APPENDIX A: *ESCHERICHIA COLI* RIBOTYPING AND WATER QUALITY MONITORING FOR THE COLCHESTER, VERMONT IWRMP, FINAL REPORT, 1ST YEAR, 2009 FIELD SEASON

Escherichia coli Ribotyping and Water Quality Monitoring for the Colchester, Vermont IWRMP

Final Report
1st Year (2009 Field Season)

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INTRODUCTION

One of the most common issues facing environmental managers concerned with surface water quality is fecal-borne microbial contamination and the threat of diseases to humans who come in contact with contaminated water or shellfish. For purposes of monitoring the sanitary quality of surface waters, fecal coliforms, enterococci and *Escherichia coli* have traditionally served as indicators of water quality for classifying waters to protect public health. However, as untreated sewage from inadequately designed wastewater treatment facilities has been eliminated or reduced in significance, the residual contamination that limits uses of surface waters is typically of unknown origin. Efforts to reduce contamination have often revolved around making a best guess of what potential sources may be significant, conducting extensive sampling programs, eliminating sources and then re-sampling surface waters to see if improvements in water quality have occurred. This process is expensive and oftentimes less fruitful than desired.

Recent adoption of biotechnological techniques for application to water quality issues has spawned a number of approaches to address identification of sources of fecal-borne contamination. These new approaches, often called "microbial source tracking" (MST), have been used successfully for well over 10 years in a number of areas in the U.S. Ribotyping of *E. coli* isolates cultured from target surface waters is one approach that can provide information on a wide range of potential sources of fecal contamination.

Various studies have reported on the use of ribotyping for tracking sources of fecal-borne microbial contaminants. The UNH/JEL lab has conducted well over 30 studies, mostly in NH and ME, but also in MA, VT and NY states. Starting with the 1st UNH study conducted in the Colchester area in 2000, these ribotyping studies have been conducted in freshwater watersheds and beaches, and marine and estuarine waters (Jones 2007, Jones 2008, Nelson et al. 2008).

Because ribotyping can provide information on the identity of source species of bacteria found in surface waters, follow-up efforts to identify and eliminate contamination sources can be directed towards those types of sources where the few species responsible for the most significant amounts of contamination can be targeted for management action. Through an iterative process of then finding possible sources of fecal contamination from significant species, ribotyping can be used again to match strains for a given species to specific sources. Thus, the overall effort to improve water quality can be targeted because the most significant sources actually found in surface waters of concern are directly identified and eliminated. Such an approach also provides significant savings of time and expense compared to traditional approaches.

Colchester, Vermont is a fast growing community just outside of Burlington, the state's largest city, and is itself the second most populated municipality in the state. Colchester faces continuing development pressure on its finite natural resources. The Town has 27 miles of shoreline, and shoreline development has been an important issue in Colchester's history. Centralized wastewater service areas are limited with the majority of the community currently served by on-site wastewater disposal systems, including all of the communities' shoreline areas. There are approximately 6,200 systems throughout the community. The Town of Colchester's stormwater management program complies with the General Permit for Small Municipal Separate Storm Sewer Systems (MS-4) issued by the State of Vermont Department of Environmental Conservation. The Town has conducted surface water quality testing in Malletts

Bay for the last decade, and has documented reoccurring problems with coliform pollution of surface waters.

Eight watersheds are currently listed as impaired under the Clean Water Act, Section 303(d) list. With the exception of the Lamoille and Winooski rivers on the boundaries of town, water quality impacts are mostly from within the town. Runoff into Malletts Bay – the sheltered part of Lake Champlain on Colchester's western edge – comes primarily from small streams whose watersheds are largely within Colchester and from direct overland flow. All water quality sampling associated with this project will occur in surface waters located in the Town of Colchester, Vermont.

Water quality problems with microbial contamination have caused problems in Malletts Bay in Lake Champlain, Vermont where consistent water quality problems for years have resulted in the posting of swimming beaches during summer recreation months. An earlier study (Jones 2002a) of Malletts Bay and the lower Winooski River included four sites used in the present study, where birds, pets, wild animals and human sources were identified (Table 1). Identification of the source(s) of the contamination from the water samples collected in this study, and in follow-up sampling planned for the next two years, will help direct management activities for eliminating significant sources of microbial pollution that limit recreational uses of the lake.

		2000	2004		2005		2006		2007		2008		Geomean
		Ribotyping study	Geometric	90th	Geometric	90th	Geometric	90th	Geometric	90th	Geometric	90th	order
Site#	Location	identified sources*	mean	percentile	mean	percentile	mean	percentile	mean	percentile	mean	percentile	descending
1	Delta Park Beach		24	82	13	53	34	224	22	67	37	249	2
2	Colchester Point		10	29	22	296							
3	Mills Point		8	81	9	162							
4	Porters Point		9	125	6	99	5	30	7	31	20	111	5
5	Camp Holy Cross		4	55	5	60	3	28					
6	Spalding West Beach		10	141	5	33	8	207	6	119	9	35	11
6A	Spalding West Culvert		45	1,267									
7	Moorings Stream		46	462	46	360	31	99	84	1,733	23	201	4
8	Smith Hollow Beach	cat,duck,coyote,human,raccoon	8	103	20	1,366	17	178	11	47	25	122	3
8A	Smith Hollow Creek		224	710	242	1,186							
9	60 West Lakeshore Drive	cat,gull,human,raccoon	7	43	13	96	17	101	10	39	15	29	7
10	4 West Lakeshore Drive		9	59	7	33							
11	Crooked Creek Beach		26	153	25	201	31	305	18	145	71	488	1
11A	Crooked Creek	raccoon,gull,cow	215	729	733	2,420							
B-1	Bayside Beach West	deer,raccoon,cat	11	69	14	152	8	36	28	204	18	35	6
B-2	Bayside Beach Center		25	180	22	191	7	29	24	216	14	68	8
B-3	Bayside Beach East		13	127	12	68	5	24	18	201	12	58	9
R-1	Rossetti Beach West								4	35	11	58	10
R-2	Rossetti Beach East								6	25	9	48	11

*Jones (2002a) ribotyping study; see references
Descending order of 2008 Geomean

Table 1. Geometric mean *E. coli* concentrations and identified source species at beach and watershed sites from 2000-08.

MATERIALS AND METHODS

The study area is the beach area of Malletts Bay in Colchester VT and the surrounding watersheds. Previous years of water quality monitoring provided data on *E. coli* concentrations that helped to focus the present study efforts (Table 1). Duplicate samples from 11 sites, including routine monitoring sites and sites previously shown to have high *E. coli* concentrations were collected from August 12 to September 2, 2009 by the Town of Colchester and processed for enumerating putative *E. coli* concentrations by membrane filtration at Endyne (Table 2). The MB2-BAY site at Bayside Beach was chosen from amongst three possible sites because birds were congregating there during the sample period. Up to 20 isolates from samples with *E. coli* concentrations >77/100 ml, or lower concentrations that represented the highest 3 or 4 concentrations on some sample dates, were shipped to UNH/JEL.

Monitored Sites			Ribotyping Study Samples							2009
Site #	Site Location	Site Designation	8/12/09 *E. coli* cfu/100 ml	8/17/09 *E. coli* cfu/100 ml	8/19/09 *E. coli* cfu/100 ml	8/24/09 *E. coli* cfu/100 ml	8/26/09 *E. coli* cfu/100 ml	8/31/09 *E. coli* cfu/100 ml	9/2/09 *E. coli* cfu/100 ml	Geomean* *E. coli* cfu/100 ml
1	Delta Park Beach	M1-DP	8	<2	4	4	3	27	17	6
2	Colchester Point									
3	Mills Point									
4	Porters Point	M4-PP	11	9	50	6	6	8	<2	8
5	Camp Holy Cross									
6	Spalding West Beach	M6-SW		14	<2	8	12	790	2	13
6A	Spalding West Culvert									
7	Moorings Stream	M7-MS	135	2	2	<2	160	22	<2	9
8	Smith Hollow Beach	M8-SH	6	6	8	10	4	530	11	13
8A	Smith Hollow Creek	M8A-SH	200	250	313	880	350	250	214	307
9	60 West Lakeshore Drive	M9-CT	131	2	8	3	15	93	5	12
10	4 West Lakeshore Drive									
11	Crooked Creek Beach	M11-CC	75	54	42	12	52	72	6	33
11A	Crooked Creek	M11A-CC	393	192	260	340	230	155	72	208
12	Malletts Creek	M12-MC		42	30	34	28	62	37	37
B-1	Bayside Beach West	MB1		9	27	8	6	8	4	8
B-2	Bayside Beach Center	MB2-Bayside	112	4	12	28	4	7	13	13
B-3	Bayside Beach East	MB3		6	3	6	2	8	7	5
R-1	Rossetti Beach West	MR1-Rosetti		8	6	<2	2	550	2	8
R-2	Rossetti Beach East	MR2-Rosetti		5	<2	<2	6	630	5	8

*All reported data below detection limits (<2 cfu/100 ml) were converted to 1.8 cfu/100 ml for calculating geometric mean concentration

Shaded cells represent samples with high enough *E. coli* concentrations that justified keeping isolates for possible ribotyping

Table 2. *E. coli* concentrations at beach and watershed sites during the study period.

Fecal samples from local source species were collected on November 11, 2009 and shipped on ice to UNH/JEL. Fecal samples were decimally diluted to 10^{-8}. Aliquots (2.5 ml) from the dilution tubes and water sample were filtered through membrane filters (0.45 µm pore size) and the filters placed onto mTEC agar. The agar plates were incubated at 37°C for 2 h then 44.5°C for 22 h. Yellow colonies were counted as fecal coliforms. Following urease testing on urea substrate, remaining yellow colonies were counted as *E. coli* and plates giving countable colonies were used for selection of *E. coli* strains for ribotyping analysis.

The *E. coli* colonies chosen were subject to a battery of biochemical tests to confirm their identity as *E. coli*. The procedures used for isolating and identifying *E. coli* strains for this study were according to standard lab protocols (Jones 2002b, Jones and Bryant 2004). After inspection of *E. coli* concentration trends at sites on different sample dates, a subset of samples were chosen

for ribotyping. The study team decided to use only five isolates from each source species and water sample to be ribotyped to allow for analysis of as many samples as possible.

The confirmed *E. coli* isolates were then analyzed for determining ribopatterns. *E. coli* isolates were stored in cryovials at -80°C and re-cultured onto trypticase soy agar (TSA). Cultures on TSA were incubated overnight at room temperature (20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C. The culture was then ready for ribotyping.

A RiboPrinter was used to analyze *E. coli* cultures for ribotype determinations. After preparation of the samples, the automated process involved lysing cells and cutting the released DNA into fragments via the restriction enzyme EcoR1. These fragments were separated by size through gel electrophoresis and then transferred to a membrane, where they were hybridized with a DNA probe and mixed with a chemiluminescent agent. The DNA probe targeted 5S, 16S and 23S ribosomal RNA genes. A digitizing camera captured the light emission as image data, from which the system extracted a RiboPrint® pattern. This pattern was initially compared to others in the RiboPrinter database for characterization and identification based on densiometry data. However, our source species identification analysis approach has conformed to other ribotyping studies in using banding patterns as the basis for comparing patterns.

Band Identification

The images were transferred from the RiboPrinter into GelComparII (Applied-Maths) analytical software. The bands in lanes containing the standard were labeled and entered into the memory for optimization of gel pattern images. The densiometry data were processed for band identification. The ribopattern data for each water sample isolate were then selected for identification of source species. The analysis of the Colchester water sample isolates for identification of source species was based first on the local source species database then on a more comprehensive regional database (Table 3). The local database consisted of 22 unique ribopatterns for *E. coli* isolates collected from 5 species and a septic system, all located in the study area watersheds. The regional database consisted of 1166 unique ribopatterns from 35 species and sources collected from the Northeast US. The term ‘unique (ribo)pattern’ reflects the occurrence of identical patterns for closely related strains and clonality of *E. coli* strains in individual animals or sources. The redundant patterns are not counted under ‘unique patterns’.

Species	REGIONAL			COLCHESTER		
	# Samples	# Ribotypes	# Unique Ribotypes	# Samples	# Ribotypes	# Unique Ribotypes
Alpaca	1	3	2	-	-	-
Buffalo	2	10	8	-	-	-
Cat	7	44	21	-	-	-
Chicken	5	33	25	-	-	-
Cormorant	8	48	25	-	-	-
Cow	11	89	68	-	-	-
Coyote	10	41	31	-	-	-
Deer	44	170	104	-	-	-
Dog	24	163	84	1	5	5
Duck	8	21	14	-	-	-
Fox	19	75	53	-	-	-
Goat	2	10	8	-	-	-
Goose	22	135	90	2	5	4
Horse	14	65	54	1	5	2
Human	8	115	54	-	-	-
Landfill Trash	4	20	20			
Mouse	1	3	2	-	-	-
Muskrat	5	32	17	-	-	-
Otter	3	14	9	-	-	-
Oxen	1	10	4	-	-	-
Pig	1	16	5	-	-	-
Pigeon	2	7	4	-	-	-
Rabbit	5	30	24	-	-	-
Racoon	31	79	61	-	-	-
Robin	1	4	2	-	-	-
Seagull	33	180	111	1	5	3
Septage	6	43	26	1	5	3
Sheep	2	8	5	-	-	-
Skunk	1	6	4	-	-	-
Sparrow	1	4	3	-	-	-
Starling	1	3	1	-	-	-
Unidentified Avian	2	20	14	-	-	-
Unidentified Wildlife	6	45	31	-	-	-
Wastewater	37	193	169			
Wild Turkey	3	17	13	1	5	5
Totals	331	1756	1166	7	30	22

Table 3. Local Colchester and regional source species databases.

Data Analysis

All data were analyzed with GelComparII software on a Dell computer, where the source species database was also stored. Similarity indices between the unknown isolates and the known source isolates were determined by using Dice's coincidence index. For this study, 1% band tolerance and 1.5% optimization settings were used. Both of these parameters are used to adjust the ability to differentiate between bands for the degree of accuracy desired, and also to compensate for possible misalignment of homologous bands caused by technical problems.

The source species profile with the best similarity coefficient at a given set of optimization and tolerance settings was accepted as an indication of the possible source species for the water sample isolate. For this study, the predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 90%. Thus, the identification of the source species was considered successful if the value calculated for a given water isolate was equal to or greater than the threshold value; if the calculated value was below the threshold similarity index, the water sample isolate was considered to be of unknown origin.

The decision for using a 90% similarity index threshold was based on the inter-gel variability within cumulative Dice's coincidence indices determined for *E. coli* positive controls run for every ribotyping study. Useful information is gained through this process to help guide management decisions and resource allocation for pollution source identification and elimination in the Colchester area.

Cluster analyses were performed to determine the relationships among isolates from the same source species and the same sites, as well as banding patterns that were identical for different isolates. The cluster analyses were based on the un-weighted pair group method by arithmetic averaging (UPGMA) or the neighbor joining algorithms.

The last step in data analysis is visual inspection of the band matching results. Hard copies of ribotype patterns and similarity coefficients for the unknown and most closely related source species are printed for interpretation. Interpretation and accompanying tabular representations of the data were done using MS Excel on Macintosh computers. The results of identification of source species are summarized according to both the actual and type of source species identified.

RESULTS & DISCUSSION

2009 *E. coli* concentrations and screening of samples for possible ribotyping

Previous year monitoring showed the highest *E. coli* concentrations were found in Crooked Creek (upstream of beach-M11A-CC) and in Smith Hollow Creek (upstream of beach-M8A-SH), even though monitoring at these two sites ended in 2005 (Table 1). Otherwise, the 2008 site geometric mean *E. coli* concentrations ranged from 9 to 71/100 ml, the latter concentration at Crooked Creek beach (M11-CC).

In 2009, results were similar to 2008 in that the highest *E. coli* concentrations were again observed at M11A-CC and M8A-SH, followed by Malletts Creek (M12-MC) and M11-CC, with geometric means of 37/100 ml and 33/100 ml, respectively (Table 2). Sample *E. coli* concentrations ranged from below detection (<2/100 ml) in eight samples to a high of 880/100 ml at M8A-SH on 8/24/09. Sample concentrations on 8/31/09 at four of the sites (M6-SW, M8-SH, MRI-Rosetti beach west, MR2-Rosetti beach east) were much higher than normal.

The sample *E. coli* concentrations formed the basis for deciding which samples would be used for ribotyping to determine sources of pollution under the worst conditions. The samples with the five highest *E. coli* concentrations, and all samples collected on 8/31/09, when concentrations were uniformly high at all sites, were chosen (Table 4). All samples available for two other priority sample dates, 8/12/09 and 8/24/09 were also chosen, and a sample from M7-MS collected on 8/26/09 was also chosen. This strategy provided samples over three dates for M11A-CC and M8A-SH, and coupled beach-upstream samples for these areas on 8/31/09.

Sample Date	Site #	*E. coli* conc. cfu/100 ml	# Isolates taken	# Isolates confirmed	# Ribotyped
8/12/09	M7-MS	135	20	2	2
8/12/09	M11-CC	75	20	7	5
8/12/09	M8A-SH	200	20	9	5
8/12/09	M11A-CC	393	20	6	5
8/12/09	M9-CT	131	20	2	2
8/12/09	MB2-BAYSIDE	112	20	6	5
8/17/09	M11-CC	54	20	none	
8/17/09	M8A-SH	250	20	16	
8/17/09	M11A-CC	192	20	none	
8/17/09	M12-MC	42	20	none	
8/19/09	M8A-SH	313	20	10	
8/19/09	M11-CC	42	6	2	
8/19/09	M11A-CC	260	17	3	
8/24/09	M8A-SH	880	20	6	5
8/24/09	MB2-BAYSIDE	28	16	5	5
8/24/09	M11A-CC	340	20	17	5
8/24/09	M12-MC	34	20	19	5
8/26/09	M7-MS	160	20	11	5
8/26/09	M8A-SH	350	20	19	
8/26/09	M11-CC	52	20	17	
8/26/09	M11A-CC	230	20	18	
8/31/09	M6-SW	790	20	4	4
8/31/09	M8-SH	530	20	11	5
8/31/09	M8A-SH	250	20	10	5
8/31/09	M11-CC	72	20	5	5
8/31/09	M11A-CC	155	20	15	5
8/31/09	MR1-ROSSETTI	550	20	12	5
8/31/09	MR2-ROSSETTI	630	20	6	5
9/2/09	M8A-SH	214	20	15	
9/2/09	M11A-CC	72	20	9	
		TOTAL	579	262	83

Shaded cells are the 5 highest *E. coli* concentrations

Table 4. *E. coli* concentrations and species confirmation success for samples recommended for ribotyping.

Source species sampling and choice of isolates for ribotyping

Source species samples were collected after the summer water sampling season in November 2009, and again in February and March 2010. The 23 samples were from 16 different locally occurring species or sources (Table 5). Some yielded no *E. coli*, though most samples with high levels of *E. coli* yielded a high fraction of confirmed *E. coli* isolates.

Sample Date	Source/species	Putative *E. coli* (cfu/g WW)	# Isolates taken	# Isolates confirmed	# Ribotyped
11/11/09	Septic system-1	424	20	20	5
11/19/09	dog-1	925,000	20	20	5
11/19/09	dog-2	119,000,000	20	19	0
11/19/09	geese-1	1,276,000	20	20	1
11/19/09	geese-2	29,600,000	20	20	4
11/19/09	gull-1	747	20	19	5
11/19/09	horse-1	8,091	20	20	5
11/19/09	wild turkey-1	88,235	20	19	5
2/10/10	grouse A	NG*	0	0	0
2/10/10	grouse B	NG	0	0	0
2/10/10	rabbit	416	10	6	0
2/10/10	avian	NG	0	0	0
2/10/10	deer A	122,324	20	20	0
2/10/10	deer B	2,000	20	18	0
2/10/10	coyote	77,232	20	20	0
2/10/10	muskrat	NG	0	0	0
3/16/10	red fox	NG	0	0	0
3/16/10	coyote	17,673	20	2	0
3/16/10	muskrat-1	14,925	20	20	0
3/16/10	muskrat-2	75.05	1	1	0
3/16/10	otter	NG	0	0	0
3/16/10	mink	NG	0	0	0
3/16/10	raccoon	82,659	20	20	0

*NG = no growth

No ribotyping has been conducted on any of the 2010 scat samples from 2/10 & 3/16

Source species	# isolates available	# isolate ribotyped	Unique patterns	Shared* patterns
Septic system	20	5	3	3
Dog	20	5	5	2
Goose	20	5	4	3
Gull	20	5	3	4
Horse	20	5	2	4
Wild turkey	20	5	5	1

*Patterns that are identical to patterns for other species

Table 5. Local source species sample *E. coli* concentrations and ribotyping characteristics.

No source species isolates were chosen for ribotyping from the samples collected during 2010 because the decision of which samples to include was made prior to those sample dates. Five isolates from each of the six November 2009 sources/species were chosen for ribotyping as an initial screening of the diversity and usefulness of these sources and samples. Identical patterns from the same sample are not useful for identifying sources from water sample isolates, though all sources but horses had at least three unique patterns from the five chosen isolates (Table 5), suggesting good diversity and the potential for getting new patterns from more isolates from these samples. The wild turkey had one pattern that was 'shared', or identical to a pattern from another source isolate from the local source species database, while dog had only two shared patterns, thus having higher potential for water sample isolates to match with a single source species and not with a mix of sources sharing the same pattern.

RT analysis using the local and regional databases

All chosen water sample isolates were ribotyped and then analyzed to determine source species using the database of local sources (Table 6). Several isolates did not match well with the RiboPrinter *E. coli* database of ribopatterns and were thus not considered to be *E. coli*. These included two of the five isolates from the 8/12/09 MB2-Bay sample, and both isolates from the 8/12/09 M9-CT sample. Overall, 23 of the remaining 74 isolates were identified to sources, i.e., they matched source species patterns at $\geq$90% similarity. The remaining unidentified isolates either matched local source species patterns at <90% similarity (33 isolates) or matched multiple, or 'mixed' source patterns at $\geq$90% similarity (18 isolates). There were only single isolate matches to the septic system and horse source patterns, no matches to the gull and dog patterns. The rest of the water sample patterns (21 isolates) matched to geese (12 isolates), wild turkey (8 isolates) or a mix of gull and goose patterns (1 isolate).

Site & date	TOTAL ISOLATES	Isolates identified	Goose	Wild turkey	Gull	Mix bird	Septic	Horse	Dog	**No identification** <90%	Mixed >90%
8/12/09											
M7-MS	2	2		1			1			0	
M11-CC	5	4	3	1							1
M8A-SH	5	1						1		3	1
M11A-CC	5	1	1							4	
MB2-BAY*	3	1	1							1	1
M9-CT**											
8/24/09											
M8A-SH	5	2	1	1						3	
M11A-CC	5	2		2						2	1
M12-MC	5	2	1			1				2	1
8/26/09											
M7-MS	5	0								1	4
8/31/09											
M6-SW	4	0								4	
M8-SH	5	3	2	1							2
M8A-SH	5	1	1							3	1
M11-CC	5	1		1						2	2
M11A-CC	5	2	1	1						1	2
MR1-ROS	5	1	1							2	2
MR2-ROS	5	0								5	
TOTAL	74	23	12	8	0	1	1	1	0	33	18
		31%								45%	24%

*2 of the 5 chosen isolates were not *E. coli,* according to the RiboPrinter

**the 2 available isolates were not *E. coli*

two species matched to the water sample pattern, gull and goose

Table 6. Ribotyping analysis using the local Colchester source species database.

The lack of any identified sources for some samples (M6-SW and MR2-Rosetti on 8/31/09) suggests the source(s) was not in the local database, or that an inadequate number of isolates from the sampled sources were included for the source species database. It appears that the goose and wild turkey source patterns were very useful for identifying sources while the septic system, horse, gull and dog patterns were not. The horse and gull isolates tended to share patterns with other species, while the dog and septic system isolates used may contain specific patterns that are not contributing to the pollution. Selecting only five isolates from each source is a minimum number and more patterns from these samples could be useful in identifying sources for more water samples.

Use of the full regional database, including the local source isolates, enabled identification of sources for a total of 39 isolates, or 53% of the total 74 isolates analyzed (Table 7). This is on the low end of acceptable results, given the tendency for patterns to be either unique and not match well with known source patterns (6 isolates) or to match with a mix of

species (29 isolates). Wild turkey and goose remained significant sources, though better matches to regional database sources were found for several isolates identified to these species using the local database. The regional database included patterns that provided matches to some of the same source species in the local database, including gull, wild turkey and dog. This suggests that use of more patterns from local sources of these species could be useful for identifying sources of pollution. Wild animals, including deer and especially fox, were also useful for identifying sources.

			COLCHESTER DATABASE									*REGIONAL + COLCHESTER DATABASE*								
	TOTAL	Isolates		Wild		Mix					**No identification**		Mix wild			Mix		Wild			Landfill
Site & date	ISOLATES	identified	Goose	turkey	Gull	bird	Septic	Horse	Dog	<90%	Mixed	>90%	animal	Fox	Deer	bird	Gull	turkey	Dog	Cow	trash
8/12/09																					
M7-MS	2	2		1			1														
M11-CC	5	4	3								1						1				
M8A-SH	5	4						1			1		1			2					
M11A-CC	5	3	1							1	1			1				1			
MB2-BAY*	3	2	1								1			1							
M9-CT**																					
8/24/09																					
M8A-SH	5	4		1							1						2		1		
M11A-CC	5	3		2							2					1					
M12-MC	5	2	1			1				1	2										
8/26/09																					
M7-MS	5	0									5										
8/31/09																					
M6-SW	4	0								1	3										
M8-SH	5	3	2	1							2										
M8A-SH	5	3	1							1	2			1						1	
M11-CC	5	2									2			1	1						
M11A-CC	5	3	1	1							2		1								
MR1-ROS	5	2	1							1	2			1							
MR2-ROS	5	2								1	2			1							1
TOTAL	74	39	11	6	0	1	1	1	0	6	29		2	6	1	3	3	1	1	1	1
		53%								8%	39%										

*2 of the 5 chosen isolates were not *E. coli*, according to the RiboPrinter
**the 2 available isolates were not *E. coli*
Source species pattern from an unknown wild animal scat sample

Table 7. Ribotyping analysis using the combined regional and Colchester source species databases.

Sources for samples with elevate *E. coli* concentrations can often be from a small number or even one source. The 24 isolates from the samples with the five highest *E. coli* concentrations had 11 isolates with identified sources, and no identified sources for the 8/31/09 M6-SW sample (Table 7). There were several identified sources, however, for the other four samples, suggested no dominant source at any of those sites. The inclusion of upstream and downstream samples at Crooked and Smith Hollow creeks on 8/31/09 showed geese at both sites in Smith Hollow and wild animal sources at both sites in Crooked Creek. This suggests possible upstream sources for contamination at the beach sites at these two locations. As suspected based on observation of congregating birds, the sources for the MB2-BAY sample included Canada geese.

Some of the 'mix' source isolates included no human sources, and can be considered as coming from non-human sources while other isolates matched to bird and wild animal sources, and can be considered as coming from non-human related sources. Using only single source or single type of source results, the identified sources can be separated into five types of sources, including human, pet, bird, wild animal and domestic animal sources. These source types

correspond to different management strategies for elimination or reductions of sources, and are thus useful for gauging what type of management strategy would be best for reducing the most significant sources.

For all samples, birds were the dominant identified source type, including 34% of the total isolates analyzed by the combined source species database (Table 8). Other studies in the region have shown birds to be the most significant sources at freshwater beaches (Jones 2008). The public health significance of bird-borne fecal pollution is not well known, but may have implications even for non-human species (Nelson et al. 2008). The other significant identified source type was wild animal, with 12% of the total isolates. In terms of management, these two types are the most difficult to manage, and the other identified sources are significant in that they can be eliminated with more straightforward management strategies, once the specific sources are found. The combined results for all samples at each study site shows four sites with identified sources from only one source type, with three of these being only bird sources and the other site with only human sources (Table 9). Four sites had both bird and wild animal sources, one site had no identified sources, one site had wild animal and human and the final site had all but human type sources.

	Vermont DB analysis		Regional DB analysis	
Source type	# of isolates	% of total	# of isolates	% of total
Human	1	1%	2	3%
Pet	0	0%	1	1%
Livestock	1	1%	2	3%
Bird	21	28%	25	34%
Wild animal	0	0%	9	12%
Identified	23	31%	39	53%
Unidentified	51	69%	35	47%
TOTAL	74		74	

Table 8. Source species types identified by analysis with both source species databases.

Site	# Isolates	# Identified	Bird	Wild animal	Human	Pet	Domestic animal
M6-SW	4	0			1		
M7-MS	7	2	1				
M8-SH	5	3	3				
M8A-SH	15	11	6	2		1	2
M9-CT	0	0					
M11-CC	10	6	4	2			
M11A-CC	15	9	7	2			
M12-MC	5	2	2				
MB2-BAY	3	2	1	1			
MR1-ROS	5	2	1	1			
MR2-ROS	5	2		1	1		

Table 9. Types of source species identified at each sampling site.

CONCLUSIONS & NEXT STEPS

These results provide a preliminary indication of what types of pollution sources may be significant in the study area, and what sampling strategies may be best in the upcoming field season. It appears that birds are significant sources, and wild animals, which were not part of the local database, may also be significant. The regional database was useful in identifying sources not included in the local database, especially wild animals. It also provided more source identifications for species included in the local database. As further samples are collected to enhance the local database, these results are a useful guidance. Furthermore, it would be extremely useful for new source species samples to be collected during the water sampling to enable better identification of source species from local sources. The regional database includes many source species that are not even in the study area, and the frequency of 'mix' source identification can be reduced with an improved, more locally representative local database.

The decision on where to sample in 2010 can be based on the *E. coli* concentrations found in 2009 throughout the swimming season; those data are not available at the time of this writing. The reported results can help to indicate where and when significant pollution events occur, and where more intensive investigation of pollution sources is required. Strategic use of study resources will require careful consideration of how many isolates to analyze from both source species and water samples. The strategy used in 2009 to collect and keep isolates from all possible samples, and then to decide on which to ribotype after all have been collected, helps to focus resources toward answering the most key questions, and addressing the most significant pollution conditions.

Analysis of other data, including that from the state and any lay monitoring programs, would be useful to help identify the most critical conditions for protecting recreational uses of the study area beaches and preventing pollution impacts. Weather data is also useful for focusing sampling efforts during potential weather induced pollution events.

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