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Tracking Bacterial Pollution Sources in Stormwater Pipes

Stephen H. Jones
University of New Hampshire - Main Campus, Stephen.Jones@unh.edu

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A final report to the
New Hampshire Estuaries Project/Office of State Planning

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

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New Hampshire Estuaries Project

UNIVERSITY of NEW HAMPSHIRE

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Introduction

The New Hampshire Department of Environmental Services (DES) conducted two rounds of wet weather sampling in the Hampton Harbor watershed during 2002. Samples were collected from stormdrains, tributaries, and harbor stations for bacteria and flow in order to calculate bacteria loads. This information was needed to prioritize pollution sources as part of a Total Maximum Daily Load (TMDL) study of bacteria in Hampton Harbor (Trowbridge, 2003).

Two of the 16 monitored stormdrain pipes were selected for microbial source determination using ribotype profiling. Stormdrain pipe selection was based on the bacteria loading data from the first wet weather sampling that occurred on 7/23/02. The two sampling sites identified as HHPS069 and HHPS182 contributed 12% and 60%, respectively, of the bacteria load from the 16 monitored stormdrains during the first storm event. It was determined that these two pipes would be targeted for more intensive investigations based on the high relative loading of bacteria. Thus, samples were collected during a second storm on October 16, 2002 from these two pipes and analyzed for source species identification using ribotype profiling.

Project Goals and Objectives

The goal of this project was to determine the bacteria source species from two of the highest priority stormdrain pipes that discharge to Hampton Harbor. Specific objectives were to:

1. Collect water samples at the two selected sites during a storm of >0.25 inch total precipitation.
2. Analyze the water samples for bacteria concentrations and determine source species using ribotype profiling.
3. Issue a report for incorporation into the Hampton Harbor Wet Weather Study for the Bacteria TMDL.

Methods

Storm Selection

For this study, one storm was needed with the following characteristics: (1) onset at or around low tide; (2) >0.25 inches total precipitation; (3) occurrence during daylight hours on Monday-Thursday; and (4) very little rainfall for the prior three days. These criteria were met for the storm that DES used for this study.

The storm occurred on October 16, 2002 and was a classic "Nor'easter" with soaking rain and high winds lasting over 12 hours. A total of 1.39 inches of rain fell during the storm (Trowbridge, 2003).

Field Methods

The sampling sites were identified as HHPS182 which is located in Seabrook, west of Rt. 1A and south of Cross Beach Road and HHPS069 which is located in Hampton, west of the municipal parking lot on Ashworth Avenue. Samples were collected from the stormdrain pipe outfalls throughout the duration of the storm in accordance with the Quality Assurance Project Plan (QAPP). The samples were collected at periodic intervals to represent the entire storm. The samples were placed on ice packs in a cooler and delivered to the UNH Jackson Estuarine Laboratory.

The sampling site descriptions, photos and field collection methods for this study are described in detail in the approved QAPP, which is on file at DES.

Lab and Analytical Methods

Detection 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).

For each sample/site, yellow colonies from the best dilution (10-30 readable colonies) were counted and recorded as fecal coliforms (Rippey et al., 1987). The yellow/yellow brown colonies remaining on the membrane filter after incubation on urea substrate were recorded as confirmed *E. coli* colonies.

Sample Processing

The procedures used for ribotyping *E. coli* isolates for this study have been used previously (Jones and Landry, 2003 and Jones, 2002) 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 instead 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 separate water sample isolate were then selected for identification of source species.

Source Species Databases

The analysis of water sample isolates for identification of source species was based on two distinct databases. The first source species database used was composed of the *E. coli* strains isolated from source species sampled in the Hampton Harbor watershed. This database contained ribotypes for 11 non-human source species and wastewater, and included 120 total ribotypes (Table 1). All water ribotypes that matched the Hampton Harbor database at <90% similarity were reanalyzed using a full New Hampshire source species database. This state database was composed of 676 ribotypes from 26 different non-human source species, humans, septage and wastewater (Table 1). The state database contained ribotypes for more species and more for each shared species except for otters, cormorants and chickens, which were all from the Hampton Harbor watershed.

Table 1 Source species databases for New Hampshire and Hampton Harbor watershed.

Source species category	Source species	Number of Isolates	
		New Hampshire	Hampton Harbor
HUMANS/SEPTAGE			
	septage	16	0
	wastewater	107	25
	humans	68	0
PETS			
	cat	11	4
	dog	54	19

Source species category	Source species	Number of Isolates	
		New Hampshire	Hampton Harbor
LIVESTOCK			
	alpaca	3	0
	buffalo	10	0
	chicken	3	3
	cow	54	0
	goat	4	0
	horse	27	0
	sheep	2	0
WILDLIFE			
	coyote	19	4
	deer	59	7
	mouse	3	0
	muskrat	12	0
	otter	4	4
	raccoon	32	0
	rabbit	30	0
	red fox	25	4
	skunk	6	0
AVIAN SPECIES			
	cormorant	14	14
	duck	10	1
	geese	44	31
	gull	36	4
	pigeon	6	0
	robin	3	0
	sparrow	4	0
	starling	3	0
	wild turkey	7	0
	Total	676	120

Data Analysis

All data were analyzed with GelComparII software on a Dell computer, where the source species database was also stored. Hard copies of ribotype patterns and similarity coefficients for the unknown and its most closely related source species were printed for interpretation. Interpretation and accompanying graphical representations of the data were done using MS Excel on Macintosh computers.

Optimization was set at 1.56% and band position tolerance was set at 1.00%. Both of these parameters were 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.

Similarity indices were determined using Dice's coincidence index (Dice, 1945) and the distance among clusters calculated using cluster analysis. 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% for comparisons to the source species databases. 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. Thus, the results of the identifications reported are less than completely accurate (0% tolerance and 100% similarity). Nonetheless, useful information has hopefully been gained to help guide management decisions and resource allocation for pollution source identification and elimination in the Hampton Harbor area.

Results and Discussion

Bacteria Concentrations throughout the Storm Event

Fecal coliform and *E. coli* concentrations were measured as part of this study. The concentrations in the pipes at the time of ribotype sample collection are summarized in Table 2. The *E. coli*:fecal coliform ratio was high (94%) for all samples. *E. coli* concentrations decreased steadily with time in HHPS069, from 304,000/100 ml to 72,000/100 ml (Figure 1). In HHPS182, concentrations increased through the first four sample times, from 14,400/100 ml to 1,1120,000/100 ml, before decreasing sharply thereafter to 172,000/100 ml. The gradual rise and sharp decline in *E. coli* concentrations at HHPS182 could be a result of the stormdrain pump stations (River Street and Ocean Boulevard stations) associated with the effluent from the northern pipe of this drainage system.

Table 2 Fecal coliform and *E. coli* concentrations in stormdrain pipes and number of isolates yielding useable ribotypes.

	HHPS069		Ribotype	**HHPS182**		Ribotype
Sample time	FC/100 ml	*Ec*/100 ml	isolates	FC/100 ml	*Ec*/100 ml	isolates
10:30				15,600	14,400	8
11:30	304,000	304,000	1			
11:47				20,400	18,800	9
12:30	236,000	212,000	6			

Sample time	HHPS069 FC/100 ml	*Ec*/100 ml	Ribotype isolates	HHPS182 FC/100 ml	*Ec*/100 ml	Ribotype isolates
13:16				136,000	120,000	5
14:00	180,000	172,000	6			
14:43				1,120,000	1,120,000	8
15:45	140,000	120,000	3			
16:09				180,000	172,000	5
16:50	72,000	72,000	8			

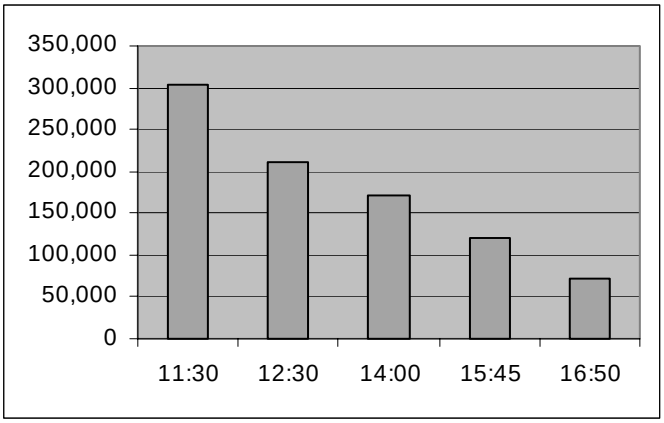

Figure 1 Site HHPS069 *E. coli* concentrations throughout the storm event.

Source Species Identification

The Hampton Harbor and New Hampshire source species databases were used to identify sources for 24 and 35 isolates from water samples taken from HHPS069 and HHPS182, respectively. Banding patterns for water sample and source species isolates were considered to be the same if there was 90% or greater similarity with reference isolates. Overall, sources for 78% of the 59 isolates were identified (Table 3).

Table 3 Identified source species (90% similarity) for 59 *E. coli* strains isolated in effluent from two stormdrain pipes.

Source	HHPS069	HHPS182	Both sites	%
human/wastewater	3	9	12	0.20
cormorant	3	8	11	0.19
goose	6	1	7	0.12
fox	3	2	5	0.08
raccoon	0	2	2	0.03
coyote	0	2	2	0.03
cat	0	2	2	0.03
seagull	1	1	2	0.03

Source	HHPS069	HHPS182	Both sites	%
dog	1	1	2	0.03
pigeon	1	0	1	0.02
Total	18	28	46	
%	0.75	0.80	0.78	
Unknowns	6	7	13	
%	0.25	0.20	0.22	

Source Species for Pollution Source HHPS069

Source species identification was successful for 18 of the 24 *E. coli* isolates (75%) from HHPS069 (Table 3). The most common source species was goose (6 isolates), followed by cormorant, fox and wastewater (3 isolates each). One isolate was identified for each of the following species: dog, pigeon and seagull. The timing of the appearance of the source species showed no clear trends, except that the goose isolates did not appear until the third sample (Table 4). Table 5 summarizes the identified source species by type. Birds were the most commonly identified source type (46%), followed by humans and wildlife (each at 13%) and pets (4%).

Table 4 Temporal identification of source species for *E. coli* in effluent from two stormdrain pipes.

Site HHPS	Time	cat	cormorant	coyote	dog	fox	goose	pigeon	raccoon	seagull	wastewater
069A	11:30										
069B	12:30		2			1		1			1
069C	14:00				1	1	2				
069D	15:45						3				
069E	16:50		1			1	1			1	2
	Total	**0**	**3**	**0**	**1**	**3**	**6**	**1**	**0**	**1**	**3**
182A	10:30	1	2			1	1			1	2
182B	11:47		1	2							3
182C	13:16	1									2
182D	14:43		4			1			2		1
182E	16:09		1		1						1
	Total	**2**	**8**	**2**	**1**	**2**	**1**	**0**	**2**	**1**	**9**
	Overall	***2***	***11***	***2***	***2***	***5***	***7***	***1***	***2***	***2***	***12***

Table 5 Identified source species types at two storm pipes in Hampton Harbor during a storm event on October 16, 2002.

Source species type	HHPS069 # of isolates	%	HHPS182 # of isolates	%	Both pipes # of isolates	%
Human (wastewater)	3	13%	9	26%	12	20%
Pets	1	4%	3	9%	4	7%
Birds	11	46%	10	29%	21	36%
Livestock	0	0%	0	0%	0	0%
Wildlife	3	13%	6	17%	9	15%
Unidentified	6	25%	7	20%	13	22%
Total isolates	**24**		**35**		**59**	

Source Species for Pollution Source HHPS182

Source species identification was successful for 28 of the 35 *E. coli* isolates (80%) from HHPS182 (Table 3). The most common source was wastewater with 9 isolates, followed by cormorant with 8 isolates, and cat, coyote, fox and raccoon with 2 isolates each. One isolate was identified for each of three other species: dog, goose and seagull. The timing of the appearance of the source species showed wastewater and cormorant sources appeared consistently through the sampling period (Table 4). The *E. coli* concentration was much higher for the fourth sample (Table 2), and cormorants were the most commonly identified source. Fox, raccoon and wastewater were also identified in the fourth sample.

Table 5 summarizes the identified source species by type. Birds (29%) and humans (26%) were the most commonly identified source types, followed by wildlife (14%) and pets (9%).

Source Species for Both Pipes

The source species identified for both pipes showed wastewater to be the most common source (12 isolates), followed by cormorant (11), goose (7) and fox (5) (Table 5). Two isolates were identified for each of the following: cat, coyote, dog, raccoon and seagull. One pigeon isolate was identified. Table 5 shows the overall most common type of source was birds (36%), followed by humans (20%), wildlife (15%) and pets (7%).

Conclusions

The present study represents the third published report on use of ribotyping to identify source species on New Hampshire estuarine waters. As such, the procedures and interpretations used have benefited from lessons learned in past studies (Jones and

Landry, 2003; Jones, 2002), and changes were made. Previous ribotyping studies in New Hampshire involved use of non-automated ribotyping procedures. The recent purchase of a fully automated RiboPrinter at UNH/JEL has provided the capacity to conduct ribotyping more rapidly, with more consistency and at a lower cost. The most striking difference resulting from use of a RiboPrinter in this study is the higher level of similarity (90%) used to provide for a reasonable percentage of identified isolates (78%). This means that the identified isolates were more accurately matched to source species than in previous reports where 80% similarity was used.

Another difference in approach used for this study compared to previous studies in New Hampshire was use of two source species databases. A local database was used first to identify sources, and then the larger state database was used to identify sources of isolates that did not meet the threshold similarity index in matching to known source ribotypes in the local database. This approach was used to see how well a small, local database works compared to a larger database. Both databases were still quite closely related from a geographic standpoint, as all ribotypes in the state database were collected from species in communities adjacent to the Great Bay Estuary, the Atlantic coast or Hampton Harbor.

There were distinct differences in identified source species for the two pipes. These differences probably reflect differences in species that are present and depositing fecal material to the drainage area. There are numerous factors that could affect the appearance of the different source species in the effluent from the two pipes. Some species may inhabit or have some presence in the pipe/drainage system prior to the storm. In the case of wastewater/human sources, these could include leaky sewer pipes underground that may cross the storm drainage pipes. The timing of the appearance of source species probably reflects time required for transport of the fecal material with runoff to the end of the pipe. The feces from birds on rooftops may take longer to reach the end of the pipes than pet waste deposited on sidewalks.

The types of source species identified were of interest. Many storm water/runoff studies have attributed fecal contamination to pet wastes. Of the four types of sources identified, pets were the least common, behind birds, humans and wildlife. It may be that pets are not common in the drainage area during October, while birds may be much more prevalent.

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