# BST? MST? CST?
# Do they help?


An example of the streak-plate method. A single bacterial colony isolate (single-species) is indicated.

# Watershed Assessment

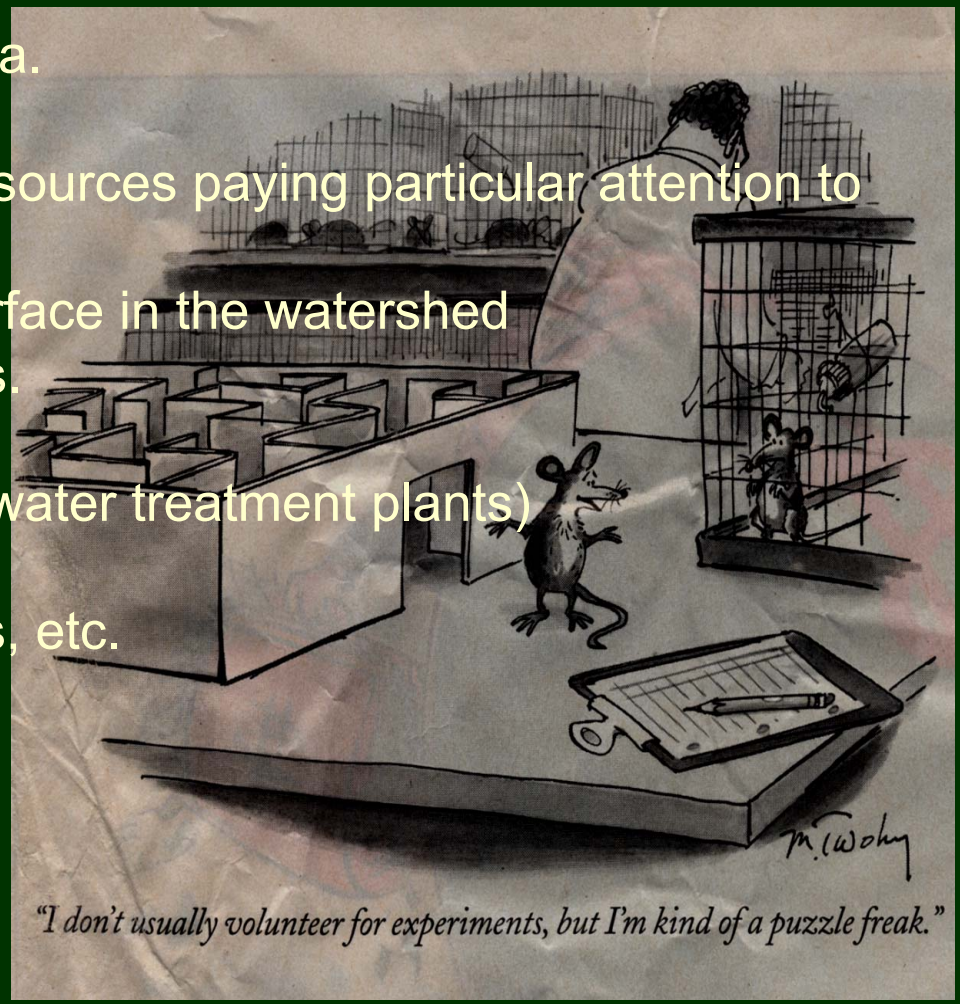
Determine the watershed DA of the survey area.

Determine Land use.

Estimate population of the survey area.

Windshield survey possible pollution sources paying particular attention to the following:

- Percentage of impervious surface in the watershed
- Marinas and docking facilities.
- Wastewater treatment plants
- Package plants (small wastewater treatment plants)
- Livestock Agriculture
- Domestic pets, i.e. cats, dogs, etc.
- Wildlife



# Watershed Assessment, cont'd. . . .

**Expand the inspection by going door-to-door**.
Pay particular attention to the following:

- Septic systems Look for Signs of Septic System Failure)
- Stormwater drains
- Ditches, canals

**Signs of Septic System Failure**

* **Wet spots or standing water** - Areas that remain wet after rain events or appear without precipitation should be investigated, since effluent can seep up from a failing system and puddle on the ground surface.
* **Odd growth patterns** - Green lines of vegetative growth on the drainfield that follow the path of the pipes are normal; however, green patches not in line with pipes, random patches of lush growth, and bulls-eye patterns (dead areas surrounded by green growth) all indicate problems in the drainfield.
* **Slow drains or backups** - If wastewater is slow to drain and the plumbing is working properly, there may be a problem with backup in the septic tank.
* **Septic odor** - Easy to detect, odor is one of the best problem indicators

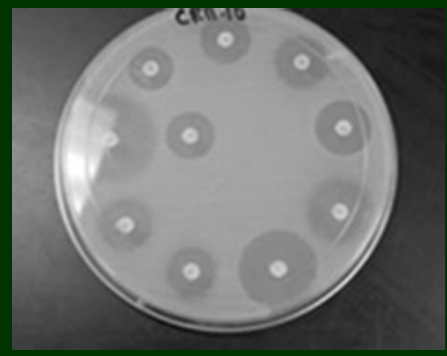
# Microbial Source Tracking Methods Classification

Non-molecular Library-dependent Methods
Antibiotic Resistance Methods
Carbon Source Profiling (Nutritional Analysis)
FAME (Sterols/Fatty Acid Methyl Ester Analysis)

Non-molecular Library-independent Methods
Fecal Bacteria Ratios
Streptococcal Population Profiles
Phenotypic Species-Specific (Host-Specific) Indicators
F+ coliphage serotyping
Enterotoxin Biomarkers

Molecular Library-dependent Methods
Rep-PCR (Polymerase Chain Reaction)
PFGE (Pulsed-Field Gel Electrophoresis)
Ribotyping
RAPD (Randomly Amplified Polymorphic DNA)
tRFLP (Terminal Restriction Fragment Length Polymorphism)

Molecular Library-independent Methods
Bacteriophage Indicators
Virus (Human Pathogen) Indicators
Genotypic Species-Specific (Host-Specific) Indicators
Bacterial Endemism and Co-Speciation



Antibiotic resistance profile analysis of an *E. coli* isolate. The white disks on the plate are impregnated with different antibiotics and the clear zones of bacterial growth inhibition are measured to determine the resistance of each bacterial isolate.

## Chemical-Based Source Tracking Methods

*Optical Brighteners/Detergents*

Whitening agents and optical brighteners are chemicals used in laundry detergents. They are often a large component of grey water discharge and have been used as indicators of sewage fallout with varying degrees of success

*Caffeine*

Caffeine detection is a fairly new source tracking method. Caffeine is generally found in highest concentrations in highly urbanized areas; however, urbanization levels have not yet been matched to known caffeine concentrations in the environment.

*Coprostanol*

Coprostanol is a byproduct of the breakdown of cholesterol and is present in human and some mammal species. Human secretion of coprostanol can be inconsistent but in general it is a good indicator of human fecal pollution.

## *The Toolbox Approach or get a bigger hammer . . .*

Methods are complimentary methods rather than opposing.

No magic bullet. Better approach may be to combine several molecular and non-molecular methods. By using this "toolbox" approach, researchers can:

- Tailor source tracking methodologies to individual study needs and limitations;

- Increase statistical confidence by obtaining similar results from different methods;

- Expand research results by identifying sources from one method that the other was unable to identify; and supplement source tracking methods that give only qualitative or quantitative information.

# Monitoring and TMDL Modeling Techniques to Assess Bacterial Loading in Estuarine Environments and Improve Management Programs

## Cooperators and Participants

NCSU College of Design
NCSU CMAST
NCSU Water Quality Group
NC Division of Health, Shellfish Sanitation Program
Duke Marine Laboratory
NOAA/NOS Center for Coastal Environmental Health and Bimolecular Research
UNC-Institute of Marine Sciences
Carteret Craven Electric Cooperative
Jumping Run Creek Watershed Citizens
Croatan National Forest
Open Grounds Farm
USDA-CSREES
NCDENR Division of Water Quality, 319 Program
NC Clean Water Management Trust Fund
NC Wetland Restoration Program

NC STATE UNIVERSITY

# Project Objectives

1. Implement and assess BST technology in NC as part of watershed-based toolbox approach to reduce bacterial loading.
2. Review TMDL process using BST / Toolbox data.
3. Review / recommend management strategies.
4. Disseminate information to coastal local governments.
5. Incorporate information into coastal environmental management / policy curriculum.

# Jumping Run

Total drainage area:
~800 acres
Mobile and RV
Housing
Low density single
family residential
Industrial
No agriculture

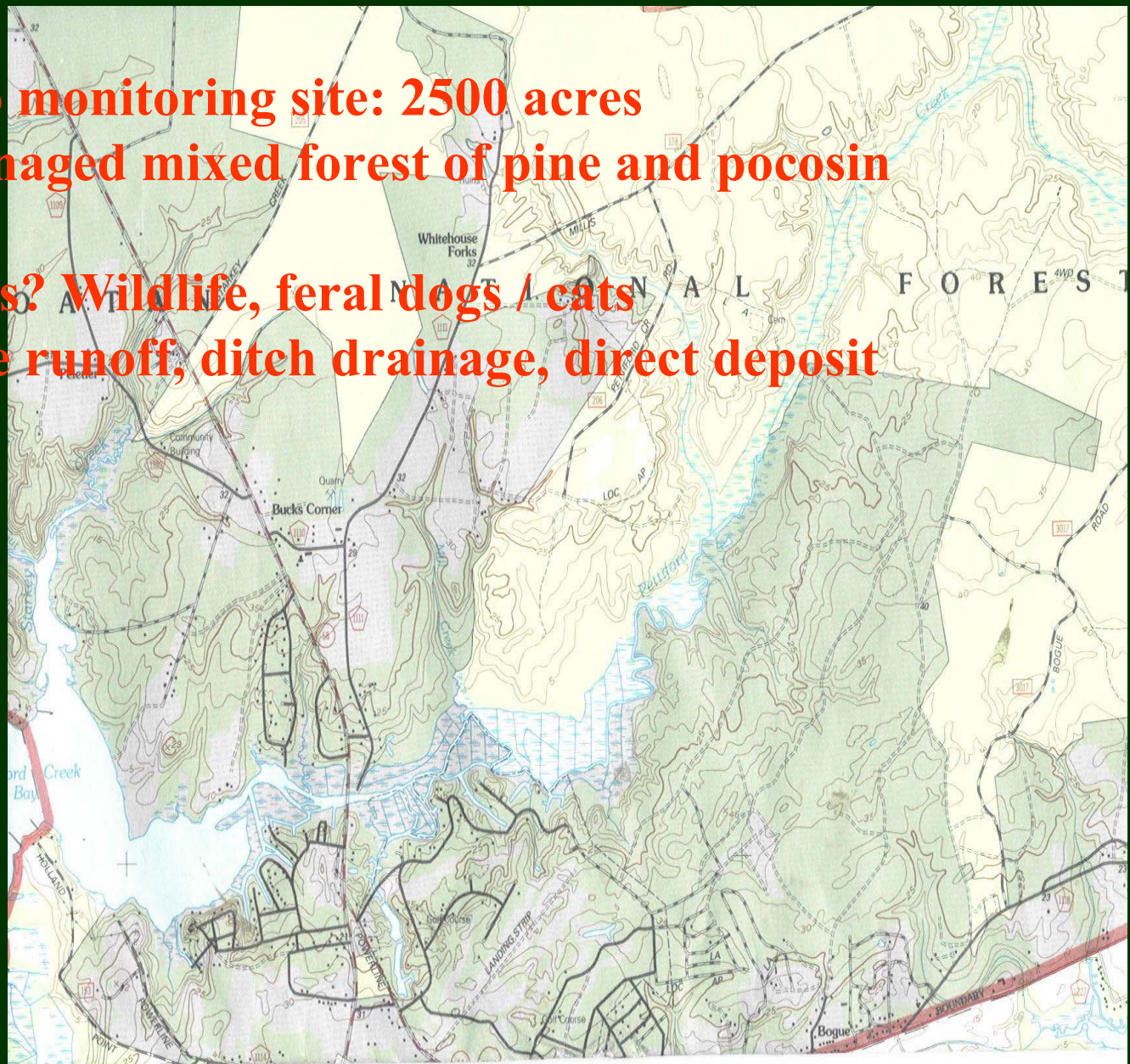
Sources? Dogs, cats, waterfowl, domestic wildlife, septic tanks.

Transport vectors? Ditches, surface runoff, surficial groundwater, airborne, direct deposit.

# Pettiford Creek-Croatan National Forest

**Drainage area to monitoring site: 2500 acres**
**Land cover: Managed mixed forest of pine and pocosin**

**Bacterial Sources? Wildlife, feral dogs / cats**
**Vectors? Surface runoff, ditch drainage, direct deposit**



# South River: Open Grounds Farm

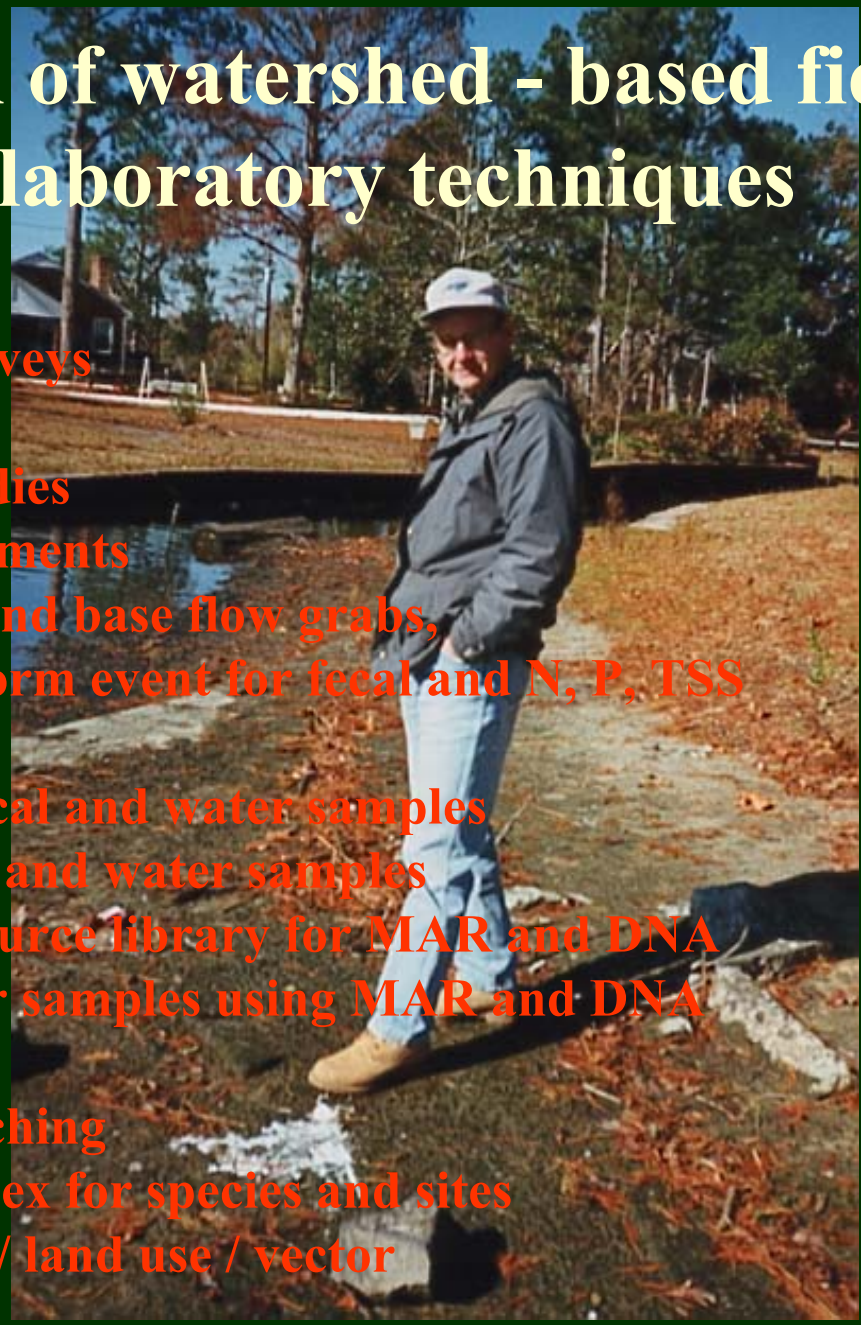
**Drainage Area to monitoring site:3000 acres**

**Land cover: Cultivated row crop agriculture**

**Bacterial Sources? Wildlife, waterfowl and other birds, rodents, dogs**

**Transport vectors? Surface runoff, ditch flow, direct deposit.**

# Methods: Integration of watershed - based field assessment and laboratory techniques



**Watershed Assessment:**

- Land use / land cover surveys
- Flow Monitoring
- Time, travel, dilution studies
- Rainfall / runoff measurements
- Water sampling: Sound and base flow grabs, flow-weighted storm event for fecal and N, P, TSS

**Laboratory:**

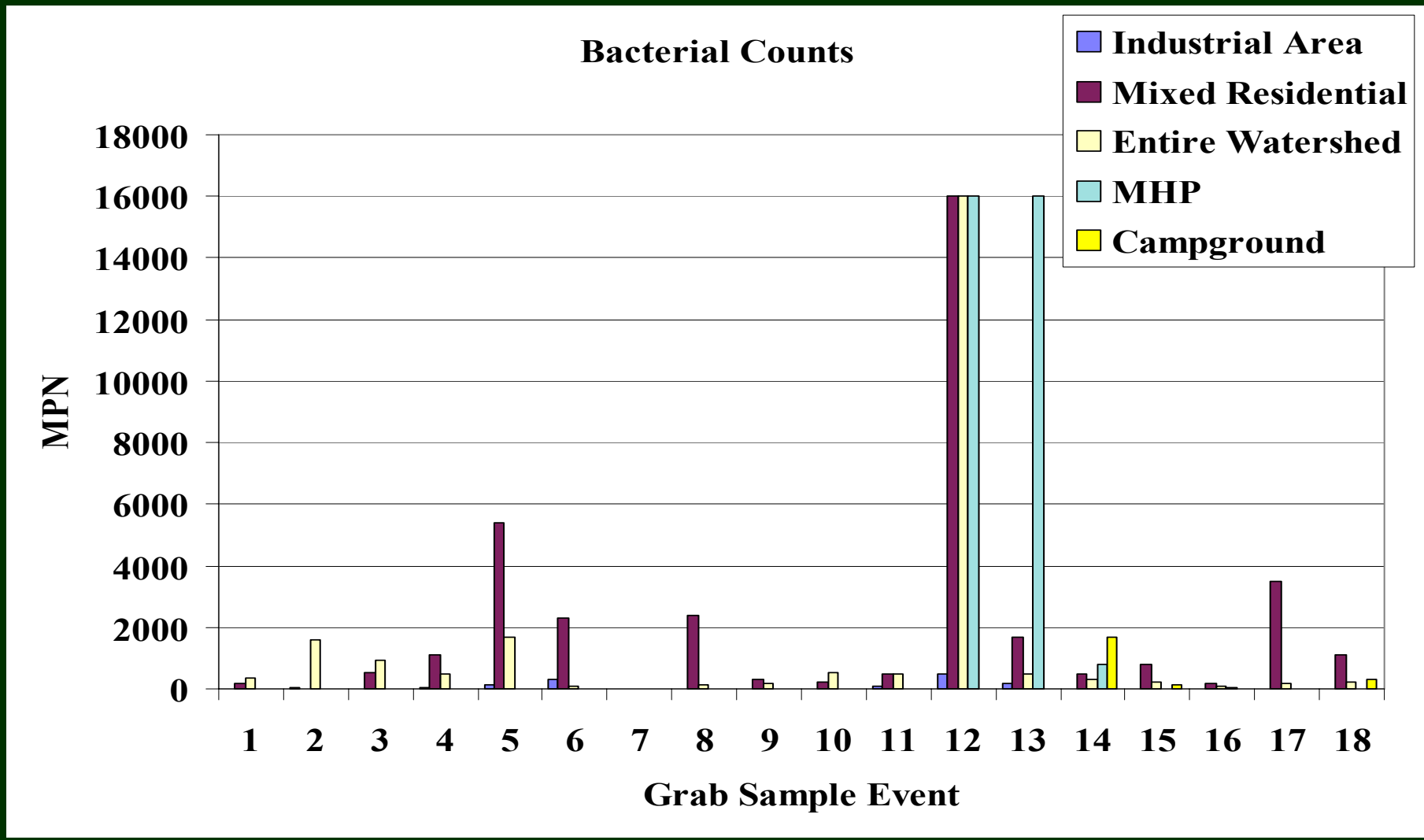
- MPN quantification of fecal and water samples
- e. coli speciation for fecal and water samples
- Watershed-based fecal source library for MAR and DNA
- Characterization of water samples using MAR and DNA

**Analyses:**

- Spatial and seasonal matching
- Resistance/ sensitivity index for species and sites
- Loading analysis by type / land use / vector

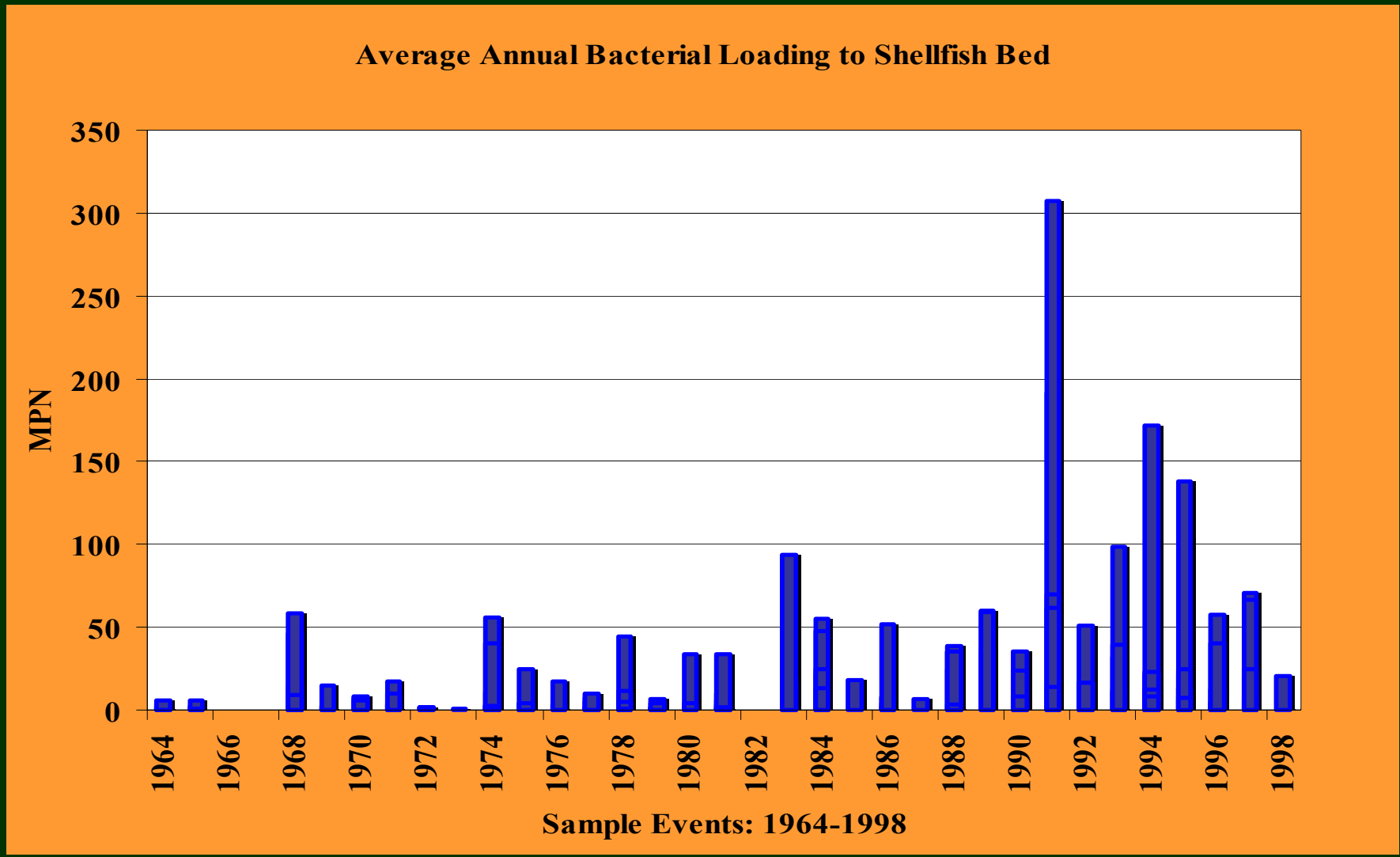
# Tailwater grab samples: Jumping Run

❖Used to quantify bacterial densities and MAR/ DNA profile for the draw-down/ baseflow component of the hydrograph.



# Sound – based grab sample data: Jumping Run

- Will be used to quantify bacterial density and the MAR/ DNA profiles in the shellfish beds.



# Watershed Assessment: Jumping Run

- Used to spatially assess bacterial sources, transport vectors, and to ground truth land use / land cover

| Location | Visits | SV | Dogs |
|---|---|---|---|
| MHP | 47 | 0 | 23 |
| Med. Density | 66 | 2 | 60 |
| Low Density | 14 | 0 | 7 |
| Campground | 1 | 0 | 0 |
| Totals | 128 | 2 | 90 |

*Does not include Roads

| Location | Cats | Other | Imperviousness |
|---|---|---|---|
| MHP | 6 | 3 | 40,000sft |
| Med. Density | 47 | 25 | 185,000 |
| Low Density | 0 | 0 | 95,000sft |
| Campground | 0 | 0 | 5,000sft |
| Totals | 53 | 28 | 325,000sft or 7.5 acres |

# Land Cover Change Assessment: Jumping Run

- Develop land use loading rates for TMDL assessment.
- Information is used to help direct and design restoration and management strategies for testing.

# Results: Dye/ Time and Travel Studies Jumping Run

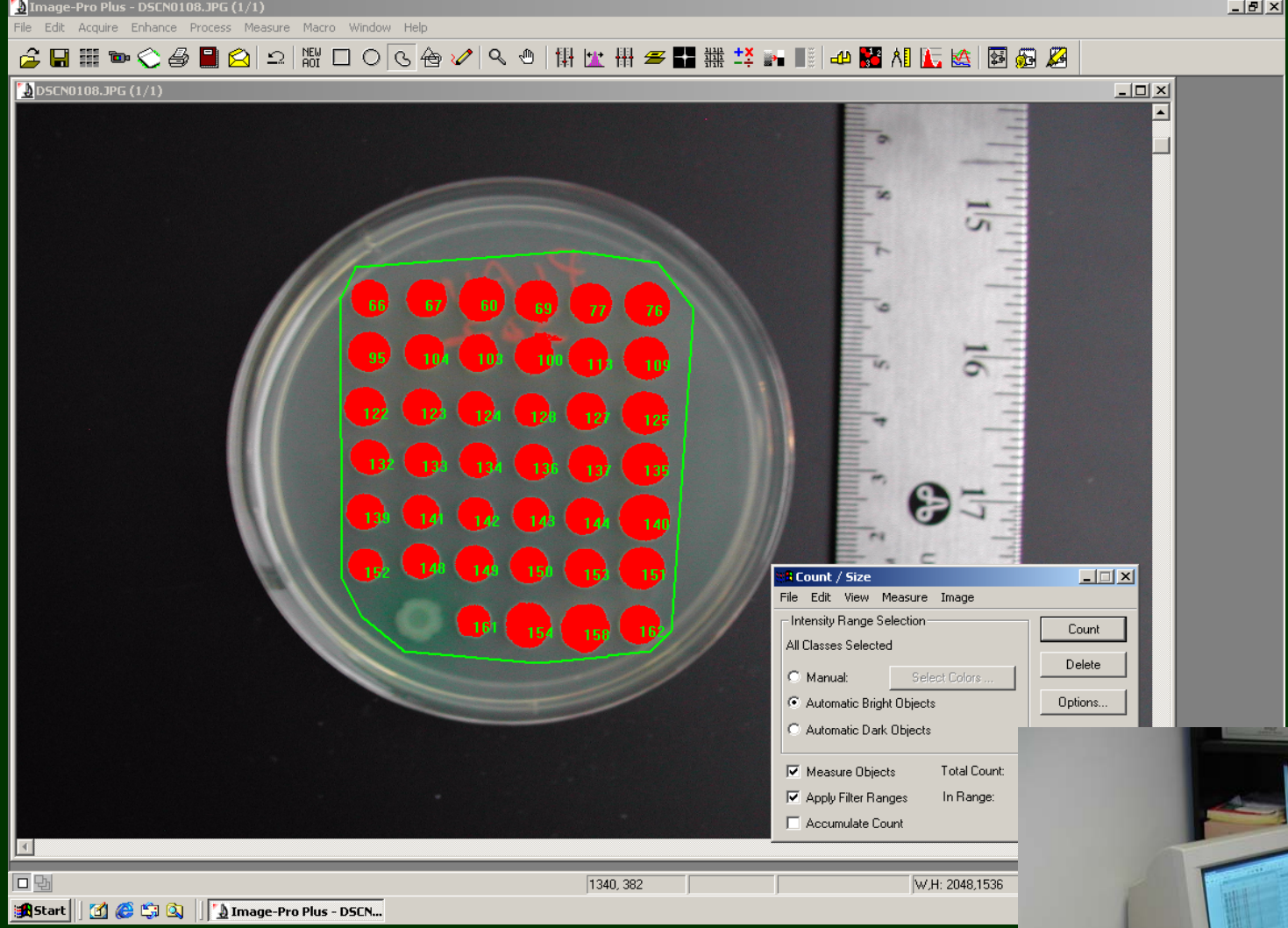
- Information is using to determine ditch drainage patterns, water movement direction and timing, as well as dilution and dispersion

| Date | Guage Ht | CFS | Time Travel |
|---|---|---|---|
| 21-Jan-98 | 1.38' | 8.4 | n/a |
| 22-Jan-98 | 1.18' | 5.8 | MHP-Outlet, 5hrs |
| 04-Feb-98 | 3' | 18.2 | MHP-Gauge, 2.5hrs |
| 23-Feb-98 | 1.7' | 10.9 | n/a |
| 03-Mar-98 | 1.13' | 3.21 | Headwaters-Gauge, 3hrs. |
| 04-Mar-98 | 1.10' | 2.73 | CmpGrnd to Outlet, 1.5hrs. |
| 03-Apr | 0.95 | 5.96 | n/a |
| 04/17/1998 | 0.9 | 10.4 | n/a |
| 04/30/1998 | 0.84 | 5.1 | n/a |
| 06/18/1998 | 0.76 | 4.6 | n/a |
| 07/14/1998 | 0.72 | 4.5 | n/a |


# Storm Event Monitoring

## MAR and DNA Data

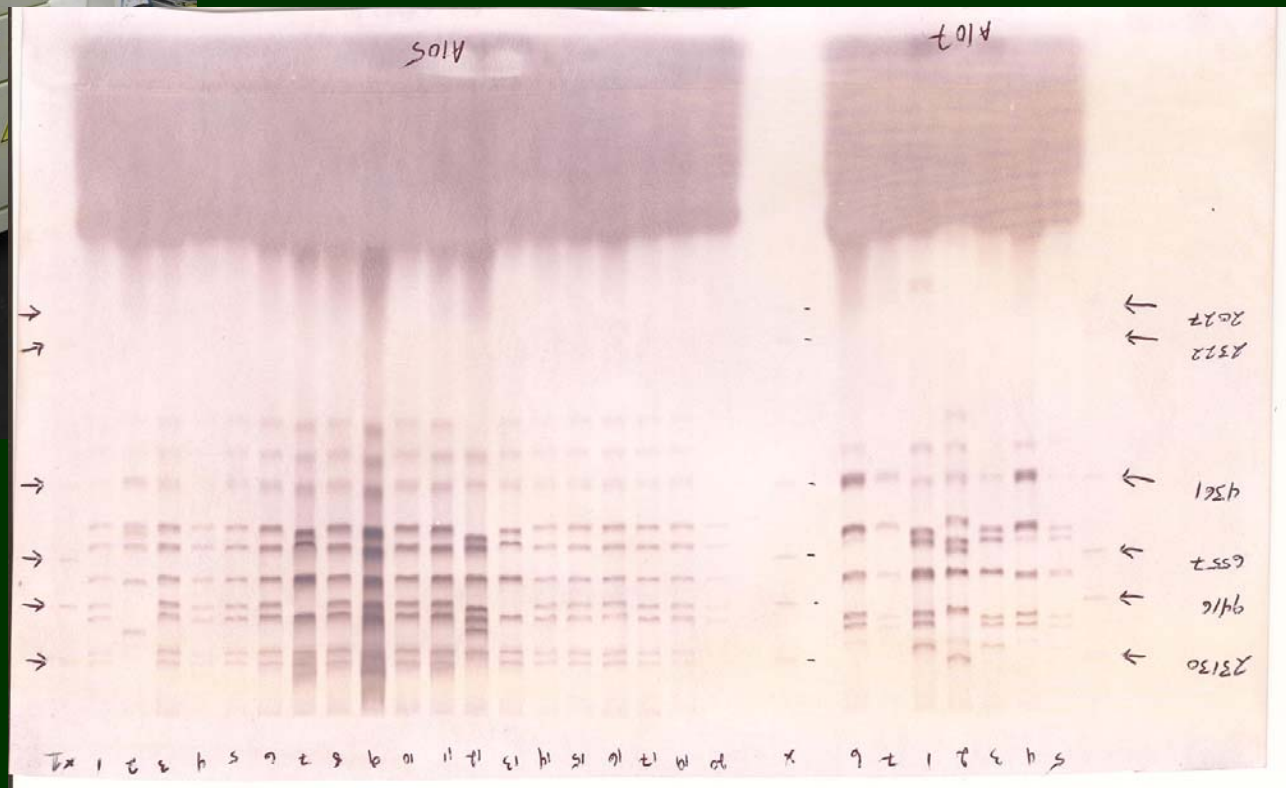
**MPN is calculated for both water / fecal library samples to link densities with water volume and flow.**




**Plates are digitally photographed and cell growth measured relative to two controls using image analysis techniques.**


**DNA analysis is conducted on all library and resistant water samples and 50% of the sensitive water samples.**

**Tracks of DNA material are delineated and banding patterns numbered to create a profile for each sample.**



## Preliminary MAR Results

**Jumping Run: 27 Water samples, Avg MPN: 3000 / 100ml, 178 isolates**

**Sensitivity index:** 48 or 26 % were 10% resistant

4 or 2% were 20% resistant

7 or 0.7% were greater than 30% resistant

**Croatan: 8 water samples, Avg MPN: 326 / 100ml, Number of isolates: 50**

**Sensitivity index:** 8 or 16% were 10% resistant

1 or 2% were 20% resistant

None greater than 20%

**Open Grounds: 6 water samples, Avg MPN 4300/ 100ml, 64 isolates**

**Sensitivity index:** 17 or 26% were 10% resistant

1 or 1% were 20% resistant

1 or 1% were 40% resistant

**In progress. . .**

**Conducting the matching and loading analyses.**
**Refining MAR profiling.**
**Adding new research components, of course!**

# Recommendations

The “toolbox” method appears to be the most effective use of these methodologies at present.
***The best approach is to evaluate land uses and sources under investigation, and tailor the research methods to fit each individual situation.***

- Source types (human, non-human, livestock, domestic pets, or wildlife);
- Pollutant loading sources and delivery vehicles (point sources, nonpoint sources);
- Sample medium (marine, freshwater, groundwater, sediments, shellfish tissue);
- Level of source resolution needed (human vs. non-human or individual categories for all); and
- Cost for each method (usually estimated on a cost per isolate basis)
- Library building needs and capabilities.

**Best comprehensive source of information:**

**Addressing microbial pollution: A reference for local governments**

**Produced by NC NERR for NC DENR DWQ Coastal Nonpoint Source Program**

**http://ncnerr.org/ccs/publications/index.html**

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