

## ***Report on the Analyses of Bacteriological Contributions to Direct Bay Tributary Streams in the Coos Estuary Using Traditional Public Health Indicators and DNA Analyses of *E. coli*.***

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The objective of this project was to characterize bacteriological loading in space and time, and to determine their species origin. The goal is to measure amounts of fecal pollution, and identify the source species causing the pollution. As initially designed, this was to be done by mapping out bacteriological regimens in direct bay tributary systems through both traditional methods in combination with innovative bacterial DNA marker analyses. Traditional analytical methods will provide amounts (most probable number, MPN) for total coliforms, fecal coliforms, and *E. coli*. The DNA analyses will identify the species contributing bacteriological load. The desired outcome from this study is an increase in knowledge about the various pathways that contribute to coliform loading and their relationship to water quality standards and management programs.

The work reported here is part of a larger project, begun in April, 2001, to use *Bacteriodes* bacteria as the microbial source tracking organism. When this technique failed to provide results by the fall of 2002, the partners involved in the project (Coos Watershed Association, South Slough National Estuarine Research Reserve, and Marshfield High School) identified a promising alternative DNA technique using *E. coli* as the microbial source species. We were able to obtain funding to test this technique at three locations (see Map 1) where we had been conducting traditional public health bacteria indicator analyses since 2001. The final funding arrangements were made in June, 2003 with sampling completed by November, 2003. The constrained time frame for this additional project limits our ability to characterize “actual bacteriological loading in space and time”, but does allow us to begin to identify the relative contributions of fecal source material in three different land uses.

### **DESCRIPTION OF THE SAMPLE LOCATIONS**

The three different sample locations shown in Map 1 represent different characteristic land uses in their watersheds. Pony Creek is an urban stream, although the headwaters of its mainstem arise in the hills above Coos Bay and are used as the municipal water supply by the towns of Coos Bay and North Bend. However, as measured at the Coos Bay/North Bend Water Treatment Plant, bacterial levels from the upper watershed are quite low. Below the water treatment plant, Pony Creek enters a canyon and receives input from three smaller tributary streams, all located in residential and commercial land uses. While some septic tanks were identified through in the *Lower Pony Creek Watershed Assessment*, the vast majority of residences and businesses in the Pony Creek watershed are served by sewers. However, there are two sewage pump stations, as well as numerous stormwater outfalls adjacent to Pony Creek.

In contrast, the Coalbank Slough sample location represents a typical rural residential characteristic land use in the Coos Bay area. Above the sample site are located a number of residences on parcels ranging from 40 acres down to about ½ acre, none serviced by sewers. In addition to septic tanks and cesspools, there are two larger farms (one cattle and one horse), as well as many “hobby farms” that have a range of livestock from cattle and horses to llamas, turkeys, sheep and goats. The watershed above the residences in the valley is forested, with elk, deer, beaver and bear as possible microbial contributors.

Finally, the Sengstaken Arm of South Slough was used as a third sample location because it represents a relatively undeveloped watershed above the sample site. The area surrounding the sample site has commercial oyster beds, so understanding bacteriological levels and source species is important in managing this resource. Land use in the adjacent hills is predominantly commercial timber, although residences and commercial development exist in Charleston where South Slough empties into Coos Bay. While samples were collected as closely as possible at low (and ebbing) tide, there is a possibility that some bacteria are being flushed up the system with incoming tides.



**METHODOLOGY**

The methodology for the *E. coli* DNA microbial source tracking follows Dr. Mansour Samadpour’s protocols, and is also consistent with the SAPP of our project partners (South Slough National Estuarine Research Reserve and Marshfield High School) for the traditional public health indicator analyses. The basic *E. coli* analysis requires identifying the source species in a minimum of 100 *E. coli* colonies taken from plated and incubated fecal coliform samples. The 100 colonies are collected from 50 plated samples (2 from each plate) during ten sample events. Five water samples (separate dips and sample bottles; however, one split sample was taken during each sample date at one site) are taken during each sample event at each of the tree locations. These samples were divided, with traditional total coliform and *E. coli* Collilert (Idexx) analyses conducted by South Slough National Estuarine Research Reserve and fecal coliform (Millipore filtration method) analyses conducted by Marshfield High School. Once counts were made, Most Probable Number (MPN) determinations were made for total coliform and *E. coli*, while the plate counts were transformed to fecal coliform levels based on the number of colony forming units (CFU) adjusted by any sample dilution prior to plating. The fecal coliform plates were sent to Dr. Samadpour’s lab, where *E. coli* colonies (isolates) were extracted and processed with his DNA species identification techniques.

**RESULTS**

For the purposes of this report, discussion will be divided between the results from the traditional public health bacteria indicators (total coliform, fecal coliform, and *E. coli*) and the results found in the DNA analysis. The results and interpretation of the *E. coli* DNA analyses.

***Results From the Traditional Public Health Bacteria Indicators***

The results from the traditional public health indicator analyses are shown in Tables 1 and 2, and Figures 1 through 3. Based on the results in Table 1, it is apparent that the three characteristic land uses have vastly different bacteria loadings (Table 2 provides data on specific sample events), although the magnitude of the differences depends upon the statistic used. Pony Creek has about twice the total coliform levels compared to Coalbank Slough using arithmetic means (averages); however, when geometric means are used, the differences between the two sites are eliminated. Standard deviations are different between the two locations (Pony Creek and Coalbank Slough), with Pony Creek having twice the variation compared to Coalbank Slough. Bacteria levels at the Sengstaken Arm of South Slough are between one-tenth (Coalbank) and one-twentieth (Pony Creek) the amounts at the other two, developed, sample locations.

Table 1. Summary statistics from the traditional public health bacteria indicators at the three *E. coli* DNA sample locations.

Statistic	Pony Creek			Coalbank Slough			Sengstaken Arm		
	TC	FC	EC	TC	FC	EC	TC	FC	EC
Arithmetic Mean	5,089	246	182	3,130	349	237	342	36	26
Standard Deviation	6,709	129	103	3,498	292	204	318	31	21
Geometric Mean*	2,297	206	139	2,105	215	138	149	14	19
Est. 90 <sup>th</sup> Percentile*	4,910	230	182	2,790	379	280	972	49	25

\* Calculated according to procedures in *Guidance Document A.7. – Estimating the Ninetieth Percentile.*

Without more detailed analysis, it is difficult to point to a causal reason for this difference in variability between the Pony Creek and Coalbank Slough sites. One possibility, however, is that storm water discharge inputs into Pony Creek raise rainfall event bacteria levels to a greater extent than runoff during storm events in Coalbank

Slough. Figures 1 through 3 show bacteria levels at the three different sample locations in relation to rainfall in the 24 hour, 3-day, and 5-day periods prior to the sample date. From a visual inspection of the charts, very little correlation is apparent between rainfall and bacteria levels, at least in the ten sample dates used for this study. More sophisticated statistical analysis of this data might provide a better understanding of causal linkages between rainfall and bacteria levels.

Table 2. Summary data for the public health bacteria indicators for the *E. coli* DNA part of the study.\*

Sample Site	Sample Date	Geometric Mean Total Coliform	Geometric Mean Fecal Coliform	Geometric Mean <i>E. coli</i>	24 Hr. Rain	3 Day Rain	5 Day Rain
<b>Coalbank Slough</b>	5/6/03	649	30	67	0.06	1.05	1.05
	5/8/03	596	269	155	0.21	0.27	1.26
	5/19/03	1,544	267	128	0.00	0.12	0.16
	5/22/03	2,215	418	434	0.00	0.00	0.07
	5/27/03	1,411	105	71	0.00	0.25	0.25
	6/2/03	2,420	412	365	0.00	0.00	0.00
	6/5/03	2,266	590	294	0.00	0.00	0.00
	10/20/03	2,481	1,003	656	0.14	0.16	0.27
	11/13/03	10,016	192	60	0.00	0.56	1.11
	11/19/03	4,802	34	12	0.01	0.96	1.37
<b>Pony Creek</b>	5/6/03	583	106	69	0.06	1.05	1.05
	5/8/03	850	283	254	0.21	0.27	1.26
	5/19/03	5,113	256	145	0.00	0.12	0.16
	5/22/03	671	114	34	0.00	0.00	0.07
	5/27/03	1,023	58	27	0.00	0.25	0.25
	6/2/03	17,188	399	301	0.00	0.00	0.00
	6/5/03	16,870	403	239	0.00	0.00	0.00
	10/20/03	1,264	268	217	0.14	0.16	0.27
	11/13/03	1,605	164	212	0.00	0.56	1.11
	11/19/03	3,369	359	300	0.01	0.96	1.37
<b>Sengstaken Arn – South Slough</b>	5/6/03	812	25	24	0.06	1.05	1.05
	5/8/03	100	12	16	0.21	0.27	1.26
	5/19/03	327	75	54	0.00	0.12	0.16
	5/22/03	68	7	8	0.00	0.00	0.07
	5/27/03	115	10	10	0.00	0.25	0.25
	6/2/03	841	81	65	0.00	0.00	0.00
	6/5/03	323	9	28	0.00	0.00	0.00
	10/20/03	3	2	4	0.14	0.16	0.27
	11/13/30	90	10	15	0.00	0.56	1.11
	11/19/03	328	15	29	0.01	0.96	1.37

\* Geometric means of the five (or six if the site had a split sample) samples collected during each sample date. Where the Collilert results indicated a > or < quantity, the numerical value was used in the calculation.

Figure 1. Results of the analysis of public health indicator bacteria counts at the Pony Creek sample location.

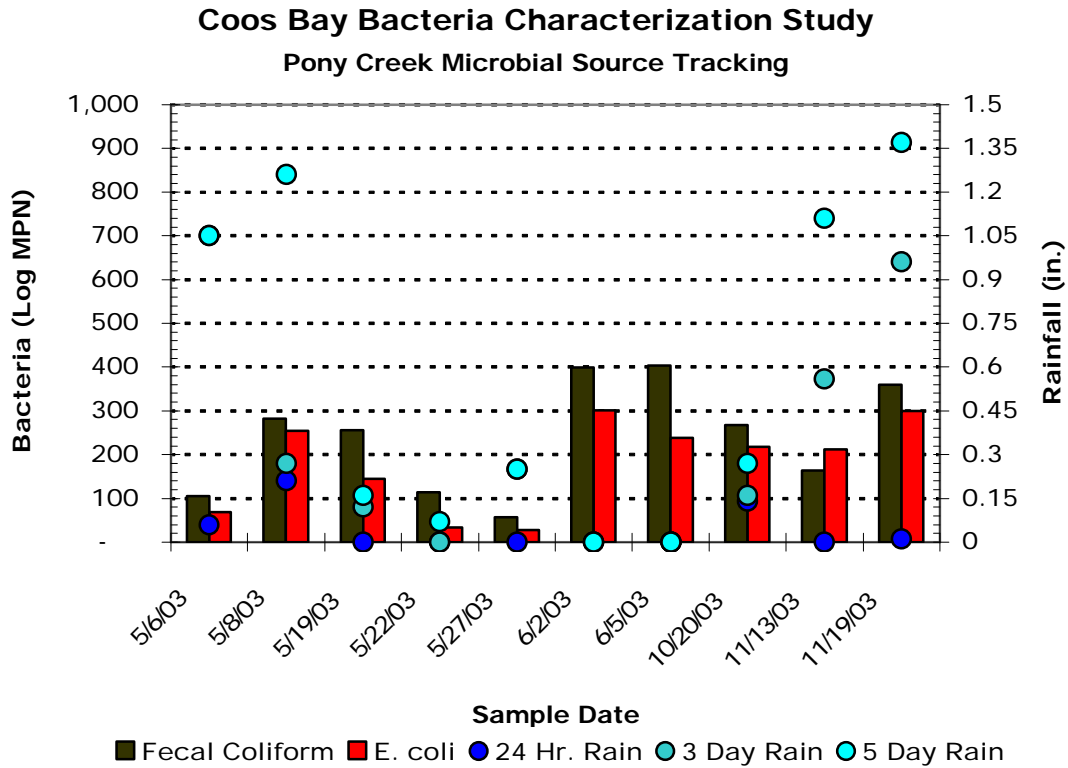


Figure 2. Results of the analysis of public health indicator bacteria counts at the Coalbank Slough sample location.

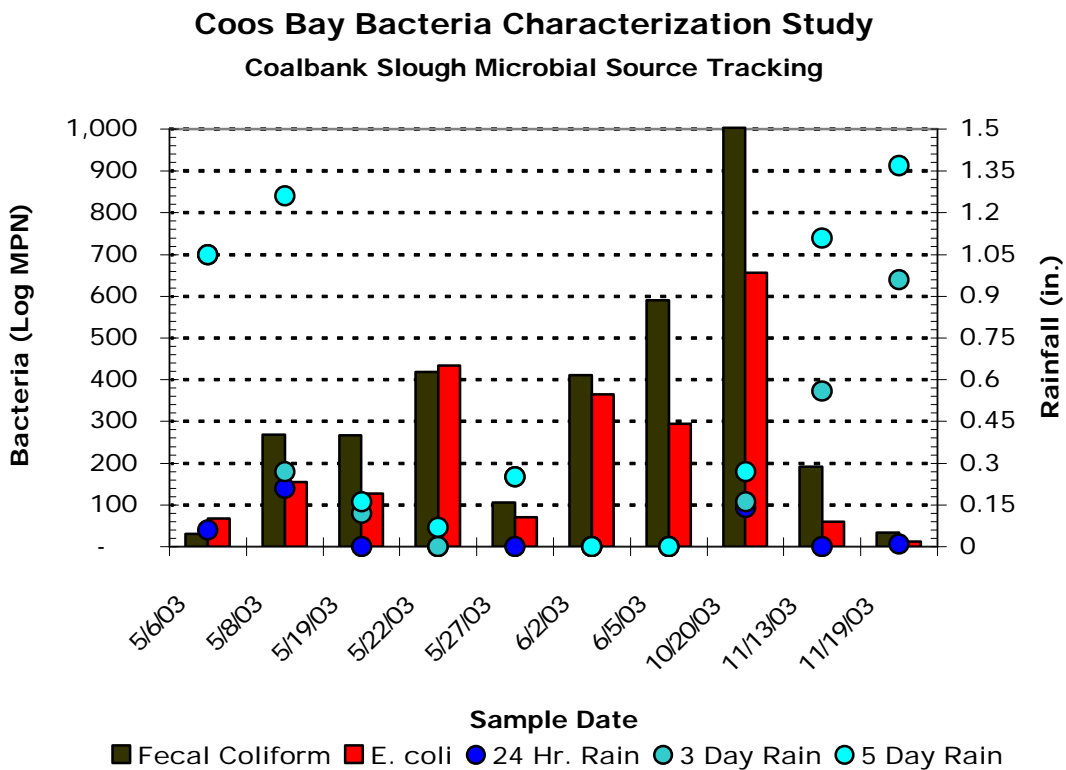
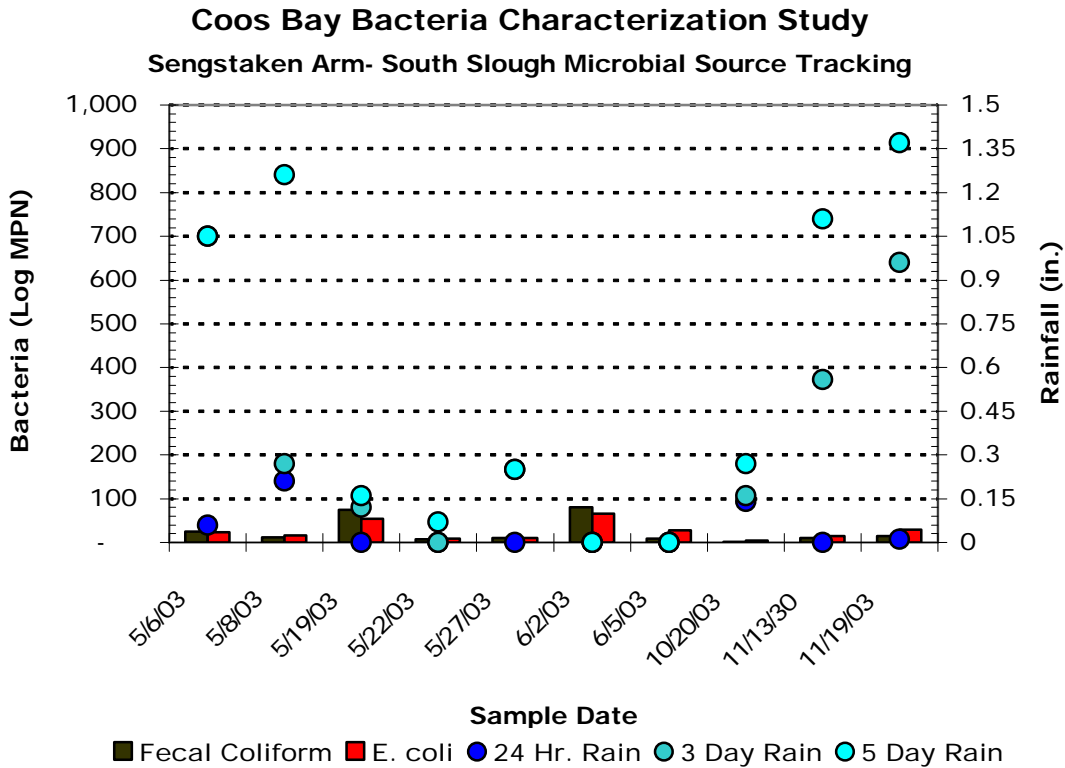


Figure 3. Results of the analysis of public health indicator bacteria counts at the Sengstaken Arm of South Slough sample location.



**Results from the DNA Analyses**

This report provides initial DNA analysis information for the ten sample events at all three sites. Table 3 provides a breakdown by species or taxonomic group for the *E. coli* DNA isolates from each of the three sample locations. In addition, an arithmetic average of the species proportions at all three sites is provided in the final column of Table 3.

Particularly noteworthy in the species identification for all sample locations is the large percentage of *E. coli* resulting from avian (including geese) sources. The total avian contribution was lowest at the Coalbank Slough site (39%), and highest at Pony Creek (46%), with the Sengstaken Arm site (43%) being about midway between the other two sites. Another source species group that is consistent among the three sample locations is canine, including dogs. Percentage levels are comparable at the Coalbank Slough and Sengstaken Arm sites (about 11%), and are about a third higher at the Pony Creek sample location where over 15% of *E. coli* results from canines, with dogs providing the largest component (9%).

Based on the ten sample events (6 base flow and 4 rainfall), inputs of *E. coli* from ruminant sources (Bovidae and Cervidae) are high at both the Coalbank Slough (13%) and Sengstaken Arm (16%) sites. In contrast, this group provided slightly over 4% of *E. coli* loading at the Pony Creek site. For both Coalbank Slough and Sengstaken Arm, wildlife (deer and elk) contributed the majority of *E. coli*, 11% at Coalbank and all the loading at the Sengstaken Arm site.

Table 3. Phylogeny of *E. coli* Microbial Sources — Coos Estuary, 2003.

Order*	Family*	Common Name(s)*	Study Site			Average
			Coalbank	Pony Cr.	Sengstaken	
Avifauna		<b>Birds</b>	<b>39.47%</b>	<b>46.48%</b>	<b>42.96%</b>	<b>42.89%</b>
		Goose	0.00%	0.70%	0.00%	0.23%
		Unspecified	39.47%	45.77%	42.96%	42.66%
Marsupialia	Didelphidae	<b>Marsupials</b>	<b>0.00%</b>	<b>0.00%</b>	<b>1.41%</b>	<b>0.46%</b>
		American Opossums	0.00%	0.00%	1.41%	0.46%
Primates	Hominidae	Human Origin	<b>16.45%</b>	<b>1.41%</b>	<b>0.70%</b>	<b>6.42%</b>
		Human Beings	10.53%	1.41%	0.00%	4.13%
		Sewerage	5.92%	0.00%	0.70%	2.29%
Lagomorpha	Leporidae	Hares, Rabbits				
Rodentia		<b>Rodents</b>	<b>7.89%</b>	<b>13.38%</b>	<b>13.38%</b>	<b>11.47%</b>
		Aplodontidae Mountain Beaver				
		Sciuridae Squirrels, Chipmonks				
		Geomyidae Pocket Gophers				
		Heteromyidae Pocket Mice, Kangaroo Rats, Mice				
		Castoridae Beavers	0.66%	2.82%	7.75%	3.67%
		Muridae Rats, Mice, Hamsters, Voles, and Gerbils				
		Erethizontidae Porcupines				
		Capromyidae Hitias and Nutria				
			<b>Rodent/Beaver</b>	1.32%	0.70%	0.00%
	Unspecified	5.92%	9.86%	5.63%	7.11%	
Carnivora	<b>Canidae</b>	Carnivores				
		<b>Dogs, Coyotes, Foxes</b>	<b>11.18%</b>	<b>15.49%</b>	<b>11.27%</b>	<b>12.61%</b>
		Dogs	1.32%	9.15%	0.70%	
		Unspecified	9.87%	6.34%	10.56%	8.94%
	Ursidae <b>Procyonidae</b>	<b>Raccoons</b>	<b>0.00%</b>	<b>9.15%</b>	<b>1.41%</b>	<b>3.44%</b>
	<b>Mustelidae</b>		<b>0.00%</b>	<b>0.70%</b>	<b>0.70%</b>	<b>0.46%</b>
		Weasels, Skunks, and Otters River Otter	0.00%	0.70%	0.70%	0.46%
	<b>Felidae</b>	<b>Domestic Cats, Bobcat, Cougar</b>	<b>4.61%</b>	<b>0.00%</b>	<b>1.41%</b>	<b>2.06%</b>
	Unspecified	4.61%	0.00%	1.41%	2.06%	
Pinnipedia	Otariidae Phocidae	Marine Mammals	<b>0.00%</b>	<b>0.00%</b>	<b>2.82%</b>	<b>0.92%</b>
		Eared Seals, Fur Seals, and Sea Lions				
		True, Earless, or Hair Seals Harbor Seals Unspecified	0.00%	0.00%	2.82%	0.92%
Perissodactyla	<b>Equidae</b>	Odd-toed Ungulates <b>Horses and Asses</b>	<b>0.66%</b>	<b>0.00%</b>	<b>0.00%</b>	<b>0.23%</b>
Artiodactyla	Suidae Camelidae	Even-toed Ungulates Pigs or Hogs Llama				
		<b>Cervidae</b>	<b>Deer, Elk</b>	<b>11.18%</b>	<b>3.52%</b>	<b>16.20%</b>
		Deer	1.97%	0.00%	3.52%	1.83%
		Deer/Elk	9.21%	3.52%	12.68%	8.49%
	<b>Bovidae</b>	<b>Cattle, Goats, Sheep</b>	<b>1.97%</b>	<b>0.70%</b>	<b>0.00%</b>	<b>0.92%</b>
		Cow Unspecified	1.32%	0.70%	0.00%	0.69%
	Unspecified	0.66%	0.00%	0.00%	0.23%	
<b>Anomalies</b>		Beaver/Otter	0.66%	0.00%	1.41%	0.69%
<b>Unknown</b>			<b>5.92%</b>	<b>9.15%</b>	<b>6.34%</b>	<b>7.11%</b>
Number of DNA Isolates (N)			152	142	142	436

\* R. M. Nowak (1991) *Walker's Mammals of the World* 2 vols. (5th Edition) (Baltimore: Johns Hopkins University Press.

Domestic livestock (bovines and cattle) provided relatively small inputs into Coalbank (2%) and Pony (<1%) Creeks. Other domestic livestock (horses) were a small contribution (<1%) in Coalbank Slough.

Not too surprisingly, rodents were a major *E. coli* source in Pony Creek and the Sengstaken Arm of South Slough. However, they were a smaller contributor (8%) at the Coalbank Slough site. The only site where the majority of the rodent contributions were identified was at Sengstaken Arm, where beaver and beaver/otter composite comprised over 8% of the 13% of total of *E. coli* loading, with opossum and raccoon adding another 3%. Raccoon contributions were over 9% at the Pony Creek site (urban land use), with another 10% of rodent contributions unclassified. Beavers comprised about 3% at Pony Creek, where known beaver dams exist just above of Newmark Avenue upstream from the Pony Creek sample location.

Human, and human-related, *E. coli* source contributions were low at the Pony Creek site (2%), and slight (<1%) at the Sengstaken Arm site for the sample events analyzed. Human-origin sources comprised about 16% of the *E. coli* in Coalbank Slough. Feline (unspecified) sources were an additional 5% at Coalbank Slough and slightly over 1% at Sengstaken Arm. Marine mammals provided another 3% of *E. coli* at the Sengstaken Arm location.

A common test of the efficacy of microbial source tracking is the percentage of *E. coli* isolates that cannot be identified to species or group. Results from the initial seven sample events had low levels of undected sources. About 6% of the *E. coli* isolates tested from the Sengstaken Arm sample location were unknown. Slightly under 6% at the Coalbank Slough site, and slightly over 9% of the isolates at the Pony Creek site were of unknown origin.

## **DISCUSSION**

The results to date indicate that the *E. coli* microbial source tracking technique holds promise for identifying the origin of fecal contamination in watersheds. At this point, our ability to analyze the data is limited by the uneven distribution of the sample events because data was available for the entire five baseflow, but only four of the rainfall, sample events. The relative percentages among the various sources will likely change with the addition of results from the other events, especially additional rainfall samples. We would also expect changes in the species composition if sampling were distributed over the entire year, rather than being concentrated in the summer and fall as was the case in this study.

At this time, the relative contribution of various fecal sources is comparable to what might be expected from different landuses above the three sample locations. The Sengstaken Arm of South Slough has no agriculture or residential development above it in the watershed — although flooding tides may transport fecal materials from developed areas in Charleston up to the sample area. However, the persistence of the fecal contributors is apparently relatively minor since only one sewerage ribotype was found in isolates from the ten sample events analyzed. Human sources were much more prevalent in Coalbank Slough, which would be expected because residences in the area are served by septic tanks instead of sewers. At present, we do not know the exact definition of “sewerage” to confirm whether septic system effluent would be included in this classification.

Relatively high “unspecified” identifications for avian, canine, and rodent source species groups limits the ability to accurately infer the specific fecal species origin. Coyotes are present in both the Coalbank Slough and Sengstaken Arm areas, and they may be a component of the unspecified canine group. The large unspecified avian component provides little assistance in targeting specific contributors, and bird species certainly vary between the Sengstaken Arm site and the two more inland and developed sites. No rats or mice were specifically identified as rodents, and undoubtedly they provide some fraction of this unspecified group. Similarly, feline fecal contributions are reasonably significant in Coalbank Slough, but none were found in Pony Creek (do all cats in this watershed use their litter boxes which are then disposed in the trash?). The small feline contribution at the Sengstaken Arm location could be either feral domestic cats or bobcats or cougar. Our expectation is that greater species specificity would be available if larger numbers of local source feces contributions were added to

the DNA library. Given the truncated time period for this study, intensive fecal source collections were not possible.

Finally, it is important to recognize that while the proportionate distribution of *E. coli* fecal contributions among various species at the three locations provides useful information, the magnitude of fecal loading is also critical in determining strategies to manage these sources. As shown in Table 1, fecal loadings (both fecal coliform and *E. coli*) are almost ten times higher in the Pony Creek and Coalbank Slough sample locations compared to the relatively undeveloped Sengstaken Arm of South Slough. Thus, for example, while deer and elk provide about 21% of the *E. coli* contribution at the Sengstaken Arm site, greater numbers of colonies of this origin can be expected at the Pony Creek site because of the ten times greater total loading in Pony Creek. Similar projections and comparisons can be made for other species and groups.