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## Tracking Bacterial Pollution Sources in Hampton Harbor

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# Tracking Bacterial Pollution Sources in Hampton Harbor

A final report to the United States Environmental Protection Agency and the  
New Hampshire Estuaries Project/Office of State Planning

Submitted by

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April 2003

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April 2003

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## **Executive Summary**

Fecal-borne microorganisms impact many shellfish-growing waters in coastal New Hampshire. Watersheds are often subject to fecal contamination by a variety of sources and efforts to improve water quality are often limited because of lack of information on which contaminant sources are most significant. Ribotyping and other microbial source tracking methods are useful new tools for providing information on the sources of fecal-borne bacterial contaminants in surface waters. New Hampshire has areas of abundant oyster (*Crassostrea virginica*) and clam (*Mya arenaria*) resources, the latter being most important in Hampton Harbor. In this study, *Escherichia coli* isolates (bacteria colonies) were obtained from water samples collected from ten sites in Hampton Harbor year-round during both dry and wet conditions. A library of known *E. coli* isolates was created from twenty different potential source species in the New Hampshire coastal watershed, including humans, livestock, pets, wildlife and avian species. The ribosomal RNA DNA of *E. coli* isolates was analyzed using ribotyping in which the patterns of ribosomal DNA were detected using chemiluminescence, then optimized and analyzed using GelCompar II software. A total of 249 isolates from the twenty known source species were used as a reference to identify sources for 390 unknown isolates from water samples taken from August 2000 through October 2001. Banding patterns for water samples and source species isolates were considered to be the same if there was 80% or greater similarity between patterns. Overall, sources for 62% of the isolates were identified.

The results suggest that the most common source species is humans. Other identified sources included deer, coyotes, horses, dogs, geese, gulls, cows, fox, ducks, chickens, a pigeon, and a robin. The results will help to focus limited resources on mitigating the most significant pollution sources identified in this study. Several pollution mitigation projects have already been completed or are currently underway. The local communities, state and federal agencies, and the University of New Hampshire are all contributing to the understanding of pollution source identification and the reduction of pollution sources.

## **Background**

The New Hampshire Department of Environmental Services (DES) is responsible for classifying shellfish growing waters in the State of New Hampshire. The purpose of conducting shellfish water classifications is to determine if growing waters are safe for human consumption of molluscan shellfish (Nash and Chapman, 2001). The DES has classified portions of Hampton Harbor, the most popular softshell clam harvesting area in the coast, as Conditionally Approved, while other portions of the harbor are closed because of previously-observed high bacteria counts, proximity to the Hampton wastewater treatment facility outfall and other pollution sources, or for lack of updated information.

The Hampton Harbor clamflats are closed for clam harvesting during the months of September and October due to elevated fecal coliform levels. The flats are open in November, April and May but close temporarily if the rainfall exceeds 0.10 inches. During the months of December, January, February and March, a rainfall that exceeds 0.25 inches triggers a closure. Each closure lasts for five days. As reported in Nash and Chapman (2001), these restrictions result in the clamflats being open for harvesting approximately 40 percent of the time during the season. Flats are not open during June, July and August based on resource management decisions by the New Hampshire Fish and Game Department. Permits for harvesting are restricted to New Hampshire residents.

The Hampton Harbor clamflats are popular, productive, and accessible to the public. Despite the construction of a new wastewater treatment facility in the Town of Seabrook, the bacteria levels in the growing waters often exceed the limits set by the DES Shellfish Program, resulting in flat closures and frustrated clam diggers.

In 2000, the DES Shellfish Program sought to determine if a revision to the current classification of Hampton Harbor was warranted (Nash and Chapman, 2001). The microbial source tracking study described in this report complemented two of the key Shellfish Program issues on which the Shellfish Program was focusing. These two areas were (1) determine the cause of intermittently high fecal coliform values noted during stretches of dry weather in September and October and (2) identify and eventually eliminate sources of contamination during wet weather that cause shellfish bed closure.

In 2000, the University of New Hampshire developed a specialized laboratory at the Jackson Estuarine Laboratory that enables researchers to identify the sources of microorganisms in the environment by comparing patterns of DNA fragments isolated, digested by restriction enzymes, and electrophoresed in agar gels to known patterns in a source library. The method requires analysis of DNA fragments of *E. coli* isolates cultured from a target watershed compared to isolates from known sources. The ribotype profile research for this study was conducted in this new laboratory.

## **Introduction**

In New Hampshire, recent studies have shown episodic events of elevated concentrations of fecal-borne bacterial contamination to be associated with runoff (Jones and Gaudette, 2001; Jones, 1998a; Jones and Langan, 1996) and its flow through urban stormwater systems. Other studies have confirmed that both runoff and dry weather flows through urban stormwater systems are significant sources of contamination to tidal waters (Jones, 2000; ANMP, 1998; Landry, 1997; NHDES, 1997). Previous studies have shown septic systems (Jones, 1998b; Jones, 1997, Jones et al., 1995; 1996), agricultural operations (Jones and Langan, 1993) and large parking lots as potentially significant sources. Responding to the results of these studies, DES has been successful in identifying and eliminating numerous contamination sources in stormwater systems, failed septic systems, and agricultural sources in the Seacoast. Despite these successes, almost all of the New Hampshire coastal waters remain contaminated to varying degrees,

and in some areas uses remain limited. Continued efforts using the similar methods (field investigations and water quality monitoring) will most likely become increasingly more difficult in successfully identifying and eliminating sources of fecal contamination because there are few remaining obvious sources.

Recent adoption of biotechnological techniques for application of water quality issues has spawned a number of approaches to address identification of sources of fecal-borne contamination. These new approaches, often called “microbial sources tracking (MST)”, have been used successfully for over ten years in a number of areas in the United States. Use of ribotyping of *E. coli* isolates cultured from target surface waters is one approach that can provide detailed information on sources of fecal contamination and has advantages over other MST methods.

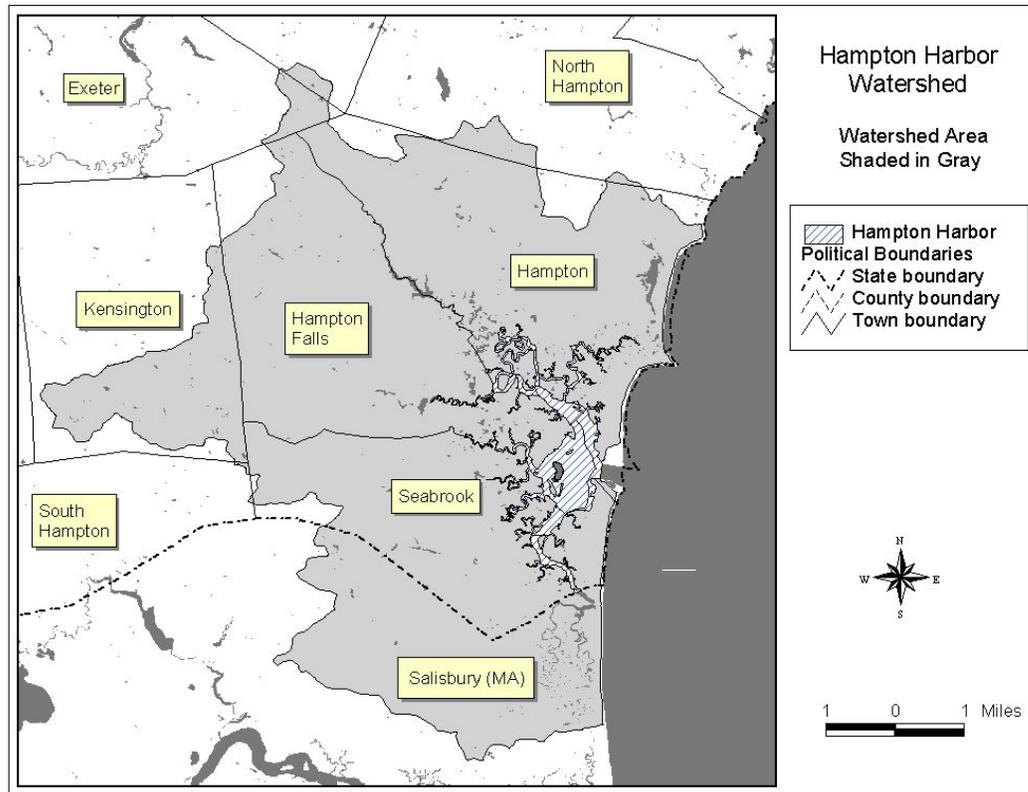
### ***Previous Work***

The most recent bacterial source tracking study in coastal New Hampshire (Jones, 2002a), analyzed ribotype profiles from samples collected in the tidal portion of the Bellamy River. This was the first report published for a ribotyping study in the estuarine waters of New England. The data were analyzed to provide information with a range of degrees of certainty for the relatedness between known source species and water sample profiles. The study results showed that there was evidence of human sources contributing to contamination; however, the wildlife species and livestock were the most commonly identified types of source species.

Other studies have reported on the use of ribotyping for tracking sources of fecal-borne microbial contaminants. Numerous ribotyping studies have been conducted in freshwater watersheds (Jones, 2002b; Carson et al., 2001; Barsotti et al., 2000; Hartel et al. 1999; Tippets, 1999; Berghoff, 1998), while others have been conducted in estuarine waters (Parveen et al., 1999; Samadpour, 1995; Simmons et al., 1995). The Jones (2002b) and Barsotti et al. (2000) studies were both conducted along the shore of Lake Champlain in northwestern Vermont.

### ***Project Setting***

Hampton Harbor is a tidally dominated, shallow estuary located at the southeast corner of New Hampshire, bordered by the New Hampshire towns of Hampton, Hampton Falls, Seabrook and the Massachusetts town of Salisbury. The watershed also encompasses small portions of North Hampton, Stratham, Exeter, Kensington and South Hampton, New Hampshire. The estuary has a total area at high tide of approximately 475 acres. The topography is relatively flat, with salt marsh comprising approximately 17 percent (5,000 acres) of the watershed area. Eighty percent of the watershed is in New Hampshire, with the remainder in Massachusetts (Jones, 1999b). The watershed map is shown in Figure 1.



**Figure 1 Watershed map of Hampton Harbor.**

The estuary receives freshwater input from the Taylor and the Hampton Falls Rivers, which converge to form the tidal Hampton River to the north. The Browns River and Mill Creek flow in from the west. Cains Brook, a major freshwater tributary, flows into Mill Creek. And, the Blackwater River flows from the south. Numerous small tidal creeks from the surrounding wetlands also drain into the estuary.

Land cover information shows a large amount of urban land concentrated near the Harbor in the town of Hampton. Jones (1999b) summarized land cover from data developed from LANDSAT Thematic Mapper imagery, 1988 and 1990 (Table 1).

**Table 1 Land cover for the New Hampshire portion of the Hampton Harbor watershed.**

Category	Acres	% of Total
Forested	10,094	40
Wetland	5,392	21
Urban	5,800	23
Agricultural	2,039	8
Disturbed	380	2
Cleared	400	2
Water	1,030	4

The Hampton Harbor area is the major summer resort area along the New Hampshire coast. Development bordering the Harbor is primarily residential and concentrated in the beach areas on the eastern shore. Commercial development consists mostly of shops, hotels and restaurants that support the tourist industry. Industrial activity in the watershed is limited (Jones, 1999b).

***Project Purpose***

The Hampton Harbor microbial source tracking (MST) survey was designed to characterize the sources of bacterial contamination in the Hampton Harbor watershed. The study was conducted by DES and researchers at the University of New Hampshire Jackson Estuarine Laboratory (UNH). Both the US Environmental Protection Agency under the 104(b)(3) program and the New Hampshire Estuaries Project provided funding.

The goal of the MST survey of the Hampton Harbor estuary was to determine the sources of bacterial contamination of the Harbor and their respective contributions. The potential sources include birds (cormorants, starlings, and gulls); domestic animals (cats, dogs, goats, and horses); and sanitary wastewater from wastewater treatment plant failures, direct and overboard discharges, and failed septic systems; and wildlife. The study involved a comprehensive sampling of the Harbor and the potential sources of fecal material from the watershed.

The intent of the survey was to provide information needed to support implementation of specific source controls and, as a result, reduce the bacterial contamination to a level that increases the number of days that the shellfish growing waters are open for recreational harvesting.

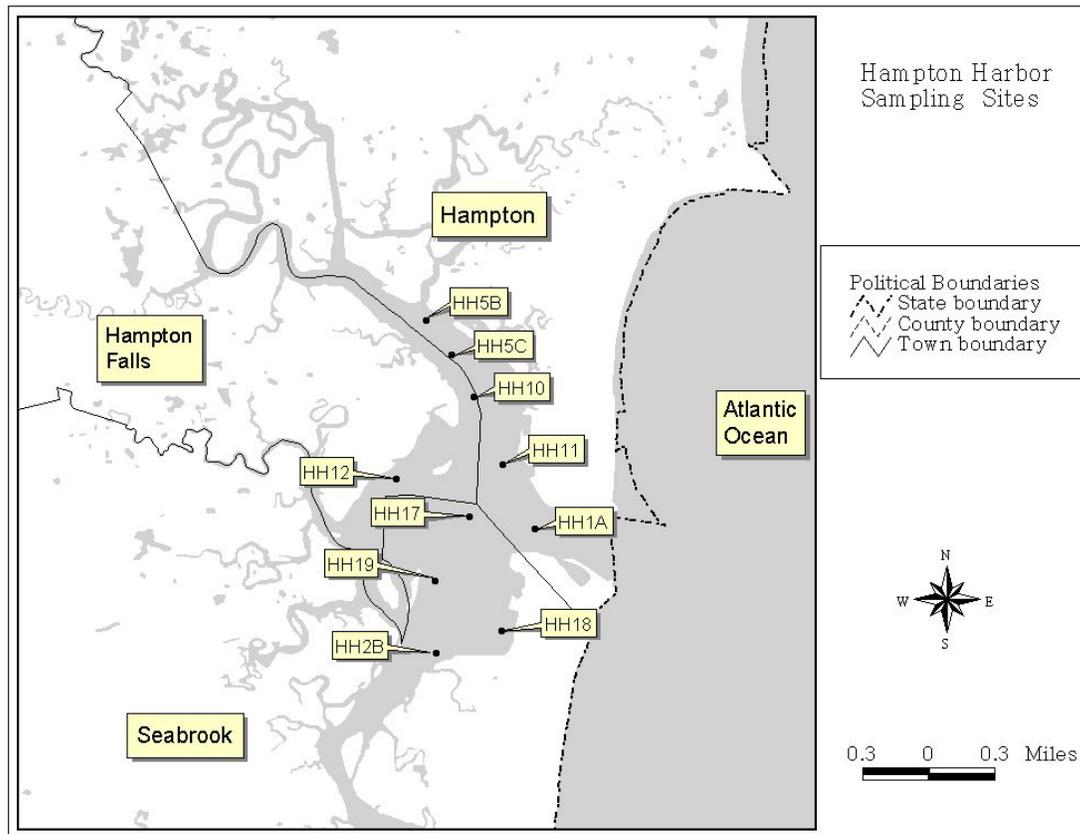
**Field and Laboratory Methods**

Sample site selection was based on the routine monitoring stations of the DES Shellfish Program (Table 2; Figure 2). All of the ten routine Harbor sites monitored by the Shellfish Program were selected for this study. Harbor water sampling was initiated in August of 2000 and continued through October 2001. Each of the ten sites was sampled at least monthly and more frequently during the autumn of 2000 and 2001.

**Table 2 Sampling site descriptions.**

Site Identification	Latitude	Longitude	Site Description
HH10	42° 54' 15.55"	-70° 49' 23.18"	In the Hampton River channel adjacent to the Hampton Marina
HH11	42° 53' 59.6"	-70° 49' 13.73"	At the confluence of the Hampton and Browns Rivers
HH12	42° 53' 57.08"	-70° 49' 47.6"	In Browns River, at the sharp bend to the south, downstream of Half Tide Rock
HH17	42° 53' 47.7"	-70° 49' 25.32"	In channel of the Blackwater River, near the northern edge of Middle Ground

Site Identification	Latitude	Longitude	Site Description
HH18	42° 53' 20.86"	-70° 49' 15.25"	In Seabrook Harbor, just past (upstream of) Yankee Fishing Cooperative
HH19	42° 53' 32.93"	-70° 49' 35.67"	In the channel of Blackwater River, near mouth of Mill Creek
HH1A	42° 53' 45.32"	-70° 49' 4.18"	Mouth of Harbor near Rt. 1A bridge, center of channel
HH2B	42° 53' 15.5"	-70° 49' 35.93"	In the Blackwater River near the main Harbor, off River Street
HH5B	42° 54' 33.97"	-70° 49' 38.29"	In Hampton River off of the mouth of Tide Mill Creek
HH5C	42° 54' 26.15"	-70° 49' 30.12"	In Hampton River near Eagle Creek



**Figure 2** Sampling site locations.

***Water Sample Collection Procedure and Sampling Dates***

Water samples were collected in accordance with procedures outlined in Chapman and Nash (2002). Use of the fecal coliform indicator was selected based on the DES Shellfish Program standard indicator and *E. coli* was selected for ribotyping analysis based on the indicator used to build the coastal New Hampshire source species library. Water samples were collected by DES personnel using 18 mL Whirlpaks™, which were

stored in a cooler with ice packs and delivered to UNH on the same day of collection. Samples were collected from 8/7/00 to 10/29/01 during wet and dry weather (Table 3).

**Table 3 Precipitation conditions at Hampton Harbor during and prior to sampling.**

Date	Weather	Rainfall used for analysis (inches)	Precipitation Day 0 (prior to sampling only)	Precipitation Day 1	Precipitation Day 2	Precipitation Day 3
8/7/2000	dry	0.05	0	0.05	0	0
9/11/2000	dry	0	0	0	0	0
9/20/2000	wet	0.48	0.46	0.02	0	0
9/21/2000	wet	0.48	0	0.46	0.02	0
9/26/2000	dry	0	0	0	0	0
9/27/2000	wet	0.33	0	0.33	0	0
10/4/2000	dry	0.01	0	0	0.01	0
10/6/2000	wet	0.99	0.51	0.48	0	0
10/16/2000	dry	0	0	0	0	0
10/25/2000	dry	0	0	0	0	0
10/26/2000	dry	0	0	0	0	0
11/15/2000	wet	1	0.02	0.98	0	0
11/29/2000	dry	0.05	0.03	0	0.02	1.08
12/5/2000	dry	0	0	0	0	0
1/22/2001	dry	0	0	<b>0.12</b>	0	<b>0.27</b>
2/20/2001	dry	0	0	0	0	<b>0.14</b>
2/27/2001	wet	0.65	0	0.02	0.63	0
3/13/2001	wet	0.73	0	0.73	0	0
3/28/2001	dry	0	0	0	<b>0.04</b>	0
4/2/2001	wet	1.69	0	0	0.01	1.68
4/9/2001	wet	0.17	0	0.17	0	<b>0.05</b>
5/21/2001	dry	0	0	0	0	0
5/29/2001	wet	0.52	0	0.01	0.51	0
6/12/2001	wet	0.9	0	0.9	0	0
7/16/2001	dry	0.01	0	0	0.01	0
9/10/2001	dry	0	0	0	0	0
9/11/2001	dry	0	0	0	0	0
9/18/2001	dry	0	0	0	0	0
10/10/2001	dry	0	0	0	0	0
10/23/2001	dry	0	0	0	0	0
10/29/2001	dry	0	0	0	0	0

**Note 1:** All precipitation that fell as snow was not included as "rainfall" in analysis. Snowfall shown in bold text. **Note 2:** Source of precipitation data was Seabrook Station, except for 5/29/01 and 9/18/01 which was NOAA Portsmouth Station.

### ***Fecal Sample Collection Procedure***

Fecal samples were collected throughout the duration of study according to Chapman (2002). Many of the wildlife and domestic animal fecal samples were collected on a large private parcel in Seabrook on several occasions, in addition to other locations near the Harbor. Human fecal samples were collected at the Seabrook and Hampton

wastewater treatment facilities from the influent on various occasions. Fecal samples from avian species were collected from the clamflats during some of the water sampling events. Fecal samples were stored and processed according to Jones and Bryant (2002). Fecal samples previously collected in the New Hampshire coastal watershed and analyzed by UNH were used in the analysis of the study data.

### ***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  $44.5 \pm 0.2$  °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, 2002a&b) and are based to a large extent on those of Parveen et al. (1998). *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.

*E. coli* isolate cultures were used for DNA extraction. Extraction was performed using Puregene (Gentra) kits and the manufacturer's instructions. Briefly, 5 ml of overnight cultures was centrifuged at 10,000 rpm for 5 minutes to concentrate the cells from the liquid medium. Three hundred (300) µl of lysis solution was added to the pelleted cells, mixed and incubated for 5 minutes at 80°C. One and one half (1.5) µl of Rnase solution was added then incubated at 37°C for 15-60 minutes. A protein precipitation solution was added, then the tube contents were mixed and centrifuged. The supernatant was transferred into a clean tube. Isopropanol and ethanol were added to remove DNA, and a hydration solution was added to re-hydrate the DNA at 65°C for 1 hour, then stored at 4°C.

The resulting DNA for each isolate was quantified by fluorometer (Turner TD700) using Hoesct's dye and calf thymus DNA at 100 µg/ml as a standard. DNA concentrations were recorded on the vials, in a lab notebook and in a computer database.

Restriction of the DNA was conducted using EcoRI (Sigma) and the manufacturer's instructions. Briefly, 2 µg of isolate DNA, 2 µl of the appropriate 1x buffer and 0.5 µl of EcoRI restriction enzyme were added to a 0.5 ml tube. Autoclaved diethylpyrocarbonate (DEPC; Sigma) water (0.1%) was added (~16 µl) to bring the total volume in the tube to 20 µl. The mixture was incubated overnight at 37°C. The next morning, 0.2 µl of EDTA was added to stop the reaction.

### ***Gel Electrophoresis, Probe Hybridization and Detection***

Restriction-digested DNA was separated by sub-marine gel electrophoresis (EC App. Corp.) in Tris-acetate-EDTA (TAE) buffer. Volumes (12 µl) of positive and negative control, isolate and standard samples were loaded into 0.8% (Nu-Sieve 3:1) agarose gels. Denaturation, neutralization and Southern blotting were performed using a Vacugene XL (Amersham). When the transfer was complete the membrane was washed, placed on blotting paper then crosslinked (Spectrolinker XL1000).

A probe was made as follows. In a 2 ml tube, 20 µl of 16S 23S rRNA (Sigma), 2 µl of DEPC water, 2 µl of reverse transcriptase (Sigma), 8 µl of 5x buffer, 4 µl of dNTP (Roche) and 4 µl of hexanucleotide mix (Roche) were mixed together. The solution was incubated overnight at 42 °C.

Prehybridization was performed in an Isotemp (Fisher) hybridization oven at 42°C for 2 h, using 30 ml hybridization solution per membrane. The probe was denatured by boiling for 10 minutes and rapid cooling in an ethanol-ice bath. The probe was added to 30 ml pre-warmed hybridization solution and incubated for 30 minutes at 68°C. The original hybridization solution was poured off the membranes and the probe solution was added and incubated overnight at 42°C.

For probe detection, the membranes were then subjected to a series of stringency washes. Blocking was done at room temperature for 60 minutes and the solution was poured off. Freshly prepared anti-DIG solution was added, incubated for 30 minutes at room temperature and poured off. Tween buffer was added and incubated for 15 minutes at room temperature. Detection buffer (Roche) was added and incubated for 2 minutes. The membranes were then placed into an acetate sheet and 20 drops of CDP-Star (Roche) was added and incubated at room temperature for 7 minutes.

### ***Image Digitization, Optimization and Band Identification***

Processed membranes were placed into the darkroom of an Epi Chem (UVP) chemiluminescence imager and the image was digitized with a 12-bit CCD camera. Each image was converted to 16-bit data, inverted and the display range set with LabWorks software (UVP).

The images were transferred into GelComparII (Applied-Maths) analytical software and the lanes for each gel were visually demarcated. Densitometry data were then processed for band identification. The bands in lanes containing the standard were labeled and entered into the memory for optimization of gel pattern images.

***New Hampshire Source Library***

Fecal samples from source species collected in the Hampton Harbor watershed were processed for isolation of *E. coli* strains. The isolates were then subjected to the previously described procedures. A database containing all of the Hampton Harbor sources and other coastal New Hampshire source species profiles with > 2 bands was used to analyze the profiles from water sample isolates. The number of isolates used to identify sources for water samples was 249, categorized by species (20) and type (5) of source in Table 4.

**Table 4 Source species database for *E. coli* from coastal New Hampshire sources.**

<b>Source species category</b>	<b>Source Species</b>	<b># of isolates</b>
<b>HUMANS/WASTEWATER</b>	wastewater	46
	humans	5
<b>PETS</b>	cat	2
	dog	9
<b>LIVESTOCK</b>	chicken	2
	cow	32
	horse	14
<b>WILDLIFE</b>	coyote	5
	deer	43
	muskrat	3
	raccoon	28
	red fox	24
<b>AVIAN SPECIES</b>	cormorant	15
	duck	4
	geese	19
	gull	5
	pigeon	2
	robin	3
<b>Total</b>		<b>261</b>

## ***Data Analysis***

All data were analyzed with GelComparII software on a Dell computer, where the New Hampshire coastal source species database was also stored. Hard copies of ribotype patterns and similarity coefficients for the unknown and 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.

The calculated similarity index for each water sample banding pattern with the most closely matching source species pattern was determined using Dice's coincidence index. For this study, the predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 80% for comparisons with the New Hampshire coastal source species isolates. 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 to use the 80% similarity index level was based on a number of criteria. For studies like this one that have relatively few (<300) source species isolates (the "Source Library"), it is difficult to get "matches" if a threshold similarity index above 80% is used. Also, there is a certain level of inter-gel variability of the Dice's coincidence index for patterns of the *E. coli* positive control, which may make it difficult to get precise matches. Also, a single mutation could cause changes in the banding pattern for what are otherwise isolates with the same ribotype profile and would result in lower levels of similarity, especially for strains that have fewer bands. And finally, the 80% similarity results were used in this study because the yield rate (60%) provided an adequate fraction of identifications to provide a useful profile of potential sources to managers.

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. Thus, the identifications reported are less than completely accurate (0% tolerance and 100% similarity). Nonetheless, useful information has been gained to help guide management decisions and resource allocation for pollution source identification and elimination in Hampton Harbor.

The band position tolerance and the optimization are settings that can be adjusted. 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.

Additional analyses included cluster analysis 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 unweighted 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 water isolate 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 the actual species identified and according to the weather conditions under which samples were collected.

### ***Data Management***

Identification of source species for water sample patterns was based on matches that had  $\geq 80\%$  as the minimum level of acceptable similarity. Use of higher similarity indexes increasing in 5% increments provided a more accurate identification of source species, but the accuracy was balanced by a decreasing percentage of identifications. The results are discussed according to identifications made using the different similarity indexes as follows 80, 85, 90, 95 and 100%.

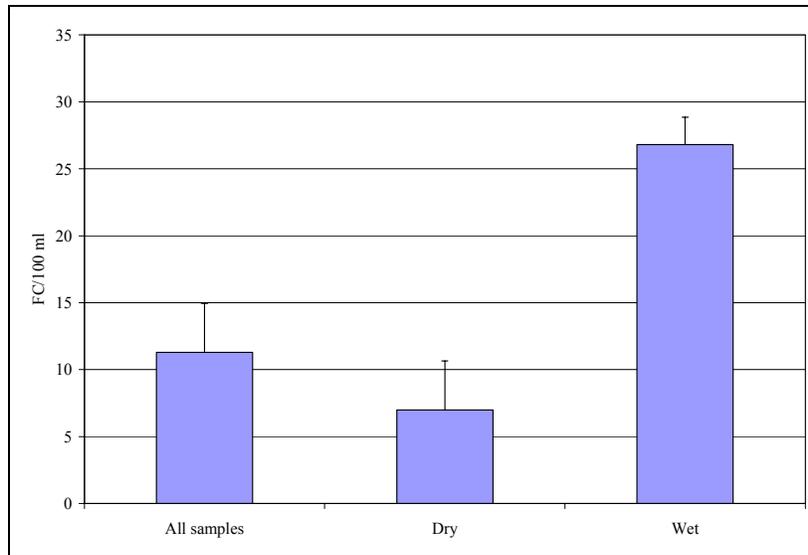
## **Results**

### ***Fecal Coliform Concentrations in Water Samples***

Fecal coliform concentrations in water samples collected as part of this study are summarized in Table 5. The geometric mean concentrations for data collected on the 31 dates for each site are also presented. Concentrations ranged from 0-168 FC/100 ml. One of the main concerns with the shellfish classification in Hampton Harbor is the fecal coliform concentrations during wet weather. The geometric means for wet and dry weather conditions showed much higher levels during wet weather at all ten sites (Table 5). Figure 3 shows the geometric mean fecal coliform levels for all sites during wet, dry and all conditions, and further illustrates the elevated concentrations during wet weather conditions. Further analysis of the total fecal coliform database showed similar concentrations during September-November (12.1 FC/100 ml) compared to December-August (10.3 FC/100 ml), and FC levels during dry weather in September-November (7.6 FC/100 ml) were similar to the full-study dry weather levels (7.0 FC/100 ml).

**Table 5 Weather conditions and fecal coliform concentrations at Hampton Harbor sampling sites during the study.**

Date	Weather	Sample site number									
		10	11	12	17	18	19	1A	2B	5B	5C
		Fecal Coliform (cfu/100 ml)									
8/7/2000	dry	0	20	0	40	80	20	80	20	20	0
9/11/2000	dry	2	6	4	6	8	4	4	10	3	4
9/20/2000	wet	36	43	42	73	34	80	123	41	34	41
9/21/2000	wet	16	23	29	56	21	18	44	22	21	23
9/26/2000	dry	18	4	10	14	6	16	15	18	4	18
9/27/2000	wet	20	11	11	23	21	27	13	21	29	23
10/4/2000	dry	18	15	23	72	23	76	63	19	14	21
10/6/2000	wet	45	26	75	118	60	104	99	80	38	44
10/16/2000	dry	22	19	29	32	31	19	39	32	19	35
10/25/2000	dry	0	3	0	4	0	1	1	1	1	2
10/26/2000	dry	3	0	1	2	1	2	1	3	4	6
11/15/2000	wet	113	58	27	108	37	92	81	11	9	16
11/29/2000	dry	130	33	33	79	49	79	79	46		79
12/5/2000	dry	6.8	9.2	4.5	2	4.5	7.8	6.8	4.5	2	2
1/22/2001	dry	11	5	12	2	10	15	6	7	11	8
2/20/2001	dry	11	5	12	2	10	15	6	7	11	8
2/27/2001	wet	17	24	23	16	8	15	13	8	15	12
3/13/2001	wet	26	21	16	20	4	29	12	5	34	26
3/28/2001	dry	12	8	11	16	11	1	15	6	29	20
4/2/2001	wet	11	26	22	71	96	53	44	11	19	17
4/9/2001	wet	14	28	35	28	31	24	24	22	4	13
5/21/2001	dry	1	1	1	0	0	0	2	2	3	2
5/29/2001	wet	22	23	18	16	16	28	19	26	34	32
6/12/2001	wet	29	26	25	43	19	127	40	31	19	21
7/16/2001	dry	7	4	4	1		2	4	11	5	4
9/10/2001	dry	9	5	28	28	9	12	32	16	8	6
9/11/2001	dry	10	18	8	20	14	9	16	12	14	5
9/18/2001	dry	10	3	7	3	2	12	6	4	17	11
10/10/2001	dry	0	5	1	11	4	1	0	4	2	1
10/23/2001	dry	10	42	38	168	60	46	41	54	27	13
10/29/2001	dry	1	1	3	2	1	2	3	1	0	2
Geometric mean	Total	10	10	10	15	11	13	14	11	10	10
	Dry	6	6	6	8	7	7	9	8	6	6
	Wet	25	26	26	41	23	42	34	19	20	22



**Figure 3 Geometric mean fecal coliform concentrations for samples collected under all-weather, dry weather, and wet weather conditions.**

### ***Ribotyping Success with Isolates from Water Samples***

The processes involved with ribotyping *E. coli* from water samples includes numerous steps that tend to reduce the numbers of initial isolates that are useable. Factors that reduce isolate usability once cultures are confirmed as *E. coli* include failure of isolates to maintain growth in the lab, lack of survival in freezing, failure to yield bands after electrophoresis of restricted DNA, and failure to yield at least 3 bands. For this study, 882 isolates were subjected to ribotyping and yielded 391 useable isolates, for a yield rate of 44%.

### ***Effects of Different Dice Similarity Indexes on Isolate Identification***

The ribotypes for 391 *E. coli* isolates from water samples were analyzed using 2% band tolerance criteria for determining the Dice similarity index for the best match when compared to the New Hampshire source species library. Overall, 60% of the 391 ribotypes matched source species at 80% similarity, 36% at 85%, 15% at 90% and 9% at >95% similarity (Table 6). The 80% similarity results were used thereafter for source species identification because the yield (60%) provides an adequate fraction of identifications to provide a useful profile of potential sources to managers. Interpreting results based on higher similarity indexes would be more accurate, but the lower yield ( $\leq 36\%$ ) for identifications would leave too large of a fraction of unidentified isolates. The overall results for identifications determined using a lower (80%) tolerance should thus be considered as a guiding summary of the most significant probable sources of fecal contamination. However, because of the lower level of accuracy of matching for source identification, the results should be interpreted as a guide and not as absolute identifications.

**Table 6 Total E. coli isolates successfully ribotyped and identified at different Dice similarity indexes.**

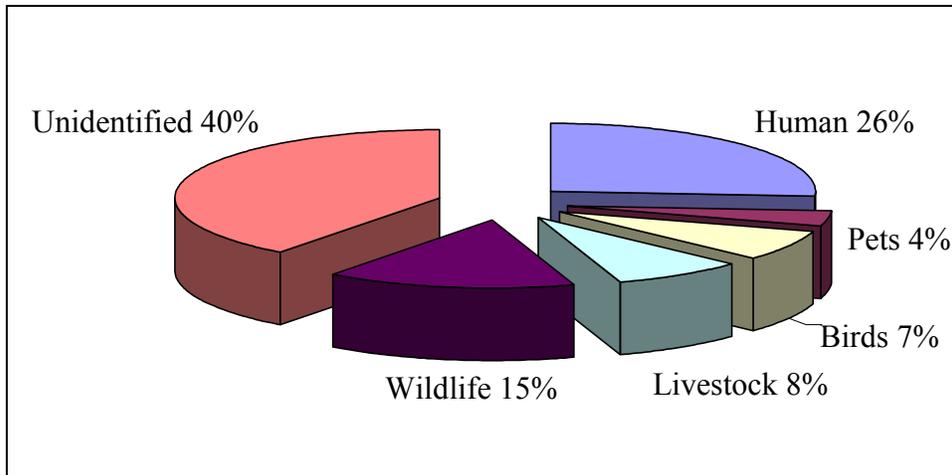
Date	Total isolates	Isolates identified at:				
		>80%	>85%	>90%	>95%	100%
8/7/2000	4	3	1	0	0	0
9/11/2000	17	11	7	5	4	4
9/20/2000	17	5	3	0	0	0
9/21/2000	38	23	14	8	5	5
9/26/2000	18	11	7	3	2	2
9/27/2000	15	4	3	0	0	0
10/4/2000	16	12	9	5	1	1
10/6/2000	9	4	4	1	1	1
10/16/2000	10	7	6	2	1	1
10/25/2000	3	3	1	0	0	0
10/26/2000	19	10	4	3	0	0
11/15/2000	11	11	8	3	3	3
11/29/2000	22	14	7	4	1	1
12/5/2000	8	5	3	1	0	0
1/22/2001	13	7	5	1	1	1
2/20/2001	19	8	1	0	0	0
2/27/2001	13	5	3	1	0	0
3/13/2001	12	3	1	0	0	0
3/28/2001	7	3	0	0	0	0
4/2/2001	22	16	10	5	4	4
4/9/2001	11	9	6	2	1	1
5/29/2001	16	10	4	2	0	0
6/12/2001	13	9	4	2	0	0
7/16/2001	13	11	4	1	1	1
9/10/2001	5	5	4	3	3	3
9/11/2001	6	4	4	2	2	2
9/19/2001	9	8	6	5	3	3
10/10/2001	8	5	5	0	0	0
10/23/2001	14	8	6	1	1	1
10/29/2001	3	2	2	0	0	0
Total isolates	391	236	142	60	34	34
% of total isolates		60%	36%	15%	9%	9%

**Source Species for Escherichia coli Isolates from Hampton Harbor**

Source species were identified for the 391 *E. coli* isolates that yielded useable ribotypes at a Dice similarity index of 80% (Table 7). Humans were by far the most frequently identified source species. The types of source species (Table 4) identified for water isolates from Hampton Harbor were as follows : 26% human, 15% wildlife, 7% birds, 8% livestock, 4% pets and 40% unidentified.

**Table 7 Source species identified for *E. coli* isolates for all sites on each sample date.**

Date	Total isolates	Total >80%	chicken	cow	coyote	deer	dog	duck	fox	goose	gull	horse	human	pigeon	robin
8/7/2000	4	3				1	1						1		
9/11/2000	17	11		2	3								6		
9/20/2000	17	5			1	2							2		
9/21/2000	38	23		2	2	1		1		1	2	1	13		
9/26/2000	18	11		2		1		1		1			6		
9/27/2000	15	4						1				1	1	1	
10/4/2000	16	12			1	3	1			1		1	5		
10/6/2000	9	4			1	1				1			1		
10/16/2000	10	7		1	2		2				1		1		
10/25/2000	3	3				2							1		
10/26/2000	19	10				1					1	4	4		
11/15/2000	11	11		1			2			1	1		6		
11/29/2000	22	14	1		1	1					1	2	8		
12/5/2000	8	5				1							4		
1/22/2001	13	7			2				3	1			1		
2/20/2001	19	8			1		2			1			3		1
2/27/2001	13	5				2					1		2		
3/13/2001	12	3								1			2		
3/28/2001	7	3					1						2		
4/2/2001	22	16	1		3	3			2		1	2	4		
4/9/2001	11	9	1		1	2				1		1	3		
5/29/2001	16	10				2						2	6		
6/12/2001	13	9				1	1					1	6		
7/16/2001	13	11				2				1	1	4	3		
9/10/2001	5	5			1								4		
9/11/2001	6	4			1		1		1				1		
9/19/2001	9	8			3	1	1					1	2		
10/10/2001	8	5				1	1		1		1		1		
10/23/2001	14	8	1			1	1	2	1	1			1		
10/29/2001	3	2											2		
Totals	391	236	4	8	23	29	14	5	8	11	10	20	102	1	1



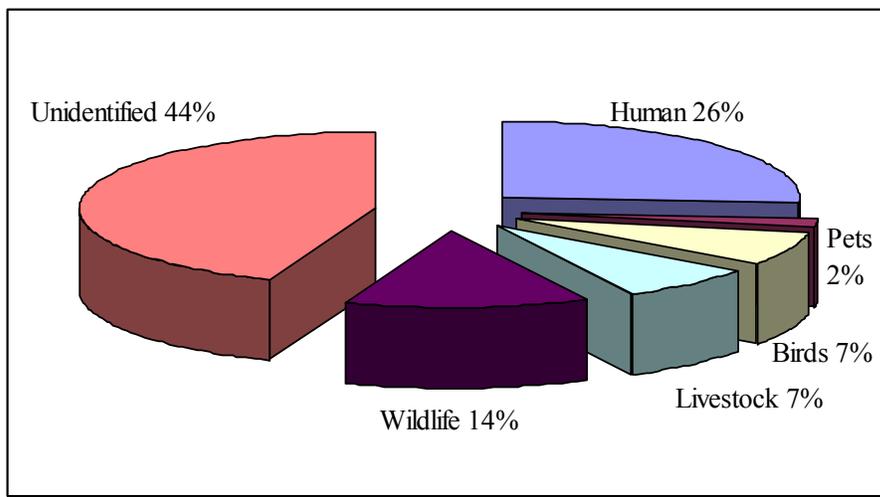
**Figure 4 Source species found in Hampton Harbor.**

The profile of types of source species identified using higher Dice similarity indexes (85, 90, 95, 100%) were essentially similar. The percent of all identifications that were human ranged from 37-51%, pets ranged from 0-6%, birds ranged from 11-14%, livestock ranged from 10-15% and wildlife from 25-30%. With an increasing threshold similarity index, isolates identified as humans increased and pet identifications decreased more than the other types.

#### ***Wet Verses Dry Weather Sources***

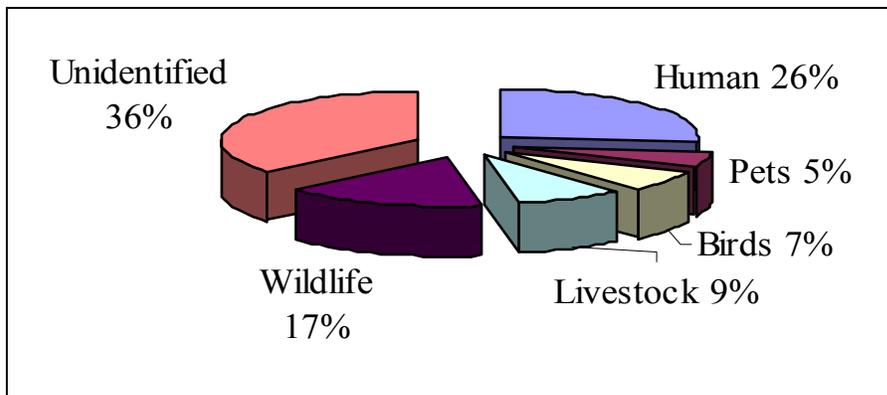
Analysis of all isolates collected throughout the study period was separated into “wet” and “dry” weather samples. Wet weather is defined as  $>0.1$ ” of rainfall during the previous two days prior to sampling including the portion of the sampling day prior to sample collection or when a large rainstorm ( $>1.5$ ”) occurred in the previous three days before sampling. Dry weather is defined as  $\leq 0.1$ ” of rainfall during the two days prior to sampling. Snowfall was not included in the rainfall amounts. Rainfall results for each sample date are summarized in Table 3.

Using the wet weather criteria described above and 177 unknown isolates from the water samples, the results of the ribotyping for all samples showed that 26% of the sources were human, 14% wildlife, 7% avian, 7% livestock, 2% pets and 44% were unidentified (Figure 5). Unidentified means that the isolates did not match with similarity greater than or equal to 80% with strains in the source library.



**Figure 5 Source Species found in Hampton Harbor during wet weather.**

The dry weather results were determined by analyzing 214 unknown isolates from the water samples. The ribotyping results were similar to the wet weather isolate analysis. The results showed that 26% of the sources were human, 17% wildlife, 7% avian, 9% livestock, 5% pets and 36% were unidentified (Figure 6).



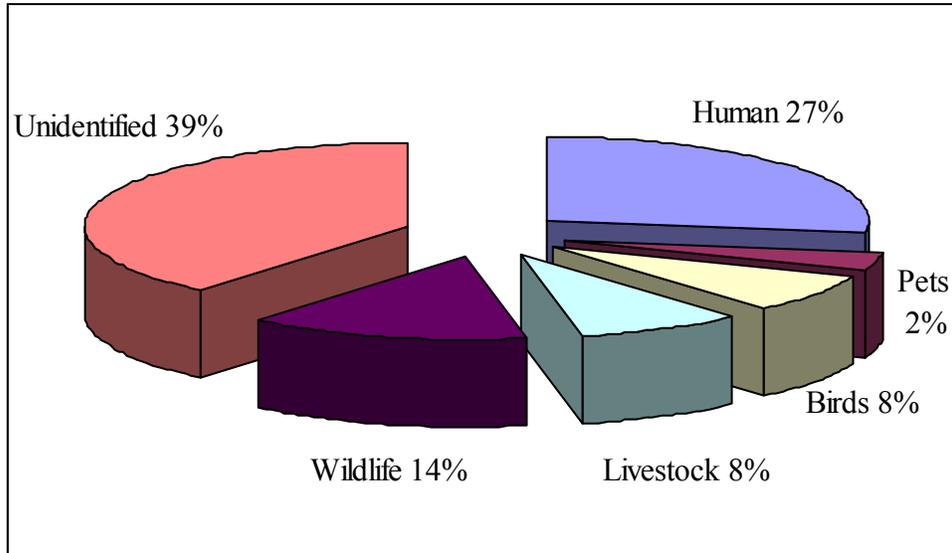
**Figure 6 Source species found in Hampton Harbor during dry weather.**

Human sources accounted for approximately one quarter of the ribotyped isolates identified during both wet and dry weather. Wildlife species also accounted for about one sixth (17%) of ribotyped isolates that were identified during dry weather, but only 14% during wet weather. Percentages of livestock and pets accounted for relatively smaller percentages (2-9%) of the identified sources during both weather conditions.

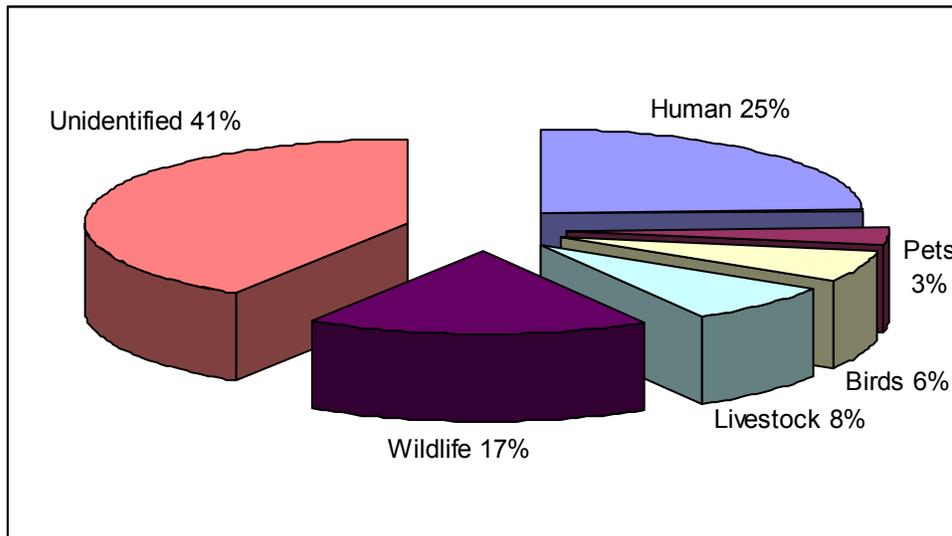
### ***Autumn Bacteria Sources***

Fecal coliform concentrations in Hampton Harbor during the study period show differences between dry weather geometric means when comparing the two periods of September through November and December through August. The fecal coliform geometric mean for samples collected during the autumn months was 67 cfu/100 mL and 9 cfu/100 mL during the other study months of December through August.

Using all September-November samples (confirmed *E. coli* isolates), the analysis shows that human sources were identified as 27% of the total isolates, 14% wildlife, 8% avian, 8% livestock and 4% pets (Figure 7). The ribotyping results for December through August, both weather types combined, yielded a 25% match to human sources, 17% wildlife, 6% avian, 8% livestock and 3% pets (Figure 8).



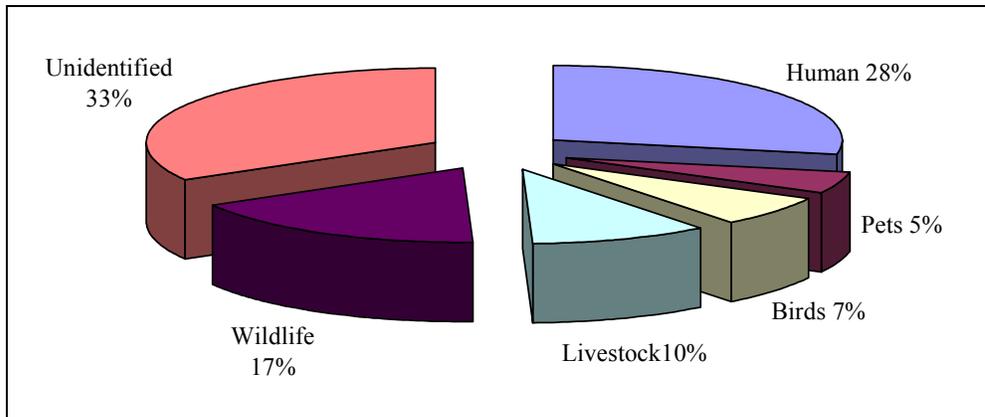
**Figure 7 Source species found in Hampton Harbor during September-November.**



**Figure 8 Source species found in Hampton Harbor during December-August.**

The sources of contamination are of most interest during September-November. The results were analyzed to show that, for 150 isolates ribotyped during dry weather,

28% of the sources were human, 17% wildlife, 7% avian, 10% livestock, 5% pets and 33% were unidentified. Thus, the profile of source species had only slight differences compared to dry weather during the whole year (Figure 9).



**Figure 9 Source species found in Hampton Harbor during September-November and dry weather.**

### ***Source Identification at Each Sampling Location***

The fraction of identified source species for each site were compared to each other to determine if some source species were more prevalent at some sites or absent from others. The purpose of this analysis is to compare results to locations of suspected or likely sources of these species.

Human, wildlife and livestock sources were identified at all ten sampling sites in the Harbor. The highest fraction of human isolates was found at site HH18, followed by HH12 and HH5C. Wildlife sources showed the highest fraction of isolates at sites HH2B, HH17 and HH1A. Livestock had the highest fraction of source species at both HH1A and HH10, followed by HH5B. The highest fraction of bird sources were identified at HH2B, HH5B and followed by HH18. Bird sources were not identified at HH17. An equal fraction of pet sources was found at sites HH5B, HH5C and HH17, followed by HH11. Pet sources were not found at HH2B, HH10 and HH19.

This analysis was not particularly useful for making comparisons of suspected sources to the results since the sources most often identified (human, wildlife, livestock) were found at all ten locations.

### **Discussion**

The purpose of conducting a separate analysis to determine the source species found during dry weather in September-November was to see if certain types of sources were more significant during conditions when elevated fecal coliform levels were measured in the Harbor. Analysis of the samples from the four autumn dates with elevated fecal coliform levels showed similar types of sources as seen for all other

analyses. The types of sources did not differ significantly during the autumn when compared to other seasons. If the source species are the same throughout the year, as the results indicate, a source or sources must be increasing the contamination load at certain times, specifically autumn and following rain events. Therefore, attention to the most prevalent sources identified with the ribotyping should decrease the bacteria concentrations during all seasons and weather events.

Bacterial contamination from humans, which was identified as the largest source of identified bacteria sources using the ribotyping method, could originate from various sources in the watershed. Potential sources include overboard discharges of human waste from boats at marinas and the harbor mooring field, both aging infrastructure and overflows/bypasses of wastewater from the Hampton wastewater treatment facility, failures from the Seabrook wastewater treatment infrastructure, and effluent from failed septic systems. Examples of infrastructure problems related to wastewater treatment could include leaking sewer pipes and cross connections with stormdrain pipes.

Pathogenic bacteria originating in human sources such as sewage or wastewater and found in surface waters include *Arcobacter butzleri*, *Enterbacter* sp., *Escherichia coli*, *Fusobacterium necrophorum*, *Mycobacterium tuberculosis*, *Pseudomonas putrifaciens*, *Salmonella enteritidis*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Vibrio cholerae* (Jones, 1999). These bacteria cause disease such as enteritis, gastroenteritis, pneumonia, dysentery and cholera. Human sources of fecal contamination also may contain pathogenic viruses, and are thus a concern for shellfish harvesting waters because of the persistence of viral pathogens.

Animal feces from livestock and wildlife can carry pathogenic bacteria species such as *Bacteroides fragilis*, *Campylobacter fetus*, and *Clostridium sporogenes*. Diseases resulting from these species include intraabdominal abscesses, septicemia, and gangrene, respectively (Jones, 1999). Source controls for livestock include the exclusion of animal pens from the salt marsh and manure storage management. Only a relatively small percentage of the land (8%) in the watershed is comprised of agricultural uses. The most impacting of these sources are animals penned on the marsh, due to their close proximity to the growing waters. Source controls for wildlife include exclusionary techniques such as spikes on bridge roosting areas and good housekeeping such as closing dumpster covers and securing garbage cans. Although some communities have gone to the extreme of removing wildlife to reduce population numbers (Simmons, 1995), it is not considered necessary in this watershed.

Because elevated bacteria counts are present throughout the harvest season following rainfall, managers need to understand the sources in order to implement source controls. Despite the large number of samples for which a source/origin was unidentified (40%), the results from this study suggest that humans are a significant source species and wildlife species are also common sources.

## **Management Actions**

Management actions should focus on reduction and elimination of human sources based on the significant relative percentage of human sources identified by the ribotyping method and the threat posed from fecal-borne pathogens that originate from humans and are found in surface waters.

The local communities in the watershed and researchers from UNH and the United States Geological Society, in addition to state agencies such as the New Hampshire Estuaries Project and the Coastal Program in the Office of State Planning, DES, the Department of Fish and Game and the Department of Health and Human Services have been working to reduce bacterial contamination in Hampton Harbor. There is a commitment by the communities and the agencies to continue this work to improve the water quality and ensure that the opportunity for shellfish harvesting is available to our citizens. Completed, on-going and planned activities to address bacterial pollution are listed in Appendix A. Many of these efforts aim to identify the significant sources of human-related bacteria such as failed septic systems, illegal boat discharges, and overflows at wastewater treatment plants. Humane efforts to reduce wildlife in the watershed are also suggested in Appendix A.

The DES Shellfish Program, through its routine water monitoring, will track any reductions through the years. Regular monitoring using bacterial indicators will provide a long term record of the improvements.

## **Conclusions**

The results suggest that of the identified sources in Hampton Harbor, the most common source species is human followed by wildlife, birds, livestock, and pets. In addition to humans, specific identified sources included deer, coyotes, horses, dogs, geese, gulls, cows, fox, ducks, chickens, a pigeon, and a robin. The results will help to focus resources on mitigating the most significant pollution sources.

The distribution of the source species type (e.g., wildlife, human) was affected only slightly by rainfall and season. The percentages remained relatively constant throughout the study period and during both wet and dry weather.

Based on the results of the ribotype profiling and the human health risk associated with pathogenic bacteria originating in human waste, it is recommended that managers focus on the reduction and elimination of human sources of bacteria. While wildlife sources were determined to be the next most prevalent source of bacteria and also present a risk to human health, the source controls are more difficult to implement based on the difficulty to target source reduction. However, there are humane steps that can be taken to reduce the numbers of deer and coyotes which reportedly increase with an increase in development (IDA, 2003).

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## **Appendix A**

The following is a summary of some projects, studies and suggestions for the Hampton Harbor watershed. The first part of the summary is a list of completed actions that were initiated as far back as 1996 by the Seabrook Conservation Commission. The current activities involve studies that research the sources and the causes of elevated bacteria levels in the shellfish growing waters. And finally, a series of management actions are recommended for community projects such as stormdrain stenciling and regulatory programs that investigate the discharges of sanitary wastewater and stormwater.

### **Completed Actions**

#### Pollution Elimination and Prevention Business Survey in Seabrook

The New Hampshire Coastal Program (NHCP) Office of State Planning and the Seabrook Conservation Commission (SCC) conducted pollution elimination and prevention surveys of commercial businesses located in the Cains Brook Watershed, on Route 1 in Seabrook, New Hampshire. The purpose of the project was to identify potential bacterial and nonpoint sources of pollution to the Cains Brook Watershed, further investigate pollution sources identified previously by the SCC (1996), make recommendations on how to minimize those sources, and educate the commercial businesses on how they can conduct business in a more water-friendly manner.

The pollution sources identified during the study included open dumpsters, trash, sediment from erosion, and lack of maintenance of swales, catch basins and water quality inlet devices. As a result of the study, the general level of environmental awareness in the business community increased. For example, the management company for a large strip mall performed catch basin cleaning within a couple weeks of receipt of the recommendations proposed to them by the SCC.

#### Installation of a Stormwater Treatment Device in Seabrook

In 2002, the Town of Seabrook and DES installed a stormwater treatment device developed by AbTech Industries of Arizona. The company claimed that their device, the SmartSponge, reduces the bacteria concentration in stormwater. The SmartSponge was installed in a water quality inlet of the storm drainage system that serves the north beach neighborhood of Seabrook. The stormwater from this storm drain discharges just south of Cross Beach Road in Seabrook. The stormwater flows into a tidal creek and enters the Harbor in the proximity of study site HH2B. The device is currently under evaluation for effectiveness.

UNH researchers will use Environmental Protection Agency evaluation protocols for determining the pollutant removal effectiveness of the SmartSponge. A minimum of

thirteen storms will be monitored and the bacterial pollutant loads entering and exiting the devices will be measured and compared.

The results of the evaluation will be used to determine if additional AbTech devices should be installed in other drain systems in the watershed. If the study finds that the device is effective and the towns consider them a feasible option for treating stormwater, consideration will be given to installation of the device in drains that transport bacteria to the shellfish growing waters.

#### Autumn Dry Weather Study by the DES Shellfish Program

In the autumn of 2000, the DES Shellfish Program began a study of intermittent and unpredictable high fecal coliform counts observed in September and October in Hampton Harbor (Nash and Chapman, 2001). Repeated low tide fecal coliform sampling was conducted through the months of September and October and only after prolonged periods of dry weather. Sampling results show high, sometimes very high, bacteria levels approximately 50% of the time during autumn dry weather conditions. Additional monitoring at tributary sites suggests that the source of contamination does not emanate from upstream locations, but is probably located much closer to the harbor itself (Nash and Chapman, 2001).

#### New Hampshire Coastal Pumpout Program

The New Hampshire Department of Environmental Services, through the authorization of the Clean Vessel Act, has funded the installation of five pumpout facilities for boat users in the coast. One of these facilities is located in Hampton Harbor at the Hampton River Marina. The pumpout facility is open 7 days a week from May 15 through October 1 from 8:30 AM to 5:00 PM. There is no fee for Marina members and a \$5.00 fee for transients.

In 2002, DES contracted with Coastal Pumpout Services to provide mobile pumpout service to boats in most coastal locations. The pumpout boat was on the water from June through December in 2002, primarily servicing boats in Little Harbor and occasionally in Hampton Harbor. The coastal pumpout boat is available on Friday, Saturday and Sunday from 10:00 AM to 4:00 PM. Boaters can call ahead for an appointment. The fee is \$10 per pumpout.

#### Assessment of Environmental Factors Affecting Fecal Coliform Concentrations in Hampton/Seabrook Harbor

Environmental factors that affect concentrations of fecal coliform bacteria were investigated by the U.S. Geological Survey, in cooperation with the New Hampshire Department of Environmental Services at Hampton Harbor (Deacon and Nash, 2002). Water samples and other environmental factors were collected from the Harbor and tributaries on a routine and precipitation-event basis from March 2000 through December

2001. Water samples were analyzed for fecal coliform concentrations. Data for other environmental factors included rainfall, salinity, turbidity, water temperature, wind speed and direction, air temperature, bird counts, and basin characteristics such as areas served by municipal sewer, on-site septic systems and population density.

The study showed that median fecal coliform concentrations increased with increasing rainfall, indicating that rainfall can directly effect concentrations of fecal coliforms in the Harbor. The report indicates that direct surface runoff may be contributing to the increase in bacteria concentrations in the Harbor during and after periods of rainfall events. Statistical correlations between fecal coliform and other environmental factors were weak or not statistically significant except for a significant correlation between fecal coliform and turbidity for the spring samples.

### Stormdrain Stenciling

In 2002, over 100 Hampton stormdrains were stenciled with a message to alert residents not to dump waste into the catchbasins. The New Hampshire Estuaries Project funded the stenciling project that was carried out by the 8<sup>th</sup> grade students at Hampton Academy Junior High with assistance from two Winnacunnet High School students. The areas covered by the project were the roadways surrounding the school, which included High Street, Academy, Mill and Winnacunnet Roads. The Hampton Department of Public Works also participated in the project.

### **Actions in Progress**

#### Hampton Harbor Total Maximum Daily Loads for Bacteria

DES is currently developing total maximum daily loads (TMDL) for Hampton Harbor in accordance with Section 303(d) of the Clean Water Act and EPA Water Quality Planning Regulations. Hampton Harbor is a priority for TMDL development because of bacteria concentrations that exceed State surface water quality standards for the consumption of shellfish. The goal of the TMDL is for the water quality in Hampton Harbor to meet specific aspects of the national standards set for shellfish growing waters by the National Shellfish Sanitation Program.

As part of the TMDL development, DES conducted a study to characterize the bacterial loading from storm drains and tributaries and also show the effects of stormwater on Harbor water quality. Bacteria loads from 23 major drains and tributaries were monitored during two storms. The study reports loading from the targeted drains and the percent of the total load contributed by each drain. This information will be useful to managers when prioritizing mitigation efforts for the drainage systems. The tributary sampling showed that the highest concentrations of fecal coliform were in Mill Creek. This pattern matches the observation that the highest weighted geometric mean fecal coliform concentration among the Harbor stations is at HH19 at the mouth of Mill Creek (Trowbridge, In Review).

Monitoring of ten Harbor stations during the storms showed that the geometric mean fecal coliform concentration across all stations increased 28-34% from pre-storm conditions to post-storm conditions; however, these apparent increases were not statistically significant as tested using the Wilcoxon Signed Rank Test for dependent samples (Trowbridge, In Review). The only large increase in fecal coliform concentration was at the mouth of Mill Creek (HH19).

## Evaluation of the Impacts of Wastewater Treatment Facilities Discharges on Estuarine Water Quality

The New Hampshire Estuaries Project *Management Plan* (NHEP, 2000) includes an action plan to investigate the impacts of wastewater treatment facilities discharges on estuarine water quality. In 2001, University of New Hampshire researchers began an investigation that initiates data collection, compilation and interpretation in support of developing a better understanding of the impacts of the discharges on estuarine water quality in New Hampshire. Specifically, the project focuses on the eleven WWTFs that are located downstream of tidal dams. Nine of these WWTFs are located in New Hampshire (Dover, Durham, Exeter, Hampton, Newfields, Newington, Newmarket, Portsmouth, and Seabrook) and two are located in Maine (South Berwick and Kittery).

The three basic questions that this project is focusing on are the following: (1) what are there impacts from chronic loading of key contaminants from WWTF effluent on estuarine waters? (2) what are the impacts of runoff-induced hydraulic overloading on estuarine waters? and (3) what are the impacts from exfiltration of aging sewer infrastructure on estuarine water quality?

Understanding these bacterial inputs should aid in the control of shellfish closures due to pathogen loading. The results of the study, expected to be released in December 2003, should provide managers with a prioritized list of problems that facilities face in controlling the discharge of untreated and partially treated wastewater.

### **Recommended Management Actions**

#### Promotion and Expansion of Boat Sewage Pumpout facilities

Local marinas, boat clubs and retailers could promote the use of pumpout facilities by distributing the New Hampshire Coastal Pumpout Program brochures printed by DES. The brochures could be made available to local businesses such as hotels, marine suppliers, and convenience stores and at rest areas and state parks. Managers should promote the use of the facilities through Great Bay Radio, at the annual chili and seafood festivals in Hampton and Portsmouth and at education centers such as the Seacoast Science Center and Sandy Point Discovery Center.

DES should investigate the potential for an additional pumpout facility location in Hampton Harbor such as the private boat club and the fishing cooperative and increase the presence of the pumpout boat in Hampton Harbor, especially in the autumn months, when there may be greater need for pumpout services during the seasonal haul-out period.

#### Implementation of the Federal Storm Water Program Phase II: MS4 General Permit

Four of the six Hampton Harbor watershed communities are required to obtain a permit under Phase II of the federal storm water program to address stormwater pollutants originating from the municipal separate storm sewer system (MS4). Hampton, Hampton Falls, Seabrook and Exeter must each develop a storm water management program plan (SWMPP) that uses appropriate best management practices (BMPs) for six minimum control measures listed in the regulation. The BMP approach will enable municipalities to develop sensible and cost-effective programs for controlling storm water runoff that are tailored to their needs. The municipalities are given a great deal of flexibility in determining BMPs and measurable goals for their MS4 service area. The EPA expects that these six minimum control measures, when implemented together, will result in significant reduction of pollutants discharged into receiving waters:

1. A public education and outreach program
2. A public involvement/participation program
3. An illicit discharge detection and elimination program
4. A construction site runoff control program
5. A post construction runoff program
6. A municipal pollution prevention/good housekeeping operation & maintenance program.

The SWMPP must include the measurable goals for each control measure that will be used to gauge the success of the overall program. It must also contain an implementation schedule that includes milestones, frequency of activities and reporting of results.

The four communities listed above were required obtain a permit from the EPA by March 10, 2003 by filing a Notice of Intent. The permit requires annual reporting of SWMPP implementation progress.

The communities have already taken some steps to address the six minimum control measures. For example, the Town of Hampton has developed stormdrain infrastructure maps as part of its Illicit Discharge Detection and Elimination Program and the Town of Seabrook has started development of its maps. And, the Town of Exeter has eliminated several illicit discharges into the MS4 system.

#### Septic System Maintenance Education

There are a few homes still remaining on septic systems in Seabrook and all residences in Hampton Falls have on-site septic disposal. A recommendation should be

made to local officials to educate home and business owners about the care and maintenance of septic systems.

### Catchbasin Stenciling

Based on the TMDL wet weather results that showed bacteria loading from the storm drains in the watershed and the MST results that showed a mix of bacterial sources in the Harbor, stenciling projects should be encouraged in other parts of the watershed where storm drainage infrastructure is present. Mitigation of non-human sources (e.g., birds, wildlife, and pets) can best be addressed by the application of nonstructural best management practices or “good housekeeping” measures such as storm drain stenciling.

### Wildlife Management

Deer enjoy eating the vegetation offered by homes in suburban and urban developments. These developments, built in formerly forested and fielded areas, provide ideal edge and winter feeding grounds for deer. Humans can take measures to limit the access deer have to such food sources. Reducing deer access to vegetation in residential developments will force deer to be more reliant on wild vegetation. When deer must rely on available wild lands for their only food source, a corresponding drop in deer population should take place.

Humans should refrain from feeding deer to keep them reliant on their own habitat for food. High fencing could be erected around plants. Fences should be at least eight feet high and buried one foot deep. Openings in the fence should be small. Contact the University of New Hampshire agricultural extension office for advice before purchasing and installing a fence. Plant native plants tolerant of deer browsing. Contact your local nursery to find out more about plants that repel deer through smell and taste.

It is often a difficult to balance the various objectives of wildlife management. For example, any reduction in the population of coyotes may have the unintended consequence of an accompanying increase in the population of rabbits, rats, and other small rodents. Coyotes can be dissuaded from staying in an area by employing a variety of humane methods. Keeping garbage cans in enclosed areas and securing the lids on the cans can dissuade coyotes from eating your garbage. If necessary, tall fences can be built around yards and property, with extensions buried underground to prevent coyotes from digging their way in.

Raccoons and skunks consume a diet consisting primarily of rodents and insects. A tempted animal may also eat trash, disrupting and even overturning garbage cans. As with other animals, the elimination of potential food source is a key to making your home a less attractive destination for these creatures. Keep garbage cans in an enclosed area. Keep dog and cat food inside. Enclose gardens with fencing. Prevent raccoons and skunks from digging below the fence by burying an extension underground.

Many of the suggestions above were provided by the In Defense of Animals website (IDA, 2003). The New Hampshire Fish and Game Department also offers suggestions for managing wildlife on the department's website (NHF&G, 2003).