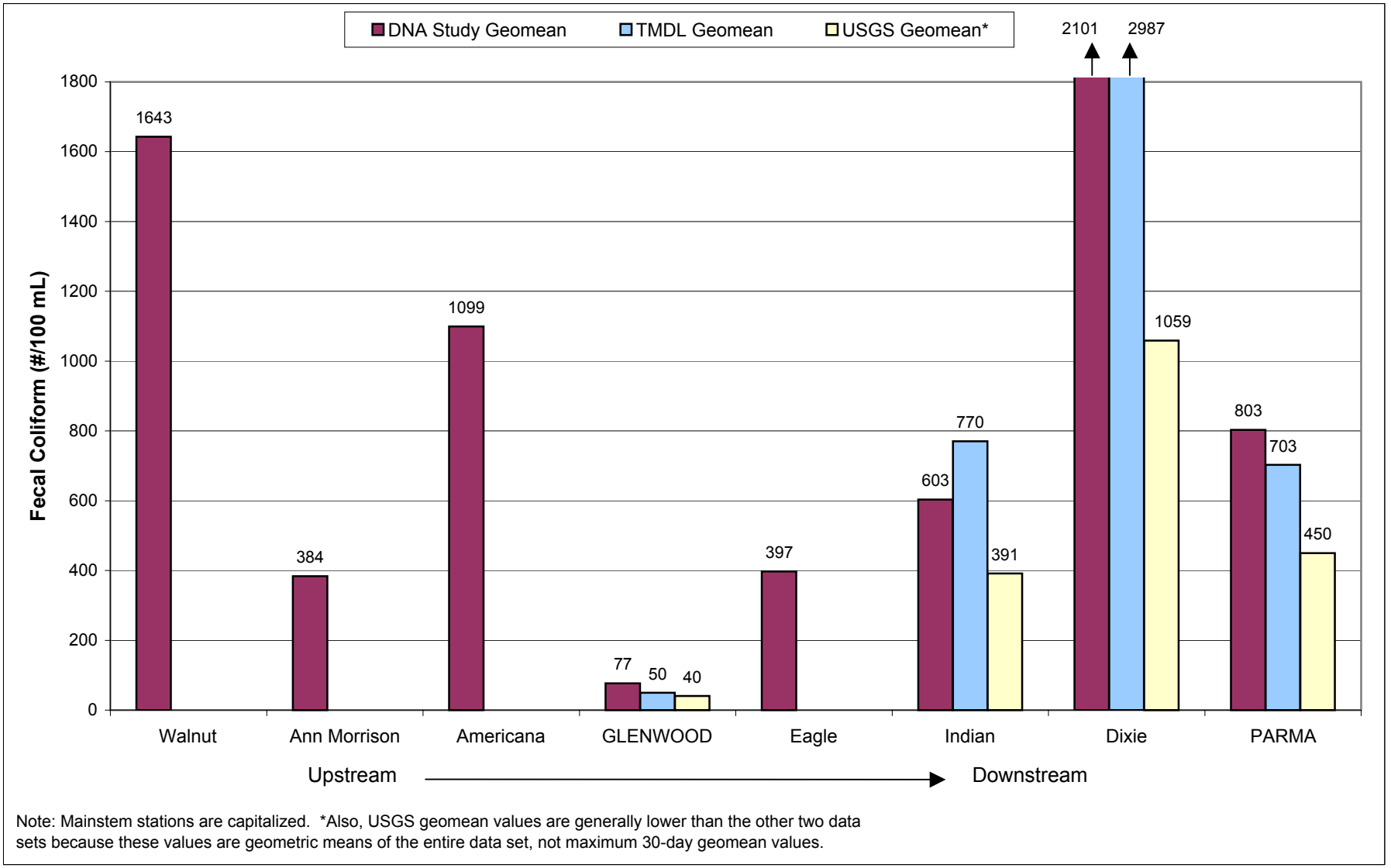
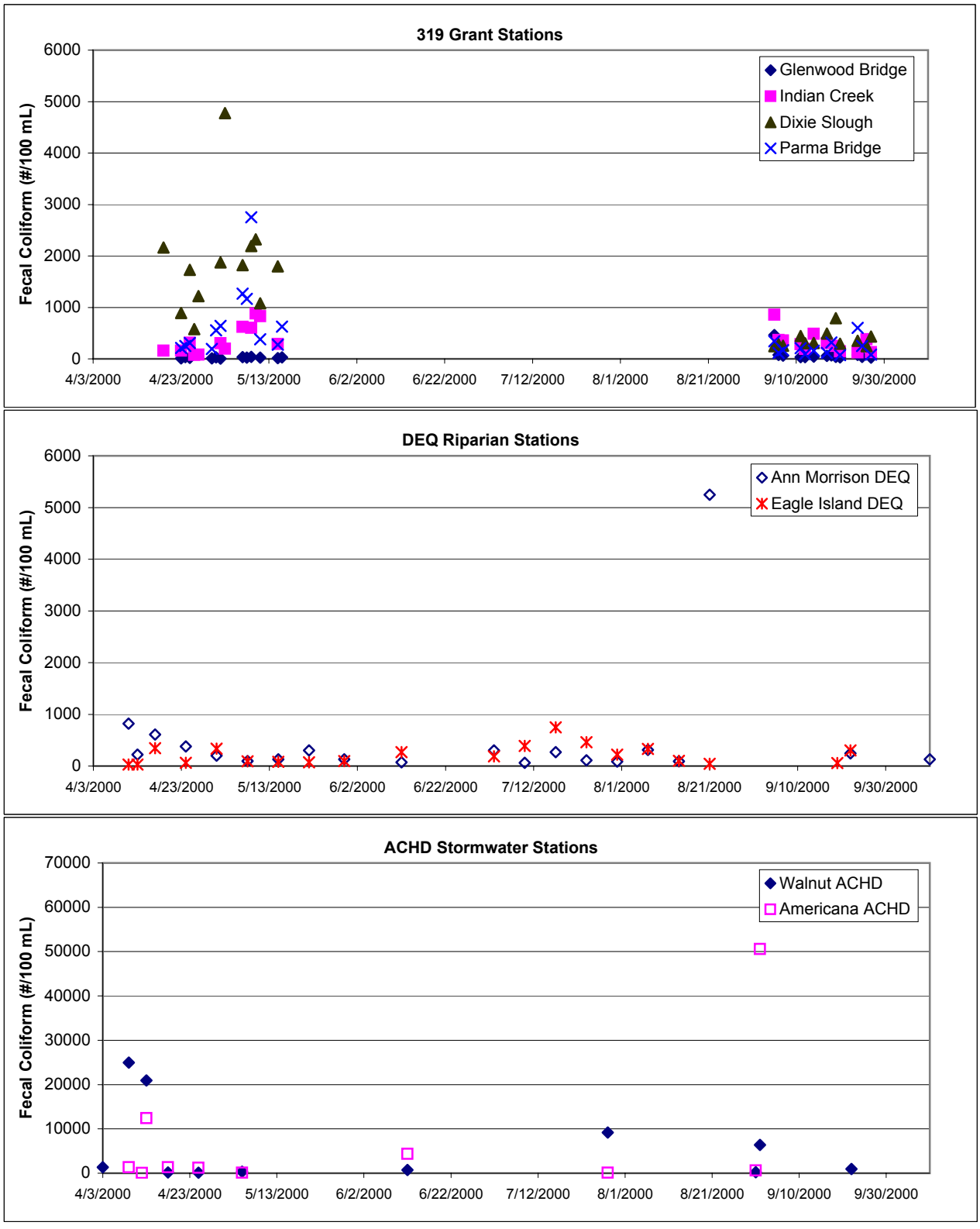



Figure 3. Station geometric mean fecal coliform concentrations.




NOTE: Different y-axis scales used.

Figure 4. Daily geometric mean fecal coliform concentrations.

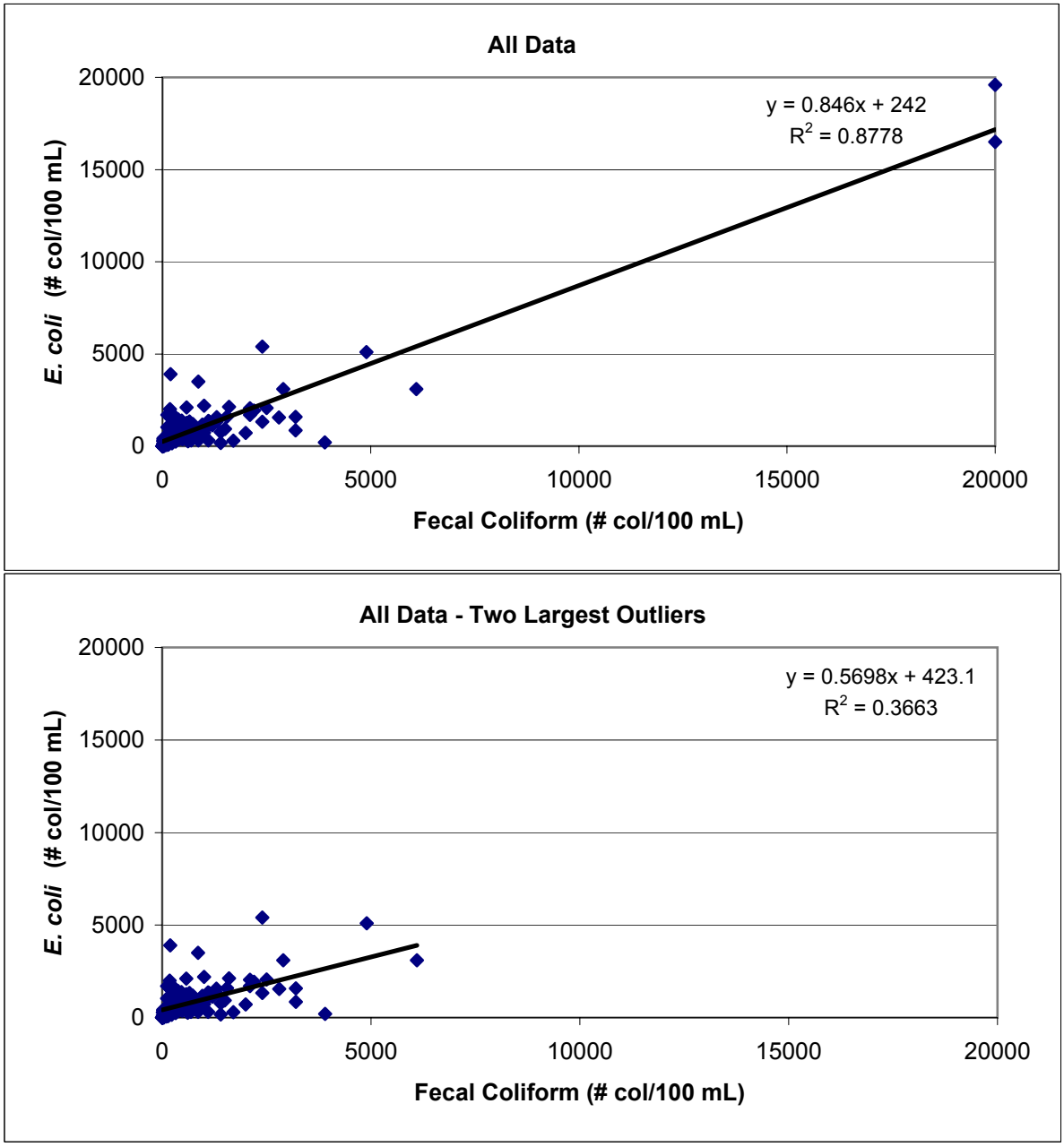



Figure 5. Comparison of fecal coliform and *E. coli* concentrations.

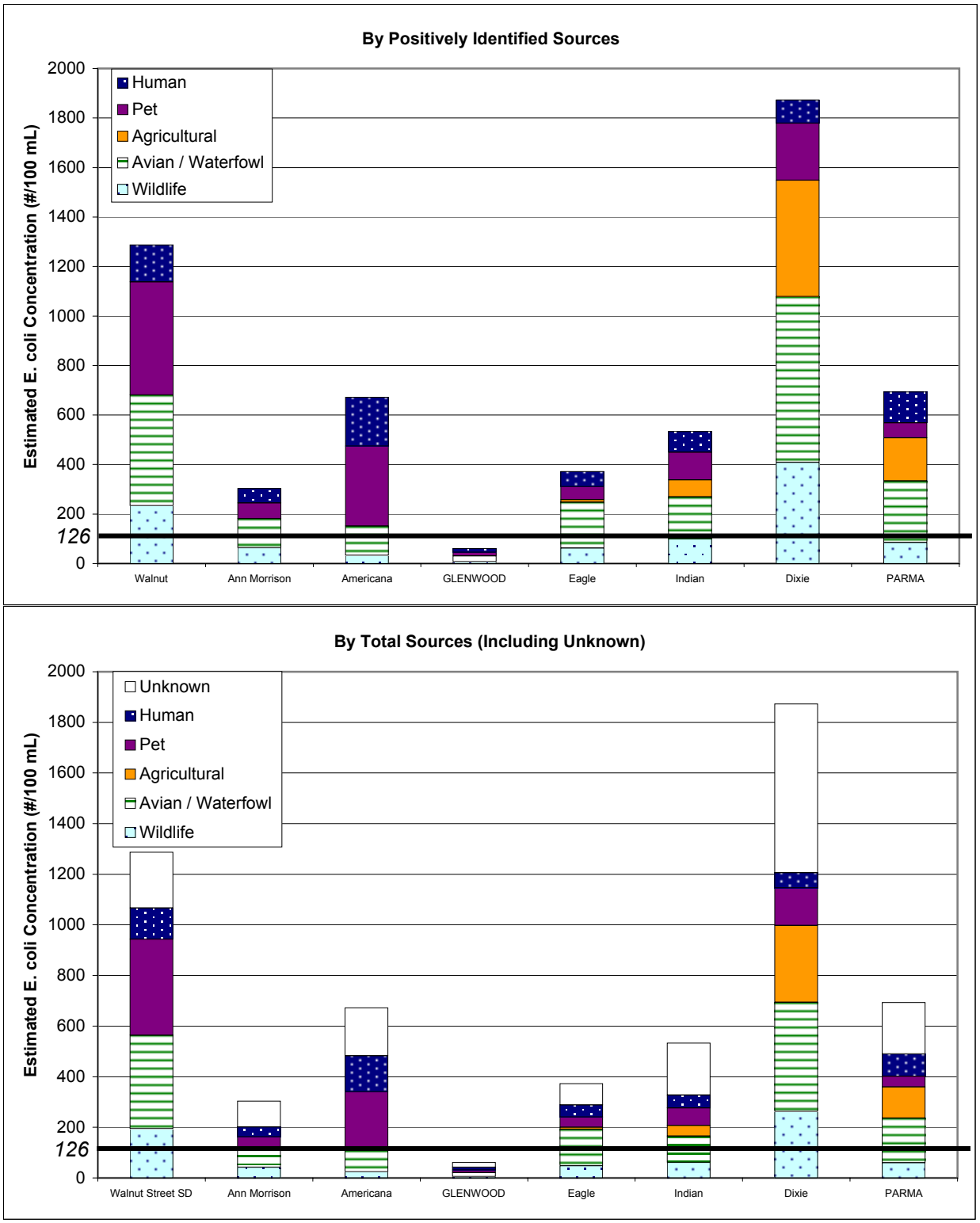



Figure 6. Schematic of estimated E. coli concentrations against new E. coli standards (126/100 mL). Upper graph shows all postively identified sources; lower graph shows all data including unidentified sources.

# Appendix A:
# Copies of Sampling Plans

Due to the size of the electronic version of the sampling plans (over 4 MB), these documents have been saved into a separate file. This file can be downloaded from the LBRWQP website:

http://www.lbrwqp.boise.id.us/dna.htm

# Appendix B:
# DNA Ribotyping Discussion and Analytical Procedures

## Overview

If we consider microbial pollution in the environment as a form of infection, then by applying the principles of epidemiology and molecular epidemiology we should be able to identify the sources of the infectious agents. While in an infectious disease outbreak we are often faced with a single source/vehicle of the infectious agent, microbial pollution in a given watershed could have originated from a large number of sources including septic tanks, wastewater treatment plant discharges, agricultural and industrial activities, wildlife, livestock, urban wildlife, avian, pets, and in certain types of climates and under some environmental conditions, re-growth of bacteria. The elements that allow us to track outbreaks of infectious (bacterial) diseases are: i) the clonal nature of bacterial populations, and ii) the unique association of the outbreak clone with the source/vehicle of the outbreak. There are two major differences between elements involved in the investigation of outbreaks of infectious diseases and microbial pollution of the environment: the magnitude of the sources, and multiplicity of the lineages of microbes involved. An infectious disease outbreak is a limited situation involving mostly a single source (at the primary level of infections) and often a single clone/lineage, while in environmental pollution a large number of point and non-point sources, and a huge numbers of microbial species, and lineages are involved.

In studying the sources of microbial pollution in the environment, since the clonal nature of the bacterial population still governs, the success of source tracking/identification largely depends on the existence of host specific lineages of bacteria (within a given species). For instance if we consider a mixed use watershed with urban and agricultural activities, identification of sources of microbial pollution would require that each group of sources have unique groups of a given bacterial species associated with them. In other words, assuming that *E. coli* is used as the surrogate for source tracking, then the human population in the watershed should harbor lineages of *E. coli* that are distinguishable (by the method used for subtyping the isolates) from cows, pigs, sheep, horses, poultry, dogs, cats, rodents, birds, etc. If indeed there are host specific lineages among bacterial species, then this would allow for the identification of the sources of microbial pollution.

The basic requirement, for the identification of the sources of microbial pollution impacting a body of water, is to establish a collection of bacterial isolates of a specific species from the impacted site that would be representative of the genetic diversity of that bacterial species in the watershed. Then, in order to identify the sources of microbial pollution, water isolates must be sub-typed and matched to a collection of bacterial isolates of the same species from known sources; this would include humans and various animal species. The only caveat is

that the subtyping method employed has to have enough sensitivity to group the bacterial isolates on the basis of their species of origin.

# Approaches

Currently there are two broad approaches to microbial source tracking studies. The first approach is the one employed in the current study, which us the epidemiological approach. The approach relies on the use of principles of epidemiology, sanitary engineering, and methods in molecular epidemiology. This approach relies on 100% match between *E. coli* strains isolated from water samples and ones from the source library. The advantage of the method is the high level of accuracy and certainty, the disadvantage is that it requires a large library of source isolates. A consequence of the use of the epidemiology approach is that there is always a percentage of water isolates for which we are unable to identify matches. In order to remedy this in the beginning of each study we estimate the level of unknowns, and we plan on taking a larger number of samples for analysis. This would allow us to identify sources for a large enough group of water isolates that the results of study are significant.

There are many ways to consider the percentage of isolates which show as unknown: one way to look at them is that they will have the same distribution as the known isolates in the study, another way is to look at them is that they represent source groups that are underrepresented (mostly wildlife) in the source library. While we believe that the reality is somewhere in between the two scenarios, we strongly recommend against speculative analysis of the unknowns and leaving the group as they are.

The second approach to source tracking investigation is to use a population genetics approach and rely on the relatedness of the isolates to identify their sources (in the absence of 100% match). A typical example of this approach is to say that strain A (isolated from water) is not a perfect match to anything in the library, however it is 75% match to strain B which is a dog isolate, therefore strain A with 75% certainty is coming from a dog. While at the first glance the argument seems credible, the argument falls apart when you consider that all *E. coli* strains (members of the species) fall within 70% of each other. Having two bacterial isolates with 75% similarity hardly puts them in one group as members of the same species. We should also consider that a human and a monkey are within 99% genetic similarity.

# Other Studies

In order to investigate the precision of the analysis and specificity of the microbial source tracking method for source identification, we conducted two studies. The objective of the first study (Samadpour, manuscript in preparation) was to investigate the existence of host specific lineages (Ecotypes) in *E. coli*. Our approach was to use ribosomal RNA typing using two restriction enzymes to study a collection of 2142*E. coli* strains isolated from 402 samples taken from known sources. The study isolates in the collection were divided into groups (ribogroups), on the basis of identical ribosomal RNA patterns obtained, for each of the two enzymes, and for the two enzymes combined (by adding the two ribotype patterns generated by the use of each of the two enzymes). Using the source data for each isolate, we

were able to assess the ability of the enzymes, individually and together, to group the isolates along the host species line. Ribogroups that were found only in one host species were labeled as resident clones (Ecotypes), those that were seen in related species (dogs and coyotes) were labeled as source related colons, and the ones that were seen in unrelated host species were called transient clones.

Tables B-1 and B-2 summarize the ribotyping results for the single enzyme analysis. Less than 50% of the total clonal groups generated on the basis of single enzyme ribotypes were resident/Ecotype clones. Human source isolates typed with *Eco*R1 and *Pvu*II had 67% and 57% residency, respectively; the residency rate increases to 94% when the results of the two enzymes ribotypes are combined (Table B-3). In the double enzyme analysis, over 76% to 95% of the total clones were resident/Ecotype clones.

**TABLE B-1.**
Pvu II Ribotype Analysis for Major Source Groups

| Source Type | Total Ribotypes | Source Specific Ribotypes | Source Related Ribotypes | Transient Ribotypes |
|---|---|---|---|---|
| Human Sources[1] | 214 | 95 (45%) | 26 (12%) | 93 (43%) |
| Bovine | 48 | 20 (42%) | 1 (2%) | 27 (56%) |
| Horse/ Llama | 50 | 27 (54%) | 0 | 23 (46%) |
| Avian[2] | 72 | 27 (37%) | 0 | 45 (63%) |
| Canine[3] | 40 | 17 (42%) | 1 (3%) | 22 (55%) |
| Feline[4] | 25 | 7 (28%) | 3 (12%) | 15 (60%) |
| Deer/ Elk | 20 | 11 (55%) | 0 | 9 (45%) |
| Farm Animals[5] | 28 | 14 (50%) | 0 | 14 (50%) |
| Wild Animal[6] | 17 | 4 (23%) | 0 | 13 (77%) |
| TOTAL | 514 | 222 (43%) | 31 (6%) | 261 (51%) |

[1] human fecal, raw sludge, digested sludge, sewage treatment plant, raw sewage, human UTI, sanitary sewer, septage
[2] avian, goose, chicken, duck, emu
[3] dog, coyote
[4] cat, bobcat
[5] pig, goat, sheep
[6] skunk, possum, deer mouse, beaver, marmot, otter, civet

**TABLE B-2.**
Eco R1 Ribotype Analysis for Major Source Groups

| Source Type | Total Ribotypes | Source Specific Ribotypes | Source Related Ribotypes | Transient Ribotypes |
|---|---|---|---|---|
| Human Sources[1] | 324 | 182 (56%) | 36 (11%) | 106 (33%) |
| Bovine | 92 | 58 (63%) | 0 | 34 (37%) |
| Horse/ Llama | 66 | 28 (42%) | 0 | 38 (58%) |
| Avian[2] | 81 | 35 (43%) | 0 | 46 (57%) |
| Canine[3] | 54 | 20 (37%) | 0 | 34 (63%) |
| Feline[4] | 32 | 14 (44%) | 2 (6%) | 16 (50%) |
| Deer/ Elk | 15 | 2 (13%) | 0 | 13 (87%) |
| Farm Animals[5] | 37 | 20 (54%) | 0 | 17 (46%) |
| Wild Animal[6] | 22 | 9 (41%) | 0 | 13 (59%) |
| TOTAL | 723 | 368 (51%) | 38 (5%) | 317 (44%) |

[1] human fecal, raw sludge, digested sludge, sewage treatment plant, raw sewage, human UTI, sanitary sewer, septage
[2] avian, goose, chicken, duck, emu
[3] dog, coyote
[4] cat, bobcat
[5] pig, goat, sheep
[6] skunk, possum, deer mouse, beaver, marmot, otter, civet

**TABLE B-3.**
EcoRI-PvuII Analysis of Major Source Groups

| Source Type | No. of Samples/ Isolates | Total Ribotypes | Source Specific Ribotypes | Source Related Ribotypes | Transient Ribotypes |
|---|---|---|---|---|---|
| Human Sources[1] | 154 / 813 | 363 | 332 (87%) | 12 (7%) | 19 (6%) |
| Bovine | 58 / 325 | 153 | 139 (91%) | 0 | 14 (9%) |
| Horse / Llama | 40 / 342 | 104 | 99 (95%) | 0 | 5 (5%) |
| Avian[2] | 42 / 183 | 107 | 93 (83%) | 2 (2%) | 12 (11%) |
| Canine[3] | 32 / 194 | 72 | 61 (85%) | 1 (1%) | 10 (14%) |
| Feline[4] | 35 / 73 | 33 | 22 (67%) | 3 (9%) | 8 (24%) |
| Deer / Elk | 13 / 53 | 21 | 17 (81%) | 0 | 4 (19%) |
| Farm Animals[5] | 12 / 100 | 41 | 39 (95%) | 0 | 2 (5%) |
| Wild Animal[6] | 18 / 59 | 25 | 21 (84%) | 0 | 4 (16%) |
| TOTAL | 402 / 2142 | 873 | 823 | 18 | 32[7] |

**TABLE B-3.**
EcoRI-PvuII Analysis of Major Source Groups

| Source Type | No. of Samples/ Isolates | Total Ribotypes | Source Specific Ribotypes | Source Related Ribotypes | Transient Ribotypes |
|---|---|---|---|---|---|

[1] human fecal, raw sludge, digested sludge, sewage treatment plant, raw sewage, human UTI, sanitary sewer, septage
[2] avian, goose, chicken, duck, emu
[3] dog, coyote
[4] cat, bobcat
[5] pig, goat, sheep
[6] skunk, possum, deer mouse, beaver, marmot, otter, civet
NOTE: The total number of transient clones is not cumulative (same clone is seen in different host species).

A summary of the subtyping data is shown in Table B-4. It is important to note that with the two enzyme ribotyping protocol, of the 873 total ribotypes, only 32 (4%) were transient. We have found that using more enzymes some of the transient ribotypes can further be divided into resident/Ecotype ribotypes (data not shown).

**TABLE B-4.**
Summary of Ribotype Totals for Single and Double Enzyme Analysis

| Enzyme | Total Ribotypes | Source Specific Ribotypes | Source Related Ribotypes | Transient Ribotypes |
|---|---|---|---|---|
| PVU II | 514 | 221 (43%) | 31 (6%) | 262 (51%) |
| ECO R1 | 723 | 368 (51%) | 38 (5%) | 317 (44%) |
| PVU II and ECO R1 | 873 | 823 (94%) | 18 (2%) | 32 (4%) |

These results clearly shows that while source specific lineages of *E. coli* do exist, their detection depends on the sensitivity of the subtyping method used to detect them. In the present study, ribosomal RNA typing was used to identify source specific (resident/Ecotype) lineages, while the use of a single enzyme ribotyping was grossly inadequate and resulted in misclassification of resident lineages as transient, about 50% of the time, the use of two ribotyping (Table B-4) reactions per isolate increased the residency/Ecotype rate to 96% for the clonal groups in the study.

In the second study (Samadpour et al., 2002, manuscript in review, FEMS Microbiology Ecology) we investigated the sensitivity, stability and reproducibility of the method and compared it to the use of antibiotic resistance analysis (ARA). The goal of this study was to compare the ability of ribotyping and ARA to group *E. coli* strains along the host species line. The methods were compared using a blinded set of 120 *E. coli* strains, isolated from cat, cows, harbor seal, horses, sea gulls, sea lions and humans. The set of 120 isolates was assembled from an original set of 40 isolates in triplicate. Ribotyping divided the isolates into

27 groups, with 100% reproducibility and host-specificity, while ARA divided the isolates into 6 groups with 90% reproducibility and 6.6% host specificity.

# Procedures

**Bacterial strains and culture conditions**. Water samples were collected from sampling stations and were processed by the membrane filtration method for fecal coliform analysis by Boise WQ Laboratory. After fecal coliform analysis was completed the m-FC plates were send to IEH in Seattle, Washington for further analysis. Colonies with appropriate morphology (round, blue, and flat) were chosen and streaked for isolation onto MacConkey media and incubated at 37° C for 24 hours.

Non-mucoid colonies that fermented lactose on MacConkey were then re-streaked onto Tripticase Soy Agar (TSA). Ten strains per sample were isolated. Biochemical analysis was done to positively identify *E. coli*. Isolates were inoculated into a tryptophane broth and onto a sodium citrate slant and incubated at 37° C for 24 hours. Isolates that produced indole from tryptophane, and did not utilize sodium citrate as a sole source of carbon were identified as *E. coli*. These isolates were then assigned an isolate number and stored in TSB-15% glycerol freezing media at -70° C. Genomic DNA preps were made from the isolates.

**Genomic DNA isolation and restriction endonuclease digestion**. Confluent growth was scraped with a sterile flat-headed toothpick and suspended in 200 µl 50mM Tris, 50mM EDTA (pH 8.0), 600 µl more of 50mM Tris, 50 mM EDTA was then added and the suspension was mixed well by pipetting up and down. Then 45 µl 20% sodium dodecyl sulfate (SDS) and then 10 µl proteinase K (20 µg/ml; Pharmacia, Piscataway, N.J.) were added. They were then incubated at 40° C for 1 hour. An equal volume of phenol was added to each tube, samples were vortexed, and then centrifuged for 5 minutes. The top layer was extracted, and an equal volume chloroform was added. The preparation was vortexed again, centrifuged , and extracted. Two and a half volumes of absolute ethanol was added and the DNA was precipitated out and spooled onto a glass capillary pipette. The DNA was washed with a few drops of absolute ethanol, dried, and re-suspended in 50µl dH2O.

Restriction endonuclease digestion reactions were set up using *EcoR*1 and *Pvu*II, 10 u/µl (Boehringer Mannheim, GmbH, Germany) as instructed by the manufacturer using 2 µl DNA extract. The preparations were incubated at 37° C overnight. The samples were centrifuged and .5µl of enzyme was added. The samples were re-incubated at 37° C for a minimum of three hours. The preparations were centrifuged again and 3 µl stop dye was added.

**Gel electrophoresis and Southern hybridization.** DNA samples were run on a 0.8% agarose gel in 1X Tris-borate-EDTA at 22 volts and 17 milliamps, for 17 hours. λ HindIII was used as a size standard along with an *E. coli* isolate designated as 3915. The DNA fragments were then transferred to a Nitran filter (Schleicher & Schuell, Keene, N.H.), baked at 80° C for one hour and probed with $^{32}$P labeled copies of *E. coli* ribosomal RNA, which were made by extension of random hexanucleotide primers using Avian Myeloblastosis Virus reverse transcriptase (Stratagene, La Jolla, Ca) under conditions specified by the supplier. Hybridization was done in 5X SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium

citrate), 0.1% SDS, 1mM EDTA, and 50% formamide at room temperature overnight. Salmon sperm DNA and blocking reagent, (Boehringer Mannheim GmbH, Germany) were used to block non-specific binding. Three washes were done with a solution of 2X SSC and .1% S.D.S., once at 25 °C for 20 minutes and twice at 65° C for 20 to wash off low-homology, non-specific binding. Blots were then exposed with an intensifying screen to X-ray film (Kodak, Rochester, N.Y.) for 24 hours at -70° C. Two to three exposures were done to ensure all possible bands would show up.

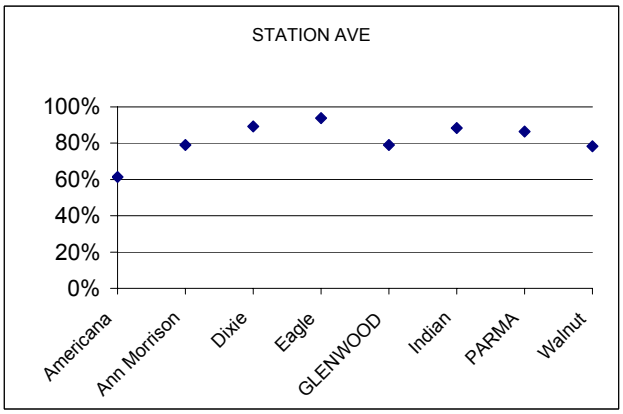
**RFLP Analysis.** Molecular characterization was then done on individual *E. coli* strains by assigning a numerical pattern to each ribotype based on how closely the bands were grouped and by size. If a band was within 3 mm of another band, then it was designated part of that set and not considered alone. If a band ran farther away than 3 mm, then it was considered alone. The groups of numbers were then listed together. Each individual isolate ribotype pattern was then entered into a database and was compared to the rest of the database. Ribotype patterns that numerically appeared to be similar were compared next to each other visually.

# Appendix C:
# IEH Dataset Used for Estimating *E. Coli* Ratios

| Site | Colonies on Mac | Colonies e. coli + | Numbered colonies | Ave E coli. | Sample Date |
|---|---|---|---|---|---|
| Americana ACHD | 54 | 53 | 33 | 0.98 | 04/09/2000 |
| Americana ACHD | 25 | 25 | 15 | 1.00 | 04/12/2000 |
| Americana ACHD | 30 | 30 | 18 | 1.00 | 04/13/2000 |
| Americana ACHD | 25 | 23 | 15 | 0.92 | 04/18/2000 |
| Americana ACHD | 24 | 8 | 8 | 0.33 | 04/25/2000 |
| Americana ACHD | 25 | 15 | 15 | 0.60 | 05/05/2000 |
| Americana ACHD | 10 | 2 | 2 | 0.20 | 06/12/2000 |
| Americana ACHD | 25 | 9 | 9 | 0.36 | 07/28/2000 |
| Americana ACHD | 10 | 4 | 4 | 0.40 | 07/28/2000 |
| Americana ACHD | 5 | 2 | 2 | 0.40 | 08/31/2000 |
| Americana ACHD | 30 | 14 | 13 | 0.47 | 09/01/2000 |
| Americana ACHD | 20 | 14 | 12 | 0.70 | 09/22/2000 |
| Ann Morrison DEQ | 10 | 10 | 6 | 1.00 | 04/11/2000 |
| Ann Morrison DEQ | 5 | 3 | 3 | 0.60 | 04/13/2000 |
| Ann Morrison DEQ | 5 | 5 | 3 | 1.00 | 04/17/2000 |
| Ann Morrison DEQ | 10 | 8 | 6 | 0.80 | 04/24/2000 |
| Ann Morrison DEQ | 5 | 5 | 3 | 1.00 | 05/01/2000 |
| Ann Morrison DEQ | 9 | 8 | 5 | 0.89 | 05/08/2000 |
| Ann Morrison DEQ | 5 | 4 | 3 | 0.80 | 05/15/2000 |
| Ann Morrison DEQ | 10 | 9 | 6 | 0.90 | 05/22/2000 |
| Ann Morrison DEQ | 5 | 4 | 3 | 0.80 | 05/30/2000 |
| Ann Morrison DEQ | 10 | 8 | 3 | 0.80 | 06/12/2000 |
| Ann Morrison DEQ | 5 | 5 | 3 | 1.00 | 07/03/2000 |
| Ann Morrison DEQ | 10 | 10 | 6 | 1.00 | 07/10/2000 |
| Ann Morrison DEQ | 5 | 5 | 3 | 1.00 | 07/17/2000 |
| Ann Morrison DEQ | 10 | 5 | 5 | 0.50 | 07/24/2000 |
| Ann Morrison DEQ | 10 | 7 | 7 | 0.70 | 07/31/2000 |
| Ann Morrison DEQ | 10 | 10 | 6 | 1.00 | 08/07/2000 |
| Ann Morrison DEQ | 10 | 6 | 6 | 0.60 | 08/14/2000 |
| Ann Morrison DEQ | 10 | 9 | 6 | 0.90 | 08/21/2000 |
| Ann Morrison DEQ | 4 | 1 | 1 | 0.25 | 09/22/2000 |
| Ann Morrison DEQ | 8 | 2 | 2 | 0.25 | 10/10/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 04/19/2000 |
| Dixie Slough | 15 | 14 | 9 | 0.93 | 04/23/2000 |
| Dixie Slough | 5 | 5 | 3 | 1.00 | 04/23/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 04/25/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 04/26/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 04/27/2000 |
| Dixie Slough | 19 | 17 | 12 | 0.89 | 05/02/2000 |
| Dixie Slough | 20 | 15 | 10 | 0.75 | 05/03/2000 |
| Dixie Slough | 20 | 19 | 12 | 0.95 | 05/07/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 05/09/2000 |
| Dixie Slough | 20 | 13 | 11 | 0.65 | 05/10/2000 |
| Dixie Slough | 20 | 19 | 12 | 0.95 | 05/11/2000 |
| Dixie Slough | 20 | 19 | 12 | 0.95 | 05/15/2000 |
| Dixie Slough | 20 | 17 | 12 | 0.85 | 09/05/2000 |
| Dixie Slough | 20 | 15 | 12 | 0.75 | 09/06/2000 |
| Dixie Slough | 20 | 19 | 12 | 0.95 | 09/07/2000 |
| Dixie Slough | 20 | 10 | 9 | 0.50 | 09/11/2000 |
| Dixie Slough | 20 | 20 | 12 | 1.00 | 09/12/2000 |
| Dixie Slough | 20 | 16 | 12 | 0.80 | 09/14/2000 |
| Dixie Slough | 16 | 11 | 9 | 0.69 | 09/17/2000 |
| Dixie Slough | 16 | 16 | 12 | 1.00 | 09/19/2000 |
| Dixie Slough | 16 | 15 | 12 | 0.94 | 09/20/2000 |
| Dixie Slough | 15 | 12 | 11 | 0.80 | 09/24/2000 |
| Dixie Slough | 16 | 15 | 12 | 0.94 | 09/26/2000 |
| Dixie Slough | 16 | 16 | 12 | 1.00 | 09/27/2000 |
| Eagle Island DEQ | 5 | 5 | 3 | 1.00 | 04/11/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 04/13/2000 |
| Eagle Creek DEQ | 10 | 10 | 6 | 1.00 | 04/17/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 04/24/2000 |
| Eagle Creek DEQ | 10 | 10 | 6 | 1.00 | 05/01/2000 |
| Eagle Creek DEQ | 5 | 4 | 3 | 0.80 | 05/08/2000 |
| Eagle Creek DEQ | 10 | 10 | 6 | 1.00 | 05/15/2000 |
| Eagle Creek DEQ | 4 | 4 | 3 | 1.00 | 05/22/2000 |
| Eagle Creek DEQ | 10 | 9 | 6 | 0.90 | 05/30/2000 |
| Eagle Creek DEQ | 5 | 3 | 3 | 0.60 | 06/12/2000 |
| Eagle Creek DEQ | 15 | 15 | 11 | 1.00 | 07/03/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 07/10/2000 |
| Eagle Creek DEQ | 10 | 7 | 6 | 0.70 | 07/17/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 07/24/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 07/31/2000 |
| Eagle Creek DEQ | 20 | 20 | 12 | 1.00 | 08/07/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 08/14/2000 |
| Eagle Creek DEQ | 5 | 5 | 3 | 1.00 | 08/21/2000 |
| Eagle Creek DEQ | 8 | 6 | 5 | 0.75 | 09/19/2000 |
| Eagle Creek DEQ | 4 | 4 | 3 | 1.00 | 09/22/2000 |
| Glenwood Bridge | 10 | 9 | 6 | 0.90 | 04/23/2000 |
| Glenwood Bridge | 10 | 10 | 6 | 1.00 | 04/23/2000 |
| Glenwood Bridge | 5 | 5 | 3 | 1.00 | 04/24/2000 |
| Glenwood Bridge | 15 | 12 | 9 | 0.80 | 04/24/2000 |
| Glenwood Bridge | 20 | 14 | 12 | 0.70 | 04/25/2000 |
| Glenwood Bridge | 20 | 19 | 12 | 0.95 | 04/30/2000 |
| Glenwood Bridge | 20 | 12 | 10 | 0.60 | 05/01/2000 |
| Glenwood Bridge | 18 | 17 | 12 | 0.94 | 05/02/2000 |
| Glenwood Bridge | 20 | 16 | 12 | 0.80 | 05/07/2000 |
| Glenwood Bridge | 20 | 16 | 12 | 0.80 | 05/08/2000 |

| | |
|---|---|
| **Colonies on Mac** | =total number of coliform colonies on plate |
| **Colonies e. coli +** | =total number of ecoli colonies on plate |
| **Numbered colonies** | =total number of ecoli colonies fingerprinted (=number of isolates) |

| | E. Coli % | SD | n |
|---|---|---|---|
| Americana | 61% | 30% | 12 |
| Ann Morrison | 79% | 24% | 20 |
| Dixie | 89% | 13% | 25 |
| Eagle | 94% | 12% | 20 |
| GLENWOOD | 79% | 20% | 27 |
| Indian | 88% | 11% | 24 |
| PARMA | 86% | 14% | 24 |
| Walnut | 78% | 24% | 15 |
| | 82% | AVE | |



| Site | Colonies on Mac | Colonies e. coli + | Numbered colonies | Ave E coli. | Sample Date |
|---|---|---|---|---|---|
| Glenwood Bridge | 20 | 8 | 8 | 0.40 | 05/09/2000 |
| Glenwood Bridge | 15 | 13 | 9 | 0.87 | 05/11/2000 |
| Glenwood Bridge | 20 | 17 | 12 | 0.85 | 05/15/2000 |
| Glenwood Bridge | 20 | 20 | 12 | 1.00 | 05/16/2000 |
| Glenwood Bridge | 20 | 14 | 11 | 0.70 | 09/05/2000 |
| Glenwood Bridge | 20 | 17 | 12 | 0.85 | 09/06/2000 |
| Glenwood Bridge | 20 | 20 | 12 | 1.00 | 09/07/2000 |
| Glenwood Bridge | 20 | 4 | 4 | 0.20 | 09/11/2000 |
| Glenwood Bridge | 20 | 15 | 12 | 0.75 | 09/12/2000 |
| Glenwood Bridge | 11 | 5 | 5 | 0.45 | 09/14/2000 |
| Glenwood Bridge | 16 | 11 | 11 | 0.69 | 09/17/2000 |
| Glenwood Bridge | 24 | 18 | 12 | 0.75 | 09/18/2000 |
| Glenwood Bridge | 4 | 3 | 3 | 0.75 | 09/19/2000 |
| Glenwood Bridge | 16 | 14 | 12 | 0.88 | 09/20/2000 |
| Glenwood Bridge | 16 | 11 | 10 | 0.69 | 09/24/2000 |
| Glenwood Bridge | 16 | 16 | 12 | 1.00 | 09/25/2000 |
| Glenwood Bridge | 16 | 16 | 12 | 1.00 | 09/27/2000 |
| | | | | | |
| Indian Creek | 20 | 20 | 12 | 1.00 | 04/19/2000 |
| Indian Creek | 20 | 20 | 12 | 1.00 | 04/23/2000 |
| Indian Creek | 20 | 17 | 12 | 0.85 | 04/25/2000 |
| Indian Creek | 20 | 18 | 12 | 0.90 | 04/26/2000 |
| Indian Creek | 18 | 9 | 12 | 0.50 | 04/27/2000 |
| Indian Creek | 19 | 18 | 12 | 0.95 | 05/02/2000 |
| Indian Creek | 19 | 17 | 12 | 0.89 | 05/03/2000 |
| Indian Creek | 18 | 15 | 11 | 0.83 | 05/07/2000 |
| Indian Creek | 20 | 18 | 12 | 0.90 | 05/09/2000 |
| Indian Creek | 20 | 16 | 12 | 0.80 | 05/10/2000 |
| Indian Creek | 20 | 18 | 12 | 0.90 | 05/11/2000 |
| Indian Creek | 20 | 20 | 12 | 1.00 | 05/15/2000 |
| Indian Creek | 20 | 18 | 12 | 0.90 | 09/05/2000 |
| Indian Creek | 20 | 18 | 12 | 0.90 | 09/06/2000 |
| Indian Creek | 15 | 14 | 9 | 0.93 | 09/07/2000 |
| Indian Creek | 20 | 13 | 11 | 0.65 | 09/11/2000 |
| Indian Creek | 20 | 19 | 12 | 0.95 | 09/12/2000 |
| Indian Creek | 20 | 16 | 12 | 0.80 | 09/14/2000 |
| Indian Creek | 16 | 15 | 12 | 0.94 | 09/17/2000 |
| Indian Creek | 16 | 15 | 12 | 0.94 | 09/19/2000 |
| Indian Creek | 16 | 15 | 12 | 0.94 | 09/20/2000 |
| Indian Creek | 16 | 13 | 10 | 0.81 | 09/24/2000 |
| Indian Creek | 16 | 15 | 12 | 0.94 | 09/26/2000 |
| Indian Creek | 16 | 16 | 12 | 1.00 | 09/27/2000 |
| | | | | | |
| Parma Bridge | 15 | 15 | 9 | 1.00 | 04/23/2000 |
| Parma Bridge | 20 | 20 | 12 | 1.00 | 04/24/2000 |
| Parma Bridge | 20 | 18 | 12 | 0.90 | 04/25/2000 |
| Parma Bridge | 20 | 15 | 12 | 0.75 | 04/30/2000 |
| Parma Bridge | 20 | 20 | 12 | 1.00 | 05/01/2000 |
| Parma Bridge | 20 | 18 | 12 | 0.90 | 05/02/2000 |
| Parma Bridge | 20 | 11 | 11 | 0.55 | 05/07/2000 |
| Parma Bridge | 19 | 19 | 12 | 1.00 | 05/08/2000 |
| Parma Bridge | 20 | 17 | 11 | 0.85 | 05/09/2000 |
| Parma Bridge | 20 | 15 | 12 | 0.75 | 05/11/2000 |
| Parma Bridge | 20 | 20 | 12 | 1.00 | 05/15/2000 |
| Parma Bridge | 20 | 18 | 12 | 0.90 | 05/16/2000 |
| Parma Bridge | 18 | 16 | 12 | 0.89 | 09/05/2000 |
| Parma Bridge | 20 | 17 | 12 | 0.85 | 09/06/2000 |
| Parma Bridge | 20 | 18 | 12 | 0.90 | 09/07/2000 |
| Parma Bridge | 25 | 13 | 9 | 0.52 | 09/11/2000 |
| Parma Bridge | 20 | 20 | 12 | 1.00 | 09/12/2000 |
| Parma Bridge | 20 | 18 | 12 | 0.90 | 09/14/2000 |
| Parma Bridge | 16 | 14 | 11 | 0.88 | 09/17/2000 |
| Parma Bridge | 16 | 11 | 11 | 0.69 | 09/18/2000 |
| Parma Bridge | 16 | 16 | 12 | 1.00 | 09/20/2000 |
| Parma Bridge | 16 | 11 | 10 | 0.69 | 09/24/2000 |
| Parma Bridge | 16 | 13 | 12 | 0.81 | 09/25/2000 |
| Parma Bridge | 16 | 16 | 12 | 1.00 | 09/27/2000 |
| | | | | | |
| Walnut ACHD | 25 | 25 | 15 | 1.00 | 04/03/2000 |
| Walnut ACHD | 39 | 36 | 23 | 0.92 | 04/09/2000 |
| Walnut ACHD | 25 | 25 | 15 | 1.00 | 04/13/2000 |
| Walnut ACHD | 20 | 20 | 12 | 1.00 | 04/18/2000 |
| Walnut ACHD | 5 | 5 | 3 | 1.00 | 04/18/2000 |
| Walnut ACHD | 5 | 5 | 3 | 1.00 | 04/18/2000 |
| Walnut ACHD | 5 | 5 | 3 | 1.00 | 04/18/2000 |
| Walnut ACHD | 25 | 12 | 12 | 0.48 | 04/25/2000 |
| Walnut ACHD | 24 | 15 | 13 | 0.63 | 05/05/2000 |
| Walnut ACHD | 25 | 6 | 6 | 0.24 | 06/12/2000 |
| Walnut ACHD | 10 | 8 | 6 | 0.80 | 07/28/2000 |
| Walnut ACHD | 15 | 12 | 9 | 0.80 | 07/28/2000 |
| Walnut ACHD | 5 | 3 | 2 | 0.60 | 08/31/2000 |
| Walnut ACHD | 25 | 17 | 13 | 0.68 | 09/01/2000 |
| Walnut ACHD | 20 | 12 | 11 | 0.60 | 09/22/2000 |
| | | | | | |
| TOTAL | 2667 | 2208 | 1570 | | |
| TOTAL | 2671 | 2212 | 1573 | | |