



**Microbial Source Tracking and Beach Monitoring  
to Determine the Origin, and Impact of  
Temperature on *Enterococcus* Concentrations,  
Persistence and Re-growth:**

***A Long Island Sound Bathing Beach Case Study***

***Boris Rukovets  
Interstate Environmental Commission***

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Atlantic City  
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# Cooperative effort based on a grant funded by the *Long Island Sound License Plate Program Connecticut Department of Environmental Protection*

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1. Interstate Environmental Commission
2. BCS Laboratories, Inc.
3. Connecticut Department of Environmental Protection



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# IEC's DISTRICT

- 3 States – NY, NJ, CT
- Created in 1936
  - 1<sup>ST</sup> Interstate Env'l Agency
- 14 million people; 797 sq. miles
- 80 WPCPs discharging 2.5 BGD
- ~650 CSOs
- EPA Regions 1 and 2
- 2 NEPs – LISS and NY/NJ HEP



# IEC'S KEY FUNCTIONAL AREAS

- Use enforcement and regulatory powers on both interstate and intrastate basis
- Provide technical assistance and support to IEC's Member States, US EPA and others
- Enhance public and legislative awareness, and disseminate information
- Coordinate interstate and region-wide programs
- Ambient and point source monitoring
- Emergency response



# IEC EFFLUENT MONITORING NETWORK

- *80 secondary WPCPs*
- *Check Compliance w/NPDES and IEC Water Quality Regulations*
- *Parameters:*
  - BOD*
  - TSS*
  - Fecal*
  - Coliform*
  - pH*



# Silver Sands State Park Study

- Investigate the dynamics of *Enterococci sp.* within the study area and factors contributing to high concentrations that resulted in beach closures
- Goal: Improve water quality and promote safe recreational use of Long Island Sound bathing beaches



# Project Background

- Prior weekly sampling by CT DEP at Silver Sands State Park revealed that *Enterococci* concentrations at the beach repeatedly exceeded bathing water criteria.
- CT DEP surveys suggested that significant sources of human sewage might not be present
- Add'l data was needed in order to enhance the understanding of localized conditions that were contributing elevated levels of indicator bacteria

# Objectives:

- Better predict and understand factors contributing to elevated bacteria levels:

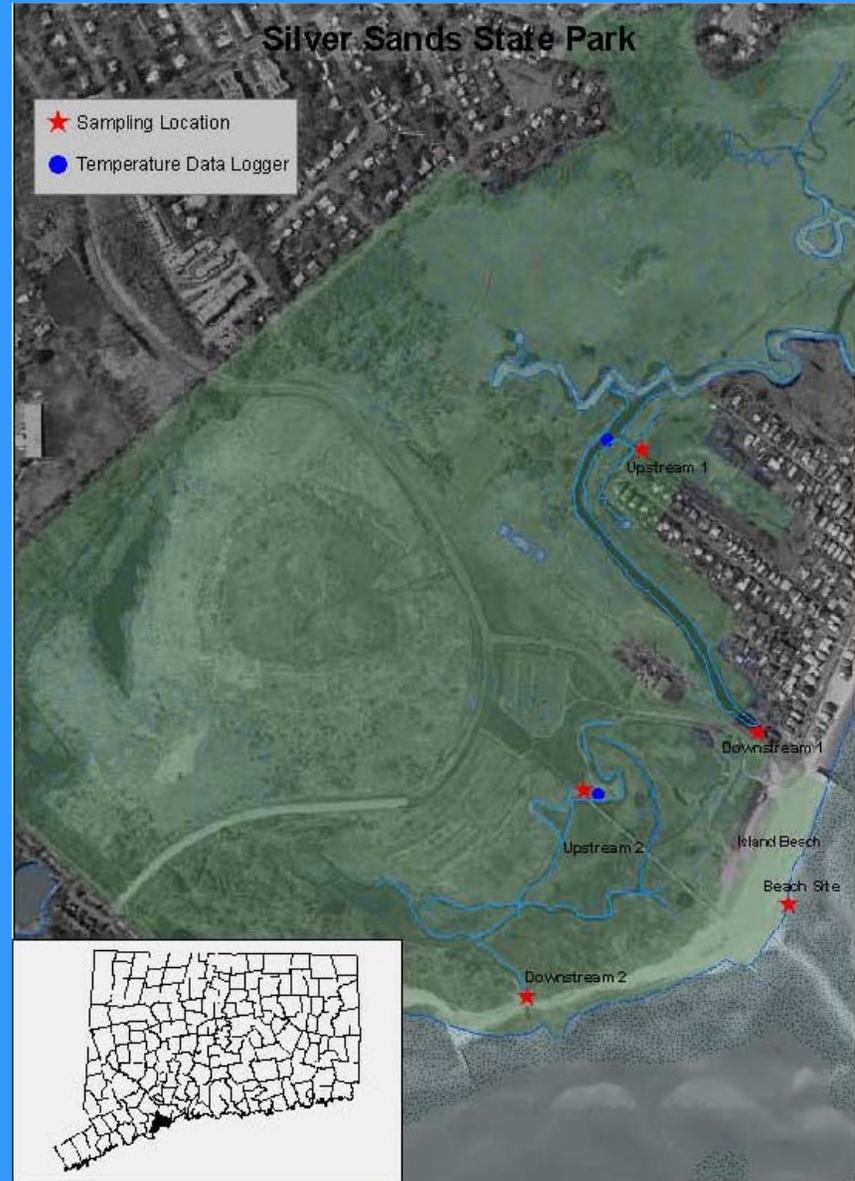
- Impact of temperature on concentration, persistence and potential re-growth (in water column and sediment)



- Determine major sources of fecal pollution using MST methodologies
- Investigate the ability of creek sediments to serve as a source of *Enterococci* to overlying waters through resuspension and remobilization

# Study Area

- Silver Sands State Park, Milford CT
- 2 Creeks w/ upstream and downstream sites:
  - Fletcher Creek
  - Great Creek
- Downstream Bathing Beach



# Using MST Methodologies to Answer our Research ?s

- 1) Characterize indicator organisms with DNA markers to investigate sources of fecal pollution
  - Water and sediment samples probed for source-specific sequences using PCR to differentiate sources of *Enterococci*:
    - Human, bird, bovine, deer, or other wildlife?
- 2) DNA fingerprinting employed to determine if indicators are re-growing or concentrating in the environment

# Field Sampling Design

- 5 Dry weather sampling events completed between June and August 2007
- Collection at 4 time intervals/event
  - early morning to afternoon at ~1.5 hr. apart
- Field measurements:
  - Temperature (water and upstream sediments), salinity, pH, depth, velocity, flow direction



# Sampling Locations

Station	Sample Media Type	Location
U1	Water	Upstream 1 (Great Creek)
U2	Water	Upstream 2 (Fletcher Creek)
D1	Water	Downstream 1 (Great Creek)
D2	Water	Downstream 2 (Fletcher Creek)
B	Water	Beach
U1S	Sediment	Upstream 1 (Great Creek)
U2S	Sediment	Upstream 2 (Fletcher Creek)



# Laboratory Analyses:

- IEC (conventional parameters)
  - *Enterococci (water and sediment)*
  - *Fecal coliform (water and sediment)*
  - *TSS*
  - *Turbidity*
- BCS (MST methodologies)
  - *Host specific PCR analyses for absence/presence of DNA sequences for source identification (subset of water samples)*
  - *DNA fingerprinting of Enterococci (sediment)*

# Temperature and *Enterococcus*

- Regression analyses showed no significant correlation between temperature and *Enterococcus* concentrations in either water or sediment
- Daily plots of time vs temperature and *Enterococcus* did not reveal any significant increase in indicator concentrations w/ corresponding temperature increase

# Source Tracking Results

DNA Markers from Most Specific DNA Tests

Location	Type	Bird Enterococcus				Human Enterococcus				Dog Bacteroides				Deer Enterococcus			
		6-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug
U1	Sediment	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
U2	Sediment	ND	Yes	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
D1	Water	Yes	ND	ND	Yes	ND	ND	Yes	ND	ND	ND	ND	ND	ND	ND	ND	ND
D2	Water	ND	Yes	ND	Yes	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
U1	Water				Yes												

Notes:

"Yes" indicates a presence of the indicated fecal pollution source was detected

"ND", not detected, indicates no presence of the indicated pollution source was detected

U1 and U2 - Upstream sampling locations of Creeks #1 and #2, respectively

D1 and D2 - Downstream sampling locations of Creeks #1 and #2, respectively

- indicates that no sample was taken (samples were included in original sampling design, based on the funds available)

# DNA Fingerprinting

- *Enterococcus* isolated from sediment samples revealed highly heterogeneous genetic population
- Coupled w/ host specific DNA markers from birds suggests some sediment organisms deposited recently and have true fecal link
- Sediments might serve as a limited fecal indicator reservoir that could have potential deleterious impact on water quality

# Conclusions:

- No significant correlation between either sediment or water temp. and *Enterococcus* levels
- Little proliferation of bacteria in upstream creek sediments
  - May serve as a sink but limited contribution to overlying and downstream waters
- Birds serve as the major source of fecal pollution to Silver Sands State Park Beach

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Final Report available on Interstate  
Environmental Commission website

<http://www.iec-nynjct.org>

