

**FINAL REPORT**

**IDENTIFYING SOURCES OF FECAL COLIFORM  
DELIVERED TO TILLAMOOK BAY**

BY

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## INTRODUCTION

It has been known for some time that different bacteria have different resistance to antibiotics. In the early 1970's Grabow et al, published an article that called for a review of the water quality standards because of the fact that some of the coliform bacteria had been identified as being drug resistant. This was the beginning of utilizing this information to expand and develop methods of identifying different sources of bacteria as pollutants in water. In 1978 two faculty members in the Department of Food Science and Technology at Oregon State University collected a total of 2445 gram negative bacteria belonging to the fecal coliform family from the rivers and bays of Tillamook, Oregon. (Kelch and Lee, 1978). The goal was to learn and provide descriptive information about the antibiotic resistance of gram-negative bacteria collected in waterways. They used 11 different antibiotics in their study. They were also interested in exploring if patterns of resistance existed among different bacteria. From this beginning they did conclude some of the differences they reported did reflect bacteria from different sources.

The science developed over the next 20 years and in 1996 a microbiologist from James Madison University published an article in which he described utilizing a statistical technique, discriminant analysis, to identify patterns of antibiotic resistance in *fecal streptococci* to differentiate human and animal sources of pollution in natural waters (Wiggins, 1996). This was the model we followed in this project.

In his early work Wiggins used 5 different antibiotics and was able to correctly identify and classify the bacterial isolates into one of six possible sources 74% of the time (Wiggins, 1996). These bacteria were from beef, chicken, dairy, human, turkey or wild sources.

In later work they used 9 different antibiotics, each at 4 different concentrations, in an attempt to improve the average rate of correct classification (ARCC) of the source of the organisms. In this study when the resistance patterns were averaged the ARCC was 96 to 100% (Wiggins, et al, 1999). Hagedorn et al, (1999) reported using this technique in a Virginia watershed to identify sources of fecal pollution. Correct source identification averaged 87% for the entire database (7,058 isolates) and ranged from 84% for deer to 93% for human isolates. In this study 11 antibiotics were used. The ARCC seems to increase as the isolate number and the number of antibiotics is increased.

In a Wisconsin study, 765 *Escherichia coli* isolates from point and non-point sources were plated on 10 different antibiotics to determine their resistance. (Parveen, et al. 1997). This showed a significantly greater resistance from point sources and they concluded the method was useful in separating the sources in Apalachicola Bay.

### Objectives

The work to date suggests that using antibiotic resistance to identify sources is a very promising technique and one worth evaluating in the Tillamook watershed where source identification is a large issue. The objectives of this study are to duplicate the techniques developed and expanded by Bruce Wiggins in the Tillamook Bay watershed. Water

samples will be collected and evaluated from the five major river mouths over the course of a year and at two sampling times during a storm event. For this work 9 different antibiotics will be used.

## MATERIALS AND METHODS

### River Sample Collection

E&S Environmental Chemistry, a private firm and the Bioresource Engineering water quality lab at Oregon State University (OSU) collected river samples as part of a joint monitoring effort. Samples were collected by employees of E&S (Sullivan et al., 1998) and analyzed for fecal coliform bacteria (FCB), total suspended solids (TSS), nutrients, conductivity and antibiotic resistance patterns for fecal streptococci bacteria (FSB). For purposes of this report only the FCB and FSB data was used to generate the results and conclusions.

Sample locations were determined by criteria such as known proximate point and non-point sources of fecal pollution, forest/agriculture interface locations, and sampling logistics. Sample sites were selected to avoid any strong influence by Tillamook Bay tides (Sullivan et al., 1998). The Trask and Tillamook rivers were selected to be storm sampled due to their previously recorded high fecal coliform loads (Sullivan et al., 1998). Twice during the course of the storm, four samples were collected along the Tillamook, three on the Trask River and one additional sample downstream of the confluence of the Tillamook and Trask at the Memaloose boat landing (Table 1). The first sampling occurred February 28, 1998, early in the storm on the rising hydrograph. The second sampling was March 3 on the falling limb of the hydrograph.

Table 1. Sampling site locations		
Site Code	Location Description	River Mile
<u>Tillamook River</u>		
TIL-RES	Rest Area	8.1
TIL TTR	Tillamook River Road	4.9
TIL BUR	Burton Bridge	4
TIL NET	Netarts Highway Bridge	0.9
<u>Trask River</u>		
TRA BTR	Below Trailer Park	3.7
TRA 5th	5th St. Boat Ramp (Above WWT)	1.5
TRA HOB	Hospital Bridge (Below WWT)	1.2
<u>Tillamook Bay</u>		
MEM-INL	Memaloose	-0.5

**Table 1. River sample locations and description**

Sample collection protocol was dependent on the logistical conditions at particular site from which it was collected. Sampling at bridge crossings were achieved by using a Van Dorn sampler or a weighted sterile bottle. Water was collected in the middle of the stream current (at a depth of about 0.5 m) where a river tends to be well mixed. Shallow sites or sites without bridge crossings were sampled from shore using a pole to submerge a Nalgene bottle directly in the stream. Samples were filled in such a way to minimize air bubbles and the bottles placed in coolers on ice and transported to the labs (Sullivan et al., 1998). A few of the non-bridge location samples were collected from a boat using a weighted sterile bottle. Fecal coliform bacteria analyzes were performed by the Kilchis Analytical Laboratory in Bay City, Oregon while fecal streptococci bacteria were processed at the OSU Water Quality Laboratory (WQL) in Corvallis.

Samples were collected using 16 autoclaved, 3.5 liter, screw top, Nalgene bottles. The bottles were autoclaved for 20 minutes at 121 °C to insure sterility. Upon the arrival of the samples to the WQL they were immediately placed in a cooler and maintained at 2-6 °C until the time of processing. The processing of all river samples was completed within 6 hours of their arrival into the WQL.

### **Sample processing using Antibiotic Resistance**

River samples were processed according to the *Discriminate Analysis of Antibiotic Resistance Patterns in Fecal Streptococci Method* established by Bruce Wiggins at James Madison University, Virginia (Wiggins, 1996).

### **Filtration**

Varying volumes of individual river samples were filtered using 0.45- $\mu$ m-pore-size filters (type GN-6; Gelman Sciences). Pre-filtering of the river samples as described in Wiggins, 1996, was not performed due to the relatively low turbidity of the samples. Volumes of up to 1000 ml were filtered without any excessive clogging of the filter apparatus. Filters were then transferred to 50-mm petri dishes containing absorbent pads soaked in 2 ml of the selective liquid media enterococcosel broth (BBL). Filters were then placed in incubation for 48 hours at 37 °C. Careful attention was paid in the filtering of samples not to contaminate them by hand or allow cross inoculations. All of the Millipore™ filtering equipment used in this process was autoclaved prior to use.

### **Fecal Streptococci Isolate Identification**

Following incubation of the plates, 96 fecal streptococci isolates were selected using sterile toothpicks and transferred to micro well plates containing 0.2 ml of enterococcosel broth (BBL). Fecal streptococci isolates were selected from the filter plates by the colony morphology described by Wiggins (Wiggins, 1996) and Standard Methods (1995). All of the isolates were screened to produce catalase and for their ability to hydrolyze esculent. The micro well plates containing the river isolates were then incubated at 37 °C for 48 hours.

To further confirm the identification of the isolated colonies, 100 isolates were randomly screened for the physiological properties that distinguish them as a member in the fecal streptococci family. Each was tested for its production of catalase, Gram reaction, growth at 37<sup>0</sup> C in brain heart infusion broth (BBL) containing 6.5% NaCl, and growth in brain heart infusion broth at 45<sup>0</sup> C.

### **Antibiotic Screening of Sample Isolates**

Nine antibiotics were selected for their wide use among animal and human populations. Ampicillin (AMP), amoxicillan (AMX), cephaolahexin (CEP), chlortertacycline hydrochloride (CTC; Sigma), erythimycin (ERY), oxytetracycline hydrochloride (OTC; Sigma), streptomycin sulfate (STR; Sigma), tetracycline (TET,) and vanomycin (VAN). Stock solutions (10 mg/ml) were prepared and stored according to protocol (Wiggins, 1996) with the exception of OTC. OTC was found to precipitate out of solution when stored as a 10 mg/ml solution and therefore was prepared fresh for each screening process.

Each antibiotic solution was filter sterilized and added to autoclaved trypticase soy agar (BBL). Varying concentrations of each of the antibiotics were then added to the agar and the agar poured in to 150 mm plastic petri dishes and allowed to cool. The following concentrations were used: 10, 15, 30, and 50 *ug/ml*, for AMP, ERY, CEP and TET; 20, 40, 60, and 80 *ug/ml* for CTC, OTC and STR; 5, 10, 15 and 20 *ug/ml* for AMX; and 5, 10, 15, and 30 *ug/ml* for VAN. Selection of antibiotics and concentration ranges were determined by Wiggins based on the results of preliminary tests. The concentration range for each of the antibiotic were deemed appropriate if isolates from one source were resistant and isolates from another source were sensitive at the highest concentration. (Wiggins, 1996)

Sample isolates were screened for antibiotic resistance using the method by Wiggins (1996). Isolates were transferred from the enterococcosel-containing micro wells, using a 96-prong replica-plater to the trypticase-soy plates containing the varying antibiotics concentrations. Each sample contained one plate of each antibiotic at one of the four concentrations. Two plates containing no antibiotics were inoculated and kept as controls for growth. Each sample set contained 36 antibiotic plates and two control plates. Plates were then incubated at 37<sup>0</sup> C for 24 hours. Isolate growth was scored for the various antibiotics at each of that antibiotic's concentrations. An isolate pattern or score to a particular antibiotic was considered to be at the highest concentration where the isolate exhibited growth. Isolates that did not grow on either of the two control plates were removed from the analysis.

### **Discriminate Analysis**

Analysis of each of the isolates and their respective growth on a particular antibiotic (36 variables per isolate) was executed using the procedure DICRIM (prior probabilities, equal; covariance matrix, pooled) in the statistical programs SAS (Windows Version 6.12: SAS Institute Inc.). Several variants of discriminate analysis were performed by varying the combination of antibiotics and the level of pooling of source types.

The DISCRIM procedure produces a classification table, which calculates the percentages of misclassified isolates and determines the average rate of correct classification (ARCC). The table is a source-by-source matrix in which the numbers and percentages of correctly classified isolates are found on the diagonal. The ARCC for a given combination of antibiotics was computed averaging the percentages along the diagonal. The percentage of misclassified isolates for a given source (false negatives) was determined by adding the percentages of misclassified isolates in the appropriate row of the table (excluding the values in the diagonal). The percentage of isolates from the other sources that was misclassified as a given source (false positives) was determined by taking the average of the percentages in the appropriate column (excluding the value in the diagonal) (Wiggins, 1996).

## **RESULTS AND DISCUSSION**

### **Average Rate of Correct Classification**

The average rate of correct classification (ARCC) is derived using the classification table produced in the SAS<sup>TM</sup> statistical program. The sources were pooled as dairy and human and the data was analyzed using parametric discriminate analysis. When using the nine antibiotics (AMP, AMX, CEP, CTC, ERY, OTC, STR, TET and VAN) the ARCC for dairy and human sources was 82%. Dairy isolates were particularly well classified (90%), while human isolates were less so with an ARCC of 74%. The number and type of antibiotic used in the analysis was varied in an attempt to increase the ARCC (as shown in Wiggins, 1996) the highest ARCC was achieved using all nine antibiotics.

### **Source Pattern Development**

The dairy source pattern was established by collecting feces from several dairy farms in the Tillamook basin. Fecal samples were collected from both individual cows as well as from slurries of several cows from each of the farms sampled. A total of 8 samples were collected which produced a total of 260 fecal streptococci isolates. These were screened to determine the antibiotic resistance pattern for dairy. Dairy isolates appeared the least resistant of the sources with isolates having low resistance levels to AMP, AMX, ERY, and VAN. Dairy isolates did show increased resistant to both CEP and STR with a majority showing resistance. Dairy isolates appears to have distinguished itself from the other sources by exhibiting the least overall range of resistance to the nine antibiotics.

The human source pattern was established by collecting wastewater samples from the Tillamook and Garibaldi municipal treatment facilities located in the towns of Tillamook and Garibaldi, Oregon. Six filtrate samples were collected prior to the primary treatment process with yielded a total of 309 isolates. Human source isolates showed the greatest overall resistance of the three source groups showing a relatively strong resistance to CEP, CTC, OTC, STR and TET. Human isolates did show the least resistant to AMP, AMX, ERY and VAN. Of the source groups human isolates showed the strongest resistance of the sources to the antibiotics CTC, STR, and TET.

The wild source pattern was established by collecting river water at the agriculture-forest interface at two locations on the Tillamook River. On the Tillamook River just upstream

from the Oregon Department of Fish and Wildlife fish hatchery and at the Agriculture-forest interface on Beaver Creek, a tributary to the Tillamook River. An assumption was made that there exists neither human nor dairy fecal sources above these sample points. Therefore samples taken in this location should only have fecal isolates from wild or 'back ground' sources. The most predominant wildlife in the basin would be elk, beaver, deer, and waterfowl. A total of five samples were collected which yielded a total of 261 isolates. Wild source isolates showed the least resistance to AMP, AMX, CTC and TET while showing increased resistance to CEP, OTC, STR, and VAN. Of the three source groups wild isolates showed the strongest resistance to VAN.

**Discriminate Classification of Sources**

Discriminate analysis completed on the 830 dairy, human and wild source isolates revealed an overall Average Rate of Correct Classification (ARCC) of approximately 83.3%. The ARCC is calculated by taking an average of each source group's correct classifications rate. The individual source correct classification rates varied from source to source. The individual group classification rates varied depending on source. The human source group had the lowest rate of 73%, while isolates from wild and dairy (were considerably better with correct classification rates of 88% and 89% respectively (Table 2).

Misclassifications in each group were most common between human and dairy sources. Human isolates were misclassified as being from dairy 26% while 9 % of dairy isolates were misclassified as human. Wild isolates were misclassified predominantly as dairy (11%).

<b>Rates of Correct Classification for Individual Sources</b>				
<b>Sources</b>	<b>Number of Isolates</b>	<b>Number Correctly Classified</b>	<b>Rate of Correct Classification (%)</b>	<b>Dominant Misclassification Source (%)</b>
Human	309	225	73	Dairy (26)
Dairy	260	231	89	Human (9)
Wild	261	230	88	Dairy (11)
<b>Totals</b>	<b>830</b>	<b>686</b>	<i>*The ARCC was »83%</i>	

**Table 2. Rates of Correction Classification for Individual Sources**

**Source Pattern Development**

Nine antibiotics, Ampicillin (AMP), Amoxicillin (AMX), Cephalothin (CEP), Chlortetracycline (CTC), Erythromycin (ERY), Oxytetracycline hydrochloride (OTC), Streptomycin sulfate (STR), Tetracycline (TET), and Vanomycin hydrochloride (VAN) were utilized to establish antibiotic resistant patterns for isolates from known sources. Varying these antibiotics in four concentrations screened isolates. Specific concentration

gradients varied by type of antibiotic according to protocol provided by Dr. Bruce Wiggins, JMU. Isolate responses were scored by noting growth/no growth response system at increasing concentrations. This system gives us five separate responses; ‘Conc. 0’ no growth on lowest concentration plate, ‘Conc. 1’ growth on the lowest concentration, ‘Conc. 2’ growth on the first and second concentrations, ‘Conc. 3’ growth on the first three concentrations, ‘Conc. 4’ growth at all concentrations. The sources (human, dairy, wild) varied in their responses to each of the antibiotics concentrations and the results were shown are shown in Table 3.

Sources	Source Isolates Growth Responses to Multiple Concentrations								
	Antibiotics								
Dairy	AMP	AMX	CEP	CTC	ERY	OTC	STR	TET	VAN
% Growth (CONC 0)	99	88	37	53	90	60	18	73	95
% Growth (CONC 1)	0	0	3	16	1	3	27	1	2
% Growth (CONC 2)	0	6	4	7	1	2	22	2	0
% Growth (CONC 3)	1	5	3	3	0	1	18	2	0
% Growth (CONC4)	0	1	52	21	8	33	15	22	3
<b>Total</b>	100	100	100	100	100	100	100	100	100
Human	AMP	AMX	CEP	CTC	ERY	OTC	STR	TET	VAN
% Growth (CONC 0)	88	74	5	26	58	34	6	31	96
% Growth (CONC 1)	3	5	4	5	4	6	13	2	0
% Growth (CONC 2)	3	6	5	6	5	3	17	6	0
% Growth (CONC 3)	0	2	19	4	3	8	11	3	0
% Growth (CONC4)	6	13	68	60	30	49	53	58	4
<b>Total</b>	100	100	100	100	100	100	100	100	100
Wild	AMP	AMX	CEP	CTC	ERY	OTC	STR	TET	VAN
% Growth (CONC 0)	69	95	10	62	82	9	11	84	26
% Growth (CONC 1)	14	5	3	18	3	24	51	3	19
% Growth (CONC 2)	10	0	6	5	6	18	7	0	16
% Growth (CONC 3)	7	0	20	3	0	18	8	2	15
% Growth (CONC4)	0	0	61	12	8	31	23	11	24
<b>Total</b>	100	100	100	100	100	100	100	100	100

**Table 3. Source Isolates Antibiotic Resistance Responses**

The dairy source pattern was established by collecting feces from several dairy farms in the Tillamook basin. Fecal samples were collected from both individual cows as well as from slurries of several cows from each of the farms sampled. A total of 8 samples were collected which produced a total of 260 fecal streptococci isolates. These were screened to determine the antibiotic resistance pattern for dairy. Dairy isolates appeared the least resistant of the sources with isolates having low resistance levels to AMP, AMX, ERY, and VAN (Figure 3). Dairy isolates did show increased resistant to both CEP and STR with a majority showing resistance. Dairy isolates appears to have distinguished itself from the other sources by exhibiting the least overall range of resistance to the nine antibiotics.

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### Characterization of Fecal Streptococci Isolates

A total of 180 isolates were randomly selected from both known sources and rivers samples and assayed to confirm them as fecal streptococci bacteria. All selected isolates were screened for their ability to hydrolyze esculin, for production of catalase, gram positive cocci, growth at 37<sup>0</sup>C in brain heart infusion broth (BBL) containing 6.5% NaCl, and growth on bile esculin azide agar (Table 4).

<b>Isolate Characterization Results</b>				
<b>Source (Isolates Screened)</b>	<b>Catalase Negative</b>	<b>Gram Positive</b>	<b>Growth on Esculin</b>	<b>Growth on Bile Azide</b>
<b>Dairy (20)</b>	<b>95%</b>	<b>100%</b>	<b>95%</b>	<b>100%</b>
<b>Human (20)</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>Wild (20)</b>	<b>85%</b>	<b>100%</b>	<b>100%</b>	<b>90%</b>
<b>River (120)</b>	<b>84%</b>	<b>98%</b>	<b>92%</b>	<b>87%</b>

**Table 4 Isolate characterization results**

Dairy and Human isolates generally had the higher percentages of both catalase negative and esculin-positive<sup>1</sup> responses that either of the river groups. A majority of all the isolates screened were esculin-positive, catalase-negative, and gram positive, grew in brain/heart infusion broth at 37<sup>0</sup>C and grew on bile esculin azide agar. A majority of all the isolates used in this study can be classified as fecal streptococci.

<sup>1</sup> Esculin screening was conducted by using the Enterococcosel broth (BBL), which all isolates were grown on for selection purposes.

## **STORM SAMPLING**

First there is a need to know the relative number of bacteria in the water. From each water sample collected the concentration of fecal coliform were determined in the Kilchis Analytical Laboratory. The second is to identify individual isolates of fecal streptococci. The fecal streptococci isolates were plated and identified in a split water sample. Once identified these bacteria will be plated on the antibiotic plates to determine their resistance to each of the kinds and levels of antibiotics.

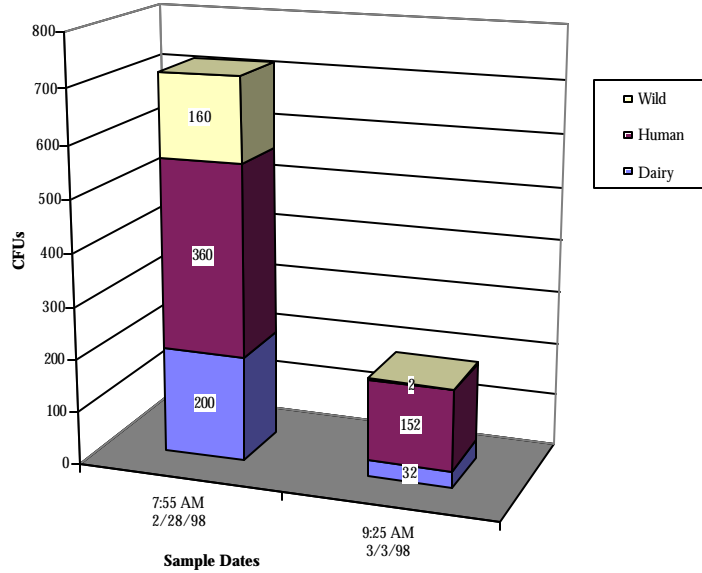
## **THE TILLAMOOK RIVER**

The sampling on the Tillamook River started at river mile (RM) 8.1 (RES) and continued downstream with samples being collected at river mile 4.9 (TTR), 4.0 (BUR) and 0.9 (NET) (Table 1). In reviewing the entire storm sampling results it is important to note that the scale of number of CFU's change with each figure. All of the data will be reviewed from the upriver site downstream. The highest concentration of bacteria on the Tillamook River during the storm sampling was recorded at RM 8.1 with a concentration of about 700 CFU's/100 ml (Figure 1). The first sampling showed that human source accounted for 50% of the organisms and 28 % coming from dairy cattle. The 22 % from wild sources was the highest percentage of wild recorded in this study. In the second sampling the total number had dropped to just 186 CFU's/100 ml with the human sources still contributing the greatest percentage at 80%. Even though the total contribution of organisms from human sources fell by over half, the percentage of human sources in the sample increased because the contribution from the dairy source dropped from 200 to only 32 CFU's/100 ml. This is logical as dairy sources are a fix amount and when they are washed into the soil or off the field their contribution will decrease sharply.

At RM 4.9 (TTR) a similar pattern is seen, but at about one third the concentration (Figure 2). At this location both the early and late storm samples show the contribution from dairy are the largest with those from the human sources a close second. Again the late storm sampling show a bacteria level of about 1/3 of the early storm concentration.

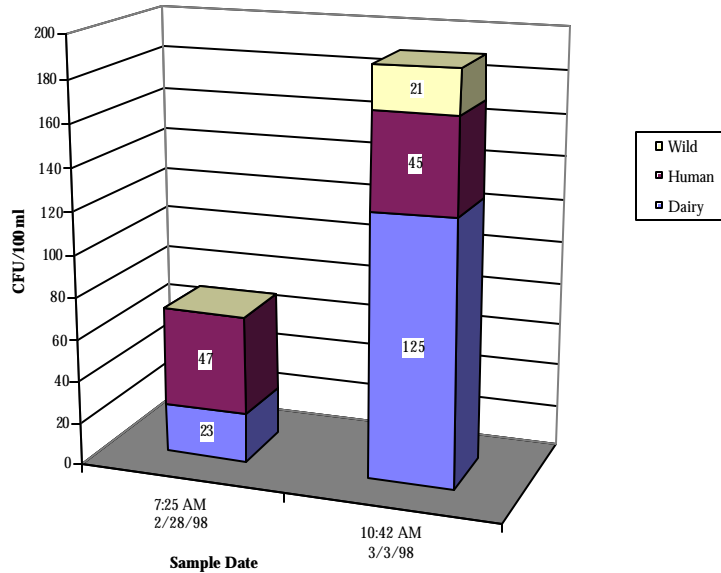
The pattern changed dramatically between RM 4.9 and 4.0 (BUR) as shown by both the magnitude of the concentrations and the relationship of the early and late samplings. The early event sampling at RM 4.9 had a concentration of almost 250 CFU's/100 ml and this had dropped to less than 60 CFU's/100 ml in less than one mile (Figure 3). This could be interpreted as a contribution of some rather clean dilution water. Both the major potential sources had dropped their contributions. Three days later on the fall of the hydrograph, the number of organisms increased by a factor of three, with the increase coming from

**TIL-RES Storm Sample Distributions Quantified (Coliform # x Distribution)**



**Figure 1 Fecal Coliform Bacteria concentration in the Tillamook River near HWY rest area**

**Til-Net Storm Sample Distribution Quantified (Coliform # x Distribution)**



**Figure 2. Fecal Coliform Bacteria concentration in the Tillamook River below trailer park**

both major sources. No apparent reason for this increase. It is difficult to know if 50 cfu's/1000m is statistically different then 150 cfu's/100m.

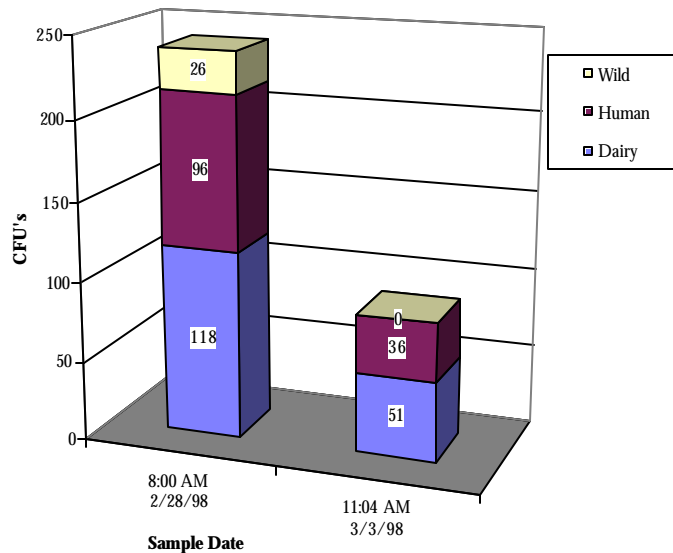
Moving on down to RM 0.9, the pattern stays quite similar to the one at RM 4.0. The total magnitude is about the same and the distribution is also quite similar, with about 2/3 from dairy and about 1/3 from human sources (Figure 4). One could draw the conclusion of little dairy contribution low in the watershed. The late sampling shows an almost three fold increase and most of that coming from the dairy source. One must be careful not to try to draw too many conclusions from data that vary from 50-to 200-CFU's/100 ml. Values in this range might be statistically the same as the exact numbers vary over a range that usually is this wide.

### THE TRASK RIVER

Samples were collected at three locations on the Trask River early and late in the storm. Samples were collected a RM 3.7(BTR), RM 1.5 (5<sup>th</sup>) and RM 1.2 (HOB) (Table 1). All the organism concentration patterns were similar in that they were highest in the early sampling and had fallen later in the storm. The early sampling at RM 3.7 was just under 400 CFU's/100 ml and both of the next downstream readings were almost 800 CFU's/100 ml (Figure 5). At all three locations the early sampling showed 63-73% of the bacteria were from dairy sources. At RM 3.7 the concentration of bacteria had fallen from over 350 CFU's/100 ml to 57 CFU's/100 ml in the three days (Figure 5). There was a large drop in the organisms from dairy sources leaving the human sources as contributing 72% of the total, even though the human sources had been reduced by half.

At the 5<sup>th</sup> street sampling site there was a small drop in total organisms between the sampling periods, but a rather dramatic shift in sources. In the first sampling 73% of the organisms were identified as coming from cattle and in the later sampling 59% of them

**Til-TTR Storm sample Distributions Quantified (Coliform # x Distribution)**



**Figure 3. Fecal Coliform Bacteria concentration in the Tillamook River at Burton Bridge**

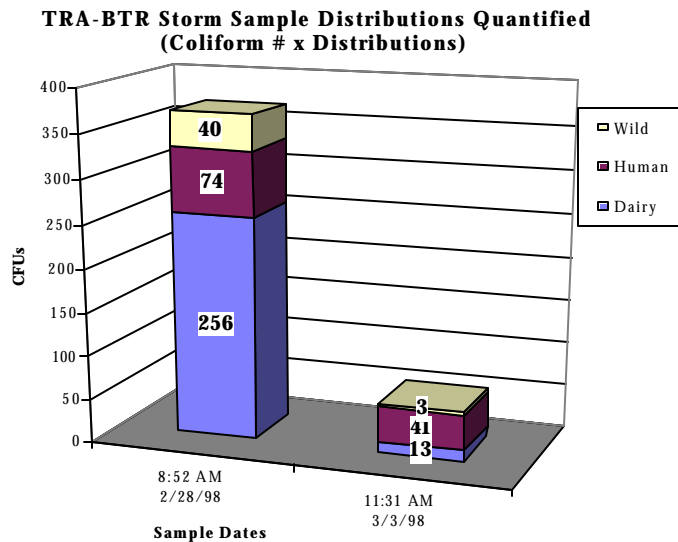
enter the river. The third site is only 0.3 miles downstream and has an almost identical early storm strength and distribution when compared to the 5<sup>th</sup> street site (Figure 7). The sampling later in the storm shows a drop from 600 CFU's/100 ml to about 400 CFU's/100 ml. Again one must be careful when drawing conclusions as these values are similar in magnitude.

**PERIODIC RIVER MOUTH SAMPLING**

Sampling of the five river mouths occurred 8 times from December 1997 until December 1998. One must be careful in the interpretation of this data when trying to draw conclusions. In the review of the percent of organisms in the Miami River that come from dairy, human and wild in Dec 1997 and April 1998, the values are shown in Table 4 and graphed in Figure 8. This data shows the sources and reports the percent of the total that are identified as coming from dairy, human or wild. The percent distribution from human and dairy just about switch in those two samplings, being 73% dairy in December and 74% human in April. What this table does not show is the total number of organism in the samples. Review of Figure 8 shows the number of organism that is associated

with each source. While the percent of the total human went from 26.4 % to 74.4% in the December and April samplings, the total number of organisms in each sample from human sources was about the same. There were just fewer organisms in the water during the April sampling. So both the distribution or percent of the total in a category and the total number of organisms in the sample are very important when reviewing the data.

Cattle are not a major source on the Trask River, based on the limited data collected in this study.

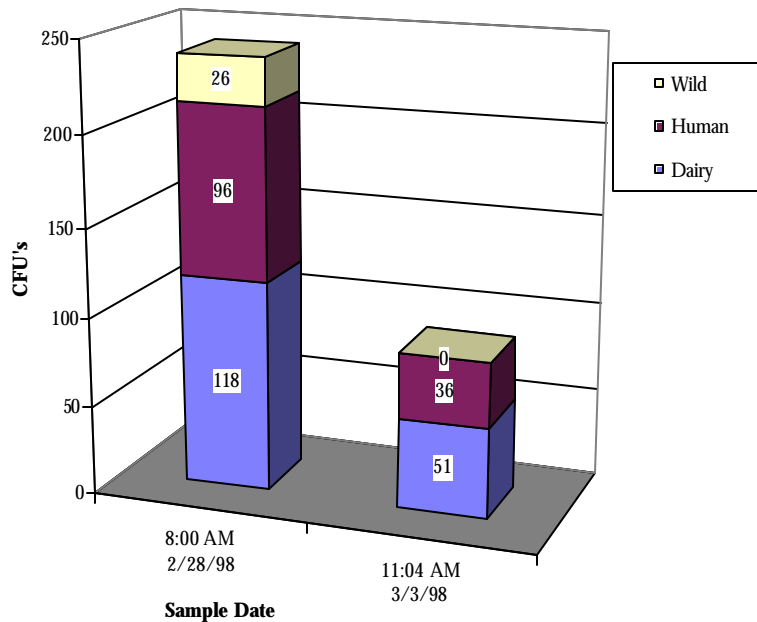


**Figure 4. Tillamook River at Netarts Highway Bridge**

Figure 8 shares the distribution of sources and the order of magnitude of organisms for all 8 sampling events. The greatest concentration of the organisms occurred in the September sampling. However this may have not been the greatest mass load of organism to the bay, if one is interested in total loading and the concentration of organisms in the bay to determine if the bay would have been closed to harvest. To determine the total loading to the bay, total flow of water and the organism concentration are needed. It is very likely that the September concentrations occurred when the flow in the river was at or near the low summer flow.

The dairy contributions, as concentrations of ~ 30 and ~ 40 CFU/100 ml, were highest during the June and September sampling. By this time the rains have tapered off and the soils are drying up. When it rains on the drier soils, it usually does not produce a runoff event that would be necessary to move organism from the pastures into the streams. The dairy sources of organisms could have come from cattle that had access to the stream and were allowed to defecate directly into the river. The high concentrations of human summer water quality problems in all the rivers. It would be interesting to collect summer water samples along a stretch of river that had fenced all the cattle from the river.

**Til-TTR Strom sample Distributions Quantified (Coliform # x Distribution)**

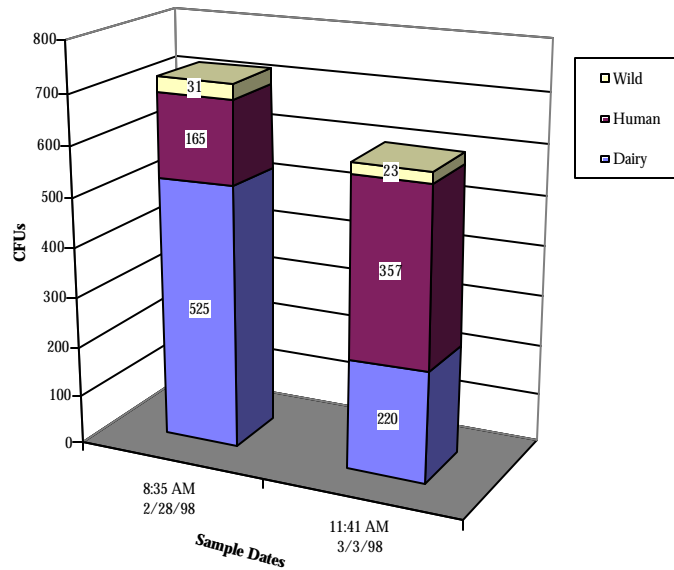


**Figure 5. Trask River below the Trailer Park**

Organisms in September and November could have resulted from failing septic tank systems which discharges septage, saturates the soil and continue to contribute organisms to the river all year long. These same constant sources of human organisms would be diluted with rainfall runoff during the winter and early spring with high flows in the river. This may account for their lower concentration seen in the December through May samplings.

When reviewing the bacterial data for all the Rivers it is important to note that the scale for the organisms on all the rivers is different. The Kilchis River has the lowest bacterial concentration of all the rivers, with only one event with over a reading of 30 CFU/100ml(Figure 3). Interesting to note this sample had the highest percentage of human source and occurred during a late summer low flow period. The next highest concentration occurred in July and recorded an elevated dairy and human source.

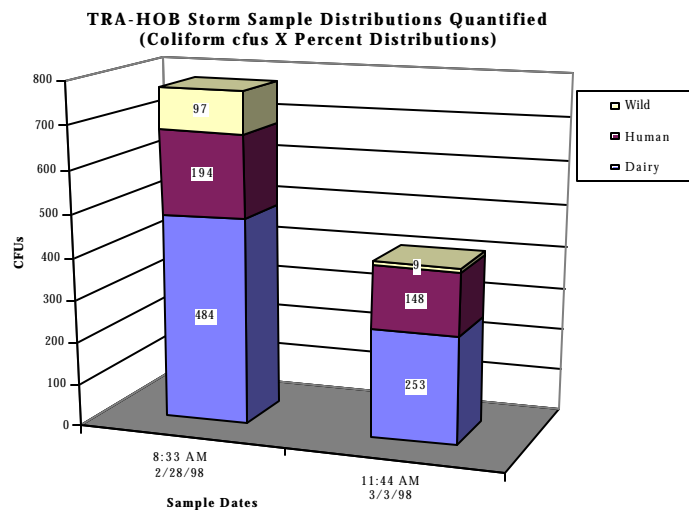
**TRA-5th Storm Sample Distributions Quantified (Coliform 3 x Distributions)**



**Figure 6. Trask River sampled at the 5<sup>th</sup> Street Bridge**

Human sources seem to be significant on the Wilson River with 4 of the 6 highest concentration recorded (Figure 4). One of the six, from the wild source, was recorded in September. Like some of the other rivers, the highest human source contribution occurred in September. The highest dairy contribution was measured in December 1997 at just over 20 CFU/100ml. The Wilson river samples did not have any organism concentrations over 30 /cfu/100ml, but 6 of the 8 samples taken had concentrations over 10 CFU/100ml.

The Trask River had the sample with the highest bacterial counts (Figure 5). In fact the highest contribution from dairy (60CFU/100ml), human (100CFU/100ml) and wild (40CFU/100ml) sources. all occurred in September. This clearly was the most polluted water sample collected in the yearlong sampling program. It is a bit puzzling as to why all three would be so high in the late summer. Cows having access to the stream as a water source, failing or faulty septic systems and wildlife, perhaps birds could be the sources. There are two sewage treatment plants that discharge their effluent into the Trask River, which might also be contributors. It appears that the contribution from dairy the second most polluted water sample collected was taken from the Tillamook River in December 1997(Figure 6). Unlike the sample taken on the Trask River, which contained high counts from all three sources, the Tillamook sample had 88% of the organisms from the dairy source. The next two highest concentrations of organisms occurred in November 1998 when the dairy sources contributed over 50CFU/100ml and the human sources were over 30 CFU/100ml. Unlike the other four river samples which all peaked in the September sampling, the Tillamook had higher counts in both the December 1997 and November 1998, the September sampling ranking third on a total organism basis.



**Figure 7. Fecal Coliform Bacteria concentration in the Trask River near Hospital Hole**

The data collected and shown above provides the reader with an idea of the general quality of the water at the five river mouths and some information about the sources of these organisms. It appears there are times when one source is significantly the primary contributor and other times when source contributions are more nearly equal. The study clearly shows that bacteria are being contributed from all three sources, with the wild being consistently the smaller fraction. The human sources seem to September, the late fall and the April and May samplings. One might have expected the animal sources to be very large in the fall when the animals have been on pasture and many operators have just emptied the storage units. As with many research efforts some questions are answered and others are questions are generated.



## Five River Study

<b>Kilchis River Distribution Data</b>			
<b>Month-Year</b>	<b>Dairy (%)</b>	<b>Human (%)</b>	<b>Wild (%)</b>
Dec-97	75.0	25.0	0.0
Jan-98	59.1	36.4	4.6
Apr-98	28.9	68.9	2.2
May-98	42.9	57.1	0.0
Jun-98	79.5	20.5	0.0
Jul-98	50.0	32.6	17.4
Sep-98	6.7	93.3	0.0
Nov-98	34.0	66.0	0.0
Dec-98	25.6	74.4	0.0

<b>Miami River Distribution Data</b>			
<b>Month-Year</b>	<b>Dairy (%)</b>	<b>Human (%)</b>	<b>Wild (%)</b>
Dec-97	73.2	26.8	0.0
Apr-98	23.3	74.4	2.3
May-98	42.5	57.5	0.0
Jun-98	67.5	30.0	2.5
Jul-98	39.0	46.3	14.6
Sep-98	41.9	55.8	2.3
Nov-98	34.6	65.4	0.0
Dec-98	8.3	89.6	2.1

<b>Wilson River Distribution Data</b>			
<b>Month-Year</b>	<b>Dairy (%)</b>	<b>Human (%)</b>	<b>Wild (%)</b>
Dec-97	50.0	42.9	7.1
Jan-98	32.6	67.4	0.0
Apr-98	36.4	63.6	0.0
May-98	59.1	40.9	0.0
Jun-98	50.0	45.0	2.5
Jul-98	51.1	31.9	17.0
Sep-98	19.2	70.2	10.6
Nov-98	8.5	44.7	46.8
Dec-98	34.5	48.3	17.2

**Table 5. Distribution of sources in the five-river study (continued on next page)**

<b>Trask River Distribution Data</b>			
<b>Month-Year</b>	<b>Dairy (%)</b>	<b>Human (%)</b>	<b>Wild (%)</b>
Dec-97	53.9	38.5	7.7
Jan-98	58.7	39.1	2.2
Apr-98	13.0	84.8	2.2
May-98	28.9	71.1	0.0
Jun-98	13.5	83.8	2.7
Jul-98	59.6	38.3	2.1
Sep-98	30.4	47.8	21.7
Nov-98	40.4	55.3	4.3
Dec-98 <sup>1</sup>	29.9	55.2	14.9

<sup>1</sup> Plates containing Antibiotic VAN contaminated, ARCC 80% for this sample.

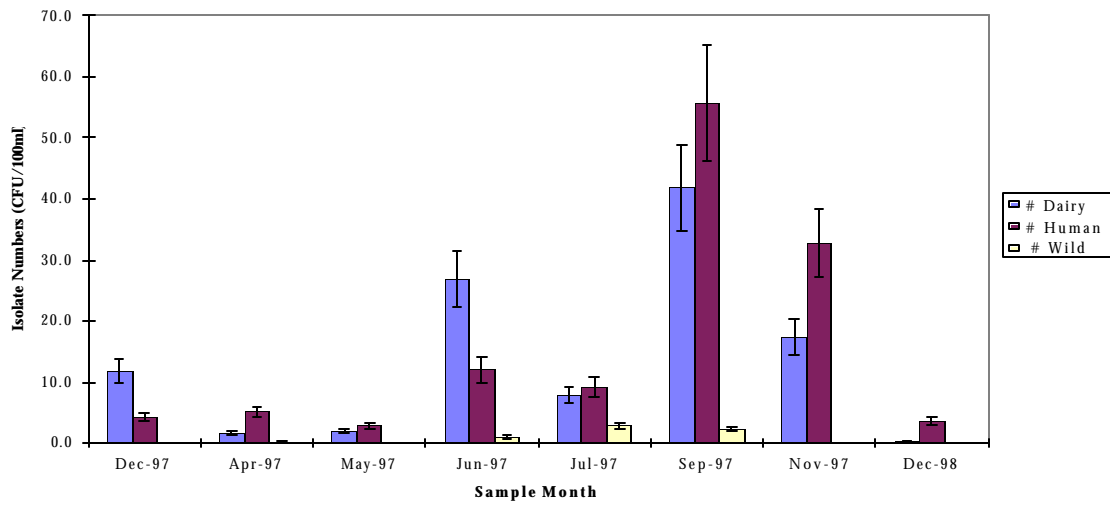
<b>Tillamook River Distribution Data</b>			
<b>Month-Year</b>	<b>Dairy (%)</b>	<b>Human (%)</b>	<b>Wild (%)</b>
Dec-97	88.5	3.9	7.7
Jan-98 <sup>1</sup>	27.7	51.1	21.3
Apr-98	24.4	75.6	0.0
May-98	32.6	67.4	0.0
Jun-98	52.5	45.0	2.5
Jul-98	41.4	58.6	0.0
Sep-98	57.5	42.5	0.0
Nov-98	60.6	39.4	0.0
Dec-98 <sup>2</sup>	15.6	71.1	13.3

<sup>1</sup> Plates containing Antibiotic CEP contaminated, ARCC 81%.

<sup>2</sup> Plates containing Antibiotic AMX contaminated, ARCC 79.5%.

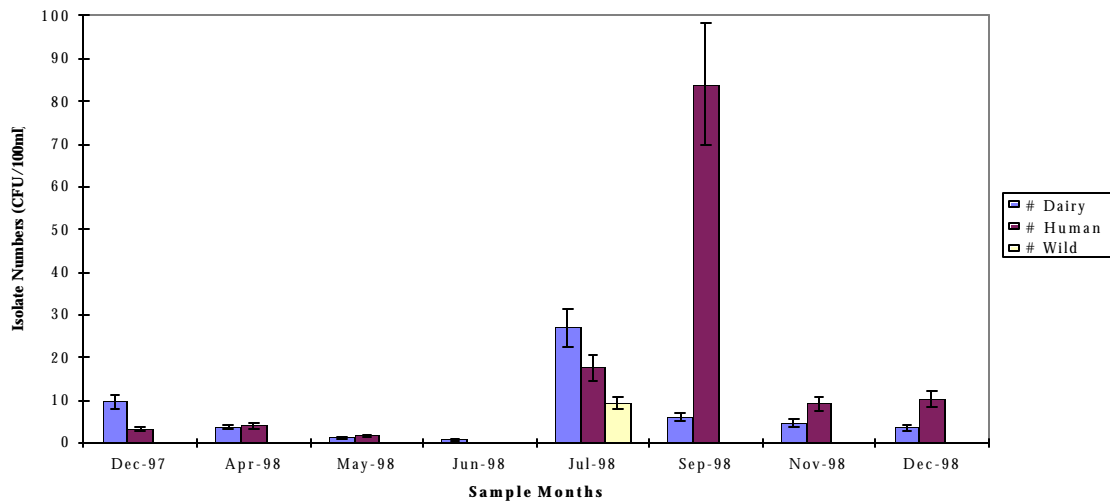
**Table 5 (continued) Distribution of sources in five-river study**

**Miami River Monthly Samples**



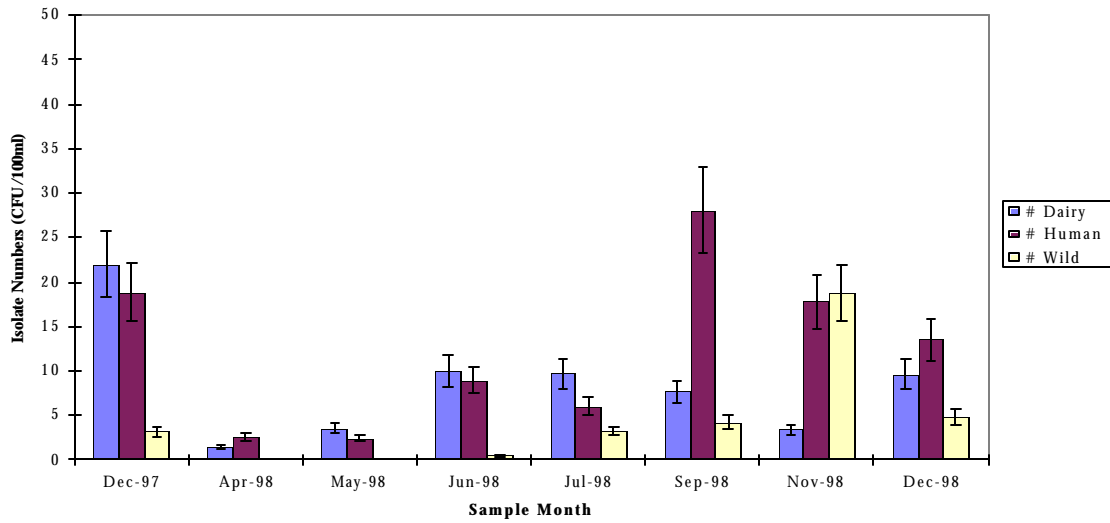
**Table 8. Distribution of sources and relative concentration of Fecal Coliform Bacteria for the Miami River**

**Kilchis River Monthly Samples**



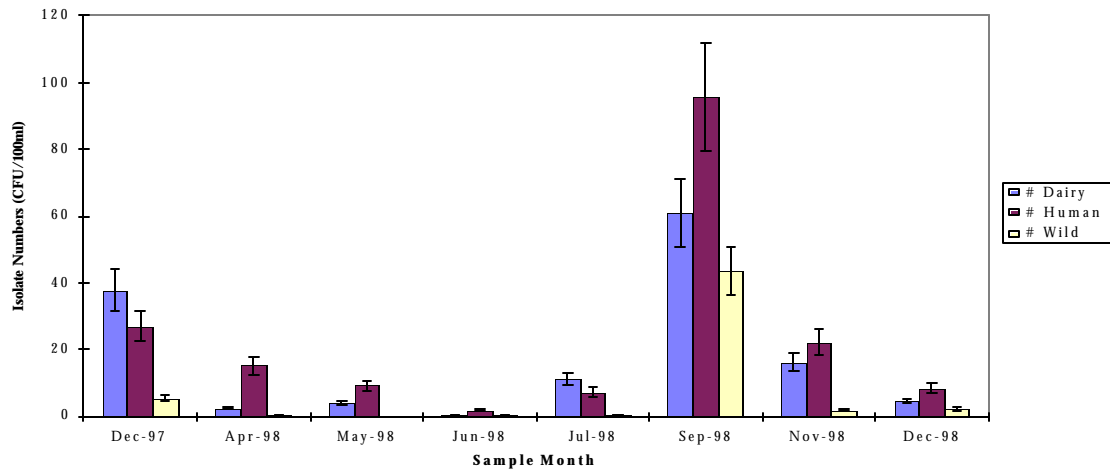
**Table 9. Distribution of sources and relative concentration of Fecal Coliform Bacteria in the Kilchis River**

**Wilson River Monthly Samples**



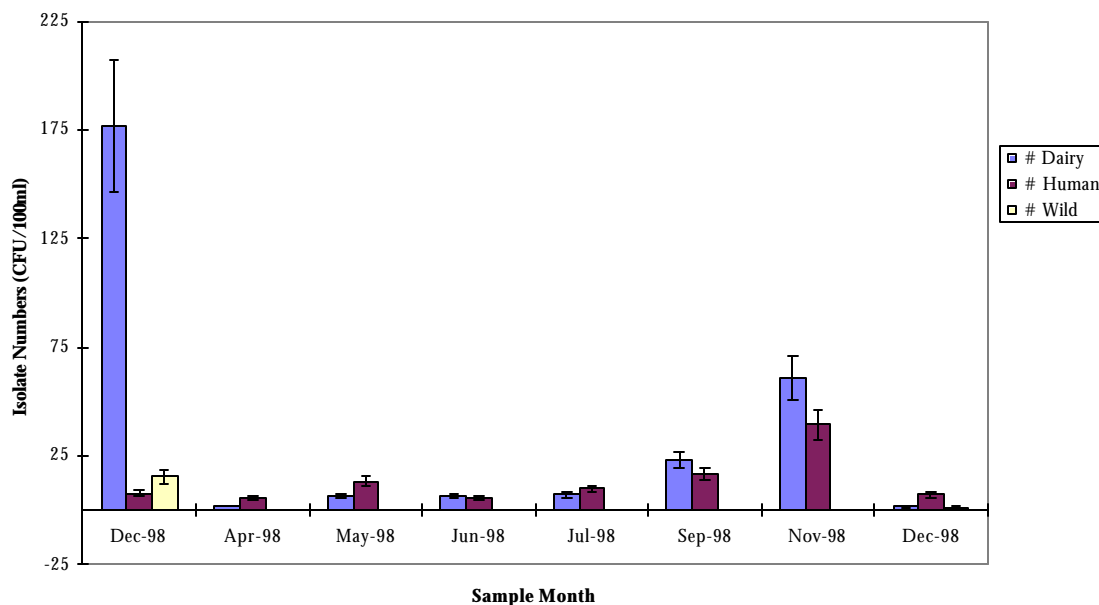
**Table 10. Distribution of sources and relative concentration of Fecal Coliform Bacteria in the Wilson River**

**Trask River Monthly Samples**



**Table 11. Distribution of sources and relative concentration of Fecal Coliform Bacteria in the Trask River**

### Tillamook River Monthly Samples



**Table 12. Distribution of sources and relative concentration of Fecal Coliform Bacteria in the Tillamook River**

#### CONCLUSIONS

- This technique proved to be a very promising as a method to identify sources of bacterial contamination in conjunction with knowing bacteria concentrations.
- This technique allows source identification of both storm event and calendar driven sampling schemes.
- Results allow the selection of best management practices that will address specific sources and transport processes over time/seasons and rainfall events.
- Concentrations of bacteria from wild sources are consistently low during all sampling.
- Bacteria concentrations typically are higher on the rising limb of the hydrograph when compared to the concentrations on the falling limb of the hydrograph.
- Bacteria concentrations can be high during late summer low flow periods.

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