Estimating Nonpoint Fecal Coliform Sources in Northern Virginia's Four Mile Run Watershed

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ABSTRACT

Pulsed Field Gel Electrophoresis (PFGE) was conducted on *E. coli* DNA from seasonally-varied stream and sediment samples in the ultra-urban Four Mile Run watershed in Northern Virginia. This study found:

1) nonhuman species are the dominant sources of *E. coli* to Four Mile Run and its tributaries; 2) waterfowl contribute over one-third (37%) of those isolates that could be identified; 3) the presence of human *E. coli* is localized; 4) the predominant nonhuman sources are wildlife species that have intimate association with the waterways; 5) the major nonhuman mammal contributors are raccoon, dog, deer, and Norway rat; and, 6) the combined human and canine contribution is approximately 25% of those isolates that could be identified. Finally, circumstantial evidence suggests that without regard to specific host animals, *E. coli* bacteria seem to regrow, through cloning, within the storm drains and stream sediments, which in turn perpetuate elevated fecal coliform levels within the connected surface waters of Four Mile Run.

The continued high levels of *E. coli* suggest an ecosystem out of balance irrespective of the source. It is neither desirable nor practical to eliminate wildlife animal species in the watershed. Rather, it is suggested that, wherever possible, nutrient loadings be controlled to restore a more balanced microbial community to the stream network.

Keywords: urban streams, bacteria, *E. coli*, Pulsed Field Gel Electrophoresis (PFGE), DNA, storm drains, regrowth, nonpoint source pollution

INTRODUCTION

Since 1990, at least five separate organizations have cumulatively collected over 500 fecal coliform samples from the Four Mile Run watershed. Approximately 50% of these were found to have a Most Probable Number (MPN) greater than 1,000, which exceeds the state's water quality standard of fecal coliform density for the watershed (SWCB, 1997). Four Mile Run is listed as one of the streams on Virginia's 303(d) list of impaired stream segments because of the elevated levels of fecal coliform bacteria (Virginia DEQ, 1998). In addition to violating the fecal coliform standard, the Four Mile Run watershed is given a "high priority" ranking for potential nonpoint source pollution by the Virginia Department of Conservation and Recreation (Virginia DEQ and DCR, 1998), and is designated as a nutrient-enriched waterway by the State Water Control Board (1997).

In the 1992 re-authorization of the federal Clean Water Act, considerable emphasis was placed on developing watershed-based strategies that have potential to reduce nonpoint source pollution in impaired streams. The Northern Virginia Planning District Commission has initiated a phased approach for meeting the mandates of the Clean Water Act for Four Mile Run through a 604(b) Water Quality Grant to

Virginia DEQ (NVPDC, 1998). This research serves as a starting point toward achieving this goal. The purpose of this research project was to determine potential animal sources for fecal coliform contamination of Four Mile Run and its tributaries in Northern Virginia.

Watershed Characteristics

The Four Mile Run watershed (12,600 acres, 19.7 square miles) is a densely populated urban watershed where the dominant land use is medium to high density residential housing. Approximately 165,000 people live in the watershed, resulting in a population density of 13 people per acre (over 8,000 people per square mile) (NVPDC, 1996a). There are two NPDES-permitted point source discharges in the watershed; a concrete batch plant near Shirlington and the Arlington Waste Water Treatment Plant (WWTP) near Route 1. The Arlington WWTP discharges into the tidal portion of Four Mile Run near its confluence with the Potomac River. There are no combined storm/sanitary sewer lines by design, and testing by NVPDC and Arlington County to determine the extent of cross-connections between the sanitary sewer system and the storm sewer system confirms the overall integrity of these separate sewer systems, with only minor problems occasionally discovered.

A very large pet population accompanies a very dense human population in the watershed. An NVPDC analysis from 1994 estimated the canine density of the watershed to be approximately one dog for every 10 people, resulting in a density of 1.3 dogs/acre (over 800 per square mile). The analysis further estimated that more than 2,400 kg (over 5,000 pounds) of fecal waste is deposited in the watershed on a daily basis, which is conservatively based on 150 g of solid waste per dog (one-third of a pound) [1.3 dogs/acre * 12,600 acres]. It was not assumed that all canine waste would make its way into the stream, but that the potential exists for some of this waste to serve as a source of fecal coliforms. Besides humans and dogs, the watershed contains a variety of mammals and waterfowl that have adapted to an urbanized landscape.

METHODS

Details of the sampling protocol and procedures related to Quality Assurance and Quality Control (QA/QC) are contained in a separate QA/QC Plan. Pulsed Field Gel Electrophoresis (PFGE) is a widely used technique to resolve microbial strain recognition in clinical and natural environments (Goering, 1993; Maslow, et al., 1993; Edberg, et al., 1994; Buchrieser, et al., 1995; Tynkkynen, et al., 1999). Details of isolate selection for DNA analyses using the *Not*I restriction enzyme are summarized in the QA/QC document.

Sample Collection, Locations and Times

A total of 55 samples were collected in this study. Fecal coliform density was measured by the Fecal Coliform Direct Test using A-1 medium and the five tube, three dilution technique (Amer. Publ. Health Assoc., *et al*., 1992). Samples were taken from the water column, water-sediment slurries, and sediment cores. The locations for the samples used in this study are presented in Figure 1. Station location and their respective identification numbers are presented in Table 1.

Four seasonally varied sampling periods were used to characterize potential nonpoint fecal coliform sources to the Four Mile Run watershed. These were: August 1998 (summer period); May 1999 (spring period); November 1999 (fall period); and February 2000 (winter period). In addition, fecal coliform density samples were taken in June 2000, but DNA results from this sampling period are not included in this study.

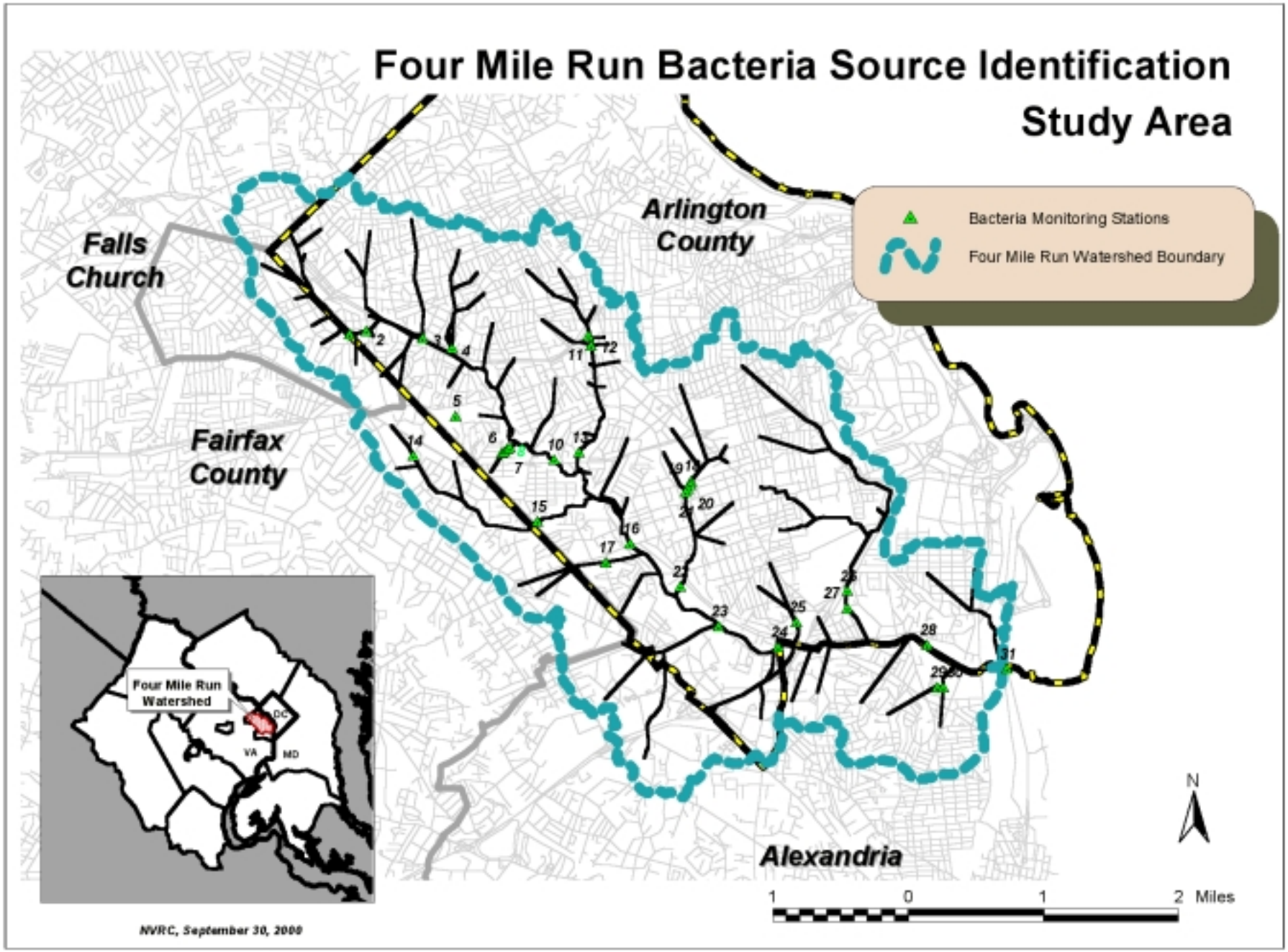

Figure 1. Map of Four Mile Run Watershed with Sample Locations

Table 1. Sample Locations and Identification Numbers

I.D.	Location	Alternate I.D.
1	Upper Four Mile Run at Falls Church line (Van Buren Street)	NVPDC#7
2	Upper Four Mile Run at Sycamore Street	
3	Ohio Street Branch at I-66 outfall	FM200 or FM210, Arlington
4	Westover Branch at I-66, outfall (twin box culvert to right of 2 m [78 in] circ.)	FM230, Arlington
5	Powhatan Run at N. Livingston Road, pristine site	u/s of FM300, Arlington
6	Manchester Street 1.1 m (42 in) outfall (Glencarlyn Branch)	FM 330, Arlington
7	46 m (150 ft) downstream (d/s) of Manchester Street outfall	d/s of FM 330, Arlington
8	91 m (300 ft) d/s of Manchester Street outfall	d/s of FM 330, Arlington
9	137 m (450 ft) d/s of Manchester Street outfall	d/s of FM 330, Arlington
10	Middle Four Mile Run, bike trail crossing just u/s of Rt. 50	NVPDC#6
11	Ballston Beaver Pond, along open channel	Near LR112, Arlington
12	Box culvert under Ballston just d/s of Beaver Pond	
13	Lubber Run at Route 50	NVPDC#5
14	Upper Long Branch d/s of Patrick Henry Drive	
15	Upper Long Branch at Carlin Springs Road	NVPDC#4
16	Four Mile Run at Columbia Pike	1AFOU004.22, Va. DEQ
17	Baileys Branch at S. Frederick Street	FM350, Arlington
18	Doctors Run at S. 6th Street & S. Quincy Street, biggest outfall	DB100, Arlington
19	Doctors Run 61 m (200 ft) d/s of S. 6th Street & S. Quincy Street	d/s of DB100, Arlington
20	Doctors Run 122 m (400 ft) d/s of S. 6th Street & S. Quincy Street	d/s of DB100, Arlington
21	Doctors Run 183 m (600 ft) d/s of S. 6th Street & S. Quincy Street	d/s of DB100, Arlington
22	Doctors Run at Barcroft Park Footbridge	NVPDC#8
23	Lucky Run outfall at Four Mile Run	NVPDC#3
24	Four Mile Run at Shirlington Road	NVPDC#2
25	Nauck Branch	FM450, Arlington
26	Lower Long Branch at I-395 near 28th Street S., outfall—quad box culvert	274 m (900 ft) d/s of LL180, Arlington
27	Lower Long Branch in Arna Valley, 26th Street S.	NVPDC#1
28	Arlington Sewage Treatment Plant outfall	
29	Alexandria trib behind Cora Kelly Community Center, u/s of outfall	
30	Alexandria trib behind Cora Kelly Community Center, corrugated metal pipe outfall	
31	Four Mile Run at George Washington Parkway	1AFOU000.19, Va. DEQ

Statistical Comparison of Populations:

The χ^2 Goodness-of-fit analysis for populations was used to test statistical differences between the *E. coli* clonal populations from the different animal groups based on their PFGE patterns. For these analyses, the entire banding profile (from 780-20 kilobase pairs) was divided into six equal units and the frequency of bands within each unit was used for comparative purposes at $\alpha = 0.10$. The percent of bands within each unit was also presented as a histogram in a separate document to visually display differences in banding patterns between *E. coli* populations of the different animal groups.

Computer-based Search of DNA Library:

The calculated numerical value of each band (molecular size as kb) was loaded into flat files (plain text, ASCII files) with respect to each animal group. All animal groups were then combined to create a single library. A TCL computer program (Tool Command Language©, an embeddable scripting language, release 8.0p2; copyright by the Regents of the University of California, Sun Microsystems, Inc., and other parties) was used to compare *E. coli* strains from field samples with *E. coli* strains from known sources in our library. A band-to-band comparison was made and expressed as a percent similarity. The program allows the investigator to adjust the lower limit of percent comparison (i.e., 75%, 78%, 80%, etc.) between known and unknown strains, and the range of kilobase pairs used for each two bands being compared (i.e. ± 5 kilobase pairs, ± 10 kilobase pairs, etc).

Libraries Used in This Study:

Several DNA libraries were used in this study. The libraries, their respective animal species, and number of PFGE patterns per species are listed in Table 2. The total number of strains used to determine potential animal sources was 843. All *E. coli* strains came from individual animals. Specifically, in the case of humans, the strains came from individuals and not from septic tanks.

Assigning Potential Sources Based on DNA Profile Analysis:

In trying to assign a "best fit," the first factor considered was similarity as measured by the degree of correlation between the strain from an unknown source and a strain from a known animal in the Virginia Tech DNA library. For example, if the DNA bands from a strain of an unknown source matched 90% of the DNA bands with an *E. coli* strain from Canada Goose, and only 82% with a strain from a canine source, it would be concluded that the unknown strain was more likely to come from a Canada Goose because there was a higher correlation with the Canada Goose strain.

However, there were instances where a strain from an unknown source correlated with a human strain and a canine strain at the same similarity (88% for example). In this case, the library provided a match but it was not be possible to differentiate between canine and human. If, however, the unknown strain matched with several human strains and only one canine strain from the library, it was considered to be more likely to come from a human source based on the number of matches. Furthermore, there are fewer human strains in the Virginia Tech DNA library than canine, and if matches were random, then a greater number of canine matches would be expected. However, because *E. coli* from dogs and humans cannot be statistically separated by this methodology used in this study, it is not possible to conclude that the unknown strain is not from a canine source.

If an unknown strain was approximately equally similar to more than one animal group and the number of matches were also approximately equal among animal groups, a visual band-to-band comparison would be made to determine which animal group might be the more likely candidate. The presence or absence

of matches in the heavier segments of DNA often provided clues as to the degree of greater similarity because there are many fewer bands in the 750-500 kilobase pair range than below this range.

Geography also played a role given that *E. coli* from known sources from several geographic areas were combined for this study, and given that there is very little known about geographic variability in *E. coli* PFGE patterns from the same animal species. Therefore, if the pattern from an unknown source matched an *E. coli* pattern from a goose in the Cornell library from the Long Island Sound area at 88%, but matched a raccoon strain from the Northern Virginia/Four Mile Run library at 84%, assignment to raccoon would probably be made, assuming a spurious correlation with the goose, and a more likely correlation with the raccoon.

Source ecology also played a factor in assigning most likely sources. In a situation where the strain from an unknown source matched approximately equally with a horse isolate collected from scat in the Rappahannock basin, a raccoon from Northern Virginia, and a pelican from the Chesapeake Bay, it would be concluded that the unknown strain was most likely from the raccoon simply because horses and pelicans are far less common in the study watershed. Another example of the way ecology was considered is a situation of similar correlation with strains from a canine source in the Cornell library and a Norway rat from the Northern Virginia/Four Mile Run library. There are very few Norway rat samples in the Virginia Tech DNA library and the fact that the unknown strain of *E. coli* matched a Norway rat strain was a compelling reason to assign a likely match. That is, all else being equal, the researchers selected matches with those animals in the watershed from which scat had been collected, especially where the researchers believed the species to be underrepresented in the DNA libraries.

However, in some cases source assignments were unclear regardless of consideration of the factors described above. For example, if a strain from an unknown source matched with an *E. coli* strain from bovine (Dr. Eugene Yagow's library from Virginia's Rappahannock basin), and that was the only match, then that animal was assigned as the possible source. In this particular case, there are several possible theories that can explain such a match. First, the match of the unknown strain to a bovine source could be false because there are no known bovines living in the Four Mile Run watershed. A second theory is that the match could be misleading because the unknown strain could be a crossover strain of *E. coli* common to multiple animal groups, perhaps picked up by birds feeding on insect larvae in bovine dung, passed through the bird's digestive tract, and deposited in the watershed by the birds while in transit. A third possibility is that the match might be correct and the data could suggest that *E. coli* from bovine are somehow making their way into the watershed through a presently unknown transport mechanism (such as leachate from restaurant dumpsters). A fourth explanation is that because the *E. coli* populations of bovine and deer are not statistically different from each other (possibly due to the complex ruminant digestive system that each animal groups possesses) the bovine signatures may be serving as surrogates for deer *E. coli*.

RESULTS

Fecal Coliform Densities

Sample locations and results of fecal coliform densities are presented in Table 3. Stormwater outfalls, fine sediments, and samples of microbial films from sediment/water mixture samples tended to have the higher densities. Most Probable Number (MPN) values of ≥1600 were scored as numerical values of 1700 for purposes of calculation.

TABLE 2. Numbers of Isolates from the Different Libraries Used in the Analysis of Potential Fecal Coliform Sources From Study Area Locations

(All library samples maintained by Virginia Tech, *n = 843*)

Eastern Shore/Chesapeake Bay Library (collected 1994 – 1997):

Muskrat	34
Raccoon	71
Deer	39
Beaver	20
Otter	22
Human	67
Canine	42
Laughing Gull	29
Herring Gull	33
Pelican	7
Tern	16
Canada Goose	45
Wood Duck	3
Merganser	5
Porcine	15
Total	*448*

Cornell Long Island Sound Library (collected 1994 – 1997):

Human	7
Raccoon	54
Deer	25
Canine	21
Horse	25
Herring Gull	24
Black Back Gull	16
Canada Goose	14
Black Duck	5
Mallard Duck	9
Mute Swan	14
Mallard Duck	11
Teal	5
Black Duck	26
Total	*256*

Four Mile Run (Northern Va) Library* (collected 1999 – 2000):

Red Fox	5
Raccoon	16
Flying Squirrel	3
Gray Squirrel	5
Opossum	7
Canine	27
Norway Rat:	6
Feline	5
Human	8
Seagull	4
Canada Goose	8
Total	*94*

Yagow (Rappahannock basin) Library (collected 1998 – 1999):

Muskrat	1
Raccoon	1
Deer	3
Beaver	1
Canine	8
Horse	8
Bovine	22
Canada Goose	1
Total	*45*

***** Number of isolates does not correspond with the number of scat samples collected for this st udy because some samples contained multiple strains of *E. coli* and other samples lacked viable *E. coli*.

DNA Profiles (PFGE Patterns) From Four Mile Run and Its Tributaries

A total of 539 bacterial isolates were removed from 55 samples of either water, a water/sediment mix, or sediment from Four Mile Run and its tributaries during this study period. Of the 539 isolates that were removed for DNA profile analysis, 100 of these could not be analyzed for reasons of taxonomic or restriction failure. The remaining 439 isolates were keyed to *Escherichia coli* (*E. coli*) using the Analytical Profile Index (API 20E) for the Enterobacteriaceae and other gram negative bacteria. These isolates provided the basis for resolving potential animal sources that could contribute to the nonpoint fecal coliform problem in Four Mile Run and its tributaries. Of the 439 isolates, 133 showed no match at 80% similarity ± 10 kilobase pairs (kbp) with any of the 843 strains of *E. coli* from known sources in the Virginia Tech DNA library (Table 2). Twenty-eight (28) isolates from the study matched at equal similarity with multiple strains in the Virginia Tech DNA library, but were inconclusive with regard to a specific species. However, within this group of 28 isolates, all suggested a nonhuman source, and nearly all suggested a nonhuman mammal source. The remaining 278 isolates did show a match at 80% similarity ± 10 kbp with a particular animal species in the library. Data in Figure 3 and Table 3 summarize these matches. Some isolates experienced taxonomic and restriction failure and others were inconclusive with regard to potential animal source. Table 4 summarizes these results.

DISCUSSION

Is the major source of nonpoint fecal coliform contamination human or non-human in origin?

The data suggested, that on the basis of the 278 isolates which did show one or more matches with strains of *E. coli* from known sources, potential contribution from human sources was moderate. Forty-six (46) isolates (17%) were considered to be of human origin, whereas 232 isolates (83%) were considered to be of nonhuman origin. The potential contribution from human sources ranged between 13-21% for all four seasonal sampling periods.

Is the human source localized?

The data suggested that possible contributions from human sources were localized. In particular, stations associated with Doctors Run (Feb '00, 13 isolates), Four Mile Run at Columbia Pike (Nov '99, 6 isolates), Donaldson Run at Military Road (Aug '98, 9 isolates), and Lucky Run (May '99, 11 isolates) suggested potential inputs of *E. coli* from human sources. Human signatures were not suggested at any of the other collecting sites.

Is the nonhuman source mammal or avian in origin?

As stated above, 232 isolates were identified as being of nonhuman origin. Of this pool (232 isolates), the data suggested that 127 isolates (55%) were from a mammalian source and 105 isolates (45%) were from one or more species of waterfowl (geese, gulls, and ducks).

Is the major mammal contribution from domestic or wild animal species?

Several animals stand out in the mammal group. Of the 127 isolates attributed to nonhuman mammal sources, raccoon were the most dominant representative of the group with 42 isolates (33%) being represented; deer were second with a total of 42 isolates (33%) (assuming that the bovine isolates served as surrogates for deer; canine isolates were third (24 isolates - 19%); and the Norway rat was fourth with 11 isolates (9%). Feline (3 isolates -2 %); opossum (3 isolates - 2%); beaver (1 isolate -1 %); and, muskrat (1 isolate -1 %) comprised the remaining matches. The dominance of raccoon in an urban watershed is consistent with findings by Hadidian, et al. (1991, 1997). These data suggested that wild

Table 3. Fecal Coliform Densities at Study Area Locations

			Fecal Coliform, MPN				
	I.D.	Alternate Station I.D.	Water	Water/ Sed.	Sedi-ment	Digital Latitude	Digital Longitude
28-Aug-98							
Note: Drought conditions							
1) Lower Long Branch in Arna Valley, 26th Street S.	27	NVPDC#1	2			38.8484	-77.0748
2) Four Mile Run at Shirlington Road	24	NVPDC#2	900			38.8431	-77.0861
3) Lucky Run outfall at Four Mile Run	23	NVPDC#3	500			38.8456	-77.0962
4) Upper Long Branch at Carlin Springs Road	15	NVPDC#4	≥1600			38.8587	-77.1268
5) Lubber Run at Route 50	13	NVPDC#5	500			38.8678	-77.1201
6) Middle Four Mile Run, bike trail crossing just u/s of Rt. 50	10	NVPDC#6	1600			38.8668	-77.1242
7) Upper Four Mile Run at Falls Church line (Van Buren Street)	1	NVPDC#7	900			38.8825	-77.1589
8) Doctors Run at Barcroft Park footbridge	22	NVPDC#8	900			38.8507	-77.1028
9) Donaldson Run at Military Road (outside of study area)	*n/a*		*500*			*38.9111*	*-77.1134*
10) Gulf Branch at Military Road (outside of study area)	*n/a*		*1600*			*38.9193*	*-77.1199*
06-May-99							
Note: Drought conditions							
1) Ballston Beaver Pond, along open channel (Lubber Run)	11	Near LR112, Arlington	900			38.8831	-77.1190
2) Powhatan Run at N. Livingston Road, pristine site	5	u/s of FM300, Arlington	50			38.8722	-77.1408
3) Manchester Street 1.1 m (42") outfall (Glencarlyn Branch)	6	FM 330, Arlington	≥1600			38.8675	-77.1330
4) Four Mile Run at Shirlington Road	24	NVPDC#2	1600			38.8431	-77.0861
5) Lucky Run outfall at Four Mile Run	23	NVPDC#3	500			38.8456	-77.0962
6) Four Mile Run at Columbia Pike	16	1AFOU004.22, Va. DEQ	900			38.8561	-77.1112

Table 3. *(continued)*			Fecal Coliform, MPN				
	I.D.	Alternate Station I.D.	Water	Water/ Sed.	Sedi-ment	Digital Latitude	Digital Longitude
23-Nov-99							
1) Upper Long Branch downstream of Patrick Henry Drive	14		80	170	80	38.8669	-77.1478
2) Upper Four Mile Run at Sycamore Street	2		30	300	30	38.8830	-77.1561
3) Box culvert under Ballston just downstream of Beaver Pond	12		900	500		38.8818	-77.1185
4) Lubber Run at Route 50	13	NVPDC#5	50	220	30	38.8678	-77.1201
5) Four Mile Run at Columbia Pike	16	1AFOU004.22, Va. DEQ	240		30	38.8561	-77.1112
6) Doctors Run at Barcroft Park footbridge	22	NVPDC#8	80		30	38.8507	-77.1028
7) Lucky Run outfall at Four Mile Run	23	NVPDC#3	900			38.8456	-77.0962
8) Four Mile Run at Shirlington Road	24	NVPDC#2	300		22	38.8431	-77.0861
9) Lower Long Branch in Arna Valley, 26th Street S.	27	NVPDC#1	≥1600		33	38.8484	-77.0748
10) Four Mile Run at George Washington Parkway	31	1AFOU000.19, Va. DEQ	130			38.8409	-77.0478
22-Feb-00							
1) Ohio Street Branch at I-66, outfall	3	FM200 or FM210, Arlington	50	900		38.8822	-77.1467
2) Westover Branch at I-66, outfall (twin box culvert to right of 2 m [78"] circular pipe)	4	FM230, Arlington	≥1600	≥1600	≥1600	38.8810	-77.1417
3) Powhatan Run at N. Livingston Road (pristine site)	5	u/s of FM300, Arlington	23	280		38.8722	-77.1408
4) Manchester Street 1.1 m (42") outfall (Glencarlyn Branch)	6	FM 330, Arlington	900	≥1600		38.8675	-77.1330
5) Baileys Branch at S. Frederick Street	17	FM350, Arlington	80	300		38.8536	-77.1152
6) Four Mile Run at Columbia Pike	16	1AFOU004.22, Va. DEQ	130	500	80	38.8561	-77.1112
7) Doctors Run at S. 6th Street & S. Quincy Street, biggest outfall	18	DB100, Arlington	1600	≥1600		38.8645	-77.1014
8) Lucky Run outfall at Four Mile Run	23	NVPDC#3	500	≥1600		38.8456	-77.0962
9) Nauck Branch	25	FM450, Arlington	500	1600	1600	38.8464	-77.0832
10) Lower Long Branch at I-395 near 28th Street S., outfall--quad box culvert	26	274 m (900') d/s of LL180, Arlington	2	21	500	38.8506	-77.0748
11) Arlington Sewage Treatment Plant outfall	28	FM545?, Arlington	0			38.8438	-77.0613
12) Four Mile Run at George Washington Parkway	31	1AFOU000.19, Va. DEQ	14	300		38.8409	-77.0478

Table 3. *(continued)*			**Fecal Coliform, MPN**				
	I.D.	**Alternate Station I.D.**	**Water**	**Water/ Sed.**	**Sedi-ment**	**Digital Latitude**	**Digital Longitude**
19-Jun-00							
Note: Samples from June 19, 2000 at Stations 5 - 12 were taken at 5 minute intervals at all four stations approximately simultaneously (in late morning). DNA results for June 19 not available for this study.							
1) Alexandria trib behind Cora Kelly Community Center, CMP outfall	**30**		900			38.8383	-77.0584
2) Alexandria trib behind Cora Kelly Community Center, upstream of outfall	**29**		≥1600			38.8383	-77.0594
3) Arlington Sewage Treatment Plant outfall	**28**	FM545?, Arlington	0			38.8438	-77.0613
4) Four Mile Run at Columbia Pike	**16**	1AFOU004.22, Va. DEQ	1600			38.8561	-77.1112
5) Doctors Run at S. 6th Street & S. Quincy Street, biggest outfall	**18**	DB100, Arlington	≥1600, ≥1600, ≥1600			38.8645	-77.1014
6) Doctors Run 61 m (200 ft) downstream of S. 6th Street & S. Quincy Street	**19**	d/s of DB100, Arlington	900, ≥1600, 900			38.8640	-77.1015
7) Doctors Run 122 m (400 ft) d/s of S. 6th Street & S. Quincy Street	**20**	d/s of DB100, Arlington	500, 900, 500			38.8635	-77.1019
8) Doctors Run 183 m (600 ft) d/s of S. 6th Street & S. Quincy Street	**21**	d/s of DB100, Arlington	900, 300, 900			38.8630	-77.1022
9) Manchester Street, 1.1 m (42 in) outfall	**6**	FM 330, Arlington	900, 500, ≥1600			38.8675	-77.1330
10) 46 m (150 ft) d/s of Manchester Street outfall	**7**	d/s of FM 330, Arlington	≥1600, 1600, ≥1600			38.8677	-77.1325
11) 91 m (300 ft) d/s of Manchester Street outfall	**8**	d/s of FM 330, Arlington	1600, 1600, ≥1600			38.8680	-77.1321
12) 137 m (450 ft) d/s of Manchester Street outfall	**9**	d/s of FM 330, Arlington	1600, 900, ≥1600			38.8682	-77.1317

TABLE 4. Number of Isolates by DNA Match with Best Species

Animal Species	FIELD DATES 28Aug98	6May99	23Nov99	22Feb00	TOTALS
Non-*E. coli* fecal coliforms[1]	0	37	4	11	52
No API Code	3	1	31	2	37
No Restriction	3	3	3	2	11
No Matches	18	9	67	39	133
Human	9	11	11	15	46
Raccoon	4	5	22	11	42
Canine	1	0	10	13	24
Deer	10	0	1	18	29
Bovine	0	0	3	10	13
Norway Rat	10	0	0	1	11
Feline	0	0	3	0	3
Opossum	0	0	0	3	3
Beaver	0	0	1	0	1
Muskrat	0	0	1	0	1
Herring Gull	6	18	1	0	25
Mallard Duck	0	18	13	1	32
Black Duck	0	0	6	2	8
Laughing Gull	8	0	1	0	9
Canada Goose	8	0	8	3	19
Black Back Gull	5	0	1	0	6
Tern	0	0	3	3	6
Undetermined	4	8	8	8	28
TOTALS	***89***	***110***	***198***	***142***	***539***

[1] Non-*E. coli* fecal coliforms = NECFC

Isolates Analyzed:

133 No Matching Records
52 NECFC
37 No API Code
11 Failed Restriction
28 Inconclusive Identification
278 Acceptable Matches
539 Total Number of Isolates Considered

Acceptable Matches:

46	Human
42	Raccoon
29	Deer
24	Canine
13	Bovine
11	Norway Rat
8	Other Mammals
105	Waterfowl
278	***Total***

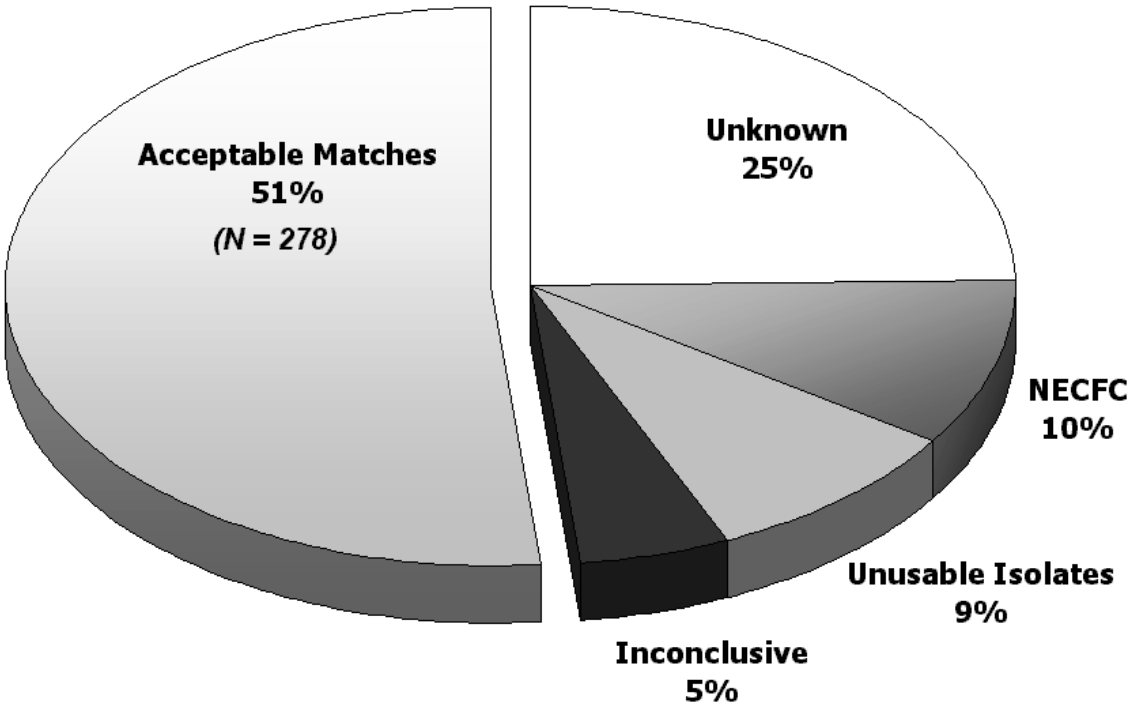

Figure 2. Success of Isolate Matching, N = 539

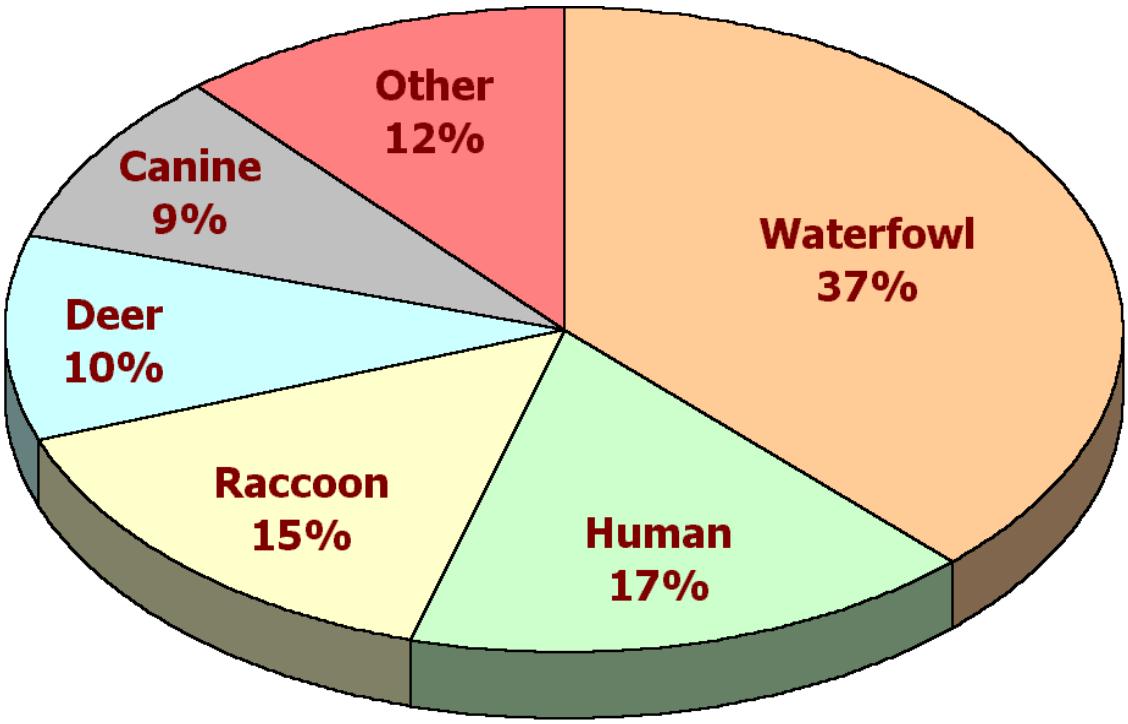

Figure 3. Distribution of Acceptable Matches by Animal Group, N = 278

animal species, rather than domestic animal species, contributed the greater percentage of fecal coliform isolates to Four Mile Run and its tributaries.

The fact that deer signatures were much more frequent than would have been suspected can be explained in several ways. One explanation has to do with frequency of occurrence of isolates, and the other explanation deals with assignment to a particular source. In the August 1998 samples, all ten isolates at Station 7 had the same profile. Assignment was made to "deer" as a result of band-to-band comparisons, but herring gull was a strong second choice. In the Feb '00 samples, all 10 isolates from Station 4 showed the same identical profile and, again, band-to-band comparisons suggested a "deer" signature, but Black Back Gull, raccoon, and canine were also possible choices. Stations 8 and 10 each had one isolate that suggested "deer," but muskrat and Canada goose were also reasonable choices. At Station 2, however, five isolates all had the same pattern, and "deer" was the only match suggested. Even if the other possible choices are considered, except in one case, the alternate choice is a wild animal source.

At the present time, the most limiting aspect of this research effort, aside from the modest size of the library, is the fact that canine and human *E. coli* populations cannot be separated statistically, despite this study's efforts to expand the source library for these two species. Caugant (1981) demonstrated that certain strains of bacteria can move freely between humans and canines that share the same living space. However, of the total pool of identifiable isolates, only 70 isolates (25%) could be assigned to human or canine and 208 (75%) isolates were assigned to wild animal sources.

The subject of urban wildlife ecology is still in its infancy and much still remains to be understood about the relationship of certain wildlife species to expanding urban environments (Murphy 1988).

The data **do not suggest** that there were more wildlife individuals in the watershed than canine or human individuals. The data **do suggest** that certain wildlife species have a greater, disproportionate, representation and effect on fecal coliform density in the watershed because of their direct contact and intimate association with the waterways. Furthermore, the frequency of occurrence of a wild animal species is not necessarily occur in direct relationship to the frequency of occurrence of their fecal coliform signature. Survival and regrowth of specific strains from a given animal also have to be considered as well as the specific time of collection.

The conclusion, suggested from the data in this study, that wildlife animal sources were a major contributor to the fecal coliform problem, has also been corroborated by fecal coliform studies in tidal creeks and estuaries in the southern Chesapeake Bay (Simmons, 1994; Simmons and Herbein, 1995; Simmons, et al, 1995; Herbein et al, 1996).

What is the role of sediments?

Two sampling periods (November 1999, and February 2000) focused on the contribution of water/sediment slurries and sediments to the fecal coliform problem. The MPN geometric mean for all sites in November for the fecal coliform densities in water was 149.3; for water/sediment slurries 239.7; and, for sediments 32.6. Estimates of sediment MPN density for this period consisted of adding 1 gm of sediment in 99 mls of buffered water, and the sediments consisted of very coarse sand and/or gravel. Some of the water/sediment slurries came from inside stormwater pipes and contained little/no sediment. While these data suggest that the greatest

number of fecal coliforms existed in the water column and as a microbial film attached to substrate, additional research using sonication is recommended to confirm this.

This exercise was repeated in February 2000. At this time, the composition of the sediments and amounts added to buffered water was different than in the November exercise. In February, two samples of very fine sediments were collected at each stormwater outfall and 1.0 gm was added to 100 ml of buffered water. In two other samples, 6.0 and 15.0 gms of sediment were added to the buffered water because the sediments were so coarse that it was not possible to weigh out 1.0 gram exclusive of residual water in the syringe. The MPN geometric mean in February for the fecal coliform densities in water was 132.3; for water/sedime nt slurries 592.9; and for sediments 574.3.

The role of sediments as potential reservoirs has been documented by other researchers (Van Donsel and Geldreich, 1971; Gerba and McLoed, 1976; Hood and Ness, 1982; Stephenson and Rychert, 1982; Sherer, et al., 1992; Davies, et al., 1995; and, Reay, 2000). The February data showed that microbial films and sediments can serve as reservoirs and potentially contribute to the nonpoint fecal coliform problem in Four Mile Run. This contribution could be through the addition of cells to the water column from regrowth of either microbial films or from the sediments. Contributions through regrowth and subsequent sampling of clonal populations from the water column could explain the low strain diversity found by this inve stigation in many of the samples collected from stormwater outfalls.

What is the role of non-*E. coli* fecal coliforms (NECFC)?

Non-*E. coli* fecal coliforms (NECFC) are those bacteria that also are characterized as part of the Enterobacteriaceae along with *E. coli*. NECFC species not only inhabit the intestinal tract of animals along with *E. coli*, but also they may occur as free-living organisms in aquatic systems as well. In routine examination of freshwaters using gas formation as a method of identificati on, these other Enterobacteriaceae species may give a false reading. Therefore, in trying to determine nonpoint *E. coli* sources, detailed identification of isolates must be made to rule out the presence of non-*E. coli* fecal coliform species.

The role of NECFC was not as significant in the final analysis of sources as originally believed, and the data suggested that NECFC contributed only in a minor way to the overall nonpoint fecal coliform source question. However, in some cases and based on the numbe r of isolates analyzed at random, the data suggested that NECFC could be significant in isolated or localized situations. For example, at Station 3 in the May 6, 1999 sampling period, the 20 isolates removed for restriction analysis were all *Citrobacter freundii*. Likewise, on the same date at Station 6, 16 of the 20 isolates removed were *Enterobacter cloacae*. At Station 6 for the February 22, 2000 sampling, five of the 10 isolates removed were *C. freundii*. Even though the data suggested that NECFC occurred at a low density level, they did contribute to the overall fecal coliform density.

Of the 539 isolates removed from samples for restriction analysis, 89 isolates (17%) fell into the category of "NECFC" or "unidentified API profile." Of these 89 iso lates, 55 isolates were identified with the API profile system to be *C. freundii*, *E. cloacae*, *Kluyvera*, spp, *Klebsiella pneumoniae*, or *K. ozaenae*. Of these taxonomic groups, *C. freundii* and *E. cloacae* comprised the greatest number of isolates (29 and 18, respectively) that were encountered in the NECFC group.

Is there any seasonal variation?

No discernable pattern of seasonal variation among acceptable human or non-human matches was evident in this study. Furthermore, even the density of fecal coliforms was just as elevated during the winter sampling period as during the warmer months. This may point to a storm drain effect, as these drains have been previously documented to moderate baseflow temperatures within Four Mile Run (NVRC, 1996b).

What is the effect of baseflow drainage through storm drains?

Two-thirds of the watershed's original stream network has been converted to underground drainage, primarily in its headwaters. The data collected from storm drains suggested that drainage from these conduits during baseflow periods contributed significantly to the fecal coliform problem in Four Mile Run and its tributaries. For example, the MPN geometric mean of fecal coliform densities in open stretches of Four Mile Run and its tributaries was 231.1 (N=23); whereas, the MPN geometric mean of fecal coliform densities from stormwater outfalls during the same period was 400.2 (N=11). In addition to temperature moderation, storm drains also prevent die-off by shielding the bacteria from the sun's ultraviolet radiation. However, as with most *E coli* studies, these counts were highly variable and more data are needed to confirm a statistically valid correlation.

In June 2000 a study was conducted at two stormwater outfalls (Doctors Run and Manchester Street) to determine the degree to which fecal coliform density from the outfalls diminished with distance downstream. The distance downstream from each outfall was approximately 100 meters. The fecal coliform density at the Doctors Run outfall was $\geq$ 1600 and had decreased to a geometric mean of 624.0 at the downstream sampling point. At the Manchester Street outfall, the geometric mean of the fecal coliform density at the outfall was 914.5 but the density increased to a geometric mean of 1347.7 at the downstream sampling point. In the latter case, given the range of density associated with MPN values, the data demonstrate that there was little/no removal of fecal coliform density within the 100 meter stretch and that the open water portion of the stream was influenced by the discharge from the stormwater line. In the former case (Doctors Run), the data suggest that, while the stream had some filtration capacity to reduce fecal coliform densities, the density in the stream was also influenced by the stormwater discharge.

The influence of storm drains on the fecal coliform problem can be explained in two possible ways. First, the density of animal scat in the storm drains may provide a constant source of fecal coliforms as the water passes over the scat deposits. Second, and a more likely explanation, is that scat material is deposited in the storm drains, fecal coliforms are transported from the scat, become deposited in the storm drains, re-grow, and contribute to the microbial film found in the storm drains. Clonal populations lift-off, or are scoured by the moving water, and provide a continuous source, or inoculation, of fecal coliforms to the discharging water.

The importance of regrowth has been investigated by Simmons and his students (Carey and Simmons, 1995) in relation to discharge from a poultry processing plant on Virginia's Eastern Shore. Sediments are also important reservoirs for fecal coliform introduction to surface waters as noted by other investigators (cited above). Additional water chemistry data from Four Mile run and its tributaries (Northern Virginia Planning District Commission, 1996b) indicate that sufficient quantities of nutrients and carbon are available to support regrowth in the storm drains.

Additional research on urban portions of Northern Virginia (Harms and Southerland (1975); Randall, et al. (1978); and, Environmental Systems Analysis, Inc (1999)) corroborates a dominant

deleterious influence of storm drains on water quality. Detrimental urban runoff contributions of nutrients, sediment, and other pollutants are well documented in the nonpoint source literature. Environmental Systems Analysis, Inc. (1999) completed a baseline macroinvertebrate assessment of Four Mile Run and found that the substrate at most sampling site s showed dominance of a few pollution-tolerant macroinvertebrates, and stations characterized by high levels of algal growth (evidence of nutrient loading), sedimentation, and erosive flows from high storm drain discharges during wet weather.

SUMMARY

Based on the interpretation of DNA profile analyses of pulsed field gel electrophoresis patterns for those *E. coli* isolates from Four Mile Run and its tributaries that could be matched with *E. coli* strains from known sources in the Virginia Tech library; and, from fecal coliform densities of water, water/sediment slurries, and sediment, the data suggested the following:

1. nonhuman species are the dominant sources of *E. coli* to Four Mile Run and its tributaries;
2. waterfowl contribute over one-third (37%) of those isolates that could be identified;
3. the presence of human *E. coli* is localized;
4. the nonhuman sources are wildlife species that have intimate association with the waterways;
5. the predominant nonhuman mammal contributors are raccoon, dog, deer, an d Norway rat;
6. the combined human and canine contribution is approximately 25% of those isolates that could be identified;
7. the organisms contributing to the presence of *E. coli* are those animals which would normally be expected in an urban watershed;
8. discharge from storm drains during baseflow seems to play a significant role in the fecal coliform problem;
9. without regard to specific host animals, *E. coli* bacteria seem to regrow, through cloning, within the storm drains and stream sediments, which in turn per petuate elevated bacteria levels within the connected surface waters of Four Mile Run.

The data **do not suggest** there were more wildlife individuals in the watershed than canine or humans, but the data **do suggest** that certain wildlife species may have a gre ater, disproportionate, representation in the DNA profile analysis because of their direct contact and intimate association with the waterways. The DNA profile analysis is not a tool for estimating population density of any given animal species, but it ma y be an excellent method to identify those animals that have an impact on water quality.

It is neither desirable nor practical to eliminate wildlife animal species in the watershed. Ecologically speaking, the microbial community, including *E. coli*, is doing what heterotrophic microorganisms do – absorb nutrients and decompose organic compounds. The continued high levels of *E. coli* suggest an ecosystem out of balance irrespective of the source.

While the citizens of Four Mile Run and those governmental age ncies whose job it is to oversee and improve water quality in Four Mile Run deserve considerable credit for improving water quality in Four Mile Run and its tributaries, much remains to be done to reduce nutrient loading which may contribute to the regrowt h of those *E. coli* which make their way into the waterways.

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