Clean Water Services
DNA Fingerprinting of Bacteria Sources in the Tualatin Sub-basin

SUMMARY

This study was divided into two parts. The first part had two goals. One goal was to determine the sources of bacteria in the stormwater and receiving streams to help focus Clean Water Services management efforts to reduce the bacteria levels. This will allow the Best Management Practices (BMPs) to be targeted at the primary sources of bacteria and will lead to the most cost-effective implementation plan. The other goal was to provide information that would encourage the public to change behaviors that were resulting in high levels of bacteria. Many of the BMPs to reduce bacteria in receiving streams rely on changing the public's perception of the source of the problem and engaging them to solve the problem. Effectively communicating this information to the public will make it more likely that they will change their behaviors to reduce bacteria loads on Tualatin River Basin streams. The second part of this study was to determine if bacteria from human sources were present at a stormwater outfall at one of the Clean Water Services wastewater treatment plants. Both parts were done at the same time.

Clean Water Services received a 319h grant for $41,723 from DEQ during the 2002 granting cycle. The grant money, plus $40,000 from Clean Water Services, paid for the DNA fingerprinting of *Escherichia coli (E. coli)* bacteria isolates. In addition Clean Water Services covered the sampling and laboratory cost of collecting the *E. coli* bacteria samples that were sent to the DNA laboratory. The DNA fingerprinting was done by Dr. Mansour Samadpour at the Institute for Environmental Health (IEH) in Seattle, Washington using the ribosomal RNA typing method. This is one of several methods currently being used for microbial source tracking (MST). The theory behind this test is that DNA patterns can be used to match *E. coli* bacteria found in the environment with the sources of the bacteria.

The Tualatin River and its tributaries have bacteria levels that are periodically above the numeric criteria established to support the water contact recreation beneficial use. The tributaries in the Tualatin Basin have higher bacteria levels than the main stem of the Tualatin River. The criteria are based on non-pathogenic *E. coli* bacteria, which serve as an indicator of the possible presence of pathogens. In 2001 the Oregon Department of Environmental Quality (DEQ) developed a Total Maximum Daily Load (TMDL) for bacteria in the Tualatin Basin. Clean Water Services (formerly Unified Sewerage Agency) received a Wasteload Allocation (WLA) for discharges from municipal separate storm sewer systems (MS4) for *E. coli* bacteria. The TMDLs establish different summer and winter stormwater WLAs for each subbasin. To meet the WLAs, Clean Water Services must develop Best Management Practices (BMPs) to control bacteria in stormwater. While the TMDL document lists likely sources of bacteria, it is not clear whether the identified sources are relevant to the Tualatin River Basin; furthermore,

information regarding the relative contributions of the individual sources, which would enable Clean Water Services to focus its management activities, was not available.

The first part of the study focused on sites that had characteristics that existed throughout the basin. Sites were selected to represent these characteristics. These were called general sites. There were both stream sites and stormwater sites in this part. General sites were selected based on a history of high bacteria levels and a probable source of bacteria. It is important that BMPs be applied to all areas that share characteristics of the sites in the study and not just the areas upstream of the sites in the study. Samples were collected from the following general sites:

- Stream site in an area with a high percentage of septic systems
- Stream site in an area that has dog waste next to the stream
- Stream site in an older urban area with a mix of septic and sewered systems
- Stream site downstream of a lake where the public often feeds the ducks and geese
- Stream site in an area that has both older homes on septic and very new homes on the sewer system.
- Stormwater site that served a commercial site
- Stormwater site that served a new residential site
- Water quality facility effluent.

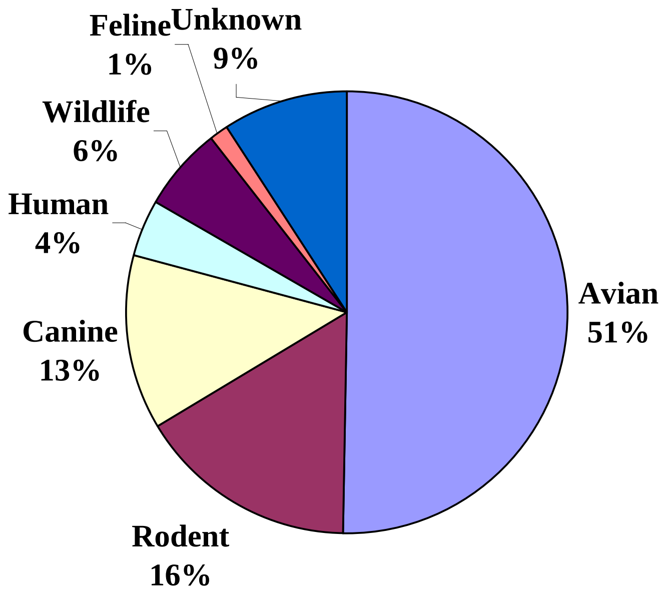
The second part of the study consisted of one site that represented a specific problem. This was called the specific site. It was a stormwater outfall at the Durham Wastewater Treatment Plant with periodically high bacteria counts and no obvious source of bacteria other than the geese that frequent the site.

Samples were collected from April 2004 to April 2005 during dry weather and during wet weather in both the summer and winter. Sites were visited between 6 and 7 times. There was an average of 116 isolates of *E. coli* bacteria evaluated for each site.

The results from part 1, the general sites, and part 2, the specific site, were evaluated separately.

A summary of the results from part 1 is shown in the following pie chart that shows the relative contribution of each source of bacteria when the results from the 924 isolates from the general sites were aggregated:

All General Site Results Combined
Percentage of *E. coli* Bacteria Sources



This pie chart shows that 51% of the 924 *E. coli* bacteria isolates came from avian sources. Rodents, canines, humans, wildlife and felines made up 40% of the isolates. Isolates of unknown origin made up 9% of the isolates. This pattern of sources (avian highest, followed by rodents and canines, and then humans) was consistent across the general sites whether aggregated together, aggregated by stream or stormwater, or aggregated by weather condition.

The following table shows the results from two other studies conducted in this geographic area that had similar aggregated results:

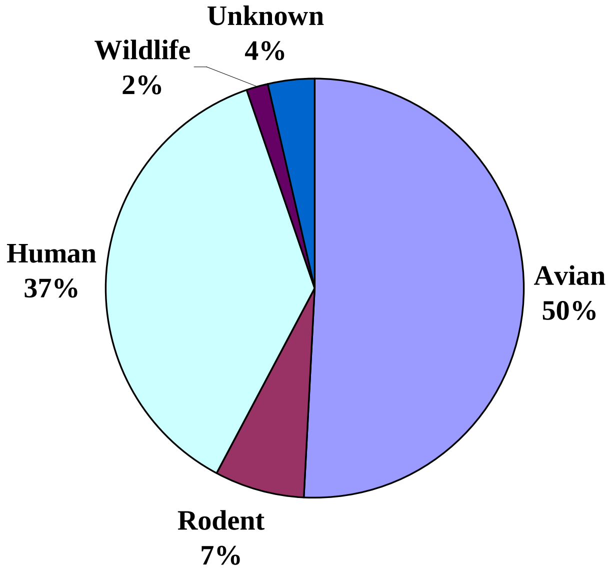
	Clean Water Services	Lower Boise River Idaho	City of Puyallup Washington
Avian	51 %	24 %	40 %
Rodent	16 %	4 %	28 %
Canine	13 %	14 %	11 %
Human	4 %	12 %	5 %
Wildlife	6 %	3 %	5 %
Agricultural		8 %	1 %
Feline	1 %	4 %	1 %
Unknown	9 %	31 %	9 %
Count of isolates	924	1565	687

The City of Puyallup and Clean Water Services had very similar results. The Boise River study was slightly different, which may be due to the fact that it had such a high percentage of unknown isolates.

This information can be used to focus the development of BMPs to reduce bacteria contributions from human activities that are detrimental to the receiving streams. It can also be used to show that anthropogenic activities have a negative impact on the bacteria levels of the receiving streams. Effectively communicating this information to the public is the best way to encourage behavioral changes that will reduce bacteria loading to streams in the Tualatin River basin.

The following pie chart shows the results for the second part of the project. It is the stormwater outfall from the Durham wastewater treatment plant.

Wastewater Treatment Plant Stormwater



The results from this site are very different from the general sites. There is a much higher percentage of bacteria from human sources (37%) and fewer types of isolates. There were only about half as many rodents and no canine isolates at this site. Now that isolates from human sources have been found at this site, options for reducing or eliminating this source are being evaluated by the treatment plant staff.

WATERSHED SETTING

The Tualatin River drains an area of 712 square miles and is situated in the northwest corner of Oregon. It corresponds to the fourth field hydrologic unit code (HUC) 17090010 and is a sub-basin of the Willamette River Basin. The headwaters are in the Coast Range and the River flows in a generally easterly direction to its confluence with the Willamette River, upstream of the Willamette Falls. The sub-basin lies almost entirely within Washington County, although there are also small portions in Multnomah, Clackamas, Columbia, Tillamook, and Yamhill counties. The Tualatin River is approximately 83 miles long. Major tributaries include: Scoggins, Gales, Dairy (including East Fork, West Fork, and McKay), Rock (including Beaverton), and Fanno creeks.

The subbasin supports a wide range of forest, agriculture and urban-related activities. The rapidly growing urban portion covers an area of 122 square miles and currently has a population of 480,000. It includes the cities of Banks, Beaverton, Cornelius, Durham, Forest Grove, Gaston, Hillsboro, King City, North Plains, Sherwood, Tigard, Tualatin, a relatively large unincorporated area, and small portions of Portland and West Linn. The Tualatin River is receiving increasing use for water contact recreation (e.g. canoeing, fishing, and swimming) as the population increases. Access to the river through parks and boat ramps has also increased.

WATER QUALITY STANDARD

The bacteria standard is designed to protect water contact recreation as a beneficial use in the Tualatin Basin.

The bacteria criterion for water contact recreation is as follows:

A 30-day log mean of 126 *E. coli* bacteria per 100 mL, based on a minimum of five (5) samples

No single sample shall exceed 406 *E. coli* bacteria per 100 mL

Water Quality Limited Determination:

More than 10% of the samples (with a minimum of 2 exceedences) exceed 406 *E. coli* bacteria per 100 mL

The following table shows the streams listed on the DEQ 1998 303(d) list, which is the basis for the 2001 TMDL.

Tualatin River Subbasin Bacteria Impaired Stream Segments			
Segment[1]	**Tributary To:**	**Listing Criterion**[2]	**Season of Violation**
Ash Ck.	Fanno Ck.	Fecal Coliform	All Year
Beaverton Ck.	Rock Ck.	E. coli	All Year
Bronson Ck.	Beaverton/Rock	E. coli	All Year
Burris Ck.	Tualatin R.	Fecal Coliform	All Year
Butternut Ck.	Tualatin R.	Fecal Coliform	All Year
Carpenter Ck.	Tualatin R.	E. coli	Summer
Cedar Ck.	Chicken Ck.	Fecal Coliform	All Year
Cedar Mill Ck.	Beaverton/Rock	Fecal Coliform	All Year
Chicken Ck.	Tualatin R.	E. coli	All Year
Christenson Ck.	Tualatin R.	Fecal Coliform	All Year
Dairy Ck. (Mouth to E/W Forks)	McKay Ck.	E. coli	All Year
Dairy Ck., West Fork	M/S Dairy Ck.	E. coli	Summer
Fanno Ck.	Tualatin R.	E. coli	All Year
Gales Ck. (Mouth to Clear Ck.)	Tualatin R.	E. coli	Summer
Hall Ck.	Beaverton Ck.	Fecal Coliform	All Year
Heaton Ck.	McFee Ck.	Fecal Coliform	All Year
Hedges Ck.	Tualatin R.	E. coli	All Year
Johnson Ck.-North	Cedar Mill Ck.	Fecal Coliform	All Year
Johnson Ck.-South	Beaverton Ck.	E. coli	All Year
McFee Ck.	Tualatin R.	Fecal Coliform	All Year
McKay Ck. (Mouth to E. Fork)	Tualatin R.	E. coli	All Year
Nyberg Ck.	Tualatin R.	Enterococci	All Year
Rock Ck.	Tualatin R.	E. coli	All Year
Summer Ck.	Fanno Ck.	Fecal Coliform	All Year
Tualatin R. (Mouth to Dairy Ck.)	Willamette	E. coli	All Year
Willow Ck.	Beaverton Ck.	Fecal Coliform	All Year

DEQ Tualatin Subbasin TMDL, August 2001, Section 4.2.3, Table 15

[1] Mouth to headwaters (unless otherwise noted)

[2] The “listing criterion” is the bacteria criterion from Table 13 (above) for which the water body had exceedances. (E.g., since “Fecal Coliform” is listed as the “listing criterion” for Summer Creek, exceedances of the fecal coliform criterion are what warranted the creek’s placement on the 303(d) list.)

The following table shows the DEQ Tualatin Sub-basin TMDL, August 2001 wasteload allocations (Section 4.2.10.3, Table 17 and 18).

Summer (May 1 – October 31) Wasteload Allocations For Discharges from Municipal Separate Storm Sewer Systems and CAFO Sources (Concentrations)			
Designated Management Agency	**5th-Field Subbasin**	**Wasteload Allocation – E. coli counts/100 mL**	
City of Lake Oswego, City of Portland, City of West Linn, Clackamas Co., Oregon Dept. of Transportation, Multnomah Co., Unified Sewerage Agency, and Washington Co.	**All Land Uses[3]/Sources Covered By MS4 Permits Except as Otherwise Noted**	**During Runoff Events[4] (Measured as an event mean concentration)**	**All other times (Measured as a grab sample)**
	Gales	9500	406
	Rock	3000	406
	Dairy	7000	406
	Scoggins/Upper Tualatin	9500	406
	Middle Tualatin	12000	406
	Lower Tualatin	12000	406
Oregon Dept. of Agriculture	All CAFO Direct Discharges	0	0

Winter (Nov. 1 – April 31) Wasteload Allocations For Discharges from Municipal Separate Storm Sewer Systems and CAFO Sources (Concentrations)			
Designated Management Agency	**5th-Field Subbasin**	**Wasteload Allocation – E. coli counts/100 mL**	
City of Lake Oswego, City of Portland, City of West Linn, Clackamas Co., Oregon Dept. of Transportation, Multnomah Co., Unified Sewerage Agency, and Washington Co.	**All Land Uses/Sources Covered By MS4 Permits Except as Otherwise Noted**	**During Runoff Events[5] (Measured as an event mean concentration)**	**All other times (Measured as a grab sample)**
	Gales	3500	406
	Rock	700	406
	Dairy	3500	406
	Scoggins/Upper Tualatin	1500	406
	Middle Tualatin	11000	406
	Lower Tualatin	5000	406
	All Septic Systems	0	0
Oregon Dept. of Agriculture	All CAFO Direct Discharges	0	0

[3] The land uses utilized in the DEQ bacteria model included forestry, meadow, open space, commercial, agricultural, industrial, residential and transportation.

[16, 17] Runoff Event is defined as the period when precipitation causes overland runoff to occur from the area of concern.

REGULATORY FRAMEWORK

Clean Water Services holds a Watershed-Based NPDES permit that covers the treated effluent produced by its four wastewater treatment plants (Rock Creek & Durham year-round and Forest Grove & Hillsboro winter-only), stormwater discharges from the Rock Creek and Durham wastewater treatment plants, and stormwater from the urban portion of Washington County (MS4 stormwater). It was originally issued on February 26, 2004 and reissued on July 27, 2005 after being reconsidered by DEQ. The wastewater treatment plant effluents have numeric limits for *E. coli* bacteria that are consistent with the bacteria criterion. The treatment plant stormwater has a benchmark of 406 *E. coli* bacteria per 100 mL. For the MS4 stormwater, Clean Water Services must evaluate bacteria levels relative to the WLA and set benchmarks in its Stormwater Management Plan (SWMP). A revised SWMP must be submitted to the DEQ on May 1, 2006.

GOALS OF THE DNA TESTING STUDY

The 2001 Tualatin Subbasin TMDL lists the tributaries and main stem of the Tualatin River as water quality limited for bacteria in both the summer (low flow period) and winter (high flow period). The tributaries are more severely impacted by bacteria than is the main stem. The summer period is more significantly impacted than the winter period. The following tables show a history of the summer and winter levels for the routinely monitored sites using the 90th percentile as the statistic. This is the statistic that is used to designate water quality listed streams on the DEQ's 303(d) list. It starts in 1995 which was the last year used to develop the 2001 Tualatin TMDL. The bold sites are part of this DNA study.

Summer *E. coli* Bacteria Levels (in *E. coli* per 100 mL)
90th Percentile

Location	River Mile	LOCCOD	1995	2000	2001	2002	2003	2004
ASH @ HEMLOCK	**1.4**	**3845014**				**2840**	**4580**	**1450**
BANSTR @ 124TH	1.0	3859010		367	8	143	536	300
BRNSN @ 143RD	5.0	3824050	2460	836	320	556	547	464
BRNSN @ 185TH	1.8	3824018	290	760	32	1030	368	1000
BRNSN @ 205TH	0.1	3824001	148	4580	980	1030	897	560
BRNSN @ BRNSN PK	2.0	3824020	304	974	130	704	784	500
BRNSN @ SALTZMAN	7.2	3824072	838	1400	68	3790	1429	1300
BRNSN @ WALKER	1.5	3824015	180	248	120	3240	248	300
BRNSN @ WU	3.2	3824032	1072	816	36	4240	515	1000
BVTN @ 170TH	5.0	3821050		834	280	1840	770	300
BVTN @ BEAMAN	0.8	3821008				2620	964	730
BVTN @ CNLUS PASS	1.2	3821012	1016	480	980	976		
CHICKEN @ SCH-SHER	2.0	3835020	732	1028	550	381	986	576
DAIRY @ HWY 8	2.1	3815021	312	860	848	978	879	476
DWSN @ AIRPORT	1.7	3850017		1950	1300	960	960	
DWSN @ BROOKWOOD	**0.6**	**3850006**		**1280**	**120**	**2080**	**3870**	**1400**
Un-named Tributary to FANNO @ WALNUT	**0.1**	**3500035**						**2160**
FANNO @ DURHAM	1.2	3840012	1460	1180	2960	821	1001	848
FANNO @ TCKRWD	7.4	3840074	1760	1160	3740	2140	2030	1900
FANNO NR ALN	**9.5**	**3840095**	**3080**	**1080**	**2420**	**3300**	**5590**	**2000**
GALES @ NEW HWY 47	1.5	3810015	254	188	526	326	300	476
JHNSN S @ DAVIS	1.4	3827014	990	1510	260	1620	2040	720
JHNSN S @ GLENBRK	1.1	3827011	4060	1200	380	1490	620	
MCKAY @ HORN	2.0	3816020	860	282	472	580	1100	336
RC@BROOK	2.2	3820022					688	1220
ROCK @ HWY 8	1.2	3820012	364	316	1610	1000		
ROCK @ QUATAMA	4.7	3820047	904	656	2360	1000	1030	740
SCOGGINS @ 47	1.7	3805017	93	52	421	25	155	72
SCOGGINS @ STIM	4.8	3805048	11	4	1	1	3	4
SUMMER @ 121ST	**0.9**	**3844009**				**5860**	**13500**	**1500**
Tualatin River @ BOONES FERRY	8.7	3701087	984	172	607	176	92	136
Tualatin River @ CHER GR	71.5	3701715	72	56	100	33	57	57
Tualatin River @ ELSNER	16.5	3701165	156	49	37	33	56	55
Tualatin River @ FARMNGTN	33.3	3701333	182	264	80	164	368	230
Tualatin River @ GOLF CRS	52.8	3701528	300	156	709	238	260	272
Tualatin River @ HWY 219	45	3701450	748	250	553	343	254	292
Tualatin River @ ROOD	39.1	3701391	1304	156	214	229	51	276
Tualatin River @ SCHOLLS	27.1	3701271	725	120	102	109	95	150
Tualatin River @ SPRINGHILL	61.2	3701612	304	212	790	100	123	164
Tualatin River @ STAFFORD	5.4	3701054	257	212	655	97	158	150
Tualatin River @ WEISS BR	0.2	3701002	214	120	100	116	101	78

The highlighted cells have values over 406 *E. coli* bacteria per 100 mL
Bold locations are in this DNA study

Winter *E. coli* Bacteria Levels (in *E. coli* per 100 mL)
90th Percentile

Location	River Mile	LOCCOD	1995	2000	2001	2002	2003	2004
ASH @ HEMLOCK	**1.4**	**3845014**				**576**	**964**	**600**
BANSTR @ 124TH	1.0	3859010		260	23	80	68	43
BRNSN @ 143RD	5.0	3824050	2187	180	279	1870	281	110
BRNSN @ 185TH	1.8	3824018	716	280	116	555	157	658
BRNSN @ 205TH	0.1	3824001	776	200	88	1240	138	208
BRNSN @ BRNSN PK	2.0	3824020	388	280	125	756	151	101
BRNSN @ SALTZMAN	7.2	3824072	868	420	1640	221	228	502
BRNSN @ WALKER	1.5	3824015	732	400	188	832	224	302
BRNSN @ WU	3.2	3824032	1142	270	159	1036	507	212
BVTN @ 170TH	5.0	3821050		620	237	1231	426	524
BVTN @ BEAMAN	0.8	3821008				228	520	298
BVTN @ CNLUS PASS	1.2	3821012	521	557	148			
CHICKEN @ SCH-SHER	2.0	3835020	240	140	142	170	195	246
DAIRY @ HWY 8	2.1	3815021	272	118	328	249	207	142
DWSN @ AIRPORT	1.7	3850017		220	660	513	228	
DWSN @ BROOKWOOD	**0.6**	**3850006**		**270**	**512**	**995**	**202**	**319**
Un-named Tributary to FANNO @ WALNUT	**0.1**	**3500035**						**464**
FANNO @ DURHAM	1.2	3840012	540	590	154	340	850	383
FANNO @ TCKRWD	7.4	3840074	1482	1082	177	468	832	545
FANNO NR ALN	**9.5**	**3840095**	**1016**	**800**	**404**	**986**	**1140**	**504**
GALES @ NEW HWY 47	1.5	3810015	213	127	84	107	136	244
JHNSN S @ DAVIS	1.4	3827014	600	310	388	1834	298	312
JHNSN S @ GLENBRK	1.1	3827011	820	420	554	1765	482	
MCKAY @ HORN	2.0	3816020				134	421	
RC@BROOK	2.2	3820022					117	504
ROCK @ HWY 8	1.2	3820012	2250	180	100	430	344	
ROCK @ QUATAMA	4.7	3820047	484	210	105	234	474	858
SCOGGINS @ 47	1.7	3805017	170	53	55	58	50	68
SCOGGINS @ STIM	4.8	3805048		5	2	9	4	2
SUMMER @ 121ST	**0.9**	**3844009**				**390**	**1080**	**845**
Tualatin River @ BOONES FERRY	8.7	3701087	280	205	82	271	362	124
Tualatin River @ CHER GR	71.5	3701715	21	15	17	24	20	17
Tualatin River @ ELSNER	16.5	3701165	160	168	87	258	314	124
Tualatin River @ FARMNGTN	33.3	3701333	170	201	61	37	198	68
Tualatin River @ GOLF CRS	52.8	3701528	300	185	216	161	118	124
Tualatin River @ HWY 219	45	3701450	240	100	278	299	173	120
Tualatin River @ ROOD	39.1	3701391	150	88	220	259	275	80
Tualatin River @ SCHOLLS	27.1	3701271	160	134	122	488	222	126
Tualatin River @ SPRINGHILL	61.2	3701612	180	468	268	264	146	155
Tualatin River @ STAFFORD	5.4	3701054	230	61	99	152	392	143
Tualatin River @ WEISS BR	0.2	3701002	260	103	66	314	328	88

The highlighted cells have values over 406 *E. coli* bacteria per 100 mL
Bold locations are in this DNA study

Clean Water Services has been assigned WLAs for stormwater for both the summer and winter time periods. Clean Water Services is required to develop a stormwater management plan, which includes BMPs to meet the WLAs.

To better focus the bacteria reduction efforts, the sources of bacteria need to be identified. This will allow the BMPs to be targeted at the primary sources of bacteria and will lead to the most cost-effective implementation plan.

The August 2001 TMDL document lists several potential sources of bacteria.

- Non-runoff sources (maximum impact during summer flow conditions)
  - Sanitary sewer cross connections and overflows to stormwater sewers
  - Confined animal feeding operations (CAFOs)
  - Direct deposition by farm animals
  - Illegal dumping of waste water
  - Failing Septic Systems

- Runoff sources (maximum impact during the winter flow conditions)
  - All of the above, although they will be diluted with rain water
  - Pet, farm animals, and wildlife waste
  - Illegal dumpsites that attract vermin

Many of the BMPs will rely on changing the public's perception of the source of the problem. By educating the public about the sources of bacteria with understandable scientific data, they will be more likely to adopt and champion the actions necessary to reduce bacteria levels in the Tualatin Sub-basin. Potential BMPs that could be used are as follows:

Connect urban areas with septic systems to the sanitary system
    (Currently underway in Clean Water Services area of responsibility)
Active pet waste management
    Signage in all parks that allow pets
    Facilities for pet waste in parks
    Require riparian areas between trails and surface water in parks
    Mailings to pet owners describing problem and how they can help
    SOLV cleanups to include pet waste collection in parks
Hobby Farms
    Require barriers between livestock and surface waters
    Establish minimum land requirements
    Educate the land owners
Duck and Goose waste
    Limit ducks and geese depending on water surface area
    Require riparian areas that do not encourage feeding of ducks and geese
    Educate the pubic
Illegal Dump Sites
    Determine responsibility; develop a procedure to discourage illegal dumping; clean up the sites that are found

Clean Water Services will use the information to develop BMPs that target human activities that result in elevated bacteria levels in the receiving streams in its Stormwater Management Plan.

The sites from this study will be monitored for *E. coli* bacteria levels over the next three (3) to five (5) years to quantify the reduction of bacteria levels that are the result of the BMPs that are implemented. After three (3) to five (5) years Clean Water Services may apply for a second 319h grant to do a follow up study to determine the effectiveness of the BMPs relative to the sources of *E. coli* bacteria. The adaptive management process will also use this information to make modifications to the BMPs. This will maximize the resources Clean Water Services uses to meet the bacteria TMDL.

SAMPLE SITE CHARACTERISTICS

Part 1 - General Sites

Stream – High Septic System Area

This is on an un-named tributary to Fanno Creek (CWS location ID 3500035) in the Tigard area. It has many septic systems and is slated for a sewer system in the next few years. It was identified as an area with high bacteria in a synoptic survey that was done in 1996. It has recently been added to the monitoring plan and continues to have high bacteria levels.

Stream – High Dog Population

This area is along Fanno Creek (CWS location ID 3840095) where there is strong evidence that people walk their dogs without picking up after them.

Stream – Older Urban

This is a neighborhood on Ash Creek (CWS location ID 3845014) that has had chronic high bacteria levels and is mostly older residential land use.

Stream – Duck Feeding Area

This is a site on Summer Creek (CWS location ID 3844009) that is downstream of Summer Lake where people feed ducks and geese. There are landscaped lawns down to the edge of the lake. This site was also the duplicate site for the stream sites.

Stream – Mixed Urban

This is a site on Dawson Creek (CWS location ID 3850006) that is a mix of older residential area and a very new residential area.

Stormwater – Commercial

This stormwater comes from a large shopping mall parking lot (CWS location ID 7301021) in the lower Tualatin Subbasin.

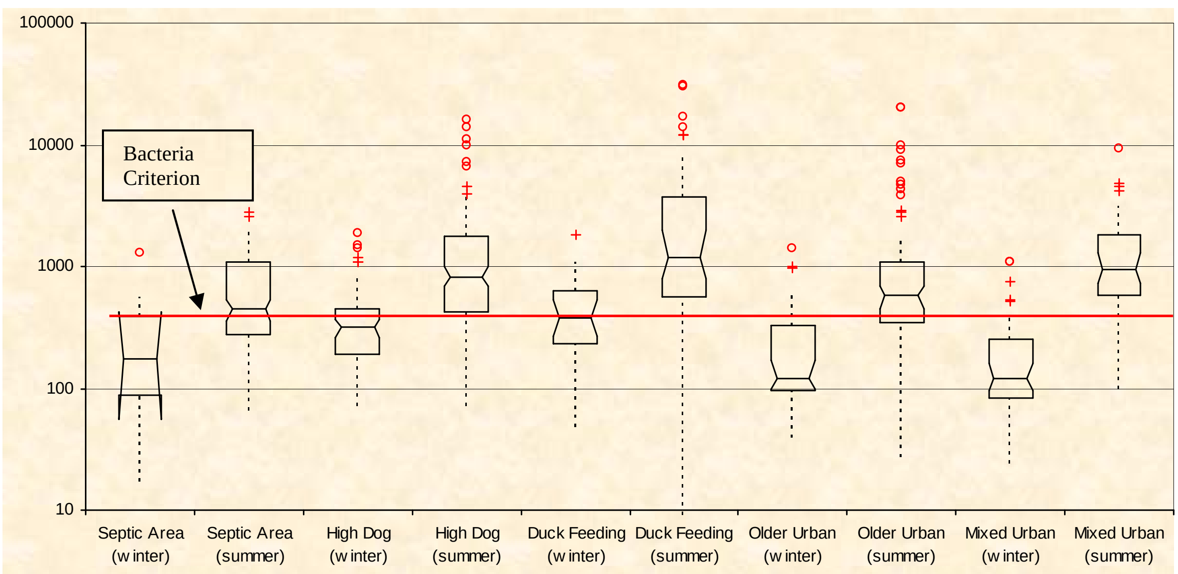
Stormwater – New Residential

This stormwater comes from a new residential area (CWS location ID 7106001) in the Rock Creek Subbasin. It is the influent to the water quality facility that is in this study. This is the duplicate site for the stormwater sites.

Stormwater – Water Quality Facility Effluent

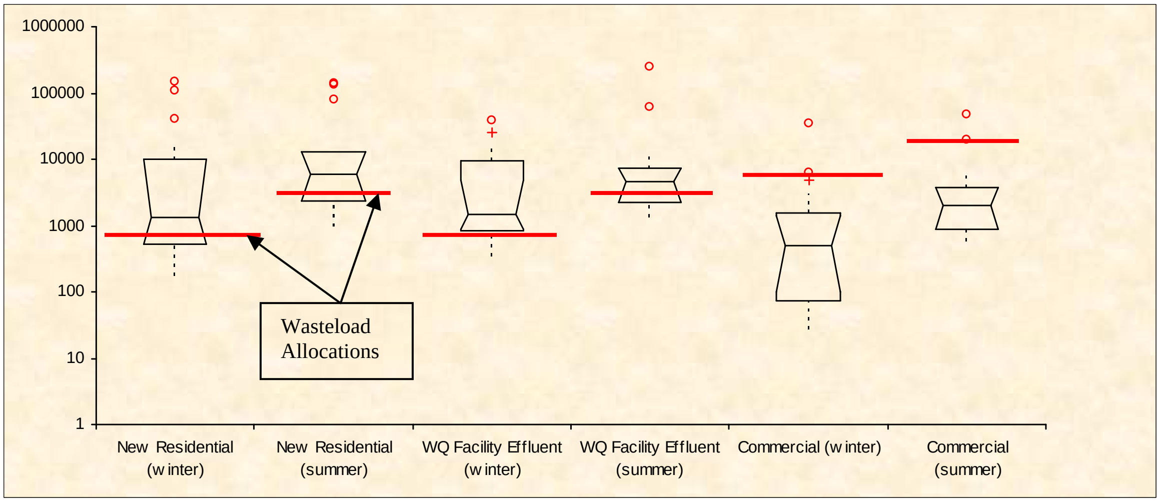
This is the effluent from an extended dry detention basin (CWS location ID 7106002) that has permanent wetland vegetation due to a spring that continually supplies it with water.

The following box plot shows the bacteria levels from the routine monitoring program for the selected stream sites between 2000 and 2005 for both winter and summer. The bacteria criterion is 406 *E. coli* bacteria per 100 mL.



Note: Units are *E. coli* per 100 mL

The following box plot shows the stormwater data from 1995 to 2005. The WLAs are set in the 2001 TMDL and vary between summer and winter and between sub-basins. The WLAs were set on stormwater to attain the geomean of 126 *E. coli* bacteria at the mouth of the 5th field watersheds that are designated in the Tualatin Basin TMDL.

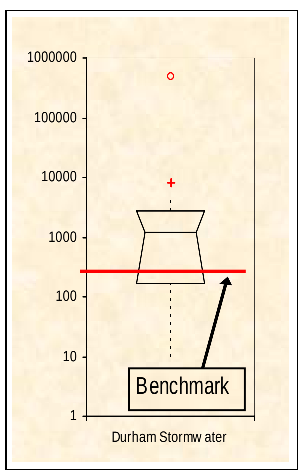


Note: Units are *E. coli* per 100 mL

Part 2 - Specific Site

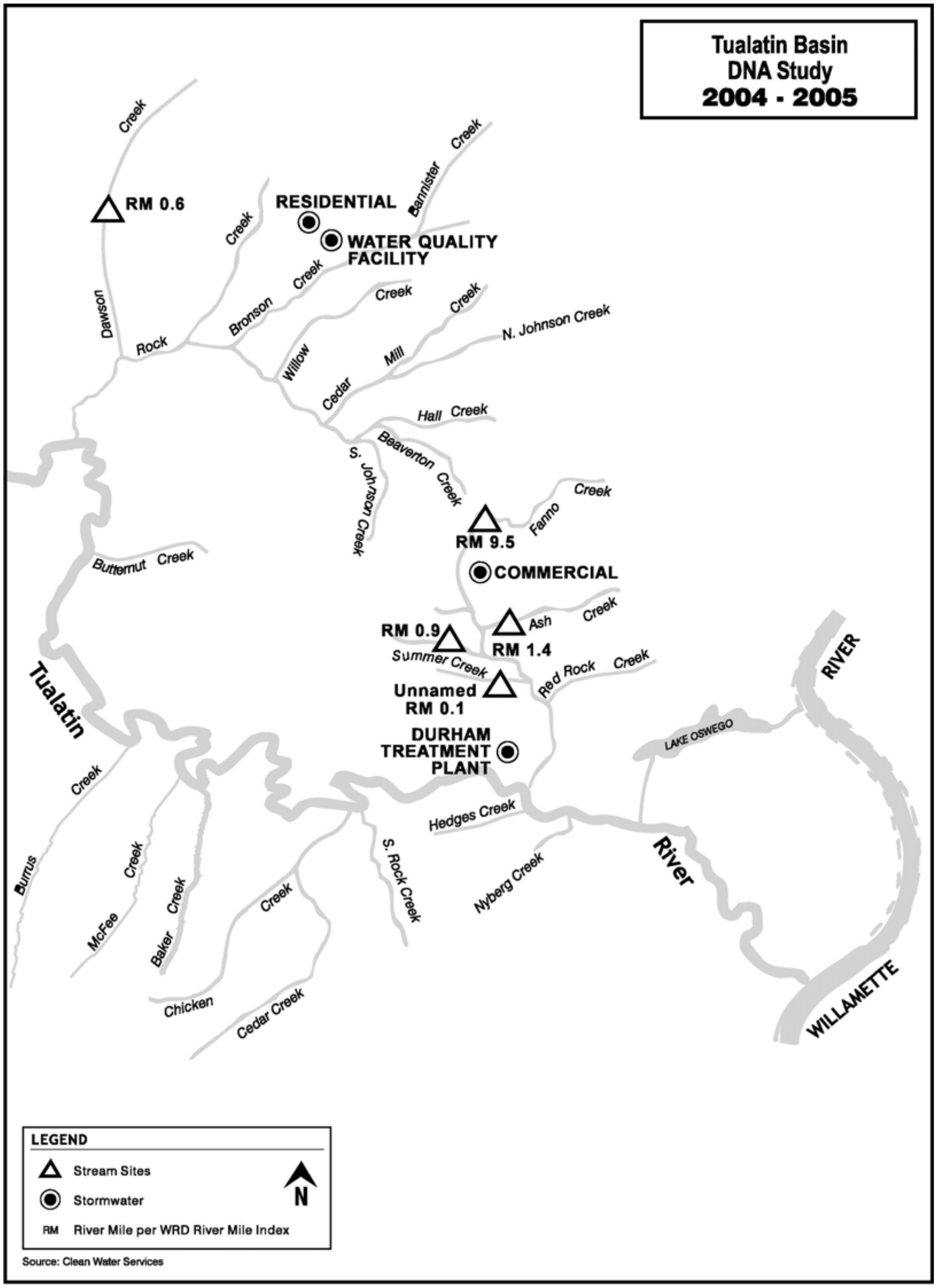
Stormwater – Manhole at the Durham Wastewater Treatment Facility

The Watershed-Based NPDES permit contains benchmarks of 406 *E. coli* per 100 mL for stormwater from its Wastewater Treatment Facilities. One of the outfalls at the Durham facility (Clean Water Services location ID 7311044) has periodic elevated bacteria levels. The only obvious source of bacteria is the geese that frequent the area. When bacteria levels exceed the benchmark, the permit requires Clean Water Services to identify the cause of the high bacteria and take necessary actions to reduce the bacteria level below the benchmark.



Note: Units are *E. coli* per 100 mL

MAP OF SITES


Tualatin Basin
DNA Study
2004 - 2005
RM 0.6
RESIDENTIAL
WATER QUALITY FACILITY
RM 9.5
COMMERCIAL
RM 0.9
RM 1.4
Unnamed RM 0.1
DURHAM TREATMENT PLANT
Dawson Creek
Rock Creek
Bronson Creek
Bannister Creek
Willow Creek
Cedar Mill Creek
N. Johnson Creek
Hall Creek
Beaverton Creek
S. Johnson Creek
Fanno Creek
Butternut Creek
Ash Creek
Summer Creek
Red Rock Creek
Tualatin River
LAKE OSWEGO
WILLAMETTE RIVER
Hedges Creek
Nyberg Creek
S. Rock Creek
Burrus Creek
McFee Creek
Baker Creek
Chicken Creek
Cedar Creek
LEGEND
Stream Sites
Stormwater
RM River Mile per WRD River Mile Index
N
Source: Clean Water Services

STUDY METHODOLOGY

Clean Water Services sampled the following weather events:

Weather Condition	Stream Samples	Stormwater Samples
Summer Dry	2	
Summer Storm	2	3
Winter Dry	1	
Winter Storm	2	3
Total Events Sampled	**7**	**6**

Each time a site was visited five, (5) samples were taken at two (2) minute intervals. During storms samples were taken on the rising hydrograph. The Clean Water Services Water Quality Laboratory used the membrane filter method to determine the number of *E. coli* bacteria in the sample. A plate from each sample with at least three (3) well separated colonies was sent to the IEH for DNA analysis. At IEH, at least three (3) isolated colonies were independently picked for DNA fingerprinting. This resulted in approximately 105 isolates per stream site (7 x 5 x 3 = 105) and 90 isolates per stormwater site (6 x 5 x 3 = 90). This met the goal of approximately 100 isolates per site for statistical validity.

Two (2) sites were tested in duplicate, one stream site (Duck Feeding) and one stormwater site (New Residential), for quality control purposes. The samples that were sent to IEH were labeled site #1 to site #11. Therefore IEH did not know the source of the samples or that two of the samples were duplicates.

A total of 1203 isolates were tested. There were 924 from general sites, 222 from duplicate sites, and 57 from the specific site.

The DNA fingerprinting was done by Dr. Mansour Samadpour at the IEH using the ribosomal RNA typing method. This is one of several methods currently being used for microbial source tracking (MST)[6]. The theory behind this test is that DNA patterns can be used to match *E. coli* bacteria found in the environment with the sources of the bacteria. The IEH has a library of DNA patterns from *E. coli* bacteria that are produced by known sources, such as specific wildlife species, pets, humans, and farm animals. The IEH matched the DNA patterns found in the isolates from the Clean Water Services study to their library of *E. coli* bacteria DNA patterns to determine the source of the bacteria in the samples.

RESULTS

The two parts of the study were evaluated separately. The duplicates were not included in the aggregates. For part 1 the general sites were evaluated in the following ways:

[6] Donald M. Stoecklt et al., *Comparison of Seven Protocols To Identify Fecal Contamination Sources Using Escherichia coli*, Environmental Science & Technology, Vol. 38, No. 22, 2004, pages 6109-6117

- Each site was evaluated separately
- All sites were aggregated
- All stream sites were aggregated
- All stream sites were aggregated and then split between weather events
- All storm sites were aggregated
- All storm sites were aggregated and then split between weather events
- Each duplicate was compared to its original

Part 2 only had one site. It was evaluated separately and then compared to all the general sites in part 1.

The study identified 19 different sources of bacteria. These were grouped in seven (7) major categories before the results were evaluated. The following shows the distribution of *E. coli* bacteria sources by major category for the 924 isolates from the part 1 sites.

Avian		Canine		Feline		Rodent		Human		Wildlife		Unknown	
Source	**Count**	**Source**	**Count**	**Source**	**Count**	**Source**	**Count**	**Source**	**Count**	**Source**	**Count**	**Source**	**Count**
avian	252	canine	22	feline	13	rodent	143	human	12	deer	12	Unknown	85
waterfowl	157	coyote	12			squirrel	5	septage	1	opossum	5		
crow	49	dog	84					sewage	26	raccoon	38		
geese	3									skunk	1		
gull	4												
	465		118		13		148		39		56		85

In some of the major categories there is a generic source designation. For instance the canine category has the generic source of canine and the more specific sources of coyote and dog. This means the canine DNA type is shared between dogs, coyotes, wolves, foxes, etc. The same is also true of the other major categories.

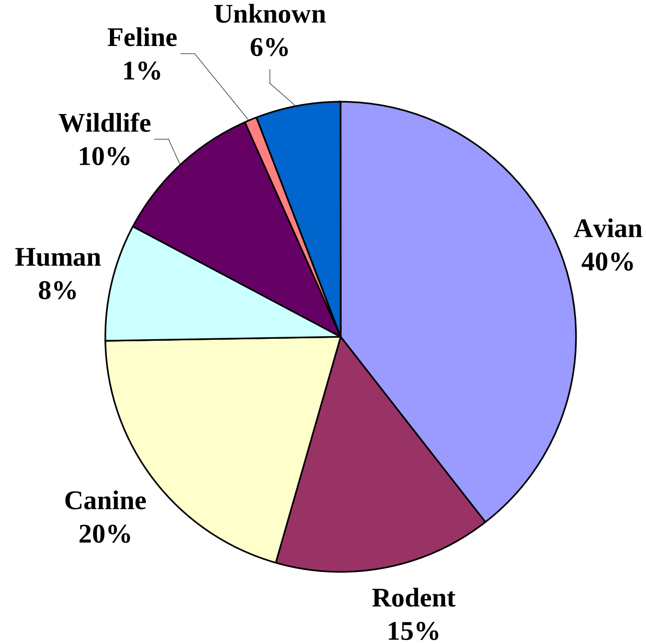
Part 1 - General Sites

Each of the general sites was evaluated using a pie chart. The following pie charts show the percentage of each *E. coli* bacteria source and the total isolates that were evaluated for that sample site. The first five (5) general sites were stream samples.

Stream

Septic Tank Area

134 Isolates

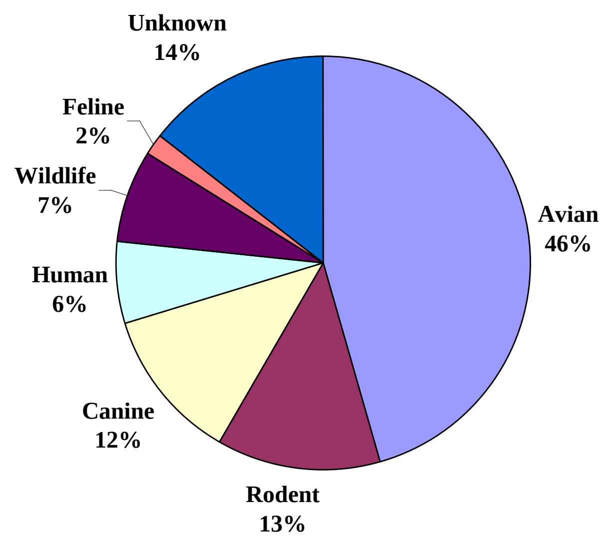


Of the general steam sites, this sample had the highest percentage of human source isolates (8% vs. 7% to 4% for stream samples). Because the site was selected based on a significant number of septic systems in the area, it was actually expected to have a higher percentage of human isolates. This site also had the highest percentage of canine source isolates (20% vs. 7% to 17%).

Stream

High Dog Population

125 Isolates

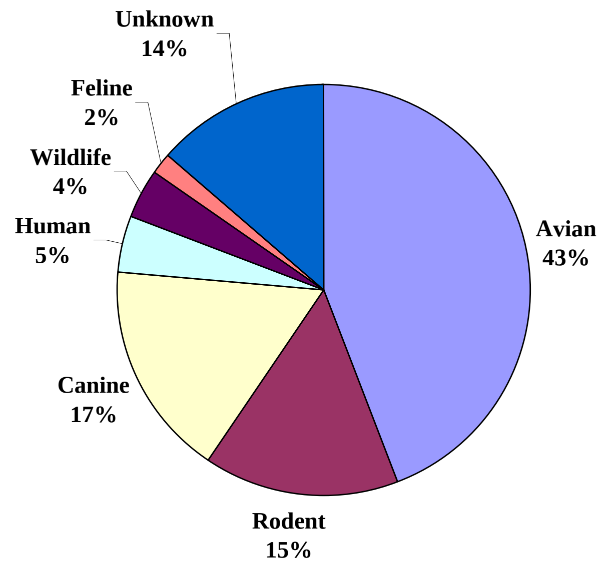


This site as expected to have the highest percentage of canine source isolates but it did not. It was in the middle of the range of isolates (12% vs. 7% to 20%).

Stream

Older Urban

131 Isolates

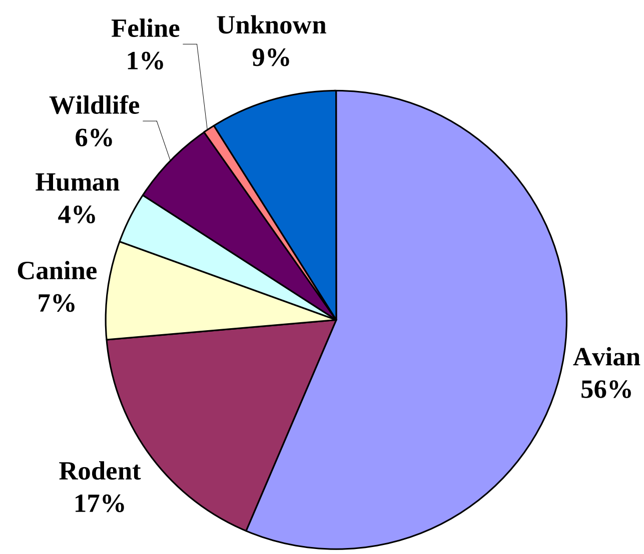


This site had a fairly average mix of bacteria sources.

Stream

Duck Feeding

133 Isolates

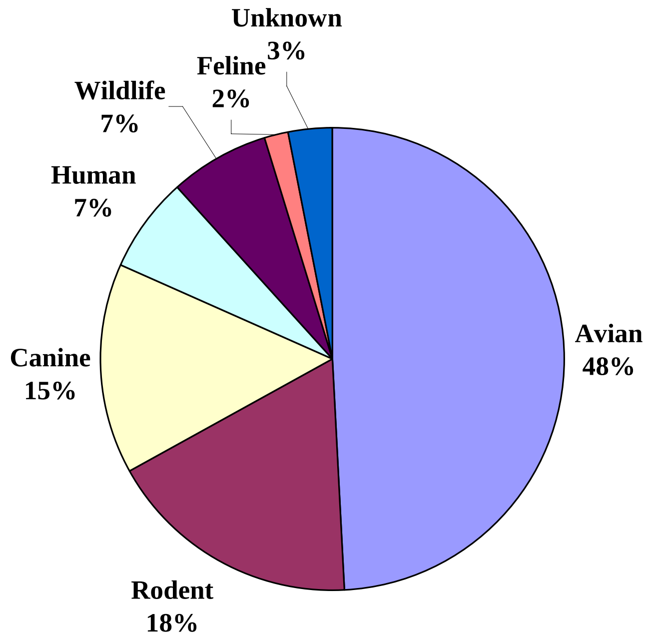


This site was expected to have a high percentage of avian sources and it did have the highest percentage for the stream sites (56% vs. 40% - 49%).

Stream

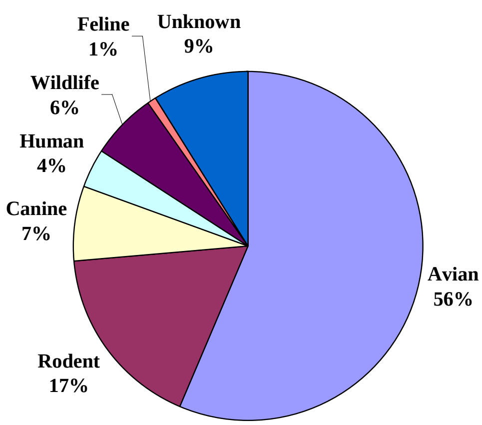
Mixed Urban

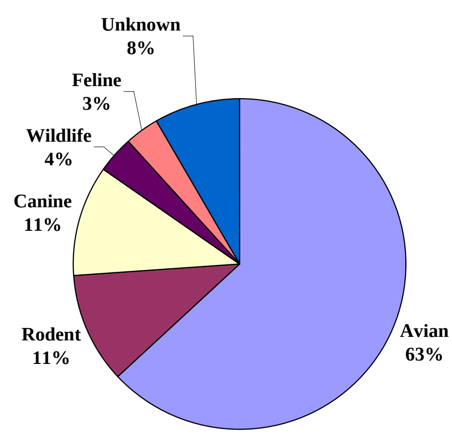
130 Isolates



This site had a fairly average mix of bacteria sources.

Duplicate Duck Feeding Area Samples





Sample **Duplicate**

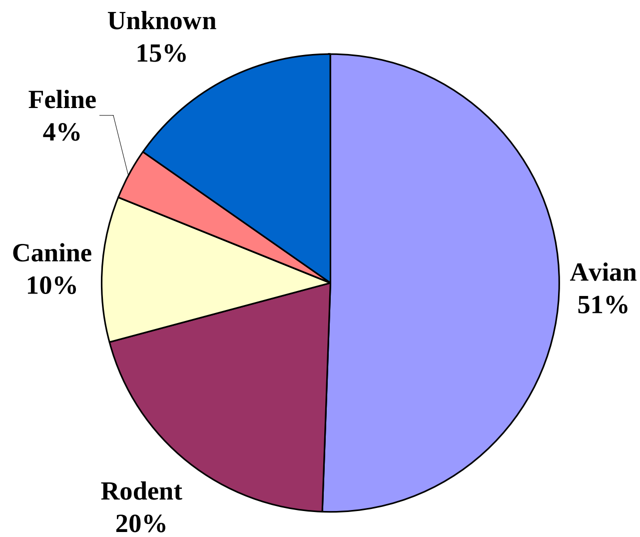
There was reasonable agreement between the sample and duplicate. However because the general sites were so similar the duplicate could have matched several other sites.

The next three (3) general sites are stormwater sites.

Stormwater

Commercial Parking Lot

79 Isolates

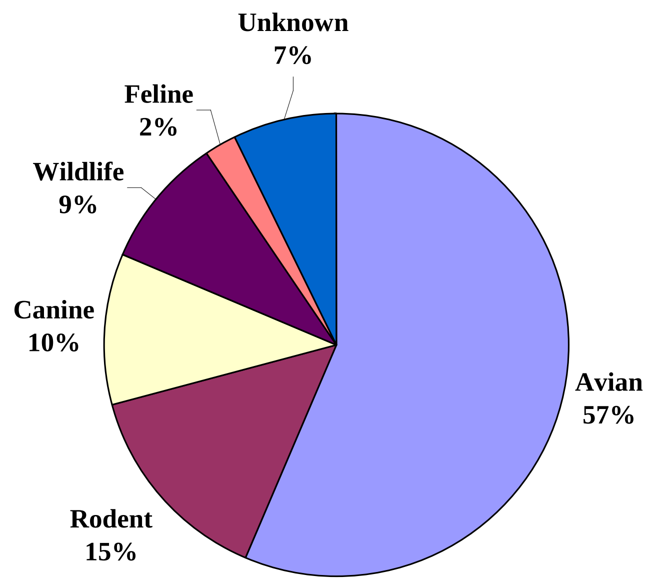


As with all the stormwater sites, this site has relatively high avian and rodent percentages and no *E.coli* bacteria from human sources.

Stormwater

New Residential

96 Isolates

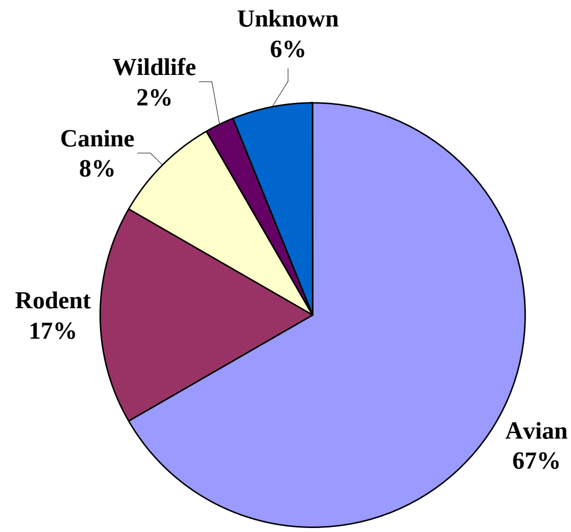


This site was similar to the commercial parking lot site.

Stormwater

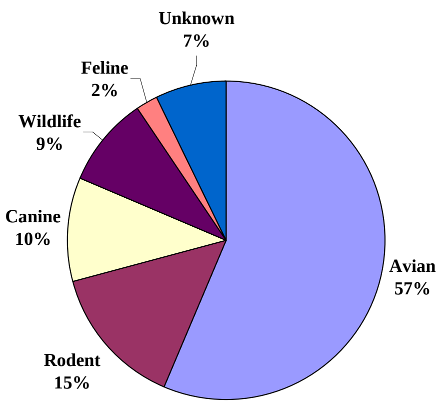
Water Quality Facility Effluent

96 Isolates

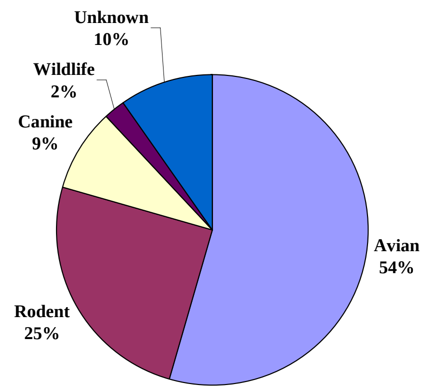


This site had the highest avian of all the sites. It is a water quality facility that is full of wetland plants.

Duplicate Stormwater – New Residential Area



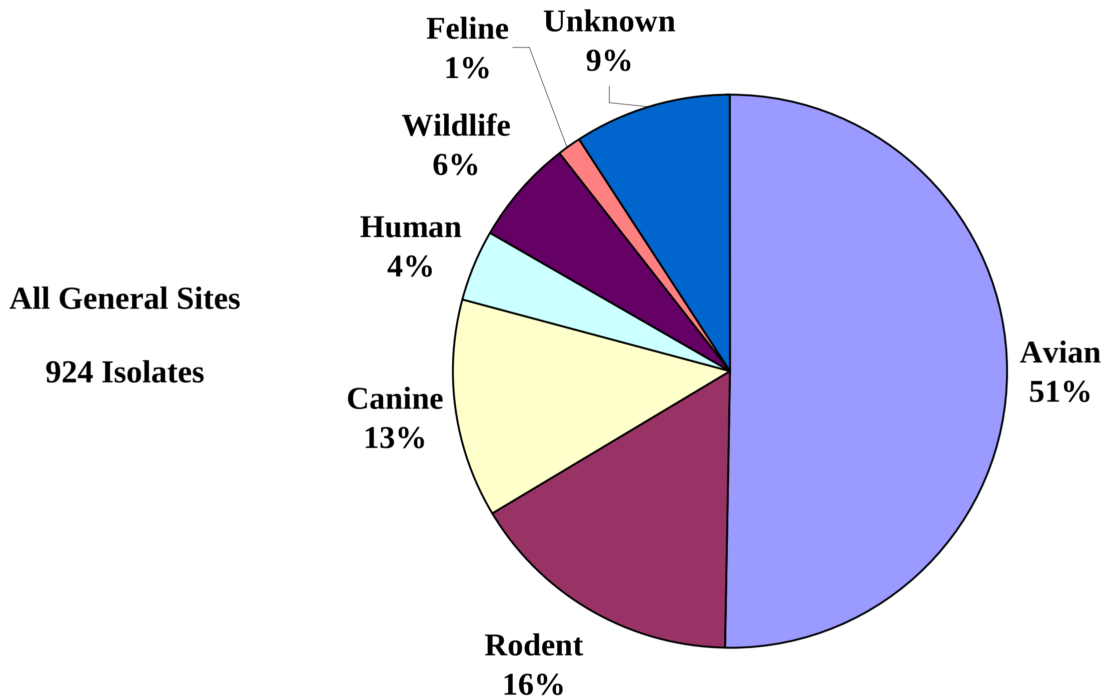
Sample



Duplicate

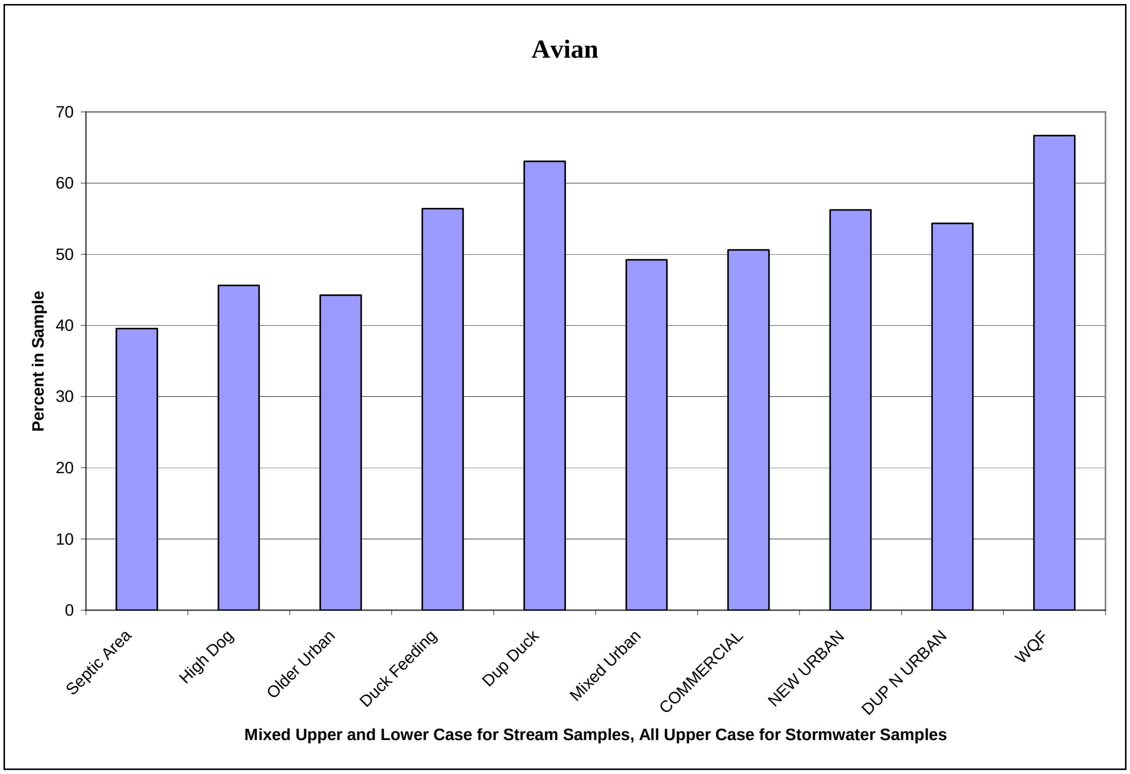
There was reasonable agreement between the sample and duplicate. However because the general sites were so similar the duplicate could have matched several other sites.

Reviewing the individual sites showed a very similar pattern. Most of the isolates were avian, followed by rodent and canine in that order. The following chart aggregates all the general sites.

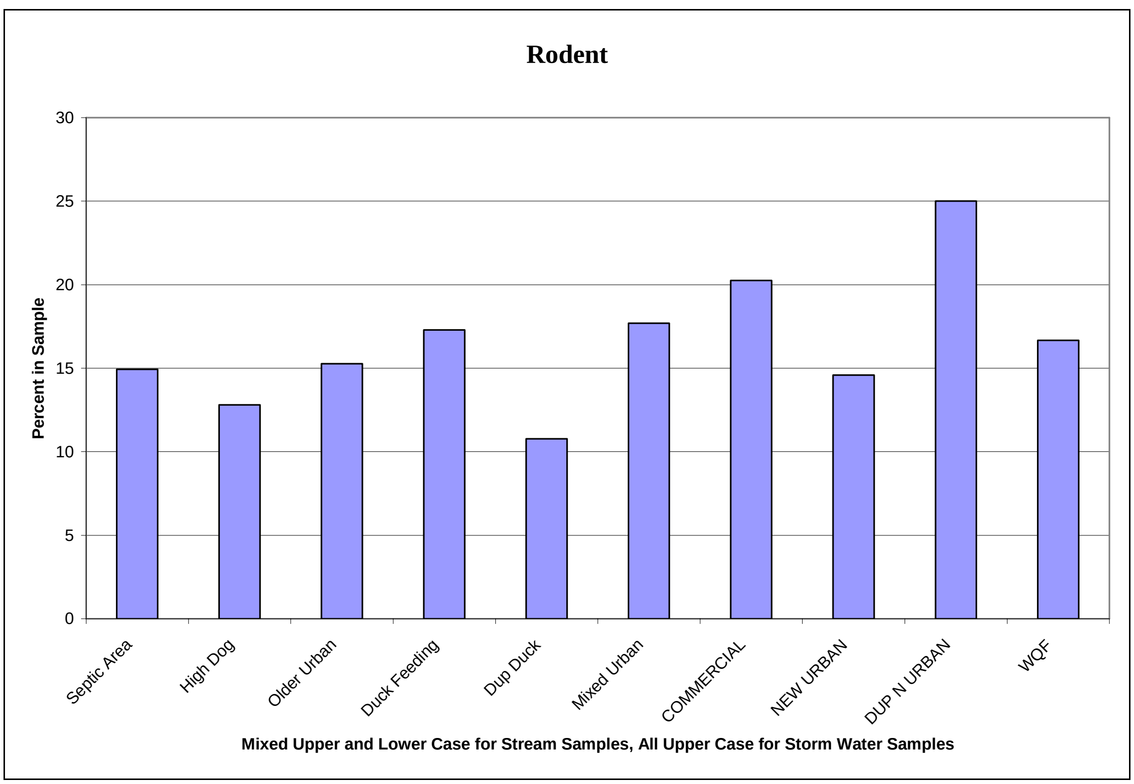


The next series of graphs show how the various sites compare for key sources. It also shows how the duplicate matches its sample and the other samples. A statistic that is used to determine if one sample of a set of samples is an outlier was used to determine if any site was statistically different from the other sites. This was done by sources. The statistic[7] is designed to be used on small data sets such as this. The data is sorted from lowest to highest percentage and then the difference between the data point in question (either the highest one in the set or the lowest one in the set) and the nearest value as a fraction of the range from the smallest to the largest value in the data set is compared to the statistic in the table. There is a 5% risk of an incorrect conclusion.

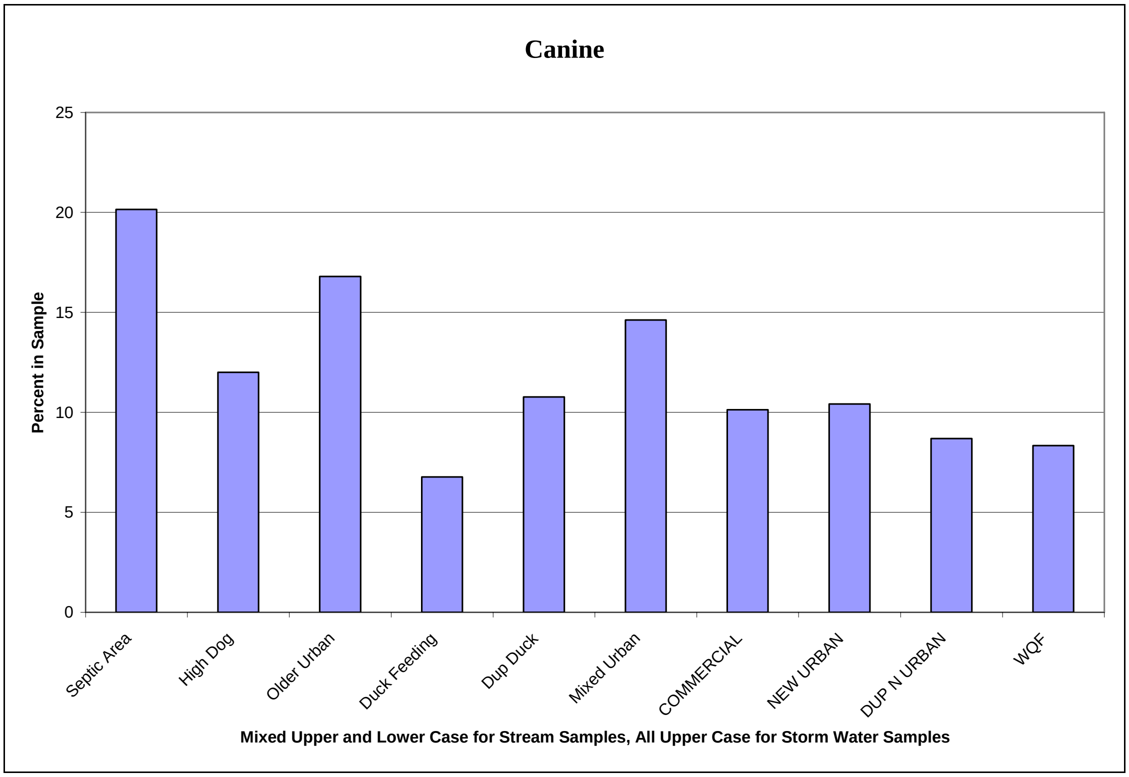
[7] W. J. Youden and E.H. Steiner, *Statistical Manual of the Association of Official Analytical Chemists*, Published by the Association of Official Analytical Chemists, 1975, pages 30 & 86



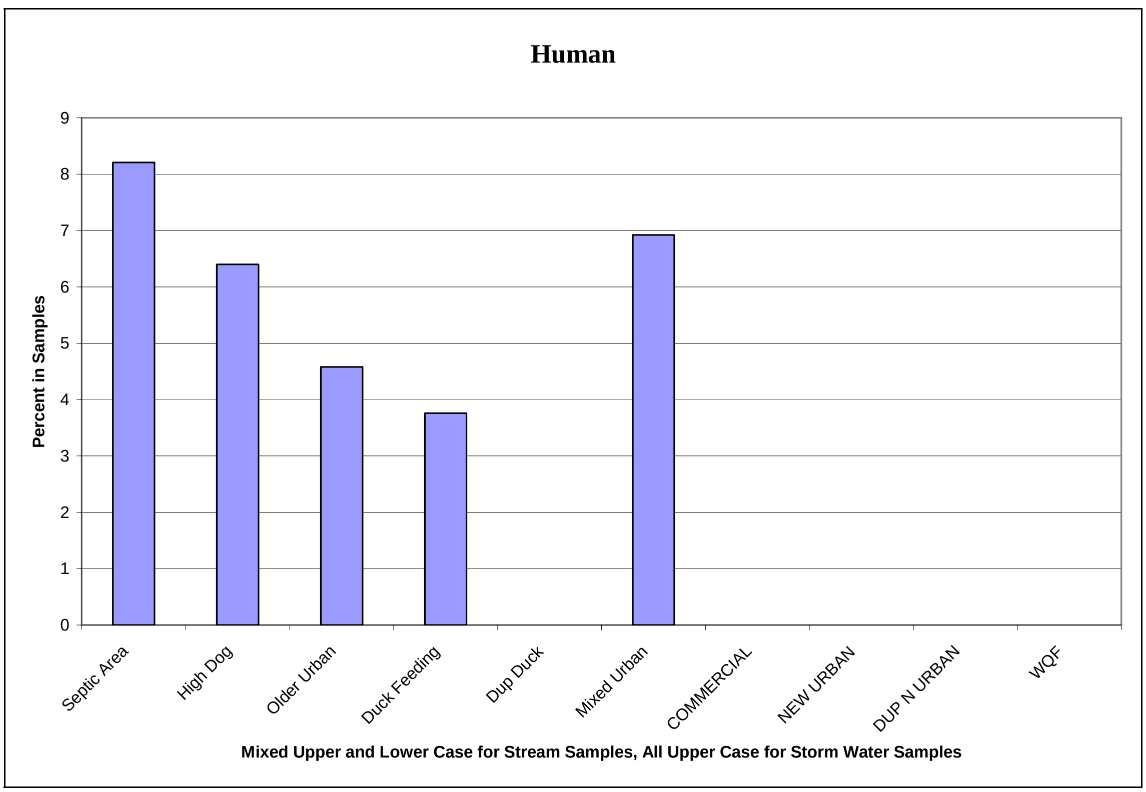
There was no statistical difference between the septic area that had the lowest percentage with the data set or the Water Quality Facility that had the highest percentage with the data set.



There were no statistically different samples in this set.



There were no statistically different sites in this data set.



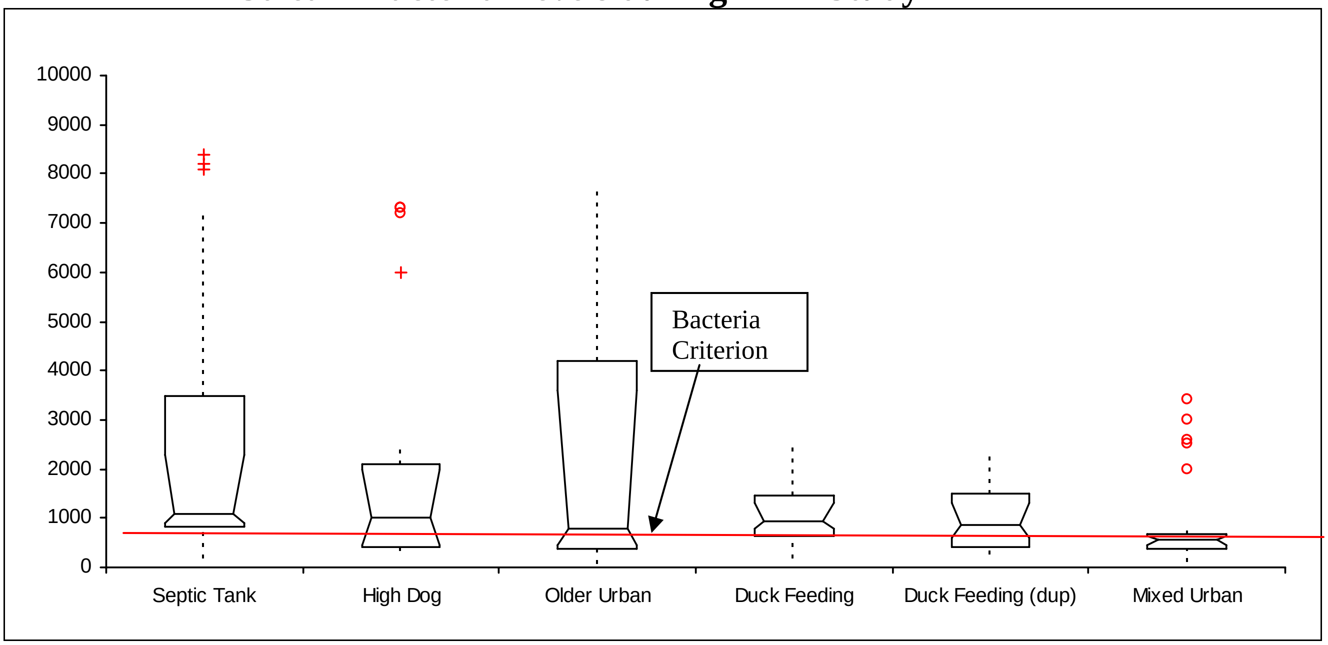
There were no statistically different sites in this data set.

From these graphs it is apparent that there is not much variation between sites. Sites that were expected to have high concentrations from a particular source did not necessarily have the highest percentage.

General Stream Sites

The next method used to evaluate the samples was to aggregate all the stream samples together. The following graph shows the bacteria levels in the samples collected for this study.

Stream Bacteria Levels during DNA Study



Note: Units are *E. coli* per 100 mL

Site Types	n	Mean	Median
Septic Tank	35	2426	1100
High Dog	35	2034	1000
Older Urban	35	2290	800
Duck Feeding	35	1063	920
Duck Feeding (dup)	35	999	880
Mixed Urban	35	800	560

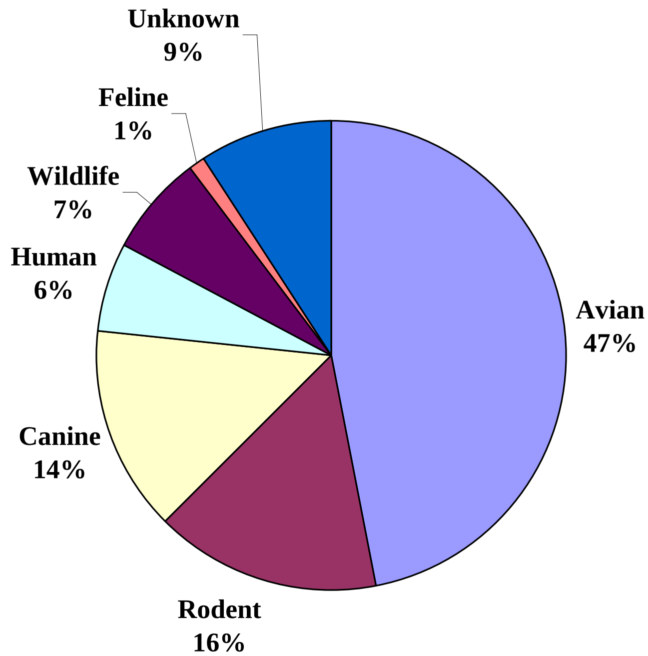
These sites were selected due to consistently high bacteria levels which were maintained during the study.

The following pie chart shows the aggregate of all the stream sites for the study.

Stream Bacteria Sources

All Sites

653 Isolates



Samples were collected during various weather conditions. The individual stream sites did not have sufficient isolates to evaluate relative to weather; however, if the sites are aggregated there are enough isolates. The following table shows the results.

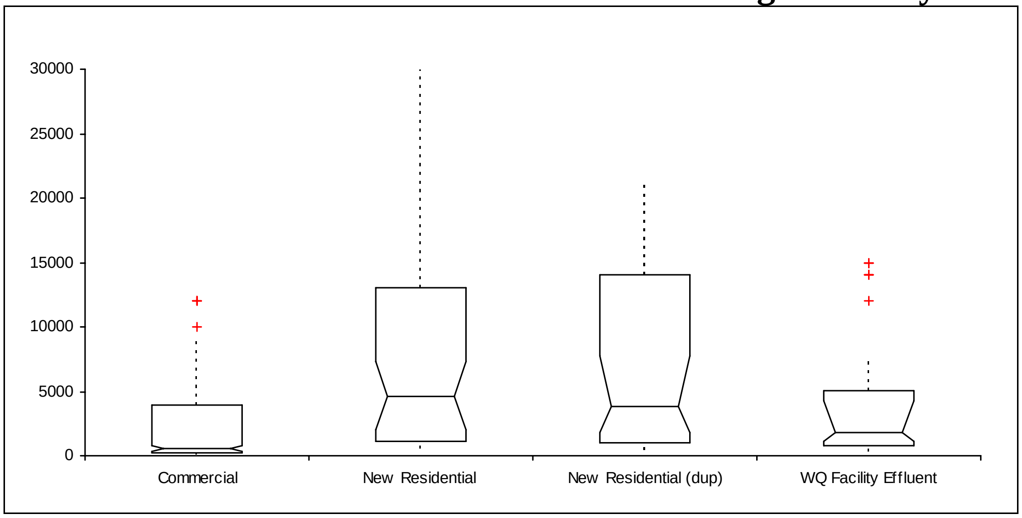
	Aggregate	Summer Dry	Summer Storm	Winter Dry	Winter Storm
Avian	**47 %**	**53 %**	**46 %**	**45 %**	**40 %**
Rodent	**16 %**	**13 %**	**17 %**	**15 %**	**18 %**
Canine	**14 %**	**14 %**	**13 %**	**16 %**	**13 %**
Human	**6 %**	**4 %**	**9 %**	**7 %**	**6 %**
Count of Isolates	**653**	**208**	**157**	**130**	**158**

The same pattern emerges when the stream samples are aggregated and evaluated relative to weather conditions. Avian is the most common followed by rodent and canine and then by human.

General Stormwater Samples

Next the stormwater samples were aggregated. The following graph shows the levels of bacteria in the samples collected for the study.

Stormwater Runoff Bacteria Levels During the Study



Note: Units are *E. coli* per 100 mL

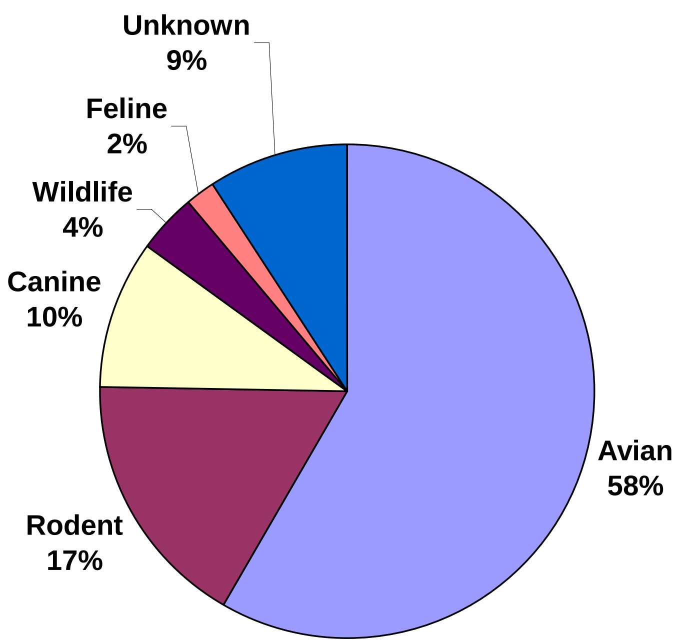
Site Types	n	Mean	Median
Commercial	33	2647	580
New Residential	33	7535	4600
New Residential (dup)	33	6957	3800
WQ Facility Effluent	33	4120	1800

The following pie chart shows the aggregated stormwater samples.

Stormwater Bacteria Sources

All Sites

271 Isolates



Samples were collected during various weather conditions. The individual stormwater sites did not have sufficient isolates to evaluate relative to weather; however, if the sites are aggregated there are enough isolates. The following table shows the results.

	Aggregate	Summer Storm	Winter Storm
Avian	**58 %**	**64 %**	**53 %**
Rodent	**17 %**	**11 %**	**22 %**
Canine	**10 %**	**10 %**	**9 %**
Human	**0 %**	**0 %**	**0 %**
Count of Isolates	**271**	**128**	**143**

Each time the data is aggregated the same pattern is present. The highest concentration is avian, followed by rodent, canine, and human.

The results of this study were also compared to two other studies. One was done for the Lower Boise River Water Quality Plan[8]. The watershed studies covered 1,300 square miles and had a population of approximately 260,000. River and stormwater sites were collected in the urban and rural areas. This study was conducted between April and September 2000. The second study was done on Clarks and Meeker Creeks near the City of Puyallup, Washington. Ten (10) sites were sampled a total of 12 times during 2002 and 2003.

	Clean Water Services	Lower Boise River Idaho	City of Puyallup Washington
Avian	**51 %**	**24 %**	**40 %**
Rodent	**16 %**	**4 %**	**28 %**
Canine	**13 %**	**14 %**	**11 %**
Human	**4 %**	**12 %**	**5 %**
Other	**7 %**	**15 %**	**7 %**
Unknown	**9 %**	**31 %**	**9 %**
Count of Isolates	**924**	**1565**	**687**

[8] Lower Boise River Coliform Bacteria DNA Testing, Prepared for the Lower Boise River Water Quality Plan, CH2MHILL, October 2003

The Lower Boise results are different but that may be due to the high percentage of unknowns and higher percentage of human sources. The higher percentage of unknowns may be due to the fact it was the first study done in this set. The higher percentage of human sources could be due to the fact that one third of the area studied was unsewered.

Part 2 - Specific Site

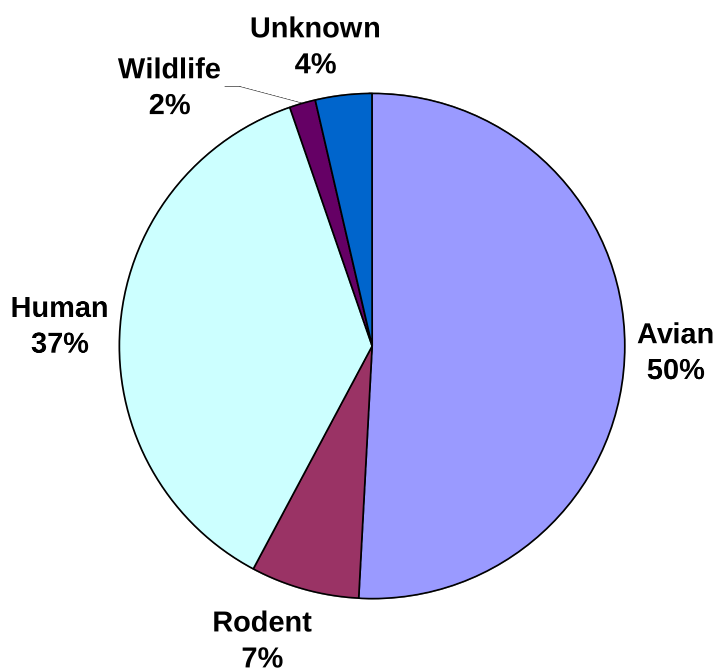
One of the stormwater outfalls from the Durham Wastewater Treatment Plant had sporadic high bacteria levels. The only likely source was the geese that frequent the wastewater treatment plant. The site was visited five (5) times during the study. The mean *E. coli* per 100 mL of each of the five samples taken during each visit were as follows: 98,000, 1, 360, < 1, 62. One of the visits had so few isolates that representative plates were not sent to IEH for analysis.

The following pie chart shows the percentage of each of the sources at this site.

Stormwater

Wastewater Treatment Plant

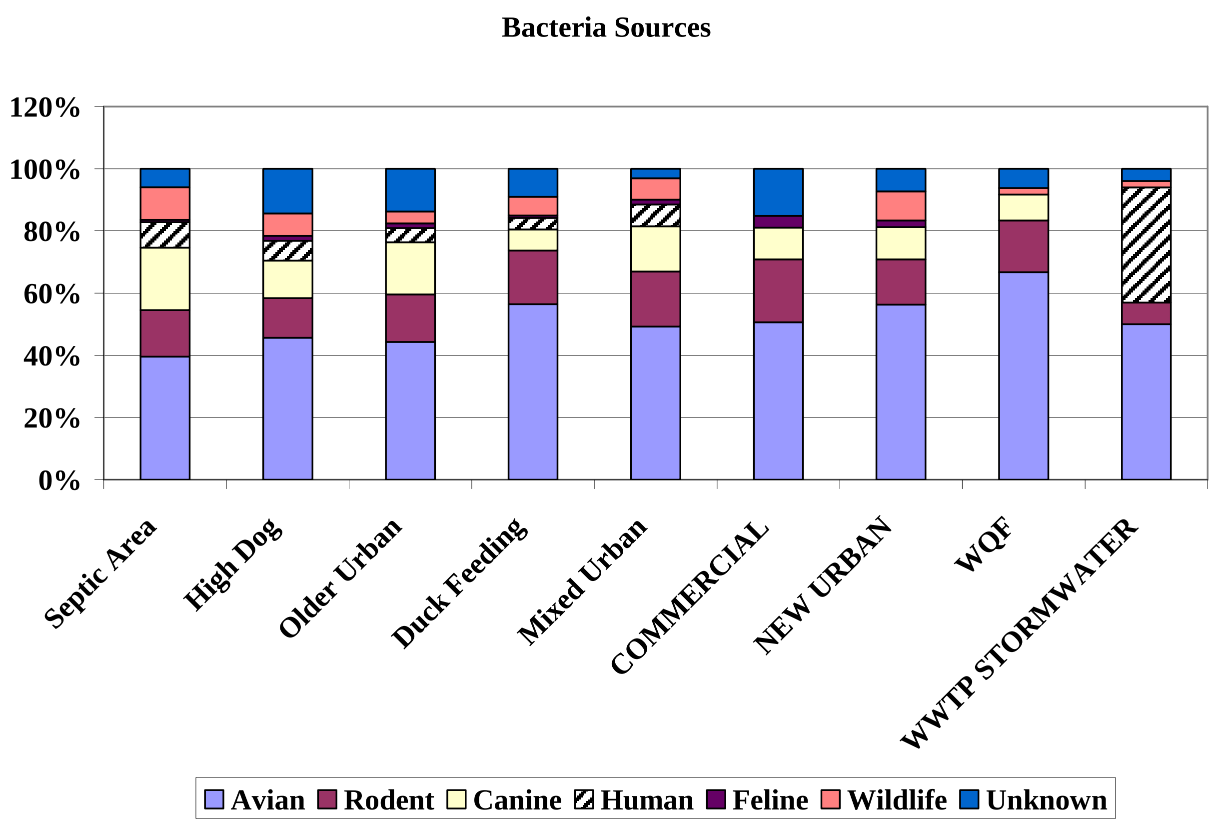
57 Isolates



This answered the original question of whether there were bacteria from human sources at this site. The human isolates represented 37% of the sources for this site.

To determine if this site was statistically different from the general sites, the data from this site was added to the data from the part 1 sites. When all sites, including the duplicates, are evaluated, there is a statistical difference between the human isolates for this site and the general sites. If the duplicates are removed from the statistical evaluation, there was a statistical difference between this site and the general sites relative to the human and rodent sources.

These differences can be seen in the following stacked bar chart.



USE OF THE DATA

This information will allow Clean Water Services to better focus its efforts to reduce the bacteria levels in stormwater and the receiving streams. The information from the first part of the study will be used in public education efforts. Clean Water Services already has a program in place to convert areas with high concentrations of septic system to sanitary sewer systems. The information from the second part will be used to reduce the bacteria in the stormwater from the wastewater treatment plant.

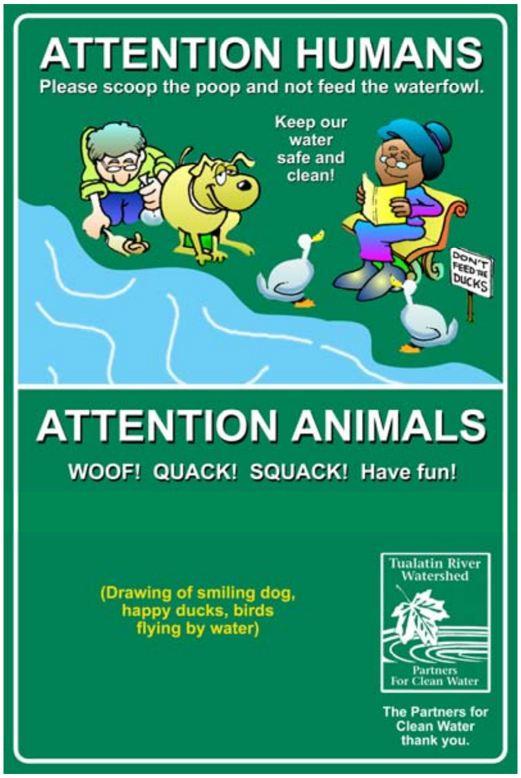
Public Education Efforts

The two areas that the public education efforts will focus on will be waterfowl feeding and dog waste management. It will be important to effectively communicate this information to the public so they understand that their actions can cause high bacteria levels in the receiving streams.

To address high avian bacteria levels, signs will be used that ask the public not to feed the waterfowl. The anticipated result is that fewer waterfowl will congregate in or near water bodies. This would not preclude waterfowl in more naturally sustained numbers being present. In addition, riparian restoration projects will be landscaped in ways that don't encourage ducks and geese to take up residence.

Another public education campaign will focus on dog waste. The City of Tigard has already prepared a news article for this purpose. In *Holy Dog Poop Batman!!!*, they compare the number of wolves that an area the size of the Tualatin Sub-basin can support with the number of dogs in the Tualatin Sub-basin. With a naturally sustainable population of 70 wolves and a dog population of 80,000 there are 79,930 dogs that need waste management!

Signage with both messages is being developed. The following is a prototype of the sign. The bottom half is still in production.



These signs along with dog-waste bags will be provided to parks in the basin. There will also be press releases that encourage the public to notice the signs and use the bags! These concepts are also incorporated into our River Ranger program that is used in the elementary schools in this area.

There are also plans to do a fun and informative postcard to licensed dog owners in the basin.

Wastewater Treatment Plant

The results of this test show a significant human contribution in the stormwater. The likely activity that is the source of this contamination has now been identified. The earlier efforts at tracking down the source focused on drainage from treatment plant processes. These were not the issue. The problem has been identified as drainage from a road that the sludge trucks drive on as they exit the treatment plant site. The best way to manage this source is currently being evaluated by Clean Water Services.

CONCLUSIONS

After evaluating a diverse set of sites, different weather events and different studies, a pattern of sources emerges: most of the bacteria identified are from avian sources, with rodents and canines being the second and third largest sources. In the Boise study human contaminants represented a higher percentage than the rodent percentage. In the other studies, humans were the fourth largest groups of isolates.

As the plans are developed to address the WLAs in the TMDLs, it is important to note that human sources are not a dominant source of bacteria. Although it is important to reduce the bacteria from human sources, this will not result in streams meeting their bacteria criterion. It is also important to note that activities that humans participate in do have an impact on the bacteria levels in both stormwater and the receiving streams. Public education will be the best way to deal with these sources. This DNA study will help convince the public that their activities have a direct and significant impact on receiving streams. Providing them with the tools necessary to do their part will be important.

Unfortunately, there is too much variability in the bacteria values and the percentages of sources to accurately predict what combination of actions will achieve the WLAs. This information should be taken into consideration as new TMDLs are developed and existing TMDLs are revised.

The routine monitoring program will continue to measure *E. coli* bacteria concentrations. This information from the study sites and the other routine sites will be used to inform the adaptive management process. It will also measure progress towards meeting the bacteria benchmarks in the SWMP.