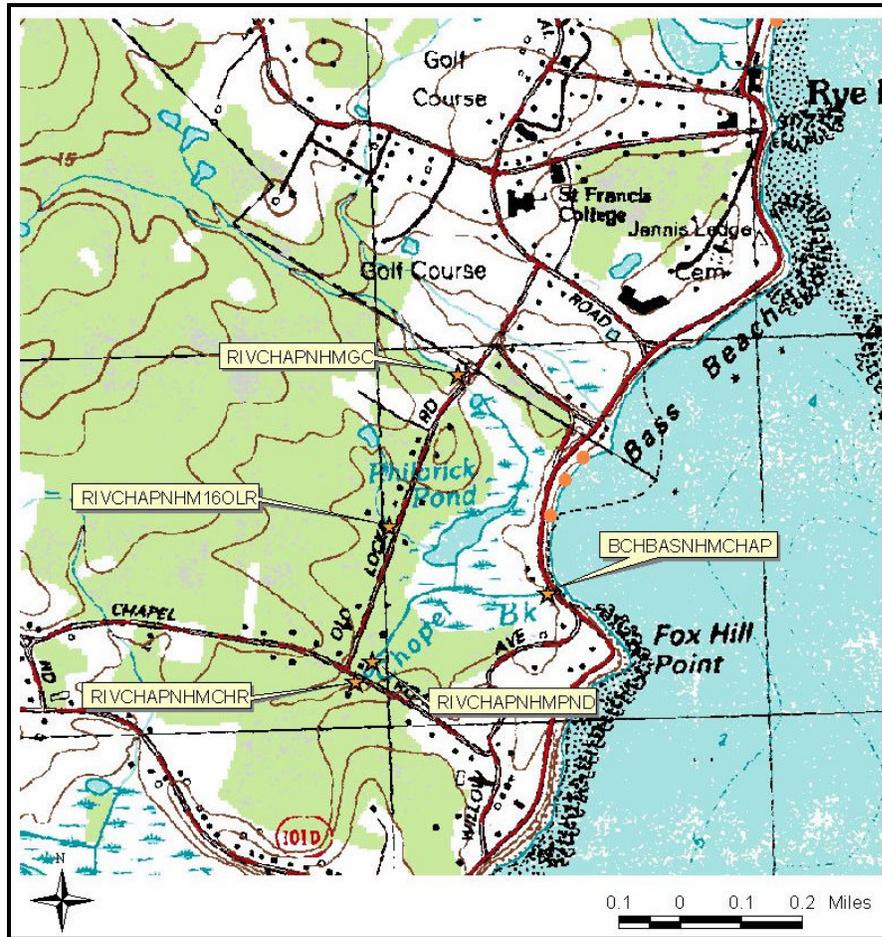


# Chapel Brook Special Study North Hampton, NH

May to September 2006



January 2008





# **Chapel Brook Special Study North Hampton, NH**

**May to September 2006**

New Hampshire Department of Environmental Services  
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January 2008

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## Introduction

In October of 2000, the United States Environmental Protection Agency (EPA) signed into law the Beaches Environmental Assessment and Coastal Health (BEACH) Act. The BEACH Act is an amendment to the Clean Water Act, which authorizes EPA to award grants to Great Lakes and coastal states. The purpose of the BEACH Act is to reduce the risk of disease to users of the nation's coastal recreational waters. BEACH Act grants support the development and implementation of monitoring and notification programs.

The New Hampshire Department of Environmental Services' (DES) coastal Beach Program has received BEACH Grant funding since 2002. The Beach Program provides a monitoring program for 15 coastal public beaches and undertakes intensive watershed and microbial source tracking (MST) studies when chronic elevated bacteria levels are measured.

Bass Beach in North Hampton is a small sheltered beach that is not well-mixed by open ocean waters (Figure 1). While the beach is not known as a popular swimming location, it is frequented by a large surfing contingent. DES has issued two advisories at Bass Beach, the first in August 2004 and the most recent in June 2006. The 2004 advisory resulted in Bass Beach being listed as impaired for primary contact recreation in the 2006 Consolidated Assessment and Listing Methodology (Comstock, 2006).



**Figure 1. Bass Beach, North Hampton, New Hampshire (aerial view).**

Chapel Brook is a tributary to Bass Beach and discharges into the Atlantic Ocean on the south side of the beach. The brook drains Philbrick Pond, as well as smaller associated tributaries. Chapel Brook was identified as an actual pollution source by the DES Shellfish Program during its Atlantic Coast Sanitary Survey (Nash and Chapman, 2000) and was the subject of a MST study by DES and the University of New Hampshire Jackson Estuarine Laboratory (Jones, Sumner and Connor, 2004). The MST study conducted in 2004 confirmed sources of *E. coli* bacteria originating from wildlife, humans, and waterfowl. Elevated enterococci results

observed during routine beach inspections prompted Beach Program personnel to design a program to further investigate bacteria sources. This report summarizes the intensive watershed study program conducted during the late spring and summer of 2006.

## Materials and Methods

### *Project Setting*

Five stations in the Chapel Brook watershed were sampled during this study (Figure 2 and Table).



Figure 2. Chapel Brook Special Study (2006) Sampling Stations.

**Table 1. Chapel Brook Special Study (2006) Sampling Station Descriptions.**

<b>Station Name and Description</b>	<b>Station ID</b>	<b>Latitude</b>	<b>Longitude</b>
<b>Chapel Brook:</b> Located on the west side of Route 1A, just north of Willow Ave. The sample is collected prior to the culvert under the road.	BCHBASNHMCHAP	42° 57' 57"	70° 46' 19"
<b>Stream on Chapel Road:</b> The stream passes under Chapel Road just southeast of the intersection with Old Locke Road.	RIVCHAPNHMCHR	42° 57' 51.7"	70° 46' 42.7"
<b>Pond:</b> The pond is accessed via the driveway of the house on the corner of Old Locke Road and Chapel Road. The sample is collected at the outlet of the pond.	RIVCHAPNHMPND	42° 57' 52"	70° 46' 40.2"
<b>16 Old Locke Road:</b> An intermittent stream passes through the property at 16 Old Locke Road. The sample is collected adjacent to the road.	RIVCHAPNHM16OLR	42° 58' 3.8"	70° 46' 37"
<b>Golf Course:</b> A stream/culvert passes under Old Locke Road and connects a detention pond on the Abeniqui Country Club property to Philbrick Pond.	RIVCHAPNHMGC	42° 58' 16"	70° 46' 28.7"

***Project Goals & Objectives***

The study objective was to provide information to identify and remediate human sources of bacteria from the Chapel Brook watershed. The study design was based on the MST study that revealed 19 percent of the *E. coli* contribution was from human sources. Chapel Brook is a complex hydrologic system that has been recently documented to contribute to the water quality of a designated public beach. The results from this project will help public health officials and DES to implement Best Management Programs to improve the water quality conditions at Bass Beach and protect the public health of those who recreate at the beach.

***Field Methods***

Weekly samples were collected between May and September 2006. Sampling variation was necessary due to weather events and flow conditions. Samples were collected from running water while sampling from stagnant pools was avoided (Appendix B). Sampling was not conducted during extreme weather events such as the 2006 Mother’s Day flooding and a June storm event that caused downed trees and power outages.

Samples were collected in sterile polypropylene bottles, placed in a cooler with ice, and transported to the DES Laboratory for analysis. All samples were returned to the laboratory within six hours of collection, as required by laboratory protocol. Samples were analyzed for the presence of *Escherichia coli* (*E. coli*) bacteria at all stations. While coastal beach samples are analyzed for Enterococci, the state standards for freshwater tributaries are based on *E. coli* concentrations. The Standard Operating Procedures (SOPs) for this study are located in Appendix A.

One inspection form was completed per sampling station for each inspection day. Parameters observed include: recent rainfall amounts, water conditions, and the presence of wildlife.

### ***Lab and Analytical Methods***

Lab methods followed the standard operating procedures of the Beach Program. All samples were analyzed for the presence of *E. coli* bacteria. SOPs for *E. coli* laboratory analysis are in Appendix A.

### **Results and Discussion**

A total of 52 samples were collected from five stations and a minimum of three samples were collected at each station (Table 2). Each station was sampled twice during the storm event of June 7, 2006.

**Table 2. Number of Samples Per Station.**

<b>Station Name</b>	<b>Number of Samples</b>
Chapel Brook	13
Stream on Chapel Road	14
Pond	14
16 Old Locke Road	8
Golf Course	3

The Pond station contributed the greatest single sample *E. coli* concentration (5,100 Cts/100mL) to the Chapel Brook watershed (Figure 3). Pond station had the highest concentration of all monitored sites on at least 50% of the sample days. The pond receives flow from the Chapel Brook Stream that originates from a forested wetland area. Field reports documented duck populations that ranged from two to 13 ducks in the pond or on the surrounding grass areas. Duck feces were often observed littering the grassy area. Waterfowl feces contribute large amounts of bacteria to waterways through surface runoff and direct deposit (WD-BB-53). The investigators noted the pond had a low water level on July 5 and again on August 16, 2006. A low dilution factor combined with warmer water temperature and decreased flushing rate typically favor increased bacteria growth. *E. coli* levels measured on these dates (910 and 800 counts per 100 mL, respectively) likely reflect the environmental conditions.

The intermittent stream at Sixteen Old Locke Road contributed the second highest concentration of *E. coli* during the study. A small pond is located upstream of the sampling location and is the likely origination point for this stream. Due to poor water flow conditions at this station, samples were only collected on approximately 50 percent of the sampling days. Slightly more than 0.25 inches of wetfall was measured prior to the August 28, 2006, sample and likely contributed to elevated *E. coli* levels. Watershed runoff from wetfall did not contribute to the *E.*

*coli* levels on July 18, 2006, and September 6, 2006 (Figure 3), and as a result, these dry weather bacteria spikes may indicate human impacts.

The stream on Chapel Road experienced elevated *E. coli* levels mid and late summer. A site walk confirmed that this stream originates from a small wooded wetland area. Wetfall was not a factor at this station. Stream flow decreased as the summer progressed but stream stagnation never became an issue with sample collection. The greatest *E. coli* measured at this station was 740 counts per 100 mL on July 5, 2006 (Figure 3). Generally, elevated *E. coli* levels measured at the Chapel Road Stream corresponded with elevated levels measured at the Pond. Five of the 14 sample days reflected a higher Pond *E. coli* level (above 300 counts per 100 mL) than that measured at the Stream on Chapel Road. .

Golf Course station *E. coli* never exceeded 46 counts per 100 mL (Figure 3). The Golf Course station does not appear to be a significant source of bacteria to Chapel Brook; however, this conclusion is only based upon two samples as stagnant conditions persisted for most of the sample period. DES recommends that continued monitoring of this site would be beneficial and would help verify that this site is not a significant bacteria source to Chapel Brook.

The ambient Chapel Brook monitoring station is located downstream from the previously discussed stations. Enterococci are historically measured at this station during low tide conditions to compare with beach Enterococci levels. Large precipitation events can negatively impact bacteria levels (Figure 4) as demonstrated on the July 13, 2006, sampling event. A three day precipitation event yielded 4.0 inches of wetfall and resulted in elevated Enterococci levels at the public beach. *E. coli* concentrations fluctuated throughout the season. *E. coli* levels were elevated on August 28, 2006, likely the result of elevated bacteria levels measured in the Stream on Chapel Road, Pond, and 16 Old Lock Road stations.

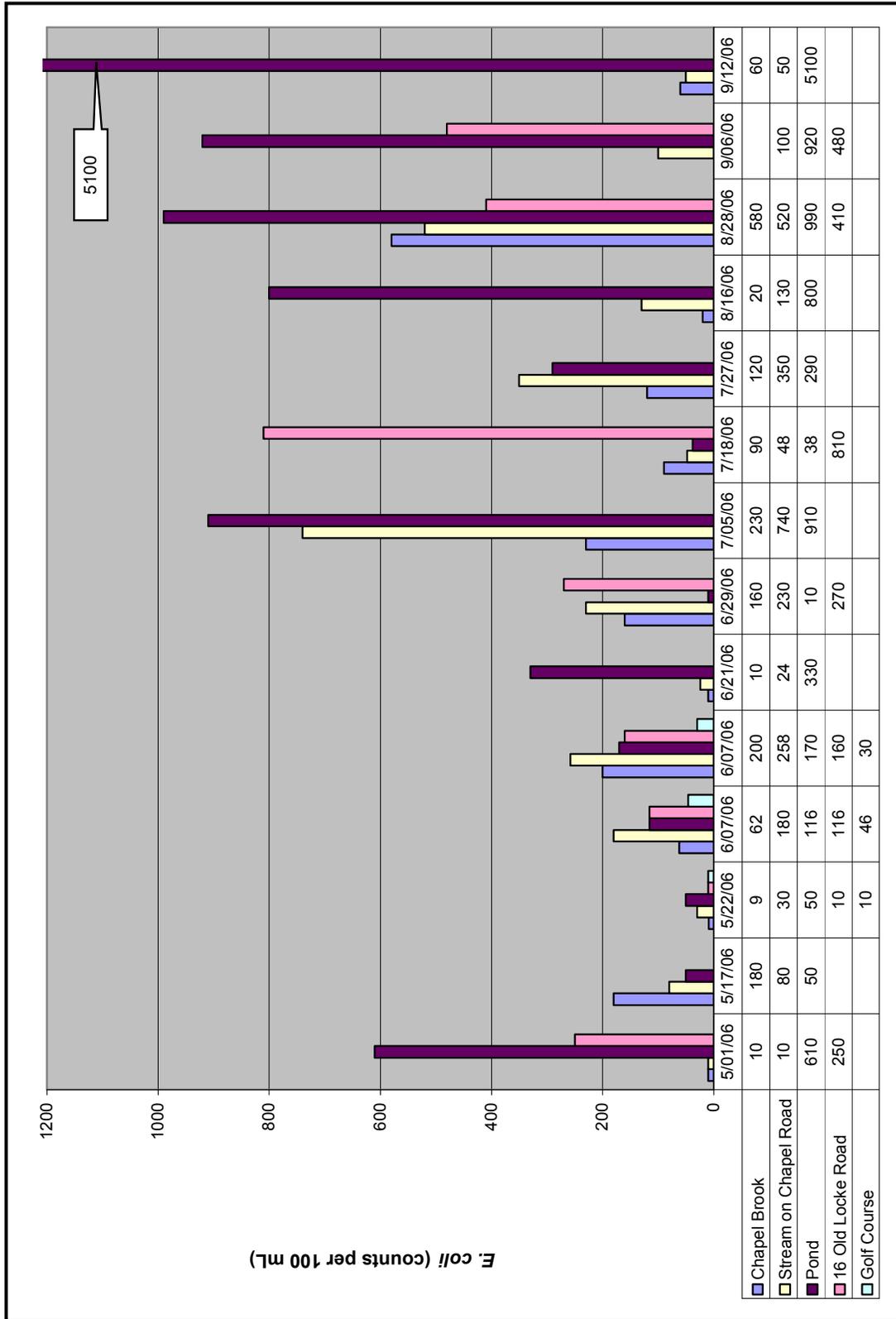


Figure 3. *E. coli* Sample Results from all sampling stations May to September 2006.

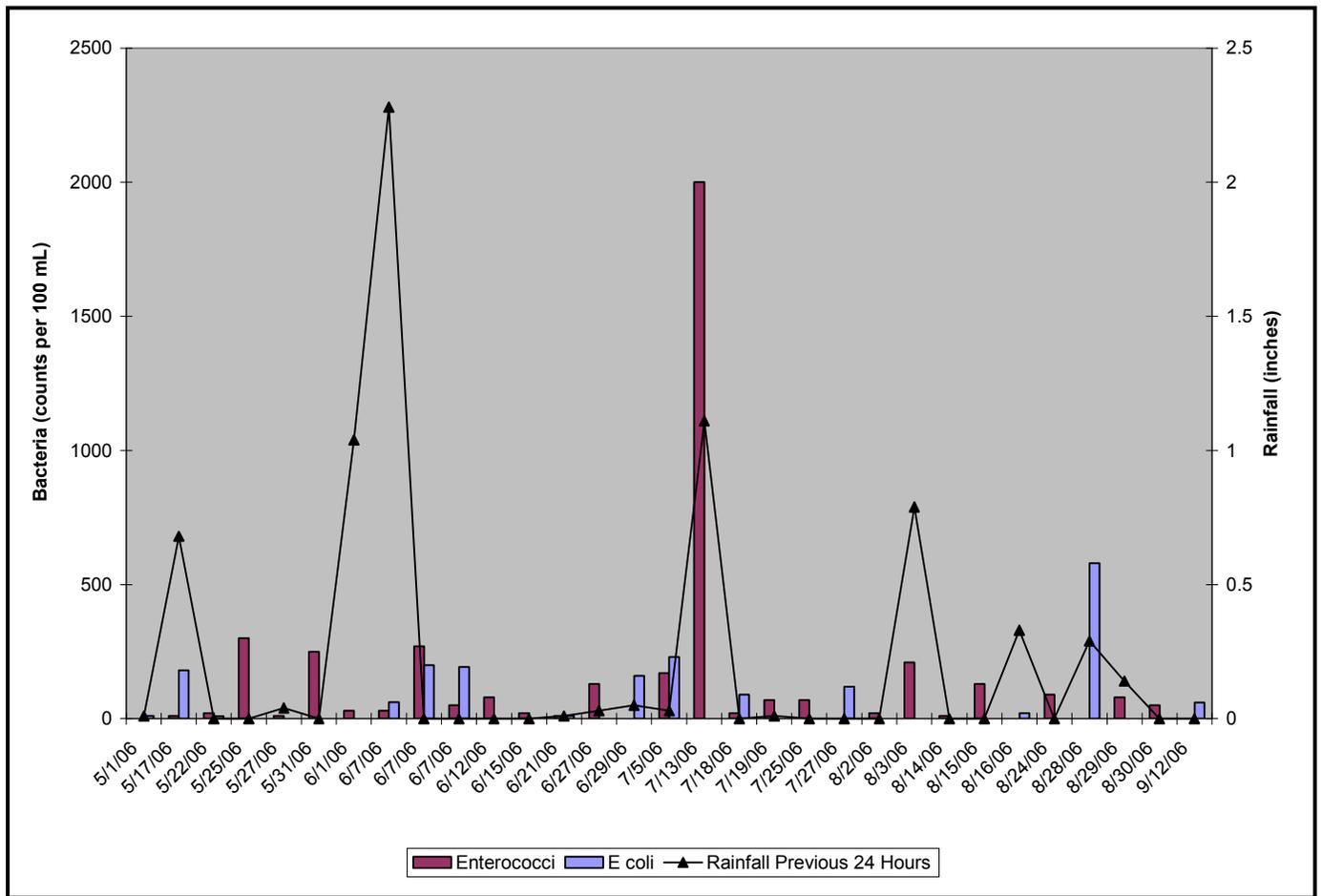


Figure 4. 2006 Chapel Brook Enterococci and *E. coli* Results with Rainfall.

### ***Wet Weather Sampling***

Weather data were obtained from a weather station located at North Hampton School, 201 Atlantic Avenue, North Hampton, New Hampshire. Weather station data indicated that 2.28 inches of precipitation occurred on June 7, 2006. Each sample location was sampled once beginning at 9:15 and again at noon time. The second round of sampling revealed higher *E. coli* concentrations at each station except at the Golf Course. The highest *E. coli* concentration was measured during the second round in the Stream on Chapel Road (258 counts per 100 mL).

High bacteria loads were not measured in Chapel Brook or other locations during the June 7, 2006, wetfall event. DES investigators missed the first flush, as precipitation began prior to their arrival. Previous recent heavy wetfall events may have flushed much of the watershed laden bacteria through the watershed.

All stations sampled on August 28, 2006 (0.43 inches wetfall) experienced elevated *E. coli* levels. The study confirms that even minimal wetfall events negatively impact the Chapel Brook system. Several dry weather sampling events also showed elevated *E. coli*. During dry weather, *E. coli* concentration is significantly increased indicating the presence of a persistent bacteria source, whereas short storm events with watershed runoff yield lower *E. coli* concentration.

### ***Sources of Bacteria***

Study results have identified two streams feeding Chapel Brook as potential bacteria sources to the public beach. The Pond/Stream on Chapel Road and the 16 Old Locke Road stations were major *E. coli* contributors. While the Golf Course may contribute other sources of pollutants like nutrients and metals, the samples did not show that this area was a significant source of bacteria.

A site walk of the Chapel Road Stream revealed the stream originates from a heavily wooded wetland. Wildlife is abundant within this wooded wetland and likely the main bacteria source.

### ***Potential Human Sources***

Conversations with people familiar with the Chapel Brook watershed indicate that 16 Old Locke Road station may be impacted by human bacteria sources. A residential development upstream of 16 Old Locke Road is serviced by older septic systems. The development is accessed via Pond Path which intersects Old Locke Road.

Septic system data for residences in the sub watershed is currently being compiled. Several systems are less than ten years old and data are readily available (Appendix C). Data for systems on Old Locke Road and Chapel Road need to be retrieved from the Town. An intern was sent to the town offices but was unable to collect the necessary data.

## **Recommendations**

The 2004 MST study indicated wildlife and humans as bacteria sources to the Chapel Brook system. Ducks were the only wildlife observed during site inspections which indicates that ducks are a bacteria source, especially to the Pond station.

- ▶ Management practices should be applied by the pond owner to discourage duck habitation. The installation of fences, employing scare tactics or planting vegetative buffers along the pond shoreline may be effective. Feces removal may also reduce bacteria loading during storm events.

To verify the potential impact by human bacteria sources, the following activities should occur:

- ▶ A watershed walk will be scheduled for the summer of 2008. Stream bracket sampling technique will be employed to locate problem areas.
- ▶ Additional MST samples should be collected at 16 Old Locke Road and additional sites upstream to determine whether human sources of bacteria are present within this system.

- ▶ Septic systems greater than 20 years old should be inspected by town officials for possible failures and/or surveys sent to homeowners for additional information

The addition of increased funding sources for watershed monitoring, source tracking and remediation would be useful to increase the scope of this program through the collection of additional samples where bacteria sources are suspected. Additional storm events incorporating a series of analytical parameters should be sampled at stations identified as potential pollution sources. Since this study only encompassed a four month period, additional samples and expanded sampling periods will provide a greater understanding of pollution sources to a designated beach.

## References

Comstock, W.G. 2006. Consolidated Assessment and Listing Methodology. New Hampshire Department of Environmental Services, Concord, New Hampshire.

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

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

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WD-BB-53. 2004. Canada Geese Facts and Management Options. Fact sheet. New Hampshire Department of Environmental Services, Concord, New Hampshire.

# Appendix I: Standard Operating Procedures

**STANDARD OPERATING PROCEDURE  
FOR BACTERIA SAMPLING**

**Prepared by:** \_\_\_\_\_ **Date:** \_\_\_\_\_  
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**Reviewed by:** \_\_\_\_\_ **Date:** \_\_\_\_\_  
**Program Manager**

**Approved by:** \_\_\_\_\_ **Date:** \_\_\_\_\_  
**Quality Assurance Officer**

**N.H. DEPARTMENT OF ENVIRONMENTAL SERVICES  
BEACH PROGRAM**

## **PROCEDURES**

### **1.0 Scope and Application**

- 1.1 This Standard Operating Procedure encompasses all aqueous sample collection for bacteria at freshwater and coastal beaches by the NHDES Beach Program. It includes all samples collected at knee and surface depth.

### **2.0 Health and Safety Warnings**

- 2.1 When sampling waters with known fecal contamination always wear disposable plastic gloves and utilize a sampling pole. Do not ingest or allow the water to come into contact with the skin. Always wash hands after sampling and do not touch hands to mouth or other exposed areas of the body before washing.
- 2.2 Ingestion of waters containing fecal contamination can cause health problems such as gastroenteritis, fever, vomiting, and diarrhea. Caution should be taken when recreating in areas where there may be a potential for fecal contamination.

### **3.0 Interferences**

- 3.1 Interferences from bacteria sampling can include cross contamination and improper sample collection.

#### 3.1.1 Cross Contamination

Avoid cross contamination by sampling with sterile bacteria bottles. Never touch the inside of the sample bottle cap or neck of the sample bottle, and always sample water that is flowing towards the body. Any bacteria that may be present on the body could contaminate the sample.

#### 3.1.2 Improper Sample Collection

Improper sample collection can include rinsing of the sample bottle, disturbance of the substrate, sampling in a disturbed area, improper sample depth, and improper sample technique.

- 3.2 Always follow standard operating procedures for sample collection to avoid these errors.

## **4.0 Equipment and Supplies**

4.1 The following supplies are needed for collection of bacteria samples:

- 8 oz. sterile plastic screw cap containers
- Sampling pole
- Cooler(s)
- Ice
- Clipboard, three ring binder, waterproof pen(s)
- Beach station list
- Station identification form
- Field data sheets, sample login and custody sheets
- Shoulder length polyethylene gloves
- Waterproof tape
- Thermometer
- Beach advisory signs
- Maps, directions, NH Atlas

4.2 Cyanobacteria collection supply list can be found in the NHDES Beach Program Standard Operating Procedures for Algal Collection and Identification.

## **5.0 Sample Collection – Preparation**

- 5.1 Determine how many beaches will be sampled that day. Based on the number of beaches to be sampled, obtain sterile bacteria bottles from the NHDES Laboratory Services Unit. Each beach will require at least three sample bottles per beach.
- 5.2 Procure a large cooler from the Limnology Center and fill cooler to about 1/2 full with ice.

- 5.3 Obtain a sterile bacteria sample bottle from the laboratory. Label the bottle with date, time and Trip Blank. In the Limnology Center, fill the bottle at least 2/3 of the way full with D.I. water being careful not to touch the inside of the bottle cap or neck of the bottle. Place the bottle in the ice filled cooler.
- 5.4 Review the equipment and supplies checklist to ensure all materials are present.
- 5.5 Once you have arrived at the sample location:
  - Introduce yourself to the beach management.
  - Observe beach and facility operations.
  - Provide necessary educational material.
- 5.6 If the beach area is less than 100 feet in length, only two samples are collected one third the distance from either end of the beach.
- 5.7 If sampling waters with known fecal contamination, always have disposable shoulder length gloves and a sampling pole available.
- 5.8 The beach area may require additional bacteria sampling. Obtain the extra sample bottle(s) and label them appropriately with date, time, location, and site.
- 5.9 Presence of a surface scum may require additional samples. Refer to the NHDES Beach Program's Standard Operating Procedures for Algal Collection/Identification.
- 5.10 Print out the pre-populated station identification forms from Cognos. Complete a station identification form for the beach and sampling points (if applicable). Fill out all shaded areas of the form.
- 5.11 Print out beach specific pre-populated inspection data sheets and sample log-in and custody sheets from the WQD.

## **6.0 Sample Collection – Method**

- 6.1 Wade into the water to knee depth. Wait for the water to be clear of debris that may have been disturbed when walking into the water. Or sample away from the disturbed area.
- 6.2 Unscrew the bottle cap making sure not to touch the inside of the cap or neck with fingers or any other object.

- 6.3 Hold the cap in one hand, and with the other hand turn the bottle upside down so the opening is facing the water surface. Make sure you never touch the opening of the bottle neck.
- 6.4 With a downward thrust moving away from your body, dip the bottle at least a foot below the surface. Fill the bottle with one sweeping motion, and discard a few milliliters to allow some head (air) space.
- 6.5 Replace the cap carefully over the bottle and tighten.
- 6.6 Mark the site location, the name of the public beach, and the date and time the sample was collected. Make sure to always use a waterproof pen or Sharpie®.
- 6.7 Measure the water temperature according the Beach Program's SOPs for Temperature Collection.
- 6.8 If the swim area is located on a naturally flowing watercourse, such as a brook or river, samples should be collected upstream, at the public beach area, and downstream. In streams or rivers in which it is difficult to collect a sample at the desired depth, locate the deepest area with a moving current. Always collect sample moving against the current to reduce the chance of contamination.
- 6.9 If there is known fecal contamination or if the area is difficult to access, use a sampling pole. Attach the sample bottle to the clamp, remove the bottle cap, and repeat step 4. Make sure to adjust the length of the pole to collect the sample as close to knee depth as possible.

## **7.0 Sample Handling and Preservation**

- 7.1 After sample collection the process is as follows:
  - 7.1.1 Place all samples in a cooler(s) with ice for preservation. Acceptable preservation temperature for *E. coli* and *Enterococci* is less than 10°C.
  - 7.1.2 Return samples to the NHDES Laboratory Services Unit within 6 hours after sampling.
  - 7.1.3 Place samples in order according to the time samples were collected on the bench in the log-in room of Laboratory Services. Complete the pre-populated login and custody sheet. If you have any questions ask the lab personnel to assist you.
  - 7.1.4 Write the beach specific EPA number on the bottle label. The EPA numbers can be found on the Beach Station List.

- 7.1.5 Place the appropriate labels on the bottle caps. These labels inform lab personnel of analyses to be run.
- 7.1.6 Sample dilution is required for suspected sewage samples. Dilutions are X1, X10, or X100. Indicate the dilution factor by listing it on the label. Login sheets must also be labeled with the dilution factor(s) in the other/notes section.
- 7.1.7 Dilution is required for Enterococci trip blank samples. Indicate X1 on the label attached to the cap and indicate in the other/notes section on the login sheet.
- 7.1.8 Sign the custody sheet to relinquish the samples to the laboratory. The lab personnel must review and sign the custody sheet. **Always notify lab personnel when you drop samples off!**

## **8.0 Data and Records Management**

- 8.1 All observations must be recorded on the Beach Program Field Data Sheet. This sheet must be filled out completely. The required observations are:
  - 8.1.1 Beach name, town, station ID
  - 8.1.2 Advisory, complaint, initial, subsequent, or safety inspection
  - 8.1.3 Beach inspector name, number of collected samples
  - 8.1.4 Date, time, weather conditions, recent storm events
  - 8.1.5 Presence or absence of toilet facilities
  - 8.1.6 Type of toilet facility: bathhouse/bathroom, outhouse, portable
  - 8.1.7 Presence or absence of enclosed trash receptacles
  - 8.1.8 Presence or absence of lifeguards, swim ropes, rafts
  - 8.1.9 Presence or absence of appropriate signage
  - 8.1.10 Number of bathers (exact number if possible, otherwise estimate)
  - 8.1.11 Water conditions (e.g. clarity, water level, water temperature, surface scums)

- 8.1.12 Presence or absence of emergency safety equipment and signage
- 8.1.13 Waterfowl, wildlife, domestic animals
- 8.1.14 Culverts, storm drains, pipes
- 8.1.15 Complaints from lifeguards or bathers
- 8.2 All inspection data must be entered into the WQD Beach Module Inspections. Data is entered on a weekly basis by the Beach Program intern. Data entry follows the WQD Beach Module Training document.
- 8.3 Station identification forms must be filled out completely for each sampling station per beach. Required fields are as follows:
  - 8.3.1 Project (program or project associated with the station a.k.a Beach)
  - 8.3.2 Station ID
  - 8.3.3 Station Type
  - 8.3.4 Latitude, longitude
  - 8.3.5 Correction of latitude or longitude if possible
  - 8.3.6 GPS unit manufacturer, model
  - 8.3.7 Method of location other than GPS
  - 8.3.8 Datum

## **9.0 Quality Control and Quality Assurance**

- 9.1 Duplicate samples are collected at a frequency of 10%. The relative percent difference (RPD) of the duplicate samples should be  $\leq 75\%$  respectively. If the RPD is exceeded, immediate re-sampling will be performed. All data generated will be accepted due to the impact, and potential health risk to the public.
- 9.2 Trip blanks are collected prior to each sampling trip using D.I. water. Trip blanks are performed twice per week for freshwater beaches and every trip for coastal beaches. Accuracy/bias of trip blanks is zero bacteria counts. If the trip blank displays bacteria colonies, immediate action is taken to identify and correct the problem.

- 9.3 Inspection data entered into the WQD are QA/QC checked by Beach Program staff. Inspection data QA/QC cannot be performed by the person responsible for entering the data.

## **10.0 References**

Water Sampling Protocol for *E. coli* Testing, Environmental Fact Sheet WD-BB-13, New Hampshire Department of Environmental Services, 1998.

## Appendix II: *E. coli* Standard Method 9213D

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Approved by:

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Laboratory Director

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Section Supervisor

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QA Officer

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#### REFERENCES:

1. Test Methods for Escherichia coli and Enterococci In Water By The Membrane Filter Procedure, EPA 600/4-85/076, Environmental Monitoring and Support Lab, Cincinnati, OH 45268.
2. Standard Methods for Examination of Water and Wastewater, American Public Health Association, 19th Edition, 1995, Method 9213D.3, p 9-28.
3. State of Maine Procedure for Escherichia coli by mTEC, Augusta, ME.

#### SAMPLING METHODOLOGY

1. Preservative: Storage at 2-4°C, 10% sodium thiosulfate if needed to inactivate chlorine.
2. Holding time: 8 hours after sampling for NPDES and other compliance samples, will accept samples up to 30 hours old for other types of monitoring.
3. Required volume: Minimum of 100 mL is best, we do accept smaller amounts if unable to get more
4. Container type: 8 oz. sterile screw cap plastic containers or 450 mL sterile sample jugs, or chlorinated samples in 4 oz. plastic bottles with sodium thiosulfate added before sterilization.

#### SUMMARY:

Escherichia coli (E. coli) is a member of the fecal coliform group and it is a good indicator of fecal contamination in water. This method analyzes water for the presence of E. coli. Results are obtained in 24 hours. The media is mTEC and the confirmation is a 20 minute Urease Test.

#### A. APPARATUS AND MATERIALS:

New Hampshire Department of Environmental Services Beach Program  
Chapel Brook Special Study

1. Autoclave
2. Vacuum Pump
3. Filter System - Funnels, Manifolds, Waste Vessel
4. UV Light Box
5. Petri Dishes, 50 x 9 mm
6. Filter Membranes 0.45  $\mu$ m pore, 0.47 mm diameter
7. Buffered water in 2 liter jugs - Section 10.36g for prep.
8. Pipettes for Dilution - Disposable sterile 1 and 10 mL.
9. pH meter with flat surface probe.
10. Hot Plate (Stirrer)
11. Incubators at 35.0°C and 44.5°C
12. Burner and forceps.
13. Sterile absorbent petri dish pads to fit 50 x 9 mm dishes.
14. Light microscope.
15. Minipet for dispensing media.

#### B. MEDIA AND REAGENTS:

##### 1. mTEC media:

##### a. To prepare 500 mL (100 plates):

- 1) 22.65 g mTEC Agar media dissolved into 500 mL of DI water.
- 2) Heat with stirrer on hot plate to dissolve completely.
- 3) Sterilize in autoclave for 15 minutes at 121°C and 15 psi.
- 4) Dispense 4-5 mL of hot media into sterile 50x9 mm petri plates.

- b. Store in refrigerator at 1-5°C in plastic bag in closed box for up to 3 months.
  - c. Check one plate for pH - 7.3+/-0.2. Record results in media log.
  - d. A positive, negative and sterility check should be done for each batch. Positive is E. coli, negative is Ps. aeruginosa. Incubate controls as you would a sample plate. Record results in media log.
2. Urea substrate:
- a. To prepare 100 mL:
    - 1) Add 2.0 g of Urea and 0.01 g of Phenol Red to 100 mL DI water.
    - 2) Stir on magnetic stirrer until dissolved. Phenol Red dissolves slowly.
  - b. Test pH - it should be between 3.0 and 4.0 Record pH in media log.
  - c. The substrate should be straw colored.
  - d. Label with pH, initials, and lot number (date of prep.). Record date removed from freezer.
  - e. Store in the refrigerator for up to one week. It may be frozen for up to 6 months. Thaw and store in refrigerator for up to one week.

### C. ANALYSIS:

1. Filtration procedure: See 10.43b Sec. G Total Coliform by Membrane Filtration.
2. Use mMTEC in place of m-Endo.
3. Incubate plates at 35.0 +/- 0.5°C for 2 hours. Put in incubator at 44.5 +/- 0.2°C for 22 +/- 2 hours.
4. Check plates for growth and record the negative plates in the log book. Suspected E. coli colonies will be yellow to yellow-brown. Plates with suspected E. coli colonies should be lined up on the bench for confirmation.
5. Remove the cover and place a sterile absorbent pad in the cover.
6. Add 1.5 to 2 mL of Urea substrate to the pad and aseptically transfer the membrane to the pad. Make sure the membrane is placed without any air bubbles.

7. Wait 15-20 minutes and look for yellow to yellow-brown colonies. These are the E. coli colonies. Negative colonies will be purple or gray.

8. Use a microscope and light source to read the plates.

#### D. CALCULATIONS:

1. The ideal range for counting is 20-80 colonies.
2. Samples may need to be diluted to stay within counting range.
3. Record the results and do the necessary calculation for diluted samples.

$$\text{counts} \times \text{dilution factor} = \text{counts}/100 \text{ mL}$$

4. If no sample results are within the 20-80 ideal counting range use the following formula:

$$(\Sigma \text{ colonies} / \Sigma \text{ volumes used for all dilutions}) \times 100 = \text{counts} / 100 \text{ mL}$$

#### E. QUALITY CONTROL:

1. See Section 10.43b Total Coliform by Membrane Filtration - L. QUALITY CONTROL.
2. Plates should be autoclaved in biohazard bags for at least 65 mins. after use.
3. Buffered rinse water blanks are run at the beginning and end of each filtering run. Resamples are requested if blanks are contaminated.
4. Duplicates are run at least every 10 samples.

## Appendix III: Chapel Brook Sampling and Analysis Plan

# Sampling and Analysis Plan For Enhanced Bacteria Monitoring at Chapel Brook

Conducted Under:

NHDES Beach Program  
Generic Quality Assurance Project Plan RFA# NH02318

Prepared By:

Sara Sumner  
New Hampshire Department of Environmental Services  
29 Hazen Dr, PO Box 95  
Concord, NH 03302-0095

---

Approved By:

\_\_\_\_\_  
Sara Sumner, NHDES Beach Program Coordinator

Date: \_\_\_\_\_

\_\_\_\_\_  
Jody Connor, NHDES Beach Program Manager

Date: \_\_\_\_\_

\_\_\_\_\_  
Scott Ashley, NHDES QA Officer

Date: \_\_\_\_\_

\_\_\_\_\_  
Vincent Perelli, NHDES Program QA Manager

Date: \_\_\_\_\_

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## **2.0 Introduction**

The New Hampshire Department of Environmental Services' (NHDES) Public Beach Inspection Program, or Beach Program, monitors coastal public beaches to protect public health and safety of New Hampshire waters. The coastal beaches are monitored on a weekly basis during the swim season for the fecal bacteria Enterococci. The award of the Beaches Environmental Assessment and Coastal Health Act (BEACH) grant money from the Environmental Protection Agency (EPA), allows the Beach Program to conduct additional sampling beyond the weekly monitoring/sampling of its beaches. One similar project titled, *Identify and Mitigate Bacterial Sources at Public Beaches* (Appendix D), provided microbial source tracking data from three tributaries that discharge to coastal public beaches. Microbial source tracking can identify host specific sources of *E. coli*. The study concluded that humans were a contributing source of elevated *E. coli* levels in the tributaries. Chapel Brook was one of the tributaries studied. Additional sampling will occur at Chapel Brook to determine if faulty septic systems are contributing to the human load of bacteria.

This Sampling and Analysis Plan (SAP) discusses the specific requirements for the Chapel Brook Sampling Project. These include sample design and collection, data management and collection, quality assurance/quality control (QA/QC) requirements, and sample analysis requirements. Project work performed for the Chapel Brook Sampling SAP will adhere to the requirements stated in the EPA-approved NHDES Beach Program Generic Quality Assurance Project Plan (QAPP) RFA# NH02318. The Beach Program Generic QAPP was written in accordance with the EPA QA/R-5 QAPP Requirements.

The Beach Program Generic QAPP is designed to meet EPA requirements and provide guidance for all program projects. The purpose of the Enhanced Bacterial Monitoring for Chapel Brook SAP is to identify the specific requirements for this project with respect to sampling, analysis, and QA/QC. The SAP will reference the QAPP for more “generic” tasks such as project quality objectives, sampling and analytical procedures, sample documentation, equipment decontamination, sample handling procedures, data management, assessment, and data review procedures. Any additional procedures and/or modifications to procedures described in the Generic QAPP will be described in the following elements.

The SAP will be prepared, reviewed, and approved in accordance with the procedures detailed in the Generic QAPP and the DES Quality Management Plan.

## **3.0 Distribution List**

The SAP will be distributed to all project personnel and stakeholders listed in Appendix A. The SAP is required reading by all personnel involved in project activities. A copy of the SAP, as well as the QAPP, will be retained electronically and in the Beach Program's files. Any deviations or modifications to the SAP will be documented using the SAP revision form included in Appendix B

## 4.0 Project Management

Project management will be documented as in the Generic QAPP. Refer to the Generic QAPP RFA# NH02318 for organization chart, communication pathways, personnel responsibilities and qualifications, and special personnel training requirements.

### 4.1 Project Team Members list

The following personnel will be involved in technical activities performed for this project:

1. Sara Sumner, Environmentalist II
2. Alicia Carlson, Environmentalist I
3. Andrew Cornwell, Program Specialist I
4. Seasonal Interns: Beach Program and Clean Vessel Act Program interns

Seasonal interns may be used for additional assistance with sampling and other technical activities. All interns will be trained in proper sample collection techniques prior to the start of the project.

## 5.0 Project Definition

### 5.1 Problem Definition

Excessive rainfall has the potential for transporting increased pathogen loads to waterbodies. Beaches located at the mouth of or adjacent to tributaries are at a greater risk of being negatively impacted during storm events.

Chapel Brook is a tributary that discharges to Bass Beach in North Hampton, NH. Chapel Brook regularly experiences elevated bacteria levels. Historical data reflects that the problem is exacerbated during storm events. The microbial source tracking study conducted in 2003 confirmed sources of *E. coli* bacteria originating from wildlife, humans, and waterfowl (*Identify and Mitigate Bacterial Sources at Public Beaches*, Appendix D). Controlling bacteria inputs from wild animal and bird populations is a difficult task however, 19% of bacteria were identified as originating from humans. Human bacteria should not be sources of contamination to public waters or the designated beaches.

Management activities and remediation of human sources can only be accomplished if the origin(s) of these sources is identified. Potential sources for human-induced contamination can include failed septic systems, sewer infrastructure failures, and illicit connections. The town of North Hampton does not have a municipal waste water treatment facility, and individual home owners must construct septic systems (source: Town of North Hampton). There are multiple homes bordering Chapel Brook, which represent possible source(s) of human fecal pollution. A

golf course also discharges to the Philbrick Pond waters draining to Chapel Brook. Runoff from wet detention ponds on the golf course will also be targeted as a potential source.

Ambient brook monitoring and a previous microbial source tracking project have provided baseline bacteria levels and identified spikes in bacteria levels during wet weather events. The goal of this project is to locate septic systems that are in failure and to identify sources of bacteria to the system. The movement of septic leachate is enhanced by wet weather events that will be used to aid DES in locating failed systems. Sample collection will occur at specific sites along the brook (before and after potential sources) during wet weather and dry weather events (for baseline). This technique, called bracketing, will identify “hot spots” of *E. coli* that should correspond to specific source areas.

The information gathered will be used to notify the town and appropriate parties of potential problems. Septic system inspections and sanitary surveys can then be conducted to confirm the existence of failure, take corrective measures, and remediate the problem.

## 5.2 Historic Data

Historical data indicate elevated *E. coli* and Enterococci levels in Chapel Brook (See Appendix D) during both dry and wet weather conditions, but are exacerbated during wet weather conditions. Septic system data will be gathered from the DES Subsurface Bureau and the town of North Hampton. Golf course hydrology data will be obtained from the DES Wetlands Bureau and/or the golf course owner.

## 5.3 Contaminants of Concern

All samples collected during wet weather monitoring will be analyzed for *E. coli*. Refer to the table in Appendix E.

# 6.0 Project Description and Schedule

## 6.1 Overview of Project Activities

### 6.1.1 Sampling Design

The Program Coordinator is responsible for sampling design. Chapel Brook, a known pollution source to coastal waters, will be targeted and assessed during wet weather events. Samples will be collected at specific station locations (Appendix C). Sample collection will target wet weather events > 0.25 inches of rainfall following at least five days of dry weather (a rain gauge will be installed on site). Dry weather events are defined as < 0.25 inches of rainfall in 24 hours. Sample collection is targeted at the start, peak storm, post-peak storm, late storm, and the end of storm events. A stream gauge will be installed to measure peak stream flow. Storm start samples, 0-5 minutes after the start of the storm, provide initial runoff data. Peak storm samples, or first flush, provide enhanced watershed runoff data. Post-peak storm samples provide data on lingering pollution sources. Late and end of storm samples provide comparative data and

indicate how persistent the sources are. Dry weather sampling will provide baseline data for the selected sites.

Rain forecasts will be monitored by referring to several weather prediction stations such as the National Weather Service, Accuweather, the Weather Channel, and Intellicast. A rain gauge will be installed along Chapel Brook to record rainfall depths. The gauge will be monitored and levels recorded during each sampling event. Rainfall data will also be collected from the weather station located at the North Hampton School, 201 Atlantic Avenue, North Hampton, NH via the website [www.24.147.107.165:357/](http://www.24.147.107.165:357/). The weather station provides data on air temperature, wind chill, wind speed, rain depths, and various other parameters. Sample collection will occur only when the water is flowing and will not be collected during stagnant conditions. This applies mainly to dry weather sampling.

Sample collection will occur from May through September. This is the target season for coastal beach and golf course usage. Vacation homes are more likely to be occupied during this season. If homes bordering Chapel Brook are used seasonally, seasonal sample targeting will capture affects from these residences. Five wet weather seasonal sampling events are targeted to provide sufficient data to pinpoint pollution sources. Dry weather event sampling will occur at least once per month during the project period.

#### 6.1.2 Sampling Tasks

All Project Team Members will assist with sampling tasks including collecting and transporting the samples to the DES Laboratory Services.

During wet weather events, Sara Sumner and a trained Beach Program intern will be responsible for sample collection at each designated brook site. These sites are easily accessible and within walking/driving distance from each other. In the event that Sara Sumner and/or the Beach Program intern are unavailable for sample collection, Alicia Carlson and/or the CVA intern will be designated to conduct the wet weather sampling. Sara Sumner will schedule dry weather sampling events for each month. Dry weather sampling will be conducted by Sara Sumner, Alicia Carlson or the Beach Program intern.

#### 6.1.3 Analysis Tasks

The DES Laboratory Services' personnel are responsible for *E. coli* analyses. Samples will be analyzed for *E. coli* according to the Standard Operating Procedure (SOP) as described in the Beach Program Generic QAPP.

#### 6.1.4 Quality Tasks

Laboratory QA/QC for *E. coli* analyses is the responsibility of the DES Laboratory Services' personnel as described in Section A 7.0 of the Beach Program Generic QAPP.

#### 6.1.5 Secondary Data

New Hampshire Department of Environmental Services Beach Program  
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No secondary data will be used for this project.

#### 6.1.6 Data Management Tasks

Data management is in accordance with Section B 10.0 of the Beach Program Generic QAPP.

#### 6.1.7 Documentation and Records

The Program Coordinator is responsible for proper documentation and record-keeping as described in Section A 9.0 of the Beach Program Generic QAPP. For the purpose of this project, the field data sheet located in Appendix D will be completed and stored as a hard copy in the project specific files.

#### 6.1.8 Data Deliverables

Data deliverables will include hard copy print outs of results and electronic transfers from the DES Laboratory database to the Watershed Management Bureau's Environmental Monitoring Database (EMD).

#### 6.1.9 Assessment/Audit Tasks

Refer to Section 16.0.

#### 6.1.10 Data Review and Evaluation

The Program Coordinator is responsible for data review and evaluation as described in Section D 1.0 of the Beach Program Generic QAPP.

### 6.2 Project Schedule

Refer to Appendix F.

## **7.0 Project Quality Objectives**

Project quality objectives, decision statements, and project acceptance limits as defined for relevant data quality indicators are documented for this project in the Beach Program Generic QAPP, Section A 7.0.

## **8.0 Sampling Design**

- 8.1 Chapel Brook and the Philbrick Pond marsh area will be bracketed to identify bacterial source origins. Sample stations are selected along Chapel Brook and at inputs to the Philbrick Pond system (Appendix C). These stations will be sampled throughout the course of a storm event. Total predicted rainfall for the event shall be > 0.25 inches. The first sample will be collected prior to the start of the storm.

The second sample will then be collected when surface runoff is visible. The third sample will be collected when it is determined that the storm's peak has occurred. The fourth sample will be collected within 15 to 30 minutes after the peak, and a final sample will be collected at storm completion. Other samples may be collected, at the discretion of the sampler, throughout the storm. There will be a total of at least five samples, but no more than eight, per sample station. Dry weather sampling will occur at each station for baseline data. Dry weather sampling will be initiated only during flowing water conditions and will not occur when it is deemed that stagnant water conditions exist.

Samples will not be collected at knee depth, as stated in the protocol for regular beach monitoring as specified in the Beach Program Generic QAPP, Section B 1.0. Instead, samples will be collected using a sampling pole. The method is included in the Beach Program Standard Operating Procedure for Bacteria Sampling (Generic Beach QAPP Appendix A).

Samples will be collected during daylight hours only. The safety of the samplers will be taken into consideration as they may be at risk during severe weather conditions including but not limited to lightning, high winds, and hurricanes. No sampling will occur if a severe weather alert is issued for the area. Program staff will monitor the National Weather Service for issuance of severe weather alerts.

- 8.2 Sample locations, frequency, matrix, analytical parameter, concentration level, sample volume, container, preservation requirements, and holding times specific to this project are detailed in Appendix G.
- 8.3 Sample locations and directions to the locations are provided in Appendix C.

## **9.0 Sampling Procedures and Requirements**

Sampling will be performed as documented in the Beach Program Generic QAPP Section B 2.0. Refer to the Generic QAPP for sampling SOPs, sample container specifications, required sample volumes and preservation techniques, cleaning and decontamination procedures, field sampling equipment calibration procedures, and field equipment maintenance, testing, and inspection procedures.

- 9.1 The following SOPs will be used for this project:

- 9.1.1 Beach Program SOP for Bacteria Sampling

## **10.0 Sample Handling, Tracking, and Custody Requirements**

Sample custody and integrity will be maintained as documented in the Beach Program Generic QAPP, Section B 3.0. Refer to the Generic QAPP for sample handling, tracking and custody procedures, and an example of the chain of custody form that will be used in the project.

### **11.0 Field Analytical Method Requirements**

There will be no field testing for this sampling program.

### **12.0 Fixed Laboratory Method Requirements**

Samples will undergo laboratory analysis in accordance with fixed laboratory methods and SOPs documented in the Beach Program Generic QAPP, Section B 4.0. Refer to the Generic QAPP for fixed laboratory analytical methods and procedures, analytical instrumentation calibration procedures, and analytical instrumentation maintenance, testing, and inspection procedures.

- 12.1 The following laboratory analytical methods and SOPs will be used in this project:

- 12.1.1 *E. coli* Standard Method 9213D

- 12.2 The fixed laboratory responsible for the analyses of the project samples will be NHDES Laboratory Services Unit located in Concord, NH. The point of contact for the laboratory is:

*E. coli*:

Mona Freese

[mfreese@des.state.nh.us](mailto:mfreese@des.state.nh.us)

Phone: (603) 271-2992

Fax: (603) 271-2997

### **13.0 Quality Control Requirements**

Quality Control activities will be performed in accordance with required frequencies described in the Beach Program Generic QAPP, Section B 5.0. Also, precision and accuracy criteria documented in the Generic QAPP, Section B 5.0, for each analytical method and SOP will be used to ensure sample analyses are within control limits. Project quality objectives and measurement performance criteria for this project are described in the Beach Program Generic QAPP, Section A 7.0.

- 13.1 Quality control samples associated with each analytical parameter and concentration level for this project are detailed in Appendix H.

## 14.0 Documentation, Records, and Data Management Activities

Project documents and records will be generated, stored, and archived as documented in the Beach Program Generic QAPP, Section B 10.0.

Also, data management activities including data manipulations, reductions, and modeling will be performed as documented in the Generic QAPP, Section B 10.0.

## 15.0 Secondary Data

Secondary data will not be used for this project.

## 16.0 Assessments and Response Actions

This table summarizes the assessment requirements approved for this project. Refer to Section C 1.0 of the Beach Program Generic QAPP for additional information regarding assessment and response actions.

**Table 1. Assessment and Response Actions**

Assessment Type	Frequency	Person Responsible for Performing Assessment	Person Responsible for Responding to Assessment Findings	Person Responsible for Monitoring Effectiveness of Corrective Actions
Field Sampling Audit	Once per season	Sara Sumner, Beach Program Coordinator, NHDES	Sara Sumner, Beach Program Coordinator, NHDES	Sara Sumner, Beach Program Coordinator, NHDES
Field Analytical Assessment	Once per season	Sara Sumner, Beach Program Coordinator, NHDES	Sara Sumner, Beach Program Coordinator, NHDES	Sara Sumner, Beach Program Coordinator, NHDES
NHDES Laboratory Services Unit Fixed Lab Audit	Weekly	Rachel Rainey, Laboratory Services QA/QC Officer, NHDES	Rachel Rainey, Laboratory Services QA/QC Officer, NHDES	Rachel Rainey, Laboratory Services QA/QC Officer, NHDES

## 17.0 Quality Assurance Management Reports

QA Reports to management and stakeholders including the Final Project Report will be generated and disseminated as documented in the Beach Program Generic QAPP, Section C 2.0.

## 18.0 Step 1 Data Review Requirements and Procedures

Project activities will be verified as documented in the Beach Program Generic QAPP, Section D 1.0.

## **19.0 Step 2 Data Review Requirements and Procedures**

### 19.1 Data Validation Requirements

Refer to Sections D 1.0 and D 2.0 of the Beach Program Generic QAPP for information regarding data verification and validation procedures and requirements.

- 19.2 The person/company responsible for reviewing the data will be the NHDES Beach Program personnel located in Concord, NH. The point of contact for validation is:

NHDES Beach Program Coordinator

Phone: (603) 271-8803

Fax: (603) 271-7894

## **20.0 Step 3 Data Usability Assessment/Reconciliations with Project Quality Objectives**

Data quality will be evaluated against project acceptance limits specified in the Beach Program Generic QAPP, Section D 3.0. Ultimately, data will be assessed to determine if it meets the needs of the user in supporting environmental decisions and conclusions.

## Appendix III-A: SAP Distribution List

### SAP Distribution List for the NHDES Beach Program

SAP Recipient Name	Title	Organization	Telephone Number	Email
Sara Sumner	Beach Program Coordinator	NHDES	603-271-8803	<a href="mailto:ssummer@des.state.nh.us">ssummer@des.state.nh.us</a>
Jody Connor	Limnology Center Director, Beach Program Manager	NHDES	603-271-3414	<a href="mailto:jconnor@des.state.nh.us">jconnor@des.state.nh.us</a>
Scott Ashley	QA/QC Program Officer	NHDES	603-271-2968	<a href="mailto:sashley@des.state.nh.us">sashley@des.state.nh.us</a>
Coastal Beach Intern	Seasonal Beach Inspector	NHDES		
Alicia Carlson	Public Beach Inspector	NHDES	603-271-0698	<a href="mailto:acarlson@des.state.nh.us">acarlson@des.state.nh.us</a>
Andrew Cornwell	Program Specialist I	NHDES	603-271-1152	<a href="mailto:acornwell@des.state.nh.us">acornwell@des.state.nh.us</a>
Rachel Rainey	NHDES Laboratory QA/QC Officer	NHDES Laboratory Services	603-271-2993	<a href="mailto:rrainey@des.state.nh.us">rrainey@des.state.nh.us</a>
Mona Freese	NHDES Laboratory Microbiology Section	NHDES Laboratory Services	603-271-2992	<a href="mailto:mfreese@des.state.nh.us">mfreese@des.state.nh.us</a>
Vincent Perelli	NHDES Quality Assurance Manager	NHDES	603-271-8989	<a href="mailto:vperelli@des.state.nh.us">vperelli@des.state.nh.us</a>
Matt Liebman	US EPA Project Officer	US EPA	617-618-1626	<a href="mailto:Liebman.matt@epamail.epa.gov">Liebman.matt@epamail.epa.gov</a>

# Appendix III-B: Sampling and Analysis Plan Revision Form

## Sampling and Analysis Plan Revision Form

*[Project Name]*

*[Project Grant Number]*

Date: \_\_\_\_\_

Revision #: \_\_\_\_\_

Revision to Project-specific SAP: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

SAP Requirement being superseded: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Original SAP Section/Paragraph/Table: \_\_\_\_\_

Justification/Reason for Revision: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Requested by (Print Name: \_\_\_\_\_

*[name, title, affiliation, date]*

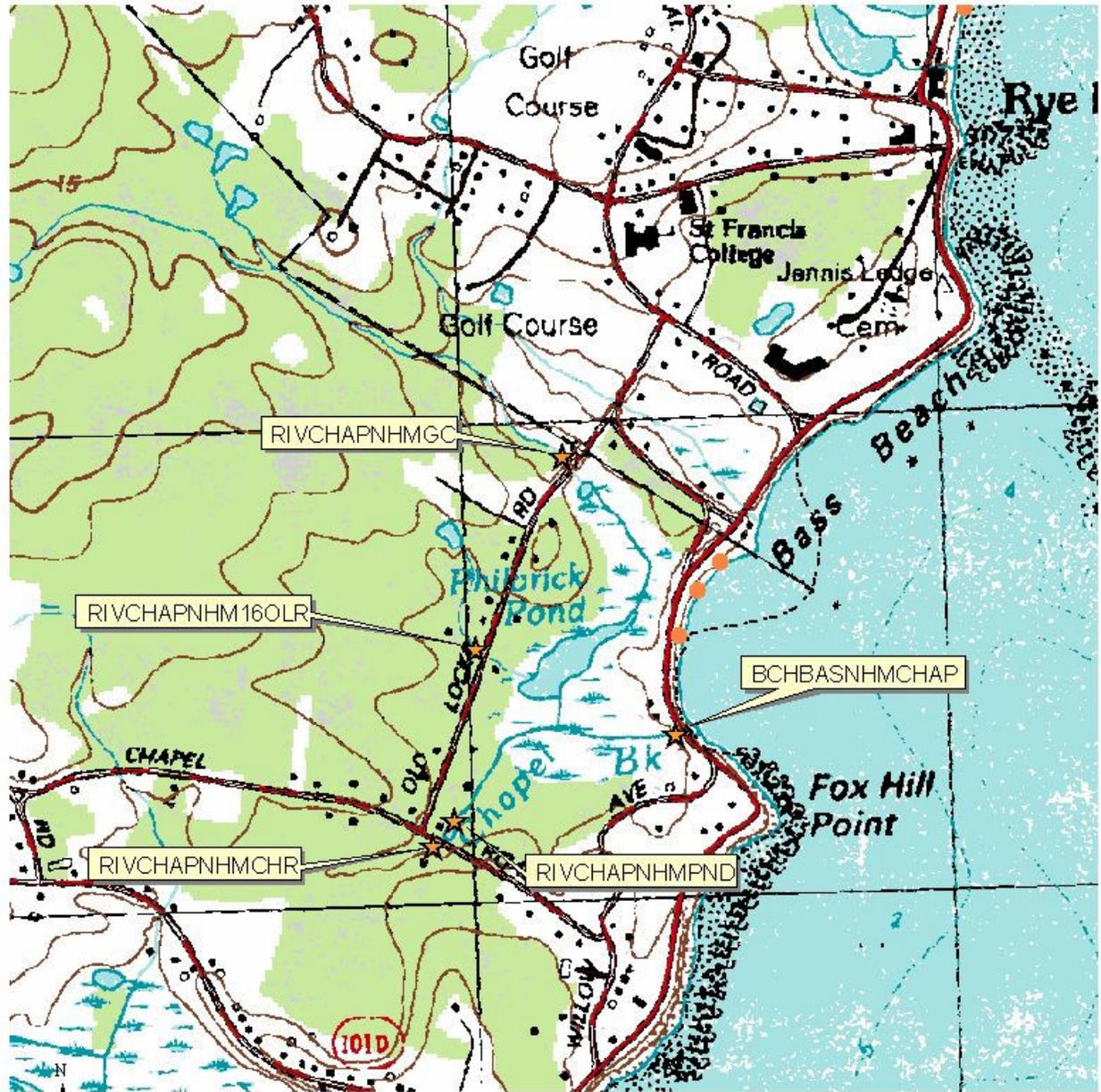
Approved by: \_\_\_\_\_

*[name, title, affiliation, date]*

# Appendix III-C-1: Site Maps

# Chapel Brook Enhanced Monitoring Stations North Hampton, NH

- Coastal Stations
- Political Boundaries
-  State boundary
-  County boundary
-  Town boundary



New Hampshire Department of Environmental Services Beach Program  
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## Appendix III-C-2: Station Detail and Directions

<b>Station ID</b>	<b>Station Name</b>	<b>Station Description</b>	<b>Station Directions</b>
BCHBASNHMCHAP	Bass Beach Chapel Brook	Upstream of culvert along Rt. 1A	101E to exit 12 to Rt. 1A North. Chapel Brook is on the left before Bass Beach.
RIVCHAPNHM16OLR	Chapel Brook 16 Old Locke Rd.	Small stream that discharges under Old Locke Rd. into Philbrick Pond Marsh. The stream runs next to house #16.	101E to exit 12 to Rt. 1A North. Left onto Willow Ave., left onto Chapel Rd., right onto Old Locke Rd. house #16 is on the left.
RIVCHAPNHMCHR	Chapel Brook Stream on Chapel Rd.	Stream flowing from wooded area into a private pond. Stream flows under Chapel Rd., sample collected on left side of rd.	101E to exit 12 to Rt. 1A North. Left onto Willow Ave., left onto Chapel Rd. The stream is on the left before you get to Old Locke Rd.
RIVCHAPNHMPNH	Chapel Brook Pond	Outlet from a private pond at a residence on the corner of Chapel Rd. and Old Locke Rd.	101E to exit 12 to Rt. 1A North. Left onto Willow Ave., left onto Chapel Rd., the house is on the right at the corner of Chapel Rd. and Old Locke Rd.
RIVCHAPNHMGC	Chapel Brook Golf Course	Discharge from a detention pond at Abeniqui CC. Discharges under Old Locke Rd. and eventually into Philbrick Pond Marsh.	101E to exit 12 to Rt. 1A North. Left onto Willow Ave., left onto Chapel Rd., right onto Old Locke Rd. Golf course is on left towards the end of the road and detention pond is on the left.

## Appendix III-D-1: Scope of Work

## **Beach Program Scope of Work Chapel Brook Wet Weather Monitoring**

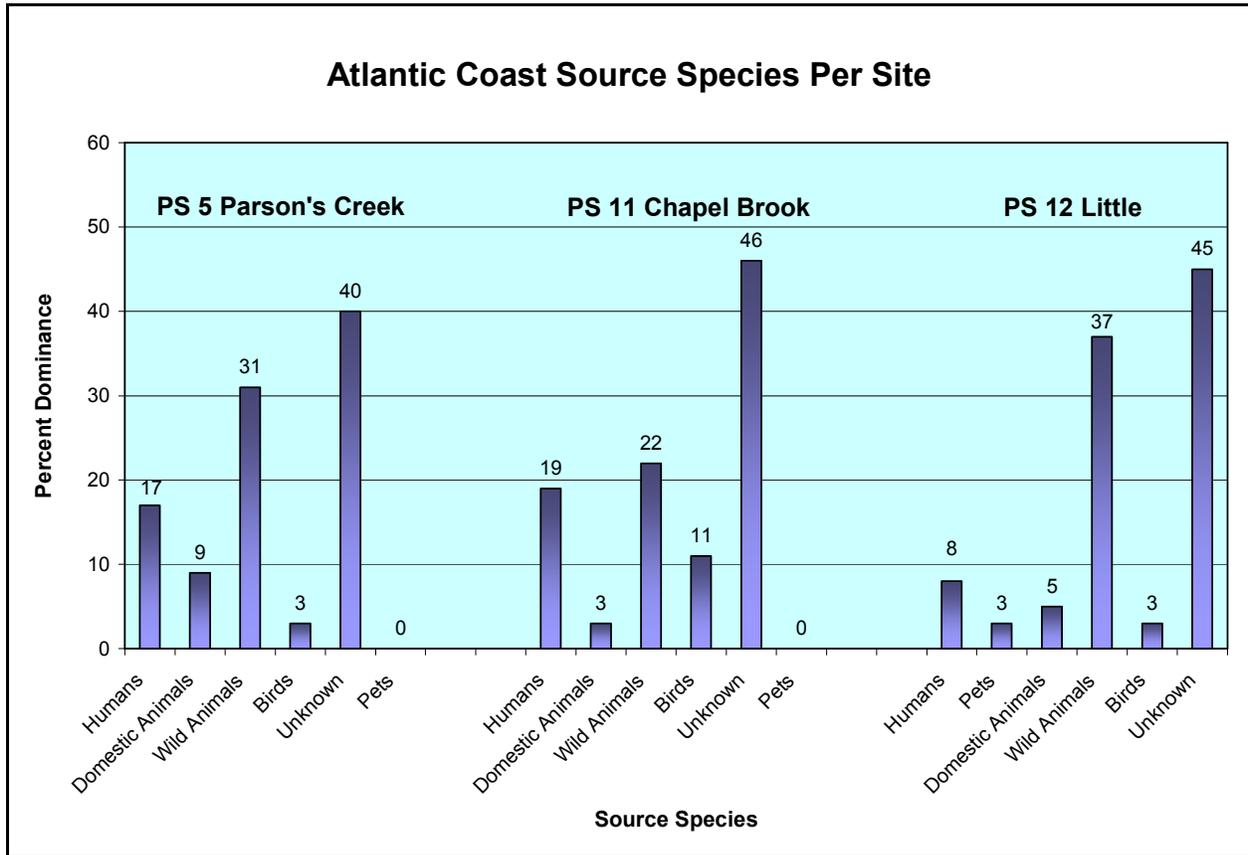
### **1.0 Program Purpose**

The New Hampshire Department of Environmental Services (NHDES) has operated a Public Beach Inspection Program, or Beach Program, for over twenty years. The goal of the Beach Program is to protect public health by expanding monitoring programs and locating sources of pollution that can cause water-borne diseases in humans. Water-borne diseases such as cholera, Giardiasis, and Hepatitis pose a serious threat to human health. The main source of these diseases is from animal/human waste. Fecal material houses a variety of coliform bacteria, the most common being *E. coli* and *Enterococci*. These bacteria are always found in fecal material, are easily cultured within 24 hours, and their presence can indicate the presence of pathogenic bacteria.

To enhance the protection of public health at coastal beaches, the Beach Program recognizes the threat of confirmed fecal pollution sources discharging to coastal beaches. These sources include rivers, streams, salt marsh discharges, and estuaries. The Beach Program conducted a microbial source tracking study in 2003 to identify the specific source species contributing to the bacteria levels in three of these tributaries, Chapel Brook, Little River and Parson's Creek. The study involved collecting wet weather samples at these sites, isolating *E. coli*, and then ribotyping the isolates to produce a DNA fingerprint of the *E. coli*. These fingerprints were compared with fingerprints of known source species such as gulls, otters, dogs, horses, cows, geese, and humans. All tributaries resulted in humans as the second most dominant source species identified (Figure 1).

Controlling and eliminating fecal pollution from wildlife populations is not a sound ecological practice and could prove to be difficult. Human fecal pollution is of larger concern because it has a greater potential to spread disease. Human fecal pollution should not be entering our waterways and beach areas. When human fecal pollution is present it indicates a larger scale problem whether it be a failing septic system, sewage infrastructure problems, or illicit connections. The first step of the problem, identifying fecal sources, has been completed. The next step is to use that information and conduct additional monitoring to identify the input of human fecal pollution.

Figure 1 Atlantic Coast Source Species



## 2.0 Program Goal

Historical data and studies provide information regarding ambient and wet weather bacteria levels and source specific information, but cannot provide the necessary data to pin-point the exact location of the bacteria source. The Beach Program will target storm events that yield storm specific information for selected Chapel Brook stations. The sampling events will aid in determining the location of bacteria sources whether they be private residences or a golf course. The enhanced monitoring will provide data to evaluate bacteria levels at specific bracketed stations along Chapel Brook. Sites displaying elevated bacteria levels will be recommended to undergo further investigation by the town or state to identify the cause.

Five sites along Chapel Brook were selected for monitoring. A sampling program will be designed to gather the necessary information. A Sampling and Analysis Plan (SAP) and Standard Operating Procedures (SOPs) will be drafted to define data quality goals and objectives. Once a SAP has been completed, wet-fall and bacteria data will be collected and evaluated. Based on the data obtained, the location of the bacteria source(s) should be evident.

Best management practices will be recommended to remediate the source(s) and implementation will be monitored.

### **3.0 Project Outline**

#### **A. Identification and Elimination of Human Bacteria Sources:**

1. Assessment of Chapel Brook.
  - a. Historical data indicate elevated bacteria levels during wet weather events.
  - b. A special study indicated that humans were a contributing source to the elevated bacteria levels.
  - c. Sampling at bracketed sites along the brook will aid in identifying the location of human bacteria sources.
    - i. *Potential sources are individual residences and a golf course.*

#### **B. Project Goals:**

1. This project will provide information to identify and remediate human bacteria from Chapel Brook.
2. This project will better protect public health at Bass Beach which is affected by the Chapel Brook discharge.

#### **C. Project Plan**

1. Select five sites along Chapel Brook.
2. Develop a monitoring regime.

*Determine necessary information. How the information will be gathered.*
3. Develop a Sampling and Analysis Plan and Standard Operating Procedures.
4. Implement monitoring plan.

5. Analyze data and determine the sites affected by elevated levels of bacteria.
7. Develop recommendations for the remediation of human bacteria.

# Appendix III-D-2: Final Report Microbial Source Tracking

# Identify and Mitigate Bacterial Sources at Public Beaches Using Microbial Source Tracking

A final report to the  
New Hampshire Department of Environmental Services

Submitted by

Dr. Stephen H. Jones  
Jackson Estuarine Laboratory/Center for Maine Biology  
Department of Natural Resources  
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Sara Summer and Jody Connor  
New Hampshire Department of Environmental Services  
Concord, NH 03301

February 2004

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New Hampshire Department of Environmental Services Beach Program  
Chapel Brook Special Study

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## 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 6 new beaches, adding to the nine previously monitored.

NH's coastal beaches have historically met state water quality standards for primary contact recreation. Monitoring programs have provided data from weekly sampling to ensure public safety. 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.

The microbial source tracking (MST) technique, ribotyping, can be used to indicate the sources of fecal pollution in the wetlands and streams flowing into the public beaches and surrounding waters. This report summarizes the MST results for three sites identified as actual pollution sources to NH marine beaches and relates results to a previous NHDES study encompassing the same sites. These results will be used to reduce and eliminate bacterial sources to the public beaches.

## Project Setting

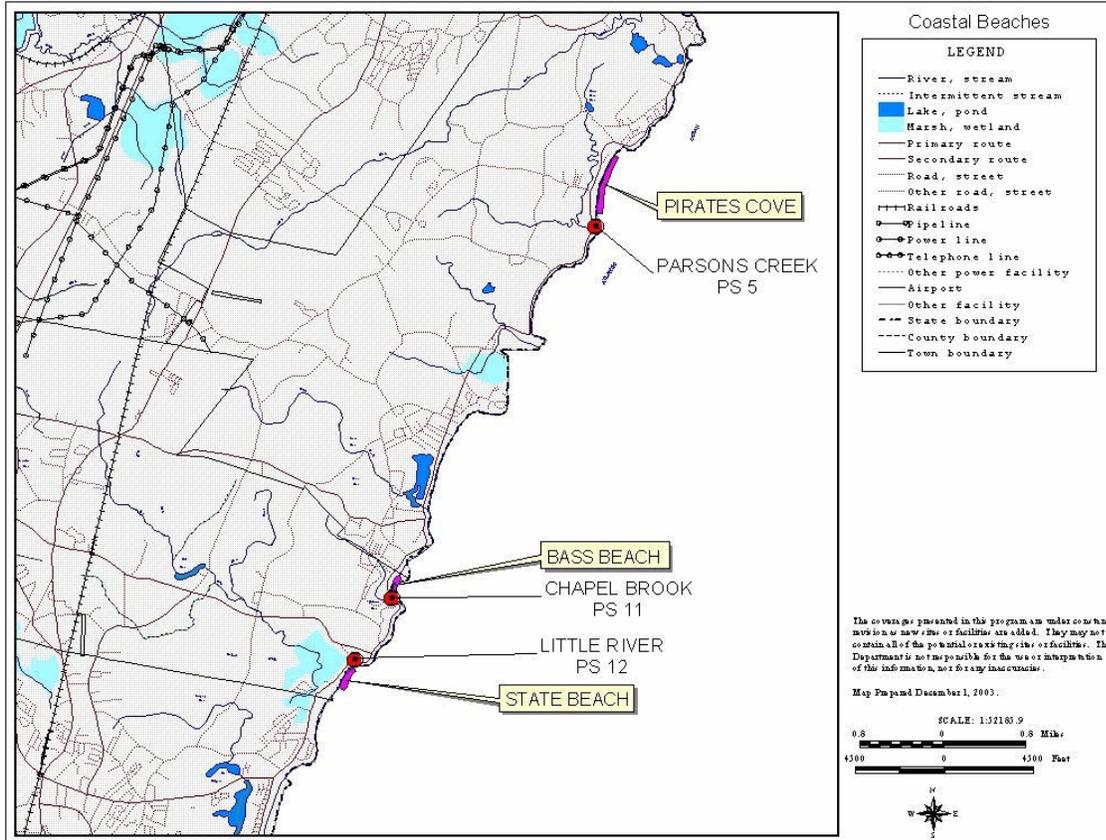
This project involved the investigation into three actual pollution sources to the Atlantic Coast. The pollution sources are PS 5, Parson's Creek, Rye; PS 11, Chapel Brook, Rye; and PS 12, Little River, North Hampton. All three sites discharge in the vicinity of coastal public beaches. Parson's Creek discharges to Pirates Cove Beach, Chapel Brook discharges to Bass Beach, and Little River discharges to North Hampton State Beach (Figure 1).

Parson's Creek is a 150 acre saltmarsh located in Rye along the western edge of Rt. 1A. The area surrounding the marsh is residential and commercial. Wildlife native to the area are: deer, muskrat, otter, mallards, shorebirds, egrets, heron, swans, and songbirds. From 1997-1999, a saltmarsh restoration project to remove tidal restrictions and control invasive species occurred. The project replaced three culverts and restored tidal flushing to the area. Final discharge of the saltmarsh is the southern end of Pirates Cove Beach where a culvert was replaced increasing flow to the beach during periods of low tide.

Chapel Brook is part of the 10.2 acre Bass Beach/Philbrick's Pond saltmarsh located along the Rye/North Hampton border. The area surrounding the marsh is mainly residential. Not much is known about wildlife common to the area. Final discharge of the saltmarsh is just south of Bass Beach. A marsh restoration project is scheduled to occur in 2003/2004 to remove tidal restrictions. When this occurs the flow to the south end of Bass Beach will increase significantly during periods of low tide.

Little River is a 193 acre saltmarsh located in North Hampton between Little Boar's Head and North Shore Rd. in Hampton. The area surrounding the marsh is mainly residential. Wildlife native to the area are: deer, muskrat, otter, mallards, shorebirds, egrets, heron, swans, and songbirds. In 2000, a saltmarsh restoration project to remove tidal restrictions occurred. The project removed an existing 48 inch culvert and replaced it with two 6X12 foot box culverts. The new culvert discharges to the northern end of North Hampton State Beach where flow has significantly increased during periods of low tide.

**Figure 1. Atlantic Coast Pollution Sources and Associated Beaches**



## Project Goals and Objectives

The goal of this project was to investigate actual and potential bacterial sources in the Atlantic Coast (NH) watershed, as identified by Nash and Chapman (2000). This study focused on wet weather sampling and is an extension of a previous MST project at the same sites that focused on dry weather sampling. Specific objectives were to:

1. Collect rain event samples from three sampling locations that were identified as pollution sources along the Atlantic Coast beaches of New Hampshire.
2. Sample each site during wet-weather events during late summer.
3. Utilize the results of the ribotyping to identify sources of bacteria to New Hampshire's coastal public beaches.
4. Provide the DES Watershed Assistance Section, Shellfish Program, New Hampshire Estuaries Project (NHEP), and the EPA with the findings of the microbial source tracking project.

## Methods

### Sample Timing and Locations

The Beach Program conducted wet weather sampling at three sites previously identified as actual pollution sources. These sites were: PS 5, PS 11, and PS 12. PS 5 (Parson's Creek), 11 (Chapel Brook), and 12 (Little River) are actual, direct pollution sources (Nash and Chapman 2000). The pollution sources listed above discharge directly to three coastal beaches. Parson's Creek discharges to the south end of Pirates Cove Beach, Chapel Brook discharges to the south end of Bass Beach, and Little River discharges to the north end of North Hampton State Beach.

Each site was sampled during storm flows twice during the summer of 2003. A minimum of 0.25 inches of rainfall triggered sample collections. There was 0.86 inches of rainfall recorded at the Portsmouth weather station on the two sample dates, August 1 and September 16, 2003. Also, Seabrook station recorded 1.16 inches of rainfall on August 1st and 0.83 on September 16, 2003. Sampling was targeted for the start, peak storm, post-peak storm, late storm and the end of storm events. Storm start samples, 5-10 minutes after start of storm, provided initial runoff data. Sampling at the peak, or first flush, provided enhanced watershed runoff data. Post-peak sampling provided data on lingering sources. End of storm sampling provided comparative data and indicate how persistent the sources were. The samples were transported to the UNH Jackson Estuarine Laboratory for bacterial indicator and ribotyping analyses.

### Laboratory and Analytical Methods

### ***Detection and Identification of Fecal Coliforms and E. coli***

The laboratory procedures for the detection and identification of fecal coliform and *E. coli* are in Appendix C along with the procedures for purification/verification of *E. coli* isolates obtained using the mTEC method.

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; 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. For some samples, fewer than 20 colonies were present on the smallest dilution analyzed, so the plate with the most numerous colonies was used. The *E. coli* isolates were subject to a battery of biochemical tests to confirm their identity as *E. coli*. The procedures used for isolating and identifying *E. coli* strains for this study were according to standard lab protocols (Jones, 2002a; Jones and Bryant, 2002). The confirmed *E. coli* isolates were then processed for determining ribopatterns. Some ribopatterns determined using the RiboPrinter<sup>®</sup> were not typical of *E. coli* and were identified by the RiboPrinter<sup>®</sup> as other species. These isolates were then 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.

### ***Sample Processing***

The procedures used for ribotyping *E. coli* isolates for this study have been used previously (Jones and Landry, 2003; Jones, 2002b) and are based to a large extent on those of Parveen et al. (1999). *E. coli* isolates were stored in cryovials at -80°C and re-cultured onto trypticase soya agar (TSA). Some of the stored isolates could not be re-cultured. Cultures on TSA were incubated overnight at room temperature (~20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C.

A RiboPrinter<sup>®</sup> 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<sup>®</sup> pattern. This pattern could be compared to others in the RiboPrinter<sup>®</sup> database for characterization and identification based on densitometry 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<sup>®</sup> 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 densitometry 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).

**Table 1. Source species database for New Hampshire sources.**

Source species	# of isolates		Source species	# of isolates	
	Coastal	State		Coastal	State
<b>DOMESTIC ANIMALS</b>			<b>HUMANS</b>		
alpaca		3	septage	6	16
buffalo		5	wastewater	42	107
chicken	3	3	humans		82
cow		56	<b>PETS</b>		
goat		4	cat	7	21
horse		28	dog	19	37
sheep		2	<b>BIRDS</b>		
<b>WILD ANIMALS</b>			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
rabbit	27	27	sparrow		3
raccoon	67	84	starling		3
red fox	23	27	wild turkey		7
skunk	5	5	<b>Total</b>		<b>358</b>

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. The

databases also included multiple isolate patterns from the same species that were identical but not from the same samples.

### ***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. 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 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. Most of 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.

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

## Storm Event Sampling

Storm event sampling occurred twice for the project. The project goal was to conduct three rounds of storm sampling. Program staff encountered difficulties in storm prediction, also, severe storm warnings hampered sample collection efforts. Four samples were to be collected at pre-storm, start, peak, and end storm stages. Due to miscalculations on storm start times and travel time to sites, pre-storm and start samples could not be collected. To compensate for the loss of those samples, post-peak storm samples were collected in hopes of capturing additional source species data. Storm first-flush data were not collected due to problems discussed above. First-flush data are an important component of storm sampling. Without it, it is unknown what source species are present in initial runoff to the tributaries.

## Bacteria Concentrations During Storm Events

Fecal coliform and *E. coli* concentrations in the water samples were measured as part of this study (Table 2). The *E. coli*:fecal coliform ratio was high (92%; excluding 8/1/03 start of storm PS 5 sample) for all samples except the 2 samples collected at the start of the 8/1/03 storm at PS 5 & 12 and one collected late during the storm at PS 12. No clear trends with storm stage were apparent for fecal coliform and *E. coli* concentrations at any site as concentrations were relatively high throughout the storms. The data for PS 5 on 8/1/03 at the start of the storm indicated a high concentration of fecal coliforms, and no *E. coli* measurements were made because the plates had confluent non-*E. coli* colonies that overgrew possible *E. coli* colonies. Excluding that date, the geometric means for fecal coliforms and *E. coli* concentrations were 855 and 771 cfu/100 ml, respectively, well above state standards for shellfishing and freshwater recreational uses.

**Table 2. Concentrations (cfu/100 ml) of fecal coliforms (FC) and *Escherichia coli* in water samples from 3 sites along the Atlantic coast, NH: 2003.**

Site	Date	Storm Stage	# of Isolates	FC	<i>E. coli</i>	EC/FC Ratio
PS 5	8/1/03	Peak	0	2300	ND	0
		Post Peak	8	140	80	0.57
		End	7	330	318	0.96
	9/16/03	Post Peak	11	620	600	0.97
		End	10	500	500	1.00
PS 11	8/1/03	Peak	10	1090	1080	0.99
		Post Peak	7	1510	1410	0.93
		End	7	1410	1380	0.98
	9/16/03	Post Peak	15	370	370	1.00
		End	15	370	370	1.00
PS 12	8/1/03	Peak	10	1740	1040	0.6
		Seep	4	2210	2190	0.99
		End	2	1280	1240	0.97
	9/16/03	Post Peak	12	1480	1410	0.95
		End	15	1460	1430	0.98
Geometric Mean				855	771	Ave. = 0.92

### Source Species Identification

There were 118 isolates from water samples collected at the 3 sites that were analyzed using the RiboPrinter<sup>®</sup>, but eight of these yielded results confirmed by biochemical tests that suggested they were a species other than *E. coli*. Source species were identified for the remaining 110 isolates. 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. Initial analysis resulted in 44 source species identifications, or 40% of the 100 isolates, using only the Atlantic coast database. However, analyses using the NH State database that included all of the Coastal isolate patterns but also had more species and overall patterns, resulted in more source species identifications. All results presented below are for analyses where the NH State database was used to improve the results found with the Coastal database.

Overall, sources for 62, or 56% of the 110 isolates were identified (Table 3). Use of lower threshold similarity indexes of 80% and 85% did not substantially increase the number of identified isolates, yielding 64% and 58% identifications, respectively. Using higher thresholds of 95% and 100% drastically reduced identifications to 41% and 27%, respectively. Thus, the results from using a threshold of 90% as used in previous studies (Jones, 2004; Jones and Landry, 2004) provided a good balance between accuracy and isolate identification

**Table 3. Source species identified for *E. coli* isolated from water samples during storm events from 3 sites along the Atlantic coast, NH: 2003.**

Site	Sample Date	Storm Stage	Total Isolates	Source Species													Identified Isolates			
				Alpaca	Cow	Coyote	Deer	Dog	Fox	Goose	Gull	Horse	Human	Otter	Raccoon	Sparrow				
PS 5	8/1/03	Peak	0																	
		Post Peak	8	1			1										1			
		End	6			1						1								
		<b>Total</b>	<b>14</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>
		Post Peak	11	1		1											1		1	
PS 11	9/16/03	End	10				2													
		<b>Date Total</b>	<b>21</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>13</b>
		<b>Site Total</b>	<b>35</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>5</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>21</b>
		Peak	10	1								2								5
		Post Peak	7								1			2						3
PS 12	8/1/03	End	6																	
		<b>Date Total</b>	<b>23</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>9</b>
		<b>Site Total</b>	<b>37</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>7</b>	<b>1</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>20</b>
		Peak	5			1								1						2
		Seep	4										1	1	1					3
PS 12	9/16/03	End	2																	
		<b>Date Total</b>	<b>11</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>5</b>
		Post Peak	12	1															7	8
		End	15						1	2				1		2				8
		<b>Date Total</b>	<b>27</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>9</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>16</b>
<b>OVERALL TOTALS</b>		<b>Site Total</b>	<b>38</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>9</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>21</b>	
		<b>Total</b>	<b>110</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>6</b>	<b>1</b>	<b>5</b>	<b>4</b>	<b>1</b>	<b>2</b>	<b>16</b>	<b>10</b>	<b>9</b>	<b>1</b>	<b>62</b>			

There were 15, or 14% of the isolates that matched database patterns at <90% similarities and were also considered to be from unknown sources. These “unknown” source isolates may be from a source species that was not included in the database, or from an included species that lacked enough diversity of ribopatterns to provide an identification of adequate accuracy.

There were also 33 (30%) isolates with ribopatterns matching database patterns shared by multiple species. These were categorized as “mixed” source species, considered successful identifications but included in the “unknown” category. There are several reasons this may occur. Some *E. coli* strains may be adaptable to multiple types of environments and be common strains in numerous different source species. Alternatively, some strains found in fecal material from different source species may be transient strains that are only there for a relatively short period of time. The mechanism of introduction could be ingestion and digestion of prey organisms, exposure to the feces of other species at landfills or sewage treatment facilities, or even coexistence of multiple species in the same area, like pets and humans or wild animals with overlapping habitats. The profile of species for some of the “mixed species” isolates included only wild animal species, suggesting one or more of the above mechanisms as a possible explanation. In the end, the existence of different strains with the same profile can also imply that ribotyping with a single restriction enzyme may give inadequate detail to differentiate all strains. One alternative strategy is the use of a second restriction enzyme in the digestion of *E. coli* DNA that cuts the chromosomal DNA at different sites. The additional information that is provided by using two profiles for each *E. coli* isolate has greatly reduced this problem and made ribotyping more useful (Jenkins et al., 2003; Hartel et al., 2002; Samadpour, 2002), although it is a more expensive overall procedure.

Overall, there were 13 different source species identified. The most commonly identified source species was humans (16 isolates), followed by otters (10) raccoons (9), deer (6), foxes (5), geese (4), coyotes and cows (3), and horses (2), with single isolates identified as coming from dogs, seagulls, sparrows and alpacas.

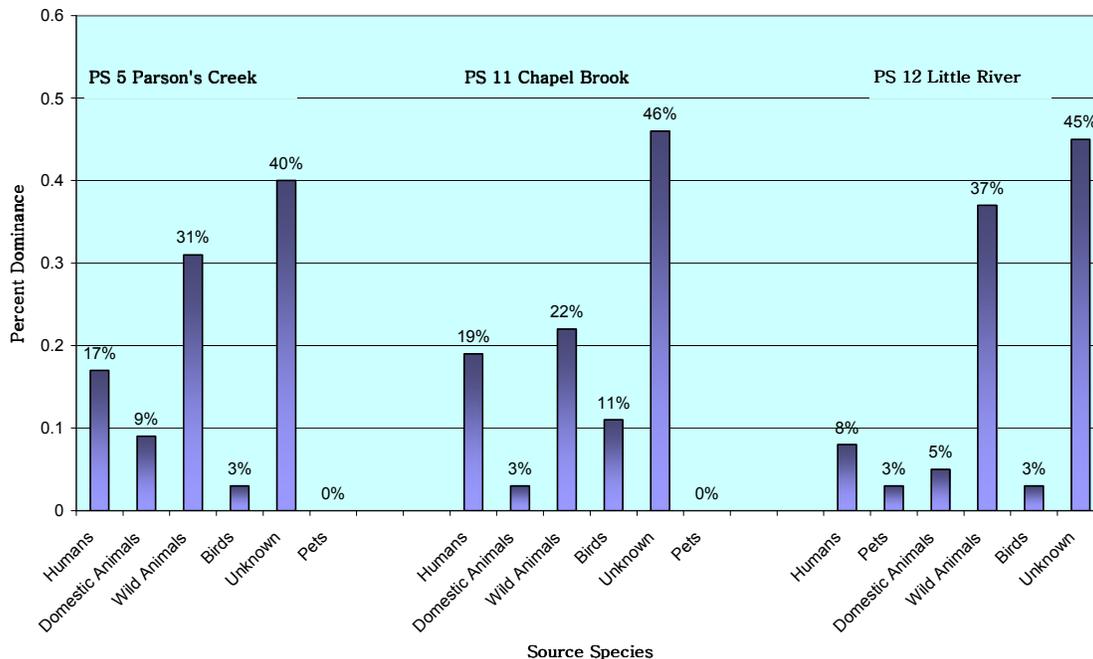
The number of isolates analyzed for each site was virtually the same (35-38 isolates), and the percentage of isolates for which source species were successfully identified ranged only from 54% for PS 11 to 55% and 60% for PS 12 and PS 5, respectively (Table 3). The number of different species identified as sources at each site was 7 for PS 5 and 11 and 9 at PS 12. Cow, deer, fox, horse, human, raccoon and sparrow isolates were identified at PS 5. Cow, deer, human and raccoon isolates were also identified at PS 11, as were coyote, goose and otter isolates. Isolates from all but cows, geese, deer and sparrows were identified at PS 12. Thus, humans and raccoons were the only source species identified at all 3 sites. Dog, goose, seagull, sparrow and alpaca isolates were only identified in one of the 3 sites. There was only one isolate identified as coming from each of these species except for geese, for which there were 4 isolates identified at PS 11.

Human isolates were more prevalent at PS 5 (17%) and PS 11 (19%) compared to PS 12 (5%), where otters were the most prevalent (24%) source species. Geese were only identified as source species at PS 11 and made up 11% of the total site isolates. Deer were most prevalent (14%) at PS 5, while raccoon isolates occurred relatively uniformly at the 3 sites. In a previous study by Jones and Landry (2004), *E. coli* isolates were also ribotyped from PS 5, 11 and 12 samples collected during mostly dry weather in 2001-02. In that study, otters were also most prevalent at PS 12 and geese at PS 11. Human isolates were much more prevalent at PS 5 (44%) and PS 12 (16%), and less prevalent at PS 11 (6%). Deer, raccoon and fox isolates were less prevalent in the previous study, but rabbit isolates were identified, in contrast to the present study where they were not identified.

### Types of Identified Source Species

Any management actions taken in response to the results of this study would hinge on what types of source species were deemed significant sources of pollution. Because of this, a useful approach for analyzing results is to group source species into types that would trigger different management actions. The different types include humans, pets, domestic animals/livestock, wild animals and birds (Table 1). Overall, wild animals were the most prevalent (30%) source species type, followed by humans (15%), birds and domestic animals (5%) and pets (1%) (Figure 2). This profile of wild animals and humans as the most prevalent source species and pets, birds and domestic animals being of lower significance has been observed in other MST studies conducted in NH, including the previous study along the Atlantic coast. Compared to the present study, Jones and Landry (2004) found a higher prevalence of human (24%), no domestic animals but similar levels of wild animal and bird isolates amongst the 59 isolates they collected from the same Atlantic coast sites.

Figure 2. Atlantic Coast Source Species Per Site

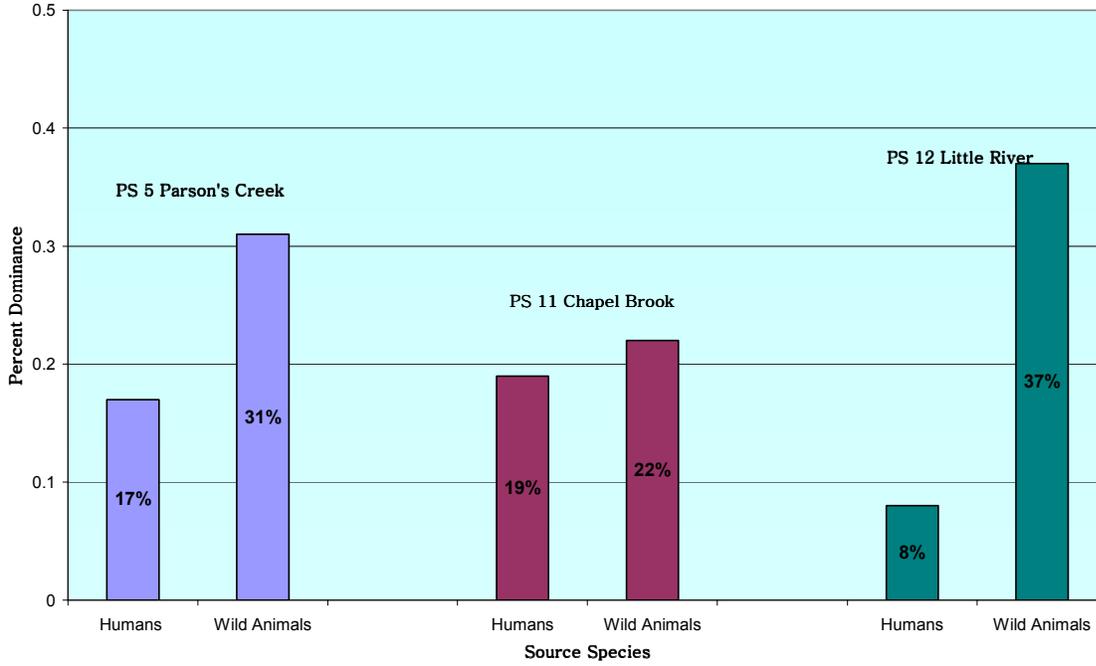


The big difference between the two Atlantic coast MST studies is that samples were collected during wet weather in this study and largely during dry weather for the previous study. As a result of this difference in weather conditions during sampling, the *E. coli* concentrations were much lower (geometric mean = 63 cfu/100 ml) than observed during the present study, where the geometric mean was 771 cfu/100 ml. Thus, the differences observed for types of source species contributing as pollution sources in the two studies probably resulted from the different weather conditions.

The types of source species identified at each site had potentially important differences, even though wild animal isolates were the most prevalent type at all 3 sites (Figure 3). Human isolates were the second most prevalent type at all 3 sites, although they were almost as prevalent (19%) as wild animals (22%) at PS 11 and they were much less prevalent (8%) at PS 12 compared to wild animals (37%). Domestic animal isolates followed human isolates in prevalence at PS 5 while bird isolates followed human isolates in prevalence at PS 11. One pet

isolate was present only at PS 12.

Figure 3. Dominant Source Species per Site



These results suggest that the most prevalent types of source species are relatively similar at the 3 sites and thus management strategies would also be similar at the different sites. Another analytical strategy is to regard human, pet and domestic animal isolates as derived from human-related sources, while birds and wild animals probably originate solely from non-human related sources. In this regard, non-human related sources still outnumber human-related sources at all 3 sites, especially at PS 12 (Table 4). Even still, human-related source species constituted ~40% of all identified sources, suggesting that they are an important type of pollution source. The reduction or elimination of human sources could provide a significant level of improvement in water quality to these sites and to the coastal beaches that receive the pollutant load.

## Conclusions

The most prevalent types of sources, wild animals and humans, were consistently present at each site. The wild animals included six species, including raccoons that were identified as source species at all 3 sites, as were humans.

There were differences between sites for less prevalent source species types. This was most striking at PS 11, the only site where bird species were more prevalent than domestic

animals because of the higher prevalence of geese, the low incidence of cow isolates and the absence of other domestic animal isolates.

The results from the present study conducted during wet weather had the same types of most prevalent source species types, wild animals and humans, but differed in some wild animal species sources for the same sites studied in 2001-02 during dry weather (Jones and Landry, 2004). There was a greater prevalence of raccoons, deer and foxes, and fewer otters and rabbits in this study. The wet weather apparently caused *E. coli* concentrations in the sampled surface waters to be higher relative to the previous dry weather samples. Because of this, the sources identified during wet weather in this study may be more important in terms of bacterial and pathogen loading to the downstream beach areas.

The results also indicate a potential impact from saltmarsh restorations on beach areas. Two sites, PS 5 and PS 12, were subject to saltmarsh restoration projects involving the removal of tidal restrictions. The removals are designed to increase tidal flow to and from saltmarsh areas, increase habitat, and restore acres of saltmarsh. The removal of tidal restrictions may have a negative impact where tidal discharge is located on or near a beach area. The increased flow rate could increase the likelihood of bacteria transport to beaches. Habitat restoration can lure additional wildlife to the area resulting in increased saltmarsh bacteria loads and wildlife source species. A recommendation to conduct a water quality study involving pre and post restoration conditions, source species, and impacts will be made for future saltmarsh restoration projects.

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## Appendix III-D-3: Historical Data

<b>Chapel Brook Historical Data</b>			
<b>Date</b>	<b><i>E. coli</i> Result (cts/100mL)</b>	<b>Enterococci Result (cts/100mL)</b>	<b>Qualifier</b>
8/1/2003	1080		
8/1/2003	1410		
8/1/2003	1380		
9/16/2003	370		
4/15/2004		10	<
5/27/2004		10	
6/15/2004		10	
7/27/2004		20	
8/10/2004		30	
8/23/2004		450	
8/25/2004		190	
5/5/2005		10	<
5/25/2005		300	
6/1/2005		30	
6/13/2005		270	
6/28/2005		50	
7/13/2005		10	
7/19/2005		10	<
7/25/2005		60	
8/9/2005		20	
8/15/2005		280	
8/24/2005		40	

# Appendix III-D-4: Field Data Sheet



# NHDES Beach Program Chapel Brook Field Data Sheet



Station ID: \_\_\_\_\_

Town: \_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Inspector: \_\_\_\_\_

Associated Beach: \_\_\_\_\_

Prior Rainfall (rain gauge): \_\_\_\_\_ in.

Ending Rainfall (rain gauge): \_\_\_\_\_ in.

Source to Beach Area (choose one)		Source Type (choose one)		Water Conditions (choose one)	Number of Samples Collected
<input type="checkbox"/> Empties directly to beach <input type="checkbox"/> Empties in proximity of beach (within 200 feet) <input type="checkbox"/> Empties outside beach area		<input type="checkbox"/> Perennial Stream <input type="checkbox"/> Tidal Creek <input type="checkbox"/> Road Culvert <input type="checkbox"/> Straight Pipe <input type="checkbox"/> Other:		<input type="checkbox"/> Clear <input type="checkbox"/> Turbid <input type="checkbox"/> Other:	
Precipitation Intensity (choose one)	Amount of Rain (if known)	Length of Storm (choose one)		Storm Comments	
<input type="checkbox"/> Light <input type="checkbox"/> Moderate <input type="checkbox"/> Heavy <input type="checkbox"/> Excessive	<input type="checkbox"/> < 0.5 in. <input type="checkbox"/> 0.5 to 1.0 in. <input type="checkbox"/> 1.1 to 1.5 in. <input type="checkbox"/> 1.6 to 3.0 in. <input type="checkbox"/> > 3.0 in.	<input type="checkbox"/> <15 min. <input type="checkbox"/> 15 to 30 min. <input type="checkbox"/> 31 to 60 min. <input type="checkbox"/> > 60 min.			
Wildlife Present (choose one)	Type and Number (estimate)	Runoff Present (choose one)		Runoff Source (choose one)	
<input type="checkbox"/> Yes <input type="checkbox"/> No		<input type="checkbox"/> Yes <input type="checkbox"/> No		<input type="checkbox"/> Parking Lot Runoff <input type="checkbox"/> Farm Runoff <input type="checkbox"/> Graywater Discharge <input type="checkbox"/> Gutter Drain <input type="checkbox"/> Other:	

Other Comments (or use back of page):



## Appendix III-E: Contaminants of Concern

**Contaminants of Concern and Other Target Analytes Table (Reference Limit and Evaluation Table)**

Analyte	Analytical method	Project Action Level	Analytical/Achievable Method Detection Limit	Analytical/Achievable Laboratory Quantitation Limit
<i>E. coli</i>	<i>E. coli</i> Standard Method 9213D	406 cts/100 mL or geometric mean of 126 cts/100 mL per 3 samples in 60 days	0+ cts/100 mL (depends on dilution and sample volume)	0+ cts/100 mL (depends on dilution and sample volume)

## Appendix III-F: Project Schedule Time Line

## Project Schedule Time Line

Activity	Dates (MM/DD/YYYY)		Deliverable	Deliverable Due Date
	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion		
SAP Preparation	02/28/2006	04/30/2006	SAP Document	4/30/2006
Laboratory Analyses	05/1/2006	09/30/2006	Analysis Results	10/31/2006
Monitoring/Sampling	05/1/2006	9/30/2006	Quantity of Wet Weather Samples Collected	10/31/2006
Final Project Report	TBD	TBD	Final Wet Weather Analysis Report	TBD

Appendix III-G:  
Sampling Locations, Sampling and Analysis  
Method/SOP Requirements

## Sampling Locations, Sampling and Analysis Method/SOP Requirements Table

<b>Sampling Location</b>	All locations
<b>Medium/Matrix</b>	Aqueous
<b>Depth</b>	Grab
<b>Analytical Parameter</b>	<i>E. coli</i>
<b>Number of Samples</b>	25-56
<b>Sampling SOP</b>	Beach Program SOP for Bacteria Sampling
<b>Analytical Method/SOP</b>	<i>E. coli</i> Standard Method 9213D
<b>Sample Volume</b>	≥ 100 mL
<b>Containers Number, size and type)</b>	100-150, 8 oz., sterile plastic
<b>Preservation Requirements (chemical, temperature, light protected)</b>	Chilled to ≤ 10°C
<b>Max. Holding Time (preparation/analysis)</b>	8 hours

# Appendix III-H: Field Sample Quality Control Summary

Biological monitoring/sampling and analyses performed by the NHDES Laboratory Services Unit or the NHDES Limnology Center will adhere to the QC guidelines listed in the NHDES Laboratory Services Unit's Quality Systems Manual (on file at EPA), the Watershed Management Bureau's Limnology Center Procedures and Protocols (Beach Program QAPP, Appendix A), and the Beach Program SOPs for Bacteria Sampling. The following table summarizes field QC procedures for this project.

### Field Sample Quality Control Summary Table

<b>Matrix</b>	<b>Analytical Parameter</b>	<b>Field QC</b>	<b>Data Quality Indicators</b>	<b>Acceptable Limits</b>	<b>Corrective Action</b>	<b>Person Responsible</b>	<b>Frequency</b>
Surface Water	<i>E. coli</i>	Field Duplicates	Precision	RPD $\leq$ 75%	Address field and lab operations and precision	Beach Program Coordinator (Sara Sumner)	10% of samples
Surface Water	<i>E. coli</i>	Trip Blanks	Accuracy/Bias	0 counts	Retest and address lab D.I. water and bottle sterilization	Beach Program Coordinator (Sara Sumner)	20% of samples

# Appendix IV: Chapel Brook Watershed Septic System Data

\*Data obtained from DES OneStop website (<http://www.des.nh.gov/OneStop.htm>)

ROAD	LOT NUMBER	SIZE (ACRES)	SEPTIC AGE
<b>OCEAN BLVD.</b>	5-12	1.33	
	5-11	12.6	
	5-10-1	1.28	8/1/1989
	5-10	1.66	
	5-9	2.46	9/14/1998
<b>WILLOW AVE.</b>	5-9-1	6.83	12/29/1998
	5-8	7.24	9/12/2003
<b>CHAPEL RD.</b>	1-136	4.06	5/14/2003
	5-24	1.5	
	5-25	0.4	5/2/2000
	5-26	0.92	
	5-27	0.93	5/14/2003
	5-28	2.8	
	5-29	2.32	
	5-30	2.44	
	5-31	2.65	
	5-32	2.69	
	5-33	2.59	
	5-34	2.94	
	1-127	4.38	
	1-126	2.53	12/14/2000
	1-125	0.65	
	1-124	0.73	
	<b>OLD LOCKE RD.</b>	5-23	4.15
5-22		6.25	
5-21		1	
5-20		11.4	
5-19-1		2.17	
5-19		2.08	
5-18		2.1	
5-17		2.3	
5-16		4.39	
5-15		4.78	
5-13		2.5	
5-76		1.15	
5-77		2.11	
5-78		2.36	
5-79		4.84	
5-80		1.7	
5-81		1.44	
5-82-1	0.47		
5-82	0.45		
5-83	1.97		
5-84	1		