



# New Jersey's Strategy for Microbial Source Tracking

Water Monitoring & Standards  
Bureau of Marine Water Monitoring

# Monitoring Areas

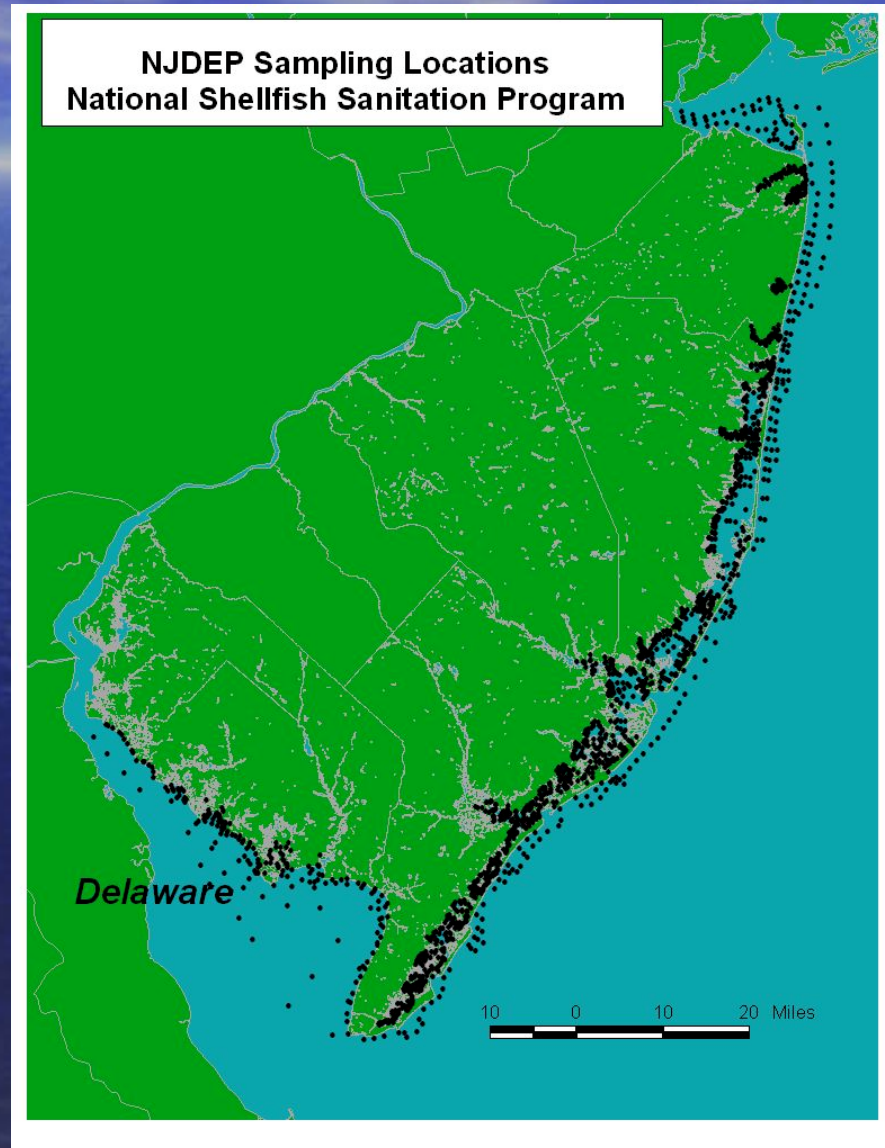
- National Shellfish Sanitation Program
- Coastal Monitoring Network
- Non-Point Runoff Studies
- Emergency Spills



<http://www.state.nj.us/dep/dsr/bmw/index.htm>

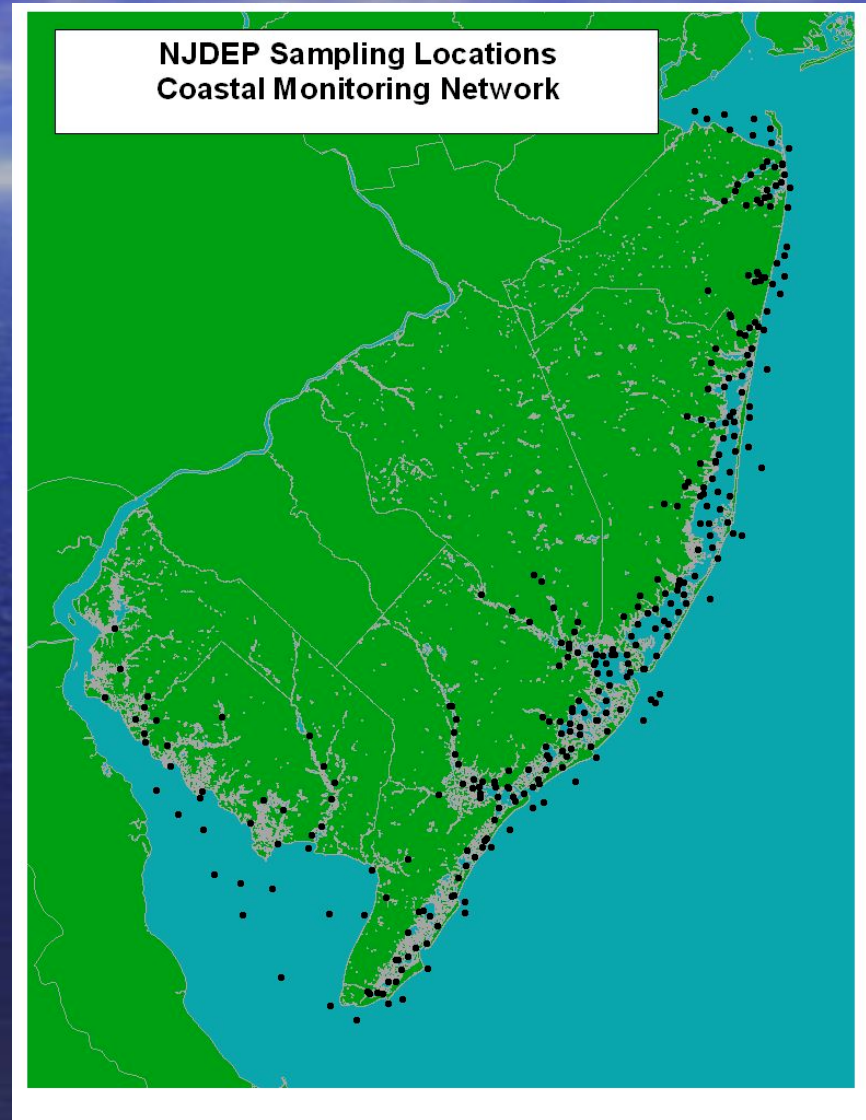
# NSSP Monitoring Network

- Approximately 2,500 sampling locations
- Samples collected 5-12x per year
- Samples analyzed for total coliform and fecal coliform bacteria - indicators of human waste.



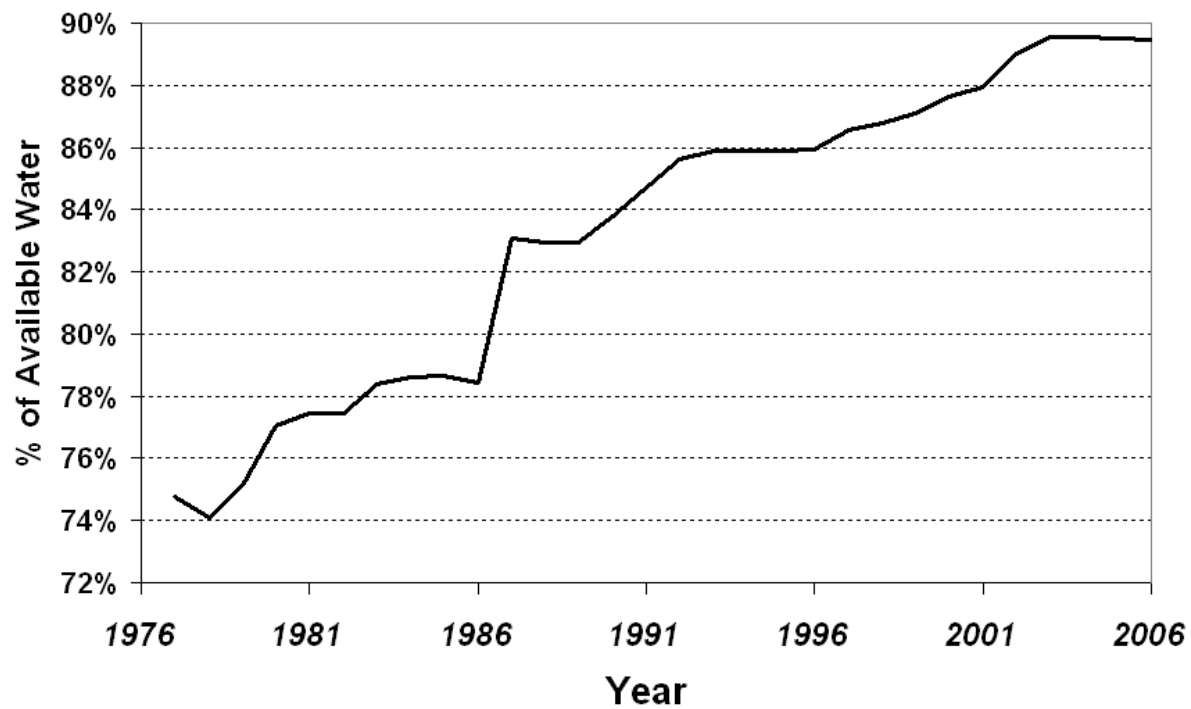
# Coastal Monitoring Network

- Established to provide consistent long-term monitoring of basic water quality
- Salinity
- DO
- Nutrients
- Temperature
- Chlorophyll
- Secchi Depth
- Enterococci



# Harvestable Acreage in NJ

*New Jersey Harvestable Shellfish Waters*



# Stormwater Impacts



Areas shaded red represent coastal waters where fecal coliform levels are elevated following a storm event.

# Stormwater Monitoring

- Developed field methods for estimating pollutant loads from storm events
- Perform monitoring during storm conditions to delineate major sources
- Use specialized tests to identify sources of contamination
- Data used to address impaired areas of the State







# Decision Criteria for MST

- Is The Problem Adequately Defined?
  - beach closures
  - TMDLs
- Has An Adequate Sanitary Survey Been Conducted?
- How Many Sources Were Identified In the Sanitary Survey?

# Decision Criteria

- Is The Watershed Of Manageable Size?
  - Generally, watersheds with drainage areas greater than 14 digit USGS code are not amenable to using MST
- What Is The Desired level of Discrimination?

# Decision Criteria

- Levels of Discrimination
  - Humans vs. all other sources
  - Species specific (humans, cows, geese, dogs, etc.)
  - Host Group (humans vs. livestock vs. wildlife)
  - Individual hosts ( cows from a certain farm vs. other farms)

# MST Strategy

- Evaluate long-term microbial monitoring results
- Perform stormwater monitoring to delineate major sources
- Use specialized tests to aid in source ID
- Evaluate data in conjunction with sanitary survey info ( GIS land use coverage, hydrographic studies, actual/potential sources)

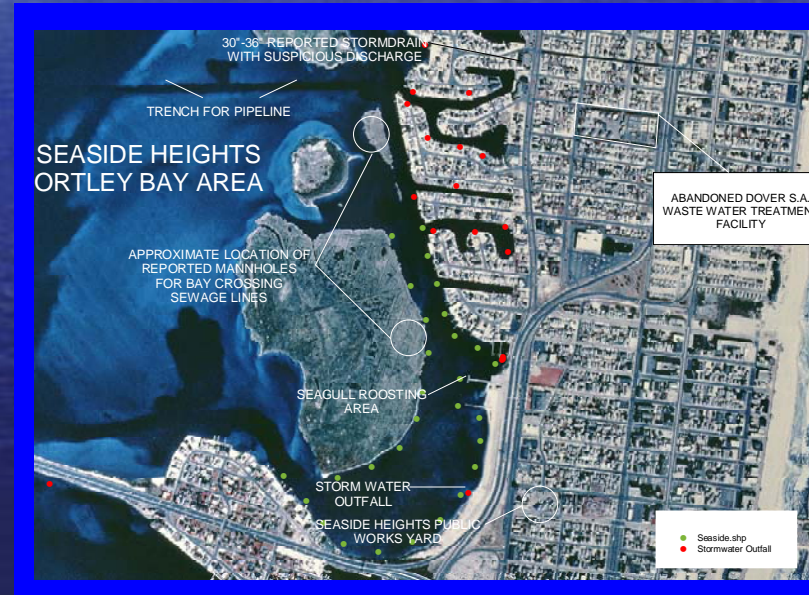
# Seaside Heights Area Study

- Area with use impairment - Bathing Beach and shellfish water closure
- Area contains numerous sources:



# Sampling Sites and Sources

- Routine sampling stations shown in green
- Stormwater pipe discharges shown in red



# Seaside Storm Water Project

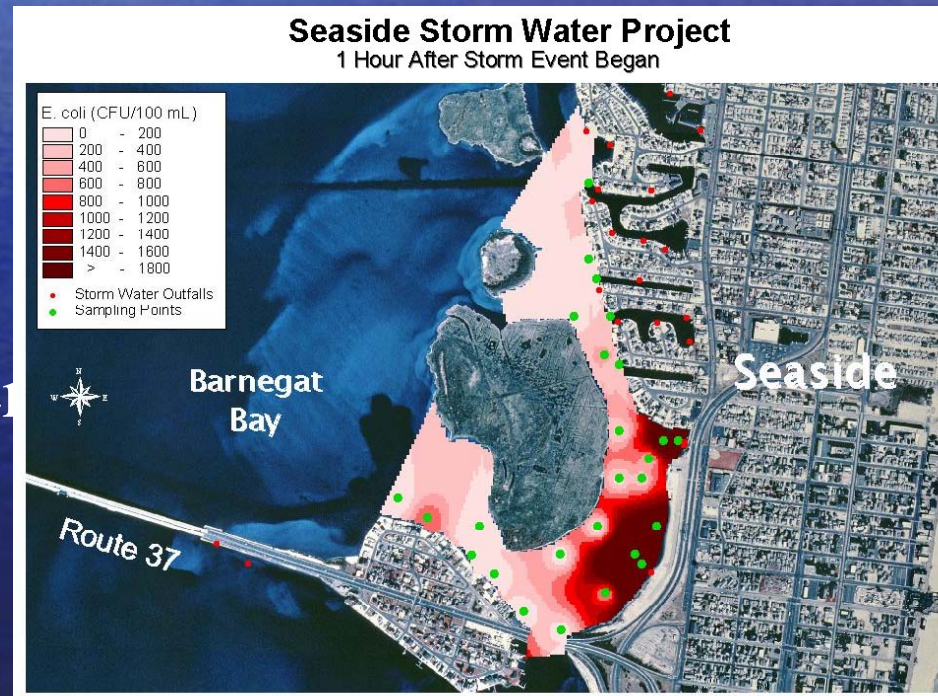
- Bacteria levels prior to rainfall
- Levels throughout the area are generally less than 100 fecal coliform per 100 mLs





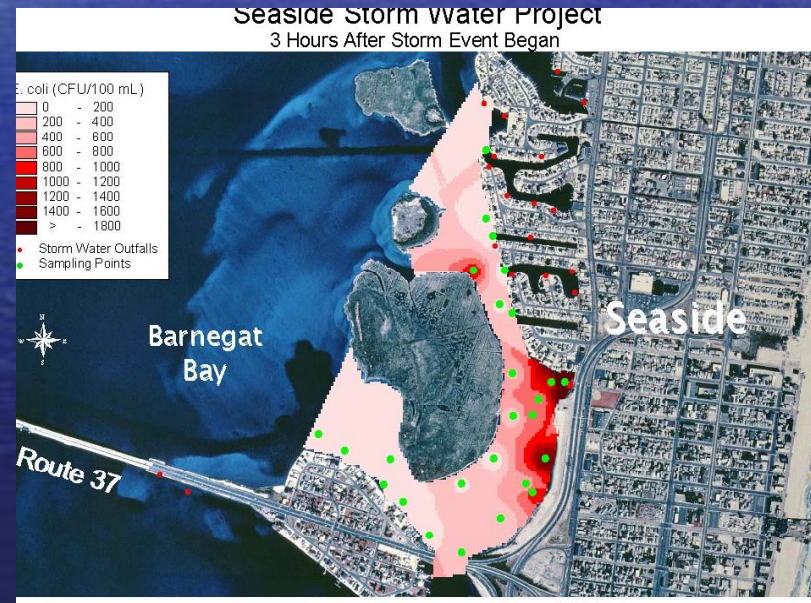
# Seaside Storm Water Project

- 1 Hour after storm event began
- Levels quickly rise - some areas with fecal coliform levels greater than 1000/100 mLs
- High values adjacent to two stormdrains in this area



# Seaside Storm Water Project

- 3 hours after storm event began
- Levels begin to subside
- High values continue to be adjacent to two of the numerous storm drains in the area



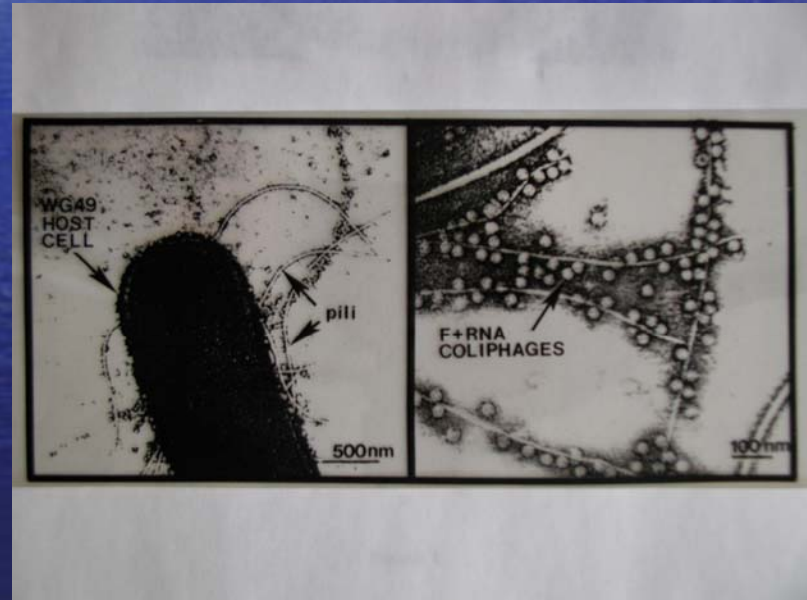
# Seaside Storm Water Project

- Stormdrain - major contribution of bacterial loading
- Coliphage testing revealed source to be of human origin
- Demonstrates concept of prioritizing sources



# F+ RNA Coliphages

- Viruses that infect bacterial cells
- Similar in size, shape and morphology to HEV including; HAV and Norwalk therefore: good viral pathogen indicator
- More resistant to chlorination than the conventional indicators
- Good wastewater effluent indicator



# Coliphage - NJDEP Findings

- Monitoring at known fecal contaminated sites
  - point human - wastewater discharge outfall
  - point animal - wildlife refuge discharge
  - non-point human - malfunctioning septic tank discharge
  - non-point animal - rural creek w/animal population
- Findings:
  - coliphage are readily detectable in most fecal contaminated sites
  - serotyping (and now, genotyping) of the phages provides a promising system for distinguishing human and animal fecal contamination

# Serotyping/Genotyping of coliphages

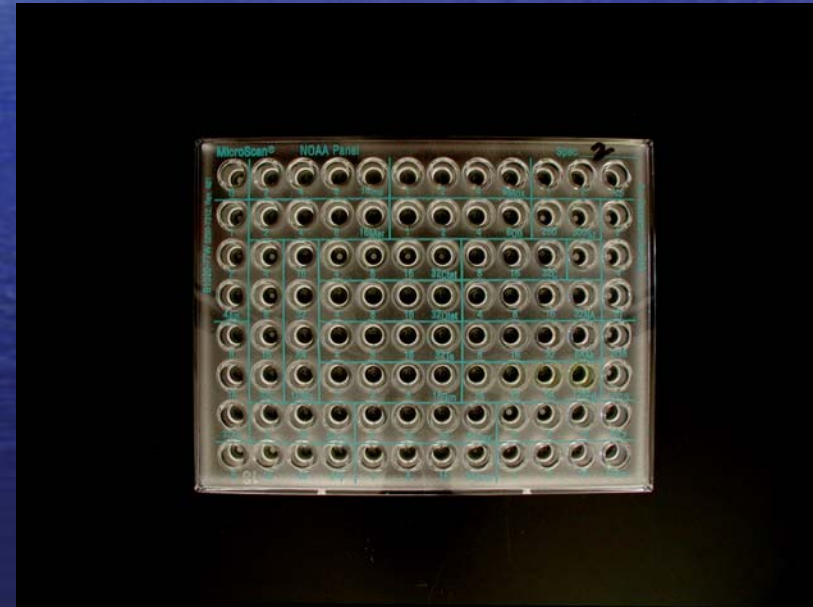
- Group I - Animal
- Group II - Human
- Group III - Human
- Group IV - Animal

# Techniques for Source ID

- **MAR - Multiple Antibiotic Resistance**
  - identifies *E. coli* that are resistant to antibiotics used to treat bacterial infections in humans. *E. coli* that is resistant to medicinal antibiotics are typically of human origin
- Used in conjunction with coliphage results
- Level of Discrimination: Human/Domesticated Animal/Wildlife

# MAR Panel

- *E. coli* isolates from water samples are inoculated into 96 well panel
- Panel contains 26 antibiotics in 3-4 dilutions
- Growth denotes resistance
- Provides a consistent, standardized process





# MAR Reporting Sheet

- Growth is recorded on a sheet which lists the antibiotics typically administered to humans and domestic animals
- Comparisons to a “reference library” is performed

| NOAA Panel | Date _____ |     |     |    |    |      | Sample # _____ |    |     |     |      |  |
|------------|------------|-----|-----|----|----|------|----------------|----|-----|-----|------|--|
| G          | 2          | 4   | 8   | 16 | 1  | 2    | 4              | 8  | 1   | 2   | 4    |  |
| 1          | 2          | 4   | 8   | 16 | 1  | 2    | 4              | 8  | 250 | 500 | 2    |  |
| 2          | 4          | 16  | 4   | 8  | 16 | 32   | 8              | 16 | 32  |     | 4    |  |
| 4          | 8          | 32  | 4   | 8  | 16 | 32   | 4              | 8  | 16  | 32  | 8    |  |
| Azi        |            |     |     |    |    | Otet |                |    |     | NA  | T    |  |
| 8          | 16         | 64  | 4   | 8  | 16 | 32   | 8              | 16 | 32  | 64  | 2/38 |  |
| 16         | 32         | 128 | 2   | 4  | 8  | 16   | 16             | 32 | 64  | 128 | 4/76 |  |
| 32         | 8          | 16  | 32  | 8  | 16 | 32   | 64             | 4  | 8   | 16  | 32   |  |
| Apr        |            |     | Cfx |    |    |      | Cax            |    |     |     | Cf   |  |
| 8          | 16         | 32  | 64  | 4  | 8  | 16   | 32             | 4  | 8   | 16  | 32   |  |
|            |            |     | P   |    |    |      | Amx            |    |     |     | Am   |  |

# MST Reference Sites

- Wildlife - Wildlife Refuge
- Domesticated Animal - Cattle Farm
- Human - WWTP (chlorinated and unchlorinated effluent)



**Legend**

- Sampling Site
- Streams



NJDEP Bureau of Marine Water Monitoring

















# WILDLIFE

NOAA Panel \_\_\_\_\_ Date 3-21-06 Coll. Time 11:00 Sample # REFUGÉ ID # EAST GATE

|           | 2       | 4         | 8         | 16<br>Imp | 1  | 2          | 4         | 8<br>Mox | 1         | 2         | 4<br>Cp     |
|-----------|---------|-----------|-----------|-----------|----|------------|-----------|----------|-----------|-----------|-------------|
| <b>G</b>  | 2       | 4         | 8         | 16<br>Mer | 1  | 2          | 4         | 8<br>Ofi | 1         | 2         | 4<br>Cp     |
| 1         | 2       | 4         | 8         | 16<br>Mer | 1  | 2          | 4         | 8<br>Ofi | 250<br>Sz | 500       | 2           |
| 2         | 4       | 16        |           |           |    |            | 8         | 16       | 32<br>C   |           | 4           |
| 4<br>Azi  | 8       | 32        | 4         | 8         | 16 | 32<br>Otet | 4         | 8        | 16        | 32<br>NA  | 8<br>T      |
| 8         | 16      | 64        | 4         | 8         | 16 | 32<br>Te   | 8         | 16       | 32        | 64<br>Ak  | 2/38        |
| 16        | 32<br>E | 128<br>St | 2         | 4         | 8  | 16<br>Gm   | 16        | 32       | 64        | 128<br>Fd | 4/76<br>T/S |
| 32<br>Apr | 8       | 16        | 32<br>Cfx | 8         | 16 | 32         | 64<br>Cax | 4        | 8         | 16        | 32<br>Cf    |
| 8         | 16      | 32        | 64<br>P   | 4         | 8  | 16         | 32<br>Amx | 4        | 8         | 16        | 32<br>Am    |

| Vol.      | <u>MODIFIED mTec</u> |              |
|-----------|----------------------|--------------|
|           | Colony<br>Count      | CFU's/100mLs |
| <u>30</u> | <u>12</u>            | <u>40</u>    |

| Phage    | PFU's/100mLs |
|----------|--------------|
| <u>—</u> | <u>&lt;1</u> |

# DOMESTICATED ANIMAL

NOAA Panel \_\_\_\_\_ Date 3-2-04 Coll. Time \_\_\_\_\_ Sample # \_\_\_\_\_ ID # ALLOWAY





























|                  | 2  | 4   | 8  | 16               | 1  | 2  | 4  | 8          | 1   | 2   | 4    |
|------------------|----|-----|----|------------------|----|----|----|------------|-----|-----|------|
| <b>G</b>         |    |     |    | <b>Imp</b>       |    |    |    | <b>Mox</b> |     |     |      |
| <b>1</b>         | 2  | 4   | 8  | <b>16</b><br>Mer | 1  | 2  | 4  | 8          | 250 | 500 | 2    |
| <b>2</b>         | 4  | 16  |    |                  |    |    | 8  | 16         | 32  |     | 4    |
| <b>4</b><br>Azi  | 8  | 32  | 4  | 8                | 16 | 32 | 4  | 8          | 16  | 32  | 8    |
| <b>8</b>         | 16 | 64  | 4  | 8                | 16 | 32 | 8  | 16         | 32  | 64  | 2/38 |
| <b>16</b>        | 32 | 128 | 2  | 4                | 8  | 16 | 16 | 32         | 64  | 128 | 4/76 |
| <b>32</b><br>Apr | 8  | 16  | 32 | 8                | 16 | 32 | 64 | 4          | 8   | 16  | 32   |
| <b>8</b>         | 16 | 32  | 64 | 4                | 8  | 16 | 32 | 4          | 8   | 16  | 32   |

|       |                      |              |
|-------|----------------------|--------------|
|       | <b>MODIFIED mTec</b> |              |
| Vol.  | Colony Count         | CFU's/100mLs |
| _____ | _____                | _____        |

|       |              |
|-------|--------------|
| Phage | PFU's/100mLs |
| _____ | _____        |

# HUMAN

NOAA Panel \_\_\_\_\_ Date 3-27-04 Coll. Time \_\_\_\_\_ Sample # ACUA ID # EFFLUENT

|   |   |   |    |   |   |   |   |   |   |   |   |    |
|---|---|---|----|---|---|---|---|---|---|---|---|----|
|    | 2   | 4   | 8  | 16  | 1   | 2   | 4   | 8   | 1   | 2   | 4   |    |
| G   |   |   |    | Imp   |   |   |   | Mox   |    |    | Cp  |    |
|    | 2   | 4   | 8  | 16  |    | 2   | 4   | 8   | 250   | 500   |    |    |
| 1   |   |   |    | Mer   |   |   |   | Ofi   | Sz  |   | 2   |    |
|    |    | 16  |    |   |   |   | 8   | 16  | 32  |    | 4   |    |
| 2   | 4   |   |    |   |   |   |   |   | C   |   |   |    |
|    |    | 32  | 4  | 8   | 16  | 32  |    | 8   | 16  |    |    | 8  |
| 4   | 8   |   |    |   |   | Otet  |   |   | 32  | NA  | T   |    |
| 8   |    | 64  | 4  | 8   | 16  | 32  | 8   | 16  | 32  | 64  | 2/38  |    |
|   |   |   |    |   |   | Te  |   |   |   | Ak  |   |    |
| 16  |    | 128   | 2  | 4   | 8   | 16  | 16  | 32  | 64  | 128   | 4/76  |    |
|   |   |   |    |   |   | Gm  |   |   |   | Fd  | T/S   |    |
| 32  | 8   | 16  | 32 | 8   | 16  | 32  | 64  |   | 8   | 16  | 32  |    |
| Apr   |   |   |    |   |   |   | Cax   |   |   |   | Cf  |    |
|  |  |  | 64 |  |  |  |  |  |  |  |  | 32 |
| 8   | 16  | 32  | P  | 4   | 8   | 16  | 32  | 4   | 8   | 16  | 32  |    |
|   |   |   |    |   |   |   | Amx   |   |   |   | Am  |    |

| MODIFIED mTec |              |              |
|---------------|--------------|--------------|
| Vol.          | Colony Count | CFU's/100mLs |
| <u>100</u>    | <u>17</u>    | <u>17</u>    |

| Phage | PFU's/100mLs  |
|-------|---------------|
| _____ | <u>11,280</u> |

### WILDLIFE

NOAA Panel Date 3-21-04 Coll. Time 11:00 Sample # REFUG ID # BEST GATE

| NOAA Panel | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 1   | 2   | 4    |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|------|
| 1          | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 250 | 500 | 2    |
|            |    |     |     | Mer |    |     |     | Off | So  |     |      |
| 2          | 4  | 16  |     |     |    |     | 8   | 16  | 32  |     | 4    |
|            |    |     |     |     |    |     |     |     | C   |     |      |
| 4          | 8  | 32  | 4   | 8   | 16 | 32  | 4   | 8   | 16  | 32  | 8    |
|            |    |     |     |     |    | Off |     |     |     | NA  | T    |
| 8          | 16 | 64  | 4   | 8   | 16 | 32  | 8   | 16  | 32  | 64  | 2/38 |
|            |    |     |     |     |    | Te  |     |     |     | AK  |      |
| 16         | 32 | 128 | 2   | 4   | 8  | 16  | 16  | 32  | 64  | 128 | 4/76 |
|            |    | E   |     |     |    | Com |     |     |     | PA  | T/S  |
| 32         | 8  | 16  | 32  | 8   | 16 | 32  | 64  | 4   | 8   | 16  | 32   |
|            |    |     | CFs |     |    |     | Can |     |     | CF  |      |
| 8          | 16 | 32  | 64  | 4   | 8  | 16  | 32  | 4   | 8   | 16  | 32   |
|            |    |     | P   |     |    |     | Amx |     |     | Am  |      |

MODIFIED mTec  
Vol. Colony CFU's/100mLs  
Count

30 12 40

Phage PFU's/100mLs  
— <1

### DOMESTICATED ANIMAL

NOAA Panel Date 3-2-04 Coll. Time \_\_\_\_\_ Sample # \_\_\_\_\_ ID # ALLOWAY

| NOAA Panel | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 1   | 2   | 4    |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|------|
| 1          | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 250 | 500 | 2    |
|            |    |     |     | Mer |    |     |     | Off | So  |     |      |
| 2          | 4  | 16  |     |     |    |     | 8   | 16  | 32  |     | 4    |
|            |    |     |     |     |    |     |     |     | C   |     |      |
| 4          | 8  | 32  | 4   | 8   | 16 | 32  | 4   | 8   | 16  | 32  | 8    |
|            |    |     |     |     |    | Off |     |     |     | NA  | T    |
| 8          | 16 | 64  | 4   | 8   | 16 | 32  | 8   | 16  | 32  | 64  | 2/38 |
|            |    |     |     |     |    | Te  |     |     |     | AK  |      |
| 16         | 32 | 128 | 2   | 4   | 8  | 16  | 16  | 32  | 64  | 128 | 4/76 |
|            |    | E   |     |     |    | Com |     |     |     | PA  | T/S  |
| 32         | 8  | 16  | 32  | 8   | 16 | 32  | 64  | 4   | 8   | 16  | 32   |
|            |    |     | CFs |     |    |     | Can |     |     | CF  |      |
| 8          | 16 | 32  | 64  | 4   | 8  | 16  | 32  | 4   | 8   | 16  | 32   |
|            |    |     | P   |     |    |     | Amx |     |     | Am  |      |

MODIFIED mTec  
Vol. Colony CFU's/100mLs  
Count

Phage PFU's/100mLs

### HUMAN

NOAA Panel Date 3-27-04 Coll. Time \_\_\_\_\_ Sample # ACHA ID # EFFLUENT

| NOAA Panel | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 1   | 2   | 4    |
|------------|----|-----|-----|-----|----|-----|-----|-----|-----|-----|------|
| 1          | 2  | 4   | 8   | 16  | 1  | 2   | 4   | 8   | 250 | 500 | 2    |
|            |    |     |     | Mer |    |     |     | Off | So  |     |      |
| 2          | 4  | 16  |     |     |    |     | 8   | 16  | 32  |     | 4    |
|            |    |     |     |     |    |     |     |     | C   |     |      |
| 4          | 8  | 32  | 4   | 8   | 16 | 32  | 4   | 8   | 16  | 32  | 8    |
|            |    |     |     |     |    | Off |     |     |     | NA  | T    |
| 8          | 16 | 64  | 4   | 8   | 16 | 32  | 8   | 16  | 32  | 64  | 2/38 |
|            |    |     |     |     |    | Te  |     |     |     | AK  |      |
| 16         | 32 | 128 | 2   | 4   | 8  | 16  | 16  | 32  | 64  | 128 | 4/76 |
|            |    | E   |     |     |    | Com |     |     |     | PA  | T/S  |
| 32         | 8  | 16  | 32  | 8   | 16 | 32  | 64  | 4   | 8   | 16  | 32   |
|            |    |     | CFs |     |    |     | Can |     |     | CF  |      |
| 8          | 16 | 32  | 64  | 4   | 8  | 16  | 32  | 4   | 8   | 16  | 32   |
|            |    |     | P   |     |    |     | Amx |     |     | Am  |      |

MODIFIED mTec  
Vol. Colony CFU's/100mLs  
Count

100 12 17

Phage PFU's/100mLs  
11,280

# MAR Results

| Antibiotic        | Waterfowl | Cattle | WWTP |
|-------------------|-----------|--------|------|
| Azithromycin      | Low       | Med    | Med  |
| Erythromycin      | Med       | Med    | Med  |
| Penicillin G or V | Med       | Low    | Med  |
| Oxytetracycline   | Low       | High   | Low  |
| Tetracycline      | Low       | Med    | Low  |
| Amoxicillin       | Low       | Low    | High |
| Ceftriaxone       | Low       | Low    | Low  |
| Ampicillin        | Low       | Low    | Med  |

Resistance Intensity

|     |     |      |
|-----|-----|------|
| Low | Med | High |
|-----|-----|------|

| Station    | Intensity | ABs   | Rank | E coli | Phage | Phage Type |
|------------|-----------|-------|------|--------|-------|------------|
| PM003      | 0.86      | 18.00 | 0.88 | 4221   | 323   |            |
| 96A        | 0.89      | 14.00 | 0.79 | 144    | 3     | Human      |
| PM002      | 0.78      | 14.00 | 0.74 | 5006   | 168   |            |
| 96B        | 0.82      | 12.00 | 0.71 | 114    | 3     | Human      |
| SMITH POND | 0.90      | 10.00 | 0.70 | 902    | < 1   |            |
| 98         | 0.92      | 9.00  | 0.68 | 54     | < 1   |            |
| 92         | 0.88      | 9.00  | 0.67 | 160    | 313   |            |
| 100        | 0.83      | 9.00  | 0.64 | 68     | < 1   |            |
| 87         | 0.66      | 11.00 | 0.61 | 1542   | 33    |            |
| 107        | 0.82      | 8.00  | 0.61 | 85     | 10    |            |
| ACUACHLEFF | 0.78      | 8.00  | 0.59 | 12     | 13640 |            |
| M.R.1-4    | 0.88      | 7.00  | 0.61 |        |       |            |
| 95         | 0.62      | 9.00  | 0.54 | 97     | 4     | Human      |
| 33         | 0.79      | 7.00  | 0.57 | 112    | 2     | Human      |
| HC-3       | 0.69      | 8.00  | 0.54 | 10     |       |            |
| 97         | 0.68      | 8.00  | 0.54 | 148    | 1     | Animal     |
| 105        | 0.68      | 8.00  | 0.54 | 568    | 182   |            |
| 67         | 0.67      | 8.00  | 0.53 | 58     | < 1   |            |
| M.R.-24    | 0.76      | 7.00  | 0.55 | 347    | < 1   |            |
| REFUGE     | 0.75      | 7.00  | 0.55 | 35     |       |            |
| M.R.3-4    | 0.81      | 6.00  | 0.55 |        |       |            |
| HC-4       | 0.73      | 6.00  | 0.52 | 370    |       |            |

Intensity = ave.  
Level of resistance  
where 1.00 means  
resistant to all ABs  
at the highest conc

ABs means # of  
antibiotics that  
E. coli were  
resistant to.

$$\text{Rank} = \frac{(\text{AB}/20 + \text{Intensity})}{2}$$



# MAR Patterns

- Fecal coliform isolates from humans were more resistant to ampicillin and amoxicillin than were animal isolates
- Fecal coliform isolates from animals were more resistant to tetracycline (oxytetracycline), erythromycin, and streptomycin than isolates from humans
- *From: Harwood, University of South Florida*



**BACTERIOPHAGE  
METHOD**

**MAR  
METHOD**

# Use of Alternate Indicators in MST

- There is no “Silver Bullet” - All microbiological methods have limitations (including fecal coliform and Enterococcus).
- Design your monitoring program to use best indicators and preferably multiple indicators.
- Combine these results with routine monitoring data and a thorough pollution source survey to develop a “weight of evidence” approach.

# Contact Information

- Eric Feerst, Section Chief -  
NJDEP Bureau of Marine  
Water Monitoring
- Eric.Feerst@dep.state.nj.us
- 609- 748-2000
- <http://www.nj.gov/dep/bmw/>

