Pollution Source Tracking at New Hampshire (USA) Ocean Beaches

Stephen Jones[1], Sara Sumner[2], Natalie Landry[3], Jody Connor[2,4]

(1) University of New Hampshire; Jackson Estuarine Laboratory; Durham, New Hampshire; 03824; USA
Tel: +(1) 603 862-5124 Fax: +(1) 603 862-1101
Email: shj@unh.edu
(2) Beach Program; NH Department of Environmental Services; 29 Hazen Dr.; PO Box 95; Concord, New Hampshire; 03302
Tel: +(1) 603 271-8803 Fax: +(1) 603 271-7894
Email: ssumner@des.state.nh.us
(3) Watershed Management Bureau; NH Department of Environmental Services; Pease International Tradeport; 50 International Drive; Suite 200; Portsmouth, New Hampshire; 03801; USA
Tel: +(1) 603 559-1507 Fax: +(1) 603 559-1510
Email: nlandry@des.state.nh.us
(4) Email: jconnor@des.state.nh.us

Abstract

Ocean beaches are an enormously popular recreational destination in New Hampshire during summer months for swimming, surfing and sunbathing. With increasing population growth and development along the coast, additional pollution sources and documented impacts to beach water quality constitute an increasingly significant threat to public health and sustainable development. Investigations for identifying pollution sources are often inconclusive, and when sources are identified and eliminated, water quality has not always been improved.

The University of New Hampshire and the New Hampshire Department of Environmental Services teamed up to assess pollution sources affecting the state's coastal beaches. Drainage areas from salt marshes behind four beaches were sampled under wet and dry weather conditions to determine concentrations of *Escherichia coli* and enterococci in water samples. *E. coli* isolates were ribotyped using a RiboPrinter and the ribopatterns were compared to patterns from known source species databases to identify the most significant source species contributing to water pollution. A threshold similarity level of 90% was used for source identifications. Enterococci concentrations in water, beach sand and seaweed wrack were also measured at one of the beaches.

E. coli concentrations were much higher in water sampled under wet weather conditions compared to dry conditions at all four study sites. There were acceptable matches for 108, or 58% of the 187 isolates that were ribotyped. The most significant type of source species was wild animals, and especially otters at one beach. Human sources were also significant, while few isolates appeared to be from pets, birds or livestock. There was little difference in the source species types identified in samples collected during wet and dry weather conditions. Beach wrack and underlying sand were found to harbor consistently high (10^3 - 10^5/g DW) levels of enterococci, raising the concern that washing of these materials during high tide could cause complications on the interpretation of apparent water quality problems and beach postings.

Further studies are underway to better assess the public health significance of these results using a more comprehensive and integrated MST approach. State agencies are applying various strategies to manage or eliminate identified sources of pollution, and the possible water quality impacts of other coastal resource management actions such as salt marsh restoration are emerging concerns. The described MST approach, used at other New Hampshire beaches and in shellfish-growing waters throughout New England, is under continuous assessment for improvements and wider applications.

Keywords: beaches, bacteria, pollution source tracking, ribotyping

Introduction

The U.S. Environmental Protection Agency (EPA) developed the Beaches Environmental and Coastal Health (BEACH) Act to better protect public health at coastal beaches in the US. The New Hampshire Department of Environmental Services (DES) Beach Program received support from EPA to enhance coastal monitoring to include six new ocean beaches, adding to the nine previously monitored. Monitoring programs have provided data from weekly sampling to notify the public when bacteria levels are elevated. The New Hampshire State water equality standard at marine swimming beaches is 104 enterococci/100 ml for a single sample and a geometric mean of 35 enterococci/100 ml in at least three samples collected in a 60-d period.

New Hampshire's coastal beaches have historically met state water quality standards for primary contact recreation. Over the past four years, enterococci concentrations have exceeded the state standard 2% of the time at most beaches. Before 2003, there had never been any advisories issued for the tidal bathing beaches in New Hampshire. Since then there have been one, three, and one beach posted for a total of three, six and one day in 2003, 2004 and 2005, respectively (Trowbridge 2006). These results may be indicative of a decline in water quality in New Hampshire's coastal waters. A sanitary survey of the Atlantic Coast area was conducted by the DES Shellfish Program in 1999 (Nash and Chapman, 2000). This study identified both actual and potential bacterial pollution sources located in Rye, North Hampton, and Hampton, in close proximity and thus potential threats to several coastal beaches.

Recent adoption of biotechnological techniques for application to water quality issues has spawned a number of approaches to address identification of sources of fecal-borne contamination. These new approaches, often called "microbial source tracking (MST), have been used successfully for over 15 years in a number of areas in the United States (USEPA 2005). Use of ribotyping of *Escherichia coli* isolates cultured from target surface waters is one MST approach that can provide information on sources of fecal contamination. Various studies have reported on the use of ribotyping for tracking sources of fecal-borne microbial contaminants. The approach involves identifying microorganisms in the environment as being from different sources by comparing patterns of (ribosomal RNA) DNA fragments isolated, digested by restriction enzymes and electrophoresed in agar gels. The method requires analysis of DNA fragments of *E. coli* isolates cultured from the target watershed and compared to isolates from known sources, including all human and animal sources suspected of being in the watershed. Samadpour and Chechowitz (1995) used ribotyping of *E. coli* from either livestock on hobby farms or on-site septic systems in Washington State. Numerous ribotyping studies have been conducted in freshwater watersheds (Tippets, 1999; Barsotti et al, 2000; Carson *et al*, 2001; Hartel *et al*, 2002), while others have been conducted in estuarine waters (Samadpour and Chechowitz, 1995; Simmons *et al*, 1995; Parveen *et al*, 1999; Jones, 2002). Jones (2002) was the first report published on ribotyping in the estuarine waters of New England.

Jones and Landry (2003) studied the pollution sources causing shellfish harvesting use impairments in Hampton/Seabrook Harbor in New Hampshire using ribotyping. Jones (2003) reported on the source species identified in a ribotyping project involving stormwater discharging from two separate storm drainage systems in coastal New Hampshire. The method has recently been used to identify fecal pollution sources near ocean beaches in New Hampshire (Jones *et al*, 2004; Jones and Landry 2004). Even though the beach water quality standard is enterococci, use of *E. coli* ribotyping is consistent with its use as a standard for freshwater pollution levels, and the fact many pollution sources are freshwater borne. In New Hampshire, the freshwater recreational water quality standard is 88 *E. coli*/100 ml, or a geometric mean of 47 *E. coli*/100 ml.

This study summarizes the findings from several pollution source investigations at New Hampshire's Atlantic coast beaches. Because ribotyping can provide information on the identity of source species of bacteria found in surface waters, follow-up efforts to identify and eliminate contamination sources can be directed towards those types of sources where the few species responsible for the most significant amounts of contamination can be targeted. Through an iterative process of then finding possible sources of fecal contamination from significant species, ribotyping can be used again to match strains for a given species to specific sources. Thus, the overall effort to improve water quality can be targeted because the most significant sources actually found in surface waters of concern are directly identified and eliminated. Such an approach also provides savings of time and overall cost.

Materials and Methods

Project Setting

This project involved the investigation into four streams flowing to the Atlantic Coast. The streams, identified as pollution sources to coastal beaches, are ACPS 5, Parson's Creek; ACPS 10, Bass Beach Brook; ACPS 11, Chapel Brook; and ACPS 12, Little River (Fig 1). All four are salt marshes that discharge in the vicinity of coastal public beaches. Parson's Creek discharges to Pirates Cove Beach, Chapel Brook and Bass Beach Brook discharge to Bass Beach, and Little River discharges to North Hampton State Beach. ACPS 10 (Bass Beach Brook) was not sampled during wet weather.

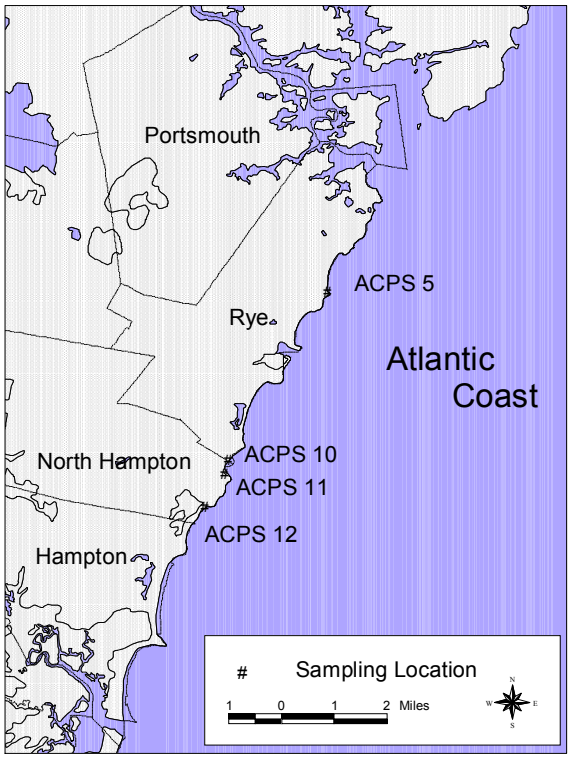

Figure 1: Coastal stream study sites near ocean beaches.

The land area along the immediate shoreline of the Atlantic Coast is sixty-four percent developed, predominantly with residential use. Approximately 31 percent of the shoreline is undeveloped, with a majority of this land permanently protected from development. Onsite (septic) systems are the most common means of sewage disposal for the shoreline properties in Rye and North Hampton (Nash and Chapman, 2000).

Field Methods

Water samples were collected in accordance with the standard procedures for collecting water samples for bacterial analysis. Each sample was collected using a sterile 250 mL plastic bottle from mid-stream, mid-depth using a telescoping sampling pole. Samples were placed in a cooler on ice packs and immediately delivered to the laboratory for analysis. Wet weather sampling occurred during three events with >21 mm of rainfall within 24 h of sampling. Precipitation data were taken from two weather stations located in coastal New Hampshire. Dry weather sampling occurred on six days under conditions with <6 mm of precipitation in the 24 h prior to sampling.

Seaweed wrack was collected monthly from the wrack line before high tide in the afternoon from June to August, 2004. Beach sand was also collected from below the wrack, midway in the intertidal zone and at 0.5 m water depth, where a water sample was also collected. All samples were placed in sterile containers and transported on ice to the lab for analysis. The air and water temperature, along with the depth of the wrack and the number of people on the beach, were recorded at the time of sampling.

Fecal samples were collected from the four Atlantic Ocean subwatersheds (Parsons Creek, Bass Beach Brook, Chapel Brook, and Little River) and from the Little Harbor watershed. The samples were collected from known sources using a sterile Whirlpak™ bag. Human sources were collected from the Portsmouth wastewater treatment facility pre-chlorination effluent. All of the fecal samples collected for this study were added to the New Hampshire Coastal and the State source species databases.

Laboratory and Analytical Methods

Detection, Identification of Fecal Coliform, E. coli and Enterococci

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 (Rippey et al., 1987).

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. The *E. coli* isolates were subject to a battery of biochemical tests to confirm their identity as *E. coli* (APHA 1995). The confirmed *E. coli* isolates were then processed for determining ribopatterns. Atypical *E. coli* isolates identified by the RiboPrinter® as other species were subject to further tests using the API 20e identification system. Those found to be *E. coli* were retained in the database while isolates giving negative results were removed.

Enterococci concentrations were determined using the Enterolert (IDEXX Laboratories, Inc., Westbrook, Maine) system. Water samples were added directly or diluted into the Quanti-tray. Clean wrack and sediment samples were added at different dilutions to polystyrene bottles and sonicated to dislodge enterococci cells from surfaces using a Branson 250 digital sonifier (Branson Ultrasonics Corp., Danbury, CT) for 30 s with pulse engaged. Volumes of supernatant were added to the Quanti-trays. All samples were incubated for 24 h at 41±0.5 °C. The 97 well Quanti-trays were then exposed to a 365-nm UV light and fluorescent wells were counted as positive. The number of positive wells was converted to a most-probable-number (MPN) value based on the dilution and the manufacturer-supplied MPN table. Sand and wrack samples were dried at 95°C for 24 h to determine dry weights.

Sample Processing

The procedures used for ribotyping *E. coli* isolates for this study is 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). 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 were processed for band identification. The ribopattern data for each separate water sample isolate were then selected for identification of source species.

Source Species Database

The analysis of the project water sample isolates for identification of source species was based initially on a New Hampshire Atlantic coast source species database and then a NH State source species database (Table 1).

Source species	# of Isolates		Source species	# of Isolates	
	Coast	State		Coast	State
LIVESTOCK (3 & 101)			**"HUMANS" (48 & 205)**		
alpaca	-	3	septage	6	16
buffalo	-	5	wastewater	42	107
chicken	3	3	humans	-	82
cow	-	56	**PETS (26 & 58)**		
goat	-	4	cat	7	21
horse	-	28	dog	19	37
sheep	-	2	**BIRDS (80 & 117)**		
WILD ANIMALS (201 & 293)			cormorant	12	12
coyote	4	29	duck	14	16
deer	49	93	geese	30	39
mouse	-	12	gull	24	28
muskrat	12	2	pigeon	-	5
otter	14	14	robin	-	4
raccoon	67	84	sparrow	-	3
rabbit	27	27	starling	-	3
red fox	23	27	wild turkey	-	7
skunk	5	5	**TOTALS**	**358**	**774**

Table 1: Source species databases for the Coast and New Hampshire (State).

The average rate of correct classification (ARCC) for the two source species databases was ~70% when all isolate patterns were included and lower when clones were excluded. The 358 Coastal and 774 State patterns included some that had identical patterns for multiple species. This is considered to reflect 'transient' (Samadpour, 2002) 'garden-variety' strains of *E. coli* that can either exist temporarily in non-source species or are adapted to multiple species. These were included to allow for identification of patterns as being from "mixed" source species.

Data Analysis

All data were analyzed with GelComparII software on a Dell computer, where the source species database was also stored. 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. Tolerance and optimization settings can be used to off set the similarity coefficient used but a balance is required between stringency of data analysis parameters and the fraction of isolates that can be identified. 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 100% matching of resulting ribopatterns using 1.5% optimization and 1.0% band tolerance. Use of lower, more stringent band tolerances gave calculated similarities of <100%, suggesting differences in banding patterns that are a function of the method, not the isolate. Thus, the 1.5/1.0% settings were best for allowing comparisons between actual banding pattern differences.

Similarity indices were determined using Dice's coincidence index (Dice, 1945) and the distance among clusters calculated using cluster analysis. The cluster analyses were based on the un-weighted pair group method by arithmetic averaging (UPGMA) or the neighbor joining algorithms. The source species profile with the best 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.

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 on Macintosh computers.

Results and Discussion

The geometric mean *E. coli* concentrations at the four study sites were all >100 cfu/100 ml, with an overall geometric mean of 141 cfu/100 ml (Table 2). Much higher *E. coli* concentrations were measured during wet compared to dry weather at three of the sites, with a smaller difference at ACPS 10. These results illustrate the detrimental impact of storm water runoff and other possible rainfall-induced conditions on water quality at these marsh discharge sites.

	E. coli CONCENTRATIONS (cfu/100 ml)		
SAMPLING SITES	All Samples	Wet Conditions	Dry Conditions
ACPS 5	109	273	51
ACPS 10	151	200	143
ACPS 11	117	784	18
ACPS 12	205	993	31
All Sites	**141**	**577**	**45**

Table 2: Geometric mean *E. coli* concentrations at the four study sites under wet and dry conditions.

Isolates of *E. coli* were selected for ribotyping from all wet and dry weather samples that were biochemically confirmed as *E.coli*. Overall, there were 187 isolates from the four sites that were successfully ribotyped using a threshold similarity level of 90% (Table 3). Identification of the source species for isolate ribopatterns by first using the Atlantic Coast database then follow-up analysis using the NH State database yielded acceptable matches for 108, or 58% of the 187 isolates. Identification of >50% of the isolates is typical (Jones 2003; Jones and Landry 2003), The US EPA MST Guide Document (USEPA 2005) cites results form an *E. coli* ribotyping study in Virginia where 65% of isolates were identified to source species. Use of 90% similarity as a threshold provides a good balance between accuracy and success in identification of source species.

Type of source species	AllSamples # of isolates	AllSamples % of total isolates	Wet Conditions # of isolates	Wet Conditions % of total isolates	Dry Conditions # of isolates	Dry Conditions % of total isolates
Humans	31	17%	20	15%	11	20%
Pets	3	2%	2	2%	1	2%
Livestock	8	4%	7	5%	1	2%
Wild animals	54	29%	38	29%	16	29%
Birds	12	6%	9	7%	3	5%
Unknowns	79	42%	56	42%	23	42%
TOTAL	187	100%	132	100%	55	100%
Total identified	*108*	*58%*	*76*	*58%*	*32*	*58%*

Table 3: Identified source species for sites near NH ocean beaches under wet and dry weather conditions.

Some of the challenges to successful source species identification included construction of a database with source species that were present in the study area and having enough ribopatterns for species that included those found in the water samples. The number of ribopatterns for a given source species varies (Table 1), and is dependent on how many samples and how many individual animals are used, as well as the resulting diversity of ribopatterns from the isolates.

The ribotyping results showed wild animal (otter, raccoon, deer) and human source species to be the most significant, with birds (geese, gulls) and especially livestock (horses, cows) and pets as less significant sources (Table 3). Human, otter and raccoon ribopatterns were present at all sites, while other source species were confirmed at only one site. Otter ribopatterns were especially prevalent in the marsh discharge at ACPS 12, the Little River near North Hampton State Beach. The actual presence of otters and their feces was field-confirmed at several sites.

Source species identified during wet weather may be more critical given the elevated levels of *E. coli* detected under wet conditions (Table 2). There were no major differences in types of source species identified during dry and wet weather (Table 3) with only a slight decrease in the significance of human sources under wet weather. The level of success for identifying source species remained the same (58%) as wild animals remained the most significant sources under both conditions.

The public health significance to bathers of fecal pollution from wild animals such as otters is largely unknown. Animals can harbor pathogenic bacteria, viruses, protozoans and other organisms that are shed with their feces and thus pose a threat to humans exposed to contaminated waters. Exposure to human-borne fecal pollution poses a known health risk, mostly from viruses. Geldrich (1996) presented a summary of the percentages of humans, cattle, sheep, pigs, dogs, cats and wild animals that excreted bacterial, parasitic and viral pathogens. The results were quite varied both for a given pathogen and for each host. Geldrich (1996) noted that pathogens in humans are usually only found in diseased individuals, whereas other animals may be more stable reservoirs of pathogens.

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Bacterial pollution sources that may not be from the marsh discharges were also a concern, and additional studies investigated enterococci concentrations in water, beach sand and seaweed wrack near ACPS 12 at North Hampton State Beach. Enterococci were detected in water at levels less than the single sample maximum of 104 cfu/100 ml except on July 26 (Table 4). Enterococci concentrations in intertidal and submerged beach sand were also relatively low.

	Overall average	6/20/04	7/26/04	8/21/04
WATER	60±47	35±12	113±46	33±17
SEDIMENT				
Submerged	46±30	69±16	12	33
Intertidal	42	ND	ND	42
Under wrack	$6.0\pm9.5 \times 10^3$	$1.7\pm1.5 \times 10^3$	ND	$1.0\pm1.4 \times 10^3$
WRACK	$2.0\pm0.9 \times 10^5$	$1.7\pm0.3 \times 10^5$	$1.3\pm0.3 \times 10^5$	$3.1\pm0.4 \times 10^5$
TEMPERATURE				
Air (°C)	27.0	26	26	29
Water (°C)	21.3	22	21	21
Wrack (°C)	33.3	31	34	35
Wrack depth (in)	10.7	10	15	7
People at beach	98	35	110	150

Table 4: Enterococci concentrations in water (MPN/100 ml), sediment and wrack (MPN/g DW) from the State Beach in North Hampton, New Hampshire.

However, beach wrack and underlying sand were found to harbor consistently high (10^3 - 10^5/g DW) levels of enterococci. The concentrations did not vary probably because of the consistent conditions in the wrack and surrounding environment, based on temperature readings. The high enterococci concentrations in the wrack and underlying sand suggest that washing of the wrack at high tide could impact water quality. Any washing of the wrack or suspension of the underlying beach sand at high tide could cause high levels of enterococci to be suspended in the water column. The public health significance of this environmental reservoir of indicator bacteria is not known as no pathogen detection studies have been conducted. Suspension of wrack-borne enterococci into the water column could cause complications in water quality monitoring and beach postings. Wrack has been suggested as a possible source of enterococci at the beach (Carlson and Sumner 2005). Others have found that algal mats accumulated along beaches in wrack lines can be sources of fecal-borne indicator bacteria (Weiskel *et al*, 1996; Whitman *et al*, 2003, Martin and Gruber 2004).

Further studies are underway to better assess the public health significance of these results using a more comprehensive and integrated MST approach. State agencies are applying various strategies to manage or eliminate identified sources of pollution, and the possible water quality impacts of other coastal resource management actions such as salt marsh restorations are emerging concerns. The described MST approach, used at other New Hampshire beaches and in shellfish-growing waters throughout New England, is under continuous assessment for improvements and wider applications.

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