Microbial Pollution Source Tracking at New Castle Beach

A final report to the
New Hampshire Department of Environmental Services

Submitted by

Dr. Stephen H. Jones
Jackson Estuarine Laboratory/Center for Maine Biology
Department of Natural Resources
University of New Hampshire
Durham, New Hampshire 03824

April 2007

This project was funded by the U.S. Environmental Protection Agency under the Beaches Environmental Assessment and Coastal Health (BEACH) Act, Assistance Agreement Number CU-9816000-1.

Acknowledgements

The author expresses gratitude to the following individuals who assisted with the collection and analysis of water samples and preparation and review of this report:

- Colin Edwards for processing scat and water samples for bacterial enumeration and ribotyping.
- The staff at NHDES for providing all the water and fecal samples.
- Sara Sumner, former DES Beach Program Coordinator, for providing review and comments to the draft report.

Table of Contents

List of Tables

List of Figures

Introduction

The Environmental Protection Agency (EPA) developed the Beaches Environmental and Coastal Health (BEACH) Act to better protect public health at our Nation's beaches. The act allows EPA to award coastal and Great Lakes states funding for development and implementation of comprehensive beach monitoring programs. New Hampshire initially received funding in 2002, with subsequent funding in later years. Funding allowed the New Hampshire Department of Environmental Services (DES) Beach Program to enhance its coastal monitoring to include 15 beaches, up from nine in previous years.

Based on historical sample data, New Hampshire's coastal beaches meet the state water quality standards for primary contact recreation. The DES Shellfish Program continually conducts and updates sanitary surveys along the Atlantic Coast. Numerous potential and actual bacteria sources have been identified. The DES Watershed Assistance Section continues to investigate these sources under base flow and wet weather conditions. The DES Shellfish Program has been proactive in investigating potential sources affecting shellfish beds along the Atlantic Coast. Specifically, the studies use a microbial source tracking technique called ribotyping to identify the specific bacterial sources. The technology results in source specific identification such as humans, dog, goose or other animal fecal sources.

Project Setting

The Beach Program initiated an investigation of a pipe that discharges bacteria laden waters to the New Castle Town Beach area using microbial source tracking (MST) conducted at the University of New Hampshire Jackson Estuarine Lab. The MST technique, ribotyping, defines sources of fecal pollution in waters. The results of the investigation will be used to reduce and eliminate bacterial sources to New Castle Town Beach.

The Beach Program has routinely monitored bacteria concentrations from the pipe since 2003. On most occasions, bacteria concentrations have exceeded state water quality standards for Enterococci at public beaches (Figure 1). The pipe outfall was either closed or obstructed by the tide during the 2005 sampling season. Therefore, only two samples were collected, both of which were well below the state standards. However, due to the excessively high bacteria concentrations previously recorded, further research was warranted.

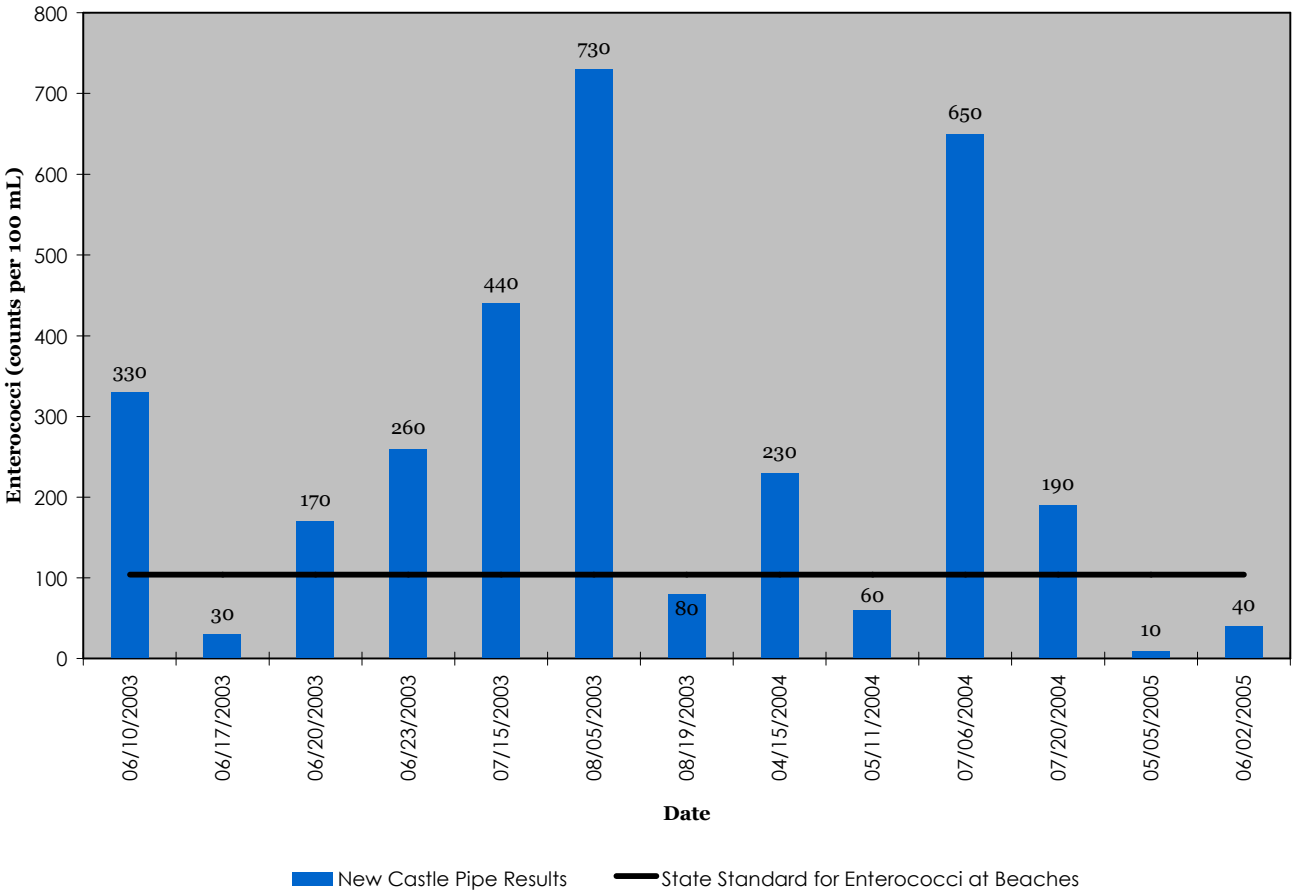

Figure 1. New Castle Town Beach: Pipe Results, 2003-2005.

The pipe discharges outside of the recognized beach area at New Castle Town Beach to a small, rocky tidal access area (Figure 2). This area can be accessed from the road or from New Castle Town Beach and is frequently used by town residents. Inspectors have observed people recreating in the water near the discharge of the pipe. During low tide, currents were observed transporting pipe discharge waters around the rock jetty to the beach area. During high tide the discharge travel distance and time are significantly decreased, increasing the potential for contamination. Because the potential for contamination exists, the Beach Program deemed it necessary to identify the sources of bacteria affecting coastal water quality.

The pipe discharges from what appears to be a small wet detention pond in the residential area adjacent to the beach. The Beach Program has not yet identified the pond watershed but the watershed for New Castle Town Beach has been delineated (Figure 3).

Figure 2. New Castle Town Beach Sampling Stations.

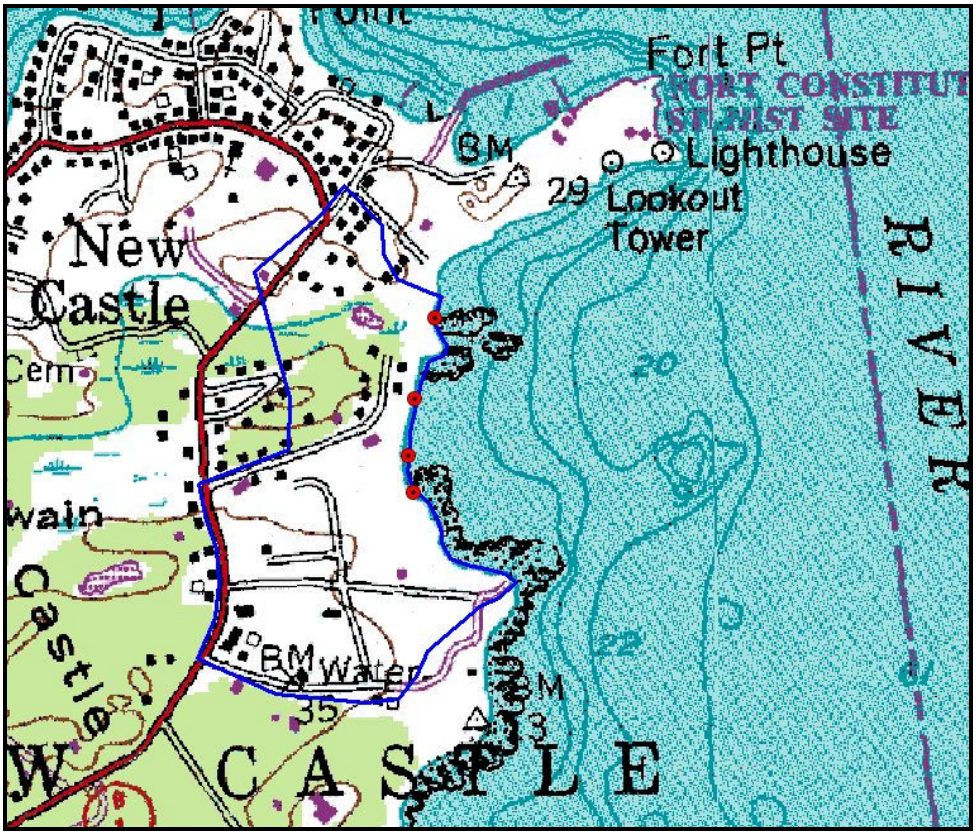

Figure 3. New Castle Town Beach Watershed.

Methods

Field Sampling

Water samples were collected on 8 dates during the summer of 2006 (Table 1; shaded cells represent samples used for ribotyping). Rainfall conditions were determined using the UNH weather station data (.http://www.weather.unh.edu/). Samples were collected during both high and low tide.

Table 1. Water sample collection dates, sites and conditions

Date	Condition	Tide	NWCLF	NWCCR	NWCRT	NWCPIPE
7/7/2010	dry	High	x	x	x	
7/19/2010	dry	Low				x
7/21/2010	dry	Low				x
8/4/2010	dry	Low	x	x	x	x
8/11/2010	dry	High	x	x	x	
8/17/2010	dry	Low	x	x	x	x
8/29/2010	wet	Low	x	x	x	x
9/7/2010	dry	High	x	x	x	

Fecal samples from suspected local source species were collected on four dates during the summer and fall of 2006 (Table 2). A total of eight samples were collected from five different source species.

Table 2. Sample dates for collection of fecal samples from local source species.

Sample ID	Local Species	Sample Date
GL3	Seagull	8/8/2006
UNK1	Deer	8/8/2006
UNK2	Deer	8/8/2006
DO1	Dog	10/9/2006
GL1	Seagull	10/25/2006
UNK1	Seagull	10/25/2006
UNK2	Unk. Wildlife	10/25/2006
DO1	Dog	12/12/2006

Laboratory and Analytical Methods

Detection and Identification of Fecal Coliforms and E. coli

Appropriate volumes of water samples were filtered to give at least 20 colonies on agar plates, where possible. The membrane filters were rolled onto mTEC agar in Petri dishes. Plates were inverted and incubated at 44.5±0.2 °C for 24 hours (USEPA, 1986). Fecal coliforms were enumerated by counting the yellow colonies after the incubation period, and *E. coli* was enumerated by counting the yellow colonies on the plate following incubation of the filter on urea substrate (Jones and Bryant 2002, Rippey et al. 1987). In several cases, the number of colonies on plates was too low to supply 20 isolates for speciation, and the remaining water in sample bags was re-filtered the next day to provide more colonies.

Following urease testing, each plate was inspected and the plate giving countable (20-60) colonies was used for selection of individual *E. coli* strains for analysis. For some samples, fewer than 20 colonies were present on the smallest dilution analyzed, so the plate with the most numerous colonies was used. The *E. coli* isolates 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 (Landry 2004, Jones 2002a, Jones and Bryant 2002). The confirmed *E. coli* isolates were then processed for determining ribopatterns.

Sample Processing

The procedures used for ribotyping *E. coli* isolates for this study have been used previously (Jones et al. 2004 a&b, Jones and Landry 2003, Jones, 2002b) and are based, to a large extent, on those of Parveen et al. (1999). *E. coli* isolates were stored in cryovials at -80°C and re-cultured onto trypticase soya agar (TSA). Some of the stored isolates could not be re-cultured. 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.

A RiboPrinter® was used to process *E. coli* culture 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 could be compared to others in the RiboPrinter® database for characterization and identification based on densiometry data, although our approach has conformed to other ribotyping studies in using banding patterns as the basis for comparing patterns.

Band Pattern 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

Table 3. Source species databases for the region, the NH Atlantic coast and locally for this study.

	REGIONAL			ATLANTIC COAST			NEW CASTLE		
Species	# Samples	# Ribotypes	# Unique Ribotypes	# Samples	# Ribotypes	# Unique Ribotypes	# Samples	# Ribotypes	# Unique Ribotypes
Alpaca	1	3	2	-	-	-	-	-	-
Buffalo	2	10	8	-	-	-	-	-	-
Cat	7	44	21	2	8	5	-	-	-
Chicken	5	33	25	3	13	12	-	-	-
Cormorant	6	14	10	6	14	10	-	-	-
Cow	11	89	68	-	-	-	-	-	-
Coyote	10	41	31	1	4	4	-	-	-
Deer	44	170	104	21	72	53	2	20	15
Dog	24	163	84	9	59	30	2	20	9
Duck	8	21	14	6	14	9	-	-	-
Fox	19	75	53	13	33	28	-	-	-
Goat	2	10	8	-	-	-	-	-	-
Goose	19	98	73	9	41	29	-	-	-
Horse	14	65	54	1	10	6	-	-	-
Human	8	115	54	-	-	-	-	-	-
Mouse	1	3	2	-	-	-	-	-	-
Muskrat	5	32	17	4	22	13	-	-	-
Otter	3	14	9	3	14	9	-	-	-
Oxen	1	10	4	-	-	-	-	-	-
Pig	1	16	5	-	-	-	-	-	-
Pigeon	2	7	4	-	-	-	-	-	-
Rabbit	5	30	24	5	30	24	-	-	-
Racoon	31	79	61	26	65	49	-	-	-
Robin	1	4	2	-	-	-	-	-	-
Seagull	15	90	58	12	71	45	3	29	16
Septage	5	32	23	3	16	14	-	-	-
Sheep	2	8	5	-	-	-	-	-	-
Skunk	1	6	4	1	6	4	-	-	-
Sparrow	1	4	3	-	-	-	-	-	-
Starling	1	3	1	-	-	-	-	-	-
Unidentified Avian	1	5	5	-	-	-	-	-	-
Unidentified Wildli	6	45	31	3	30	26	1	10	9
Wastewater	33	166	148	16	73	65	-	-	-
Wild Turkey	3	17	13	-	-	-	-	-	-
Totals	298	1522	1028	144	595	435	8	79	49

were processed for band identification using a minimum threshold for band detection of one percent. The ribopattern data for each separate water sample isolate were then selected for identification of source species.

Source Species Database

The analysis of the water isolate ribopatterns for identification of source species was based initially on a local source species database from the study sites and then on a New Hampshire source species database (Table 3). The local database for the beach study area contained ribopatterns from each of eight feces samples from three sea gulls, two deer, two dogs and one unknown wild life sample. There were 20 *E. coli* strains isolated from each sample, from which 10 were ribotyped. The Atlantic coast database contained 435 unique ribotypes from 19 different source species, including wastewater,

septage and direct human sources and unidentified wild animal (Table 3). The Regional database contained 1028 unique ribotypes from 34 different source species. Both the Atlantic coast and the Regional databases included the local database isolates

Data Analysis

All ribotyping data were analyzed with GelComparII software. Hard copies of ribotype patterns and similarity coefficients for each unknown water isolate and its most closely related source species were printed for interpretation. Interpretation and accompanying graphical representations of the data were done using MS Excel.

Optimization was set at 1.50 percent and band position tolerance was set at 1.00 percent. Both of these parameters relate to 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. Tolerance and optimization settings can be modified to influence the similarity coefficient used and result in a greater number of identified source species. However, a balance is required between stringency of data analysis parameters, the fraction of isolates that can be identified and consistency of methods between studies. The use of a QA *E. coli* strain (ATCC #51739) in the analysis for this study and comparison to past analyses of this strain gave acceptable (90%) matching of resulting ribopatterns.

Similarity indices between sample and database ribopatterns were determined using Dice's coincidence index (Dice, 1945) and the distance among clusters calculated using cluster analysis. The source species profile with the highest similarity coefficient 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%. If the value calculated for a water isolate was below the threshold similarity index, the water sample isolate was considered to be of unknown origin.

Cluster analyses were performed to determine the relationships among isolates from the same source species and the same sites, and to identify 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 was visual inspection of the band matching results. Hard copies of ribotype patterns and similarity coefficients for the unknown and most closely related source species were printed for verification of statistical analyses and further interpretation. Data analysis and accompanying tabular representations of the data were done using MS Excel.

RESULTS AND DISCUSSION

Bacteria Concentrations

In most cases, the filtration and analysis strategy for enumerating and isolating *E. coli* was effective. However, the NWCPIPE site often had unusual growth on mTEC agar using 50 ml and 5 ml dilutions. In these cases, growth of *E. coli* was inhibited by high levels of unidentified purple mucoid, lactose-fermenting bacteria that also grew at 44.5° C and completely covered the plate. Plates with high purple colony background growth also featured larger greenish yellow colonies presumed to be *E. coli* colonies that could not be verified by the urea test. These colonies were picked from the overgrown plates and streaked to mTEC agar for selective isolation of *E. coli*. Growth from the isolated presumptive *E. coli* colonies was yellow on mTEC, biochemical analyses were typical, and ribotyping identified selected isolates as *E. coli*.

In cases with high levels of background growth, *E. coli* was enumerated using a dilution of 2.5 ml of a 10^{-1} solution of sample to sterile deionized water. Plates using this dilution had countable yellow *E. coli* colonies as well as several small purple colonies in low enough concentration that they did not inhibit *E. coli* growth. NWCPIPE samples with high background growth were observed on 7/18/06, 7/20/06, and 8/3/06. In addition the 8/28/06 NWCPIPE sample had elevated levels of background growth on the 5 ml dilution plates but not enough to inhibit enumeration and isolation of *E. coli*. For NWCLF samples with low *E. coli* concentrations, filtration of more water the next day provided enough colonies for ribotyping ten isolates, except for the sample collected on 9/6/06 that yielded only 3 colonies.

E. coli concentrations in beach water samples were measured (Table 4). Concentrations ranged from <0.4 to 1,480 *E. coli*/100 ml. The *E. coli*:FC ratios for all samples combined was 99%, with individual sample ratios ranging from 50-100% (data not shown). A relatively high *E. coli* concentration (1480 cfu/100 ml) was measured in a sample from the pipe on 8/28/06. The *E. coli* concentrations in the one sample collected during wet weather events were not substantially greater than concentrations measured in the dry weather samples. Little difference in *E. coli* concentrations were observed in samples from high and low tide. These data show relatively low *E. coli* concentrations that are nonetheless in excess of standards at least some of the time at the pipe. The sampling did not reveal an extensive contamination problem at the beach sites, with only one sample exceeding the state standard.

Table 4. *E. coli* concentrations and geometric means (cfu/100 ml) for water samples collected from New Castle Beach: 2006.

*Only beach samples sites used; pipe samples excluded.
Shaded cells represent samples used for ribotyping.

Date	Condition	Tide	Site NWCLF	NWCCR	NWCRT	NWCPIPE
7/7/2010	dry	High	1	2	0.4	
7/19/2010	dry	Low				400
7/21/2010	dry	Low				800
8/4/2010	dry	Low	1	3	1	200
8/11/2010	dry	High	7	3	3	
8/17/2010	dry	Low	0.4	0.4	0.4	2
8/29/2010	wet	Low	6	14	670	1480
9/7/2010	dry	High	3	41	66	
Geometric mean						
Overall		8	2	4	5	180
Wet			6	14	670	1480
Dry			2	3	2	106

The choice of samples for ribotyping was based on *E. coli* concentrations, where samples collected on the two dates with the highest concentrations were selected along with samples collected from the pipe on three other dates where *E. coli* concentrations were also elevated (Table 4; shaded cells). A total of 10 water samples were used for ribotyping to determine source species identification.

Local Feces Samples and Source Species Database

Feces samples from the beach study area included those from three sea gulls, two samples each from dogs and deer, and a sample of an unidentified wild animal (Table 5). The *E. coli* concentrations (cfu per g wet weight) ranged from 1×10^2 for a dog sample to 3×10^{10} for the unidentified wild animal. The *E. coli* concentrations in descending order was unidentified wild animal >> deer > sea gull ~ dog > dog. The averages for all samples for a given species showed relatively similar concentrations for the sea gulls, dogs and deer (Table 5).

Table 5. Ribotyping summary for *E. coli* isolates from feces samples collected from New Castle Beach.

*Combined cluster analysis of all non-clone *E. coli* isolets from all source species

Fecal Sample Notes: GL# (8/8) sample on swab, vortexed into tube of BPW, yielded 9 colonies on 10^0 plates. UNK1 & UNK2 (8/8) plates were TNTC at the highest dilution (10^{-4} tube, 10 ml) used 200 cfu for calculation. GL1 (10/25) sample on plastic applicator in Whirlpak, rinsed into 10^0 tube, 10^{-2} plate yielded 20 colonies

Sample ID	Local Species	Sample Date	Concentration *E.coli* / g ww	# Isolates Ribotyped	# Isolates Identified as *E.coli*	# Unique Patterns Excluding Clones	Fraction Unique/ Total
GL3	Seagull	8/9/10		9	9	5	0.56
UNK1	Deer	8/9/10	2.00E+06	10	10	7	0.70
UNK2	Deer	8/9/10	2.00E+06	10	10	8	0.80
DO1	Dog	10/10/10	1.11E+02	10	10	5	0.50
GL1	Seagull	10/26/10		10	10	4	0.40
UNK1	Seagull	10/26/10	1.93E+05	10	10	7	0.70
UNK2	Unk. Wildlife	10/26/10	2.80E+10	10	10	9	0.90
DO1	Dog	12/13/10	1.31E+05	10	10	4	0.40
TOTALS				**79**	**79**	**49**	**0.62**
			POOLED ANALYSES				
	Total Species						
	Deer	2	2.00E+06	20	20	15	0.75
	Dog	2	6.56E+04	20	20	9	0.45
	Seagull	3		29	29	16	0.55
	Unk. Wildlife	1	2.80E+10	10	10	9	0.90
TOTALS			**Species combined:**	**79**	**79**	**49**	0.62
TOTALS			**All unique combined:**		**49**	**40**	

There were 20 *E. coli* strains isolated from each sample and 9-10 isolates from each feces sample were ribotyped, providing a total of 79 local ribotypes to be used for identifying source species. The ribopatterns contained 8-13 bands. Some of the resulting ribopatterns were identical amongst isolates from the same sample. These duplicate patterns were excluded from the local source species database. The final number of unique patterns used in the database was 49, or 62 percent of the total isolates (Table 5), although some of the unique patterns were shared between species, reducing the actual number of unique patterns to 40 when all isolates were included in the cluster analysis.

Ribotyping Success & Source Species Identification

There were 93 isolates from water samples collected at the pipe and the three beach sites that were analyzed using the RiboPrinter®, all of which yielded results confirmed by biochemical tests as *E. coli* (Table 6). The ribopatterns contained 8-12 bands. Cluster analysis of banding patterns from each sample showed a total of 68

unique ribopatterns. However, cluster analysis of all 93 isolates together showed 63 unique patterns because some patterns were shared between sites.

Table 6. Ribotyping success (≥90% similarity) for *E. coli* isolates from New Castle Beach.

Total unique ribopatterns from cluster analysis of all water sample isolates = 63
Two patterns included 10 or 13 isolates from the pipe, center and right beach sites

Site	Geometric mean *E.coli* cfu/100 ml	# of samples	Total isolates	Usable Ribotypes	Unique ribotype patterns	Identified ribotypes: Local database	Identified ribotypes: Atlantic Coast database	Identified ribotypes: Combined +Regional
NWCLF	2	2	13	13	13	3	10	10
NWCCR	4	2	20	20	17	4	4	7
NWCRT	5	2	20	20	12	5	6	9
NWCPIPE	180	4	40	40	26	12	18	19
Total	8	10	93	93	68	24	38	45

Banding patterns for water sample and source species isolates were considered to be the same if there was 90 percent or greater similarity. Initial analysis using only the local database resulted in 24 source species identifications, or 26 percent of the 93 isolates (Table 6). The Atlantic coast database included all of the local database patterns and also had more species and overall patterns. Further analyses using the Atlantic coast database resulted in 38 source species identifications, or 41% of the 93 isolates. The Regional database included all of the local and Atlantic coast database patterns and had more species and overall patterns. Further analyses using the Regional database resulted in 45 source species identifications, or 48 percent of the 93 isolates. For three of the seven isolates best identified using the Regional database, the source species were the same as the best (with <90% similarity) match found using the other smaller databases. All results presented are for analyses where the Regional database was used to improve the results found with the local and Atlantic coast databases.

The resulting 48 percent identification based on using a threshold of 90 percent provided a relatively good balance between accuracy and isolate identification. Comparison of these results with previous studies showed it to be on the low end of degree of identification (Table 7). This may be in part a result of not including some significant source species in the local database, as 24 percent identification using the local database is relatively low.

There were seven (8%) of the isolates that matched database patterns at <90 percent similarities and were thus considered to be from unknown sources. These "unknown" source isolates may be from source species that were not included in the database, or from included species that lacked enough diversity of ribopatterns in the database to provide an identification of adequate accuracy.

There were also 41 (44%) isolates with ribopatterns matching database patterns shared by multiple, unrelated species. These were categorized as "mixed" source species, considered successful identifications (matching at >90% similarity) but included in the "unidentified" category. There are several reasons this may occur. Some *E. coli* strains may be adaptable to multiple types of environments and be common strains in numerous

Table 7. Ribotyping success in recent microbial source tracking studies conducted by UNH in coastal New Hampshire and southern Maine.
*Non-automated ribotyping method used; RiboPrinter used in other studies

Study	Similarity index	# of isolates ribotyped	Total isolates identified
Jones and Landry (2003) Hampton Harbor study	80*	390	62%
Jones and Landry (2003) Varney Brook study	85*	192	59%
Jones 2003 Webhannet River, Wells ME	80*	270	53%
Jones (2003) Freeport ME	90	291	60%
Jones 2004 MBLR watershed, Wells ME	90	98	66%
Jones, Summer & Connor (2003) Atlantic beach study	90	110	56%
Jones & Landry, Feb 2004, Little Harbor & Atlantic Coast	90	87	54%
Jones, April 2003, stormwater pipes	90	59	78%
Jones 2004 Little Harbor TMDL study	85	44	68%
Jones, Landry, Soule. July 2004, Great Bay	90	259	60%
Jones et al. 2005 Cains Brook, Seabrook	90	283	53%
Jones 2006 Garrison Brook, Dover	90	60	78%
Jones 2006 Freshwater beaches	90	75	73%
Jones et al. 2006 Berry Brook, Rye	90	90	52%
Jones 2007 Crommet Creek, 2007	90	230	70%
This study	90	93	48%

different source species. Alternatively, some strains found in fecal material from different source species may be transient strains that are only there for a relatively short period of time. The mechanism of introduction could be ingestion and digestion of prey organisms, exposure to the feces of other species at landfills or sewage treatment facilities, or even coexistence of multiple species in the same area, like pets and humans or wild animals with overlapping habitats.

A closer inspection of the results can shed some light on the reason for the low level of identification. Twelve, or 29 percent of the "mixed" ribopatterns were similar to database ribopatterns at <100 percent similarity, suggesting that the exact matching pattern was not in the database and may be associated with yet to be identified source(s) in the local area, or, again, patterns for included species that are missing from the database. Conversely, 26 of the "mixed" ribopatterns matched at 100 percent similarity to patterns for multiple species in the local database, suggesting that one of the species included in the local database could be the source of these isolates. All of these patterns

had only 8 DNA bands, a minimal number of bands for ribotyping and many of the bands are shared by most of the other patterns.

The existence of different strains with the same profile can also imply that ribotyping with a single restriction enzyme may give inadequate detail to differentiate all strains. One alternative strategy is the use of a second restriction enzyme in the digestion of *E. coli* DNA that cuts the chromosomal DNA at different sites. The additional information that is provided by using two profiles for each *E. coli* isolate has greatly reduced this problem and made ribotyping more useful (Jones et al. 2006, Jenkins et al. 2003, Hartel et al. 2002, Samadpour 2002), however, it is a more expensive procedure.

Overall, there were 11 different source species identified, including all those sampled from the local study areas (Table 8). Two other categories were also included as successful identifications, unidentified wild animal and unidentified livestock. These two categories included actual unidentified feces isolate patterns and patterns that were shared amongst more than one species within the type of source species (wild animal, livestock).

Table 8. Source species identified for water samples collected from New Castle Beach: 2006.

NWCPIPE	**Humans**		**Wild animals**					**Birds**			**Pets**	**Livestock**		
Date	wastewater	coyote	deer	rabbit	raccoon	unidentified	duck	goose	sea gull	dog	unidentified	ID'd	Total
7/18/06	1		1						2			4	10
7/20/06	1	1		2		2						6	10
8/3/06			2						1			3	10
8/28/06						1			4	1		6	10
site total	2	1	3	2	0	3	0	0	7	1	0	19	40
NWCLF													
Date	wastewater	coyote	deer	rabbit	raccoon	unidentified	duck	goose	sea gull	dog	unidentified	ID'd	Total
8/28/06	2					1	1		3	1		8	10
9/6/06					1	1						2	3
site total	2	0	0	0	1	2	1	0	3	1	0	10	13
NWCCR													
Date	wastewater	coyote	deer	rabbit	raccoon	unidentified	duck	goose	sea gull	dog	unidentified	ID'd	Total
8/28/06			2					1	1			4	10
9/6/06			1					1	1			3	10
site total	0	0	3	0	0	0	0	2	2	0	0	7	20
NWCRT													
Date	wastewater	coyote	deer	rabbit	raccoon	unidentified	duck	goose	sea gull	dog	unidentified	ID'd	Total
8/28/06						1			1		3	5	10
9/6/06						1	1		1	1		4	10
site total	0	0	0	0	0	2	1	0	2	1	3	9	20
	wastewater	coyote	deer	rabbit	raccoon	unidentified	duck	goose	sea gull	dog	unidentified	ID'd	Total
overall total	4	1	6	2	1	7	2	2	14	3	3	45	93

The percentage of isolates for which source species were successfully identified was 48 percent (19/40 isolates) for NWCPIPE, 77 percent (10/13 isolates) for NWCLF,

35 percent (7/20 isolates) for the NWCCR and 45 percent (9/20 isolates) for NWCRT (Table 8).

The most commonly identified source species was sea gull (14 isolates), followed by unidentified wild animals (7), deer (6), wastewater/human (4), dog and unidentified livestock (3), rabbit, duck and goose (2), with single isolates identified as coming from coyote and raccoon. The number of different species identified as sources at each site was seven for the pipe, six for the left side of the beach (NWCLF), three for the center of the beach (NWCCR) and five for right side of the beach (NWCRT) (Table 8). The number of isolates identified for each source species was relatively even for the beach sites, but was dominated by sea gulls (7/19 isolates) at the pipe. Sea gulls were the only source species identified at each site; dog and unidentified wild animals were identified at three sites, while several species were identified at two sites (wastewater, deer, and duck).

Types of Identified Source Species

Any management actions taken in response to the results of this study would hinge on what types of source species were deemed significant sources of pollution. Because of this, a useful approach for analyzing results is to group source species into types that would trigger different management actions. The different types include humans, pets, domestic animals/livestock, wild animals and birds (Table 3). Overall, birds were the most prevalent (20%) source species type, followed by wild animals (18%), humans (4%), and pets and livestock (3%) (Table 9, Figure 4).

Type/Site	Overall	NWCPIPE	NWCLF	NWCCR	NWCCT	all ID'd
Human	4%	5%	15%	0%	0%	9%
Wild animals	18%	23%	23%	15%	10%	37%
Pets	3%	3%	8%	0%	5%	7%
Birds	20%	18%	31%	20%	15%	41%
Livestock	0%	0%	0%	0%	15%	7%
Unidentified	52%	52%	23%	65%	55%	0%

Table 9. Identified source species types for *E. coli* from New Castle Beach.

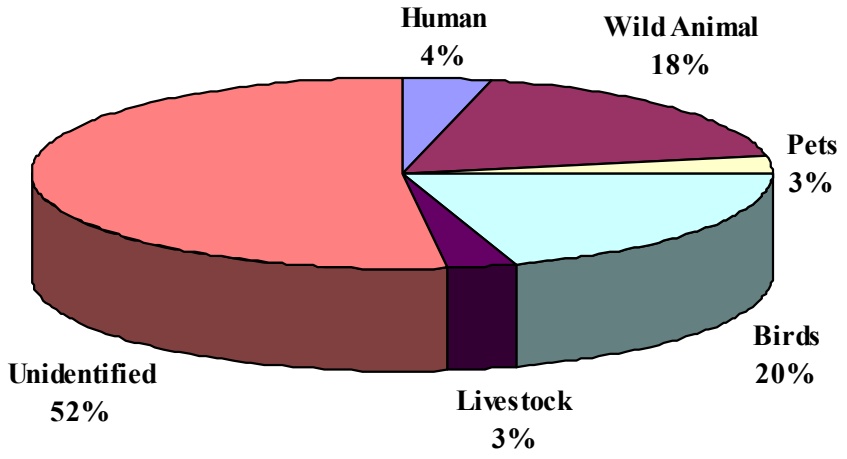

Figure 4. Identified source species for *E. coli* at New Castle beach.

Human, livestock and pet source isolates were only detected at low levels, except for the unidentified livestock isolates at NWCRT, and appear to have been insignificant sources of contamination at the beach on the sample dates. Birds and wild animals were the most significant source types at all sites. This profile of birds and wild animals being the most significant types of source species is relatively unique compared to most other MST studies conducted in the New Hampshire Seacoast area. A more common profile of wild animals and humans as the most prevalent source species and pets, birds and domestic animals being of lower significance has been observed in other coastal MST studies (Jones and Landry 2003 & 2004, Jones et al. 2004b). These results suggest that typical management actions that could be taken to reduce manageable sources (pets, humans, and livestock) will not have much effect on the pollution sources at this site.

Water Samples with Elevated *E. coli* Concentrations

Five water samples contained relatively high levels ($\geq$ 200 cfu/100 ml) of *E. coli*, ranging from 200 to 1480 *E. coli*/100 ml (Table 10). The identified source species were quite variable, although a third of the identified isolates (8/24 isolates) were from sea gulls. The profile of identified types of source species was similar to the overall results in that wild animals and birds were the most prevalent types, and humans, pets and livestock were relatively insignificant source types.

Table 10. Identified source species and types for water samples containing elevated *E. coli* concentrations.

Date	Site #	*E. coli*	Humans	Wild animals				Birds	Pets	Livestock
		concentration	wastewater	coyote	deer	rabbit	unknown	seagull	dog	unknown
7/18/06	NWCPIPE	400	1		1			2		
7/20/06	NWCPIPE	800	1	1		2	2			
8/3/06	NWCPIPE	200			2			1		
8/28/06	NWCPIPE	670					1	4	1	
8/28/06	NWCRT	1480					1	1		3
	TOTAL	**576**	**2**	**1**	**3**	**2**	**4**	**8**	**1**	**3**

Humans	2	4%
Wild animals	10	20%
Pets	1	2%
Birds	8	16%
Livestock	3	6%
Unidentified	26	52%
Total	50	

Conclusions

The local source species database was invaluable for identifying source species. Many isolates could be assigned source species using the local database alone, while the Atlantic coast and Regional databases helped to augment source species identifications for species not included in the local database.

The overall level of detection (48%) was relatively low. In other ribotyping studies conducted in New Hampshire, higher levels of identification have been observed. The EPA MST Guide Document (USEPA 2005) cites results from an *E. coli* ribotyping study in Virginia where 65% of isolates were identified to source species. The low level of detection was, to a large degree, due to the numerous isolate patterns that contained only 8 DNA bands. Patterns with 8 DNA bands represent the low end of diversity, as the 8 bands tend to be conserved regions of the genome and are therefore common to most patterns. The practical implication for this is that these patterns for water sample isolates tend to match multiple isolate patterns, and thus multiple source species, even in the local database. This makes source identification impossible.

The most common types of source species were birds and wild animals, the most difficult types of fecal sources to manage. However, the *E. coli* concentrations were relatively low for all beach samples, suggesting that these sources may not pose a large threat to human health.

Human sources of indicator bacteria at the discharge pipe and left beach station are a public health concern. Although E. coli levels at the left beach station were not elevated during the study period, historical ambient monitoring by the DES Beach Program staff revealed elevated Enterococci concentrations during several past monitoring events. Even though Enterococci are the recommended indicator organisms

for coastal waters due to their survival rate in saline waters, both indicators are fecal borne and typically occur in proportional concentrations in marine and estuarine waters, as seen with all of the monitoring programs in the New Hampshire seacoast where both are measured.

A developed lot with a single home that abuts the pipe was identified as a potential human source to the pipe. DES personnel observed that this site experienced flooding during high wetfall, likely reducing the effectiveness of the leach field operation.

The study showed that dogs were also identified as a source species in pipe and beach waters. Dogs are prohibited from on New Castle Beach during the summer beach season, however, they are allowed onto the beach during the off season. Dog walkers access the beach via a small access site by the pipe where dog feces were observed during the investigation. DES recommends that an education initiative concerning the public health risks of pet wastes may help better inform the New Castle residents of the consequences of allowing pets to access the beach area. The installation of pet waste stations at beach entrances and common area should encourage dog walkers to pick up and properly dispose of their pet's waste and reduce bacteria populations around the beach area.

The low level of *E. coli* contamination at the beach sites, despite the elevated levels more commonly observed in the pipe, suggests that the pipe may not have been a significant contamination source at the time of this study.

References

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26:297–302.

Hartel, P.G., J.D. Summner, J.L. Hill, J.V. Collins, J.A. Entry, and W.I. Segars. 2002. Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia. J. Environ. Qual. 31:1273–1278.

Jenkins, M.B., P. G. Hartel, T. J. Olexa, and J. A. Stuedemann. 2003. Putative Temporal Variability of *Escherichia coli* Ribotypes from Yearling Steers. J. Environ. Qual. 32: 305-309.

Jones, S.H. 2002a. QA Plan for the Jackson Estuarine Laboratory Microbiology Lab. USEPA approved: 2002.

Jones, S. H. 2002b. Application of Ribotyping for Tracking Bacterial Pollution Sources in New Hampshire's Shellfish Waters. A final report to the New Hampshire Coastal Program/Office of State Planning, Portsmouth, NH.

Jones, S.H. and T. Bryant. 2002. Standard procedure for detection of total coliforms, fecal coliforms, *Escherichia coli* and enterococci from environmental samples. Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH.

Jones, S.H. and N. Landry. 2004. Tracking Bacterial Pollution Sources in Little Harbor and the New Hampshire Atlantic Coast Tributaries. Final report. New Hampshire Department of Environmental Services, Concord, New Hampshire.

Jones, S.H. and N. Landry. 2003. Tracking Bacterial Pollution Sources in Hampton Harbor. Final report. New Hampshire Estuaries Project, Portsmouth, NH.

Jones, S.H., N. Landry and S. Soule. 2004a. Tracking bacterial pollution sources in the Great Bay Estuary watershed. New Hampshire Coastal Program, Portsmouth, NH.

Jones, S.H., S. Sumner and J. Connor. 2004b. Identify and Mitigate Bacterial Source at Public Beaches Using Microbial Source Tracking. Final Report. NH Department of Environmental Services, Beaches Program, Concord, NH.

Landry, N. 2004. Generic Quality Assurance Project Plan for Microbial Source Tracking. NH Department of Environmental Services, Concord, NH.

Nash, W.C. and Andrew Chapman. 2000. Sanitary Survey Report For the Atlantic Coast, Gulf Of Maine, New Hampshire. *Report Prepared for the New Hampshire Shellfish Program.* NHDES, Concord, New Hampshire.

Parveen, S., K.M. Portier, K. Robinson, L. Edmiston and M.L. Tamplin. 1999. Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. Appl. Environ. Microbiol. 65: 3142-3147.

Rippey, S.R., W.N. Adams and W.D. Watkins. 1987. Enumeration of fecal coliforms and *E. coli* in marine and estuarine waters: an alternative to the APHA-MPN approach. J. Wat. Pollut. Cont. Fed. 59: 795-798.

Samadpour, M. 2002. Microbial source tracking: Principles and practice, p. 5-10, In: Microbiological Source Tracking Workshop-Abstracts. February 5, 2002. Irvine, CA. NWRI Abstract Report NWRI-02-01. National Water Research Institute, Fountain Valley, CA.

U.S. Environmental Protection Agency (USEPA). 1986. Test methods for *Escherichia coli* and enterococci by the membrane filtration procedure. EPA 600/4-85/076. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH.