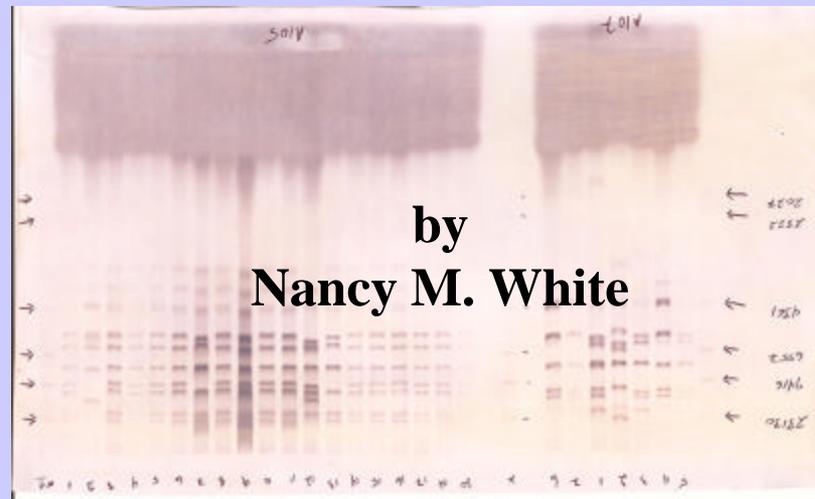


Watershed-based microbial source tracking study: From implementation and solutions to research and method evaluation



EPI-NET Symposium

Detecting Microbial Contamination in Water and Soil

January 30, 2007

2007 USDA-CSREES National Water Conference

Savannah, Georgia

Lots of 'ologists!

Cooperators and Participants

UNC Coastal Studies Institute

UNC-Institute of Marine Sciences

NCSU CMAST

NC Division of Health, Shellfish Sanitation Program

Duke Marine Laboratory

NCSU Water Quality Group

NOAA/NOS Center for Coastal Environmental

Health and Biomolecular Research

Carteret Craven Electric Cooperative

Jumping Run Creek Watershed Citizens

Croatan National Forest

Open Grounds Farm

USDA-CSREES

NC DENR Division of Water Quality, 319 Program

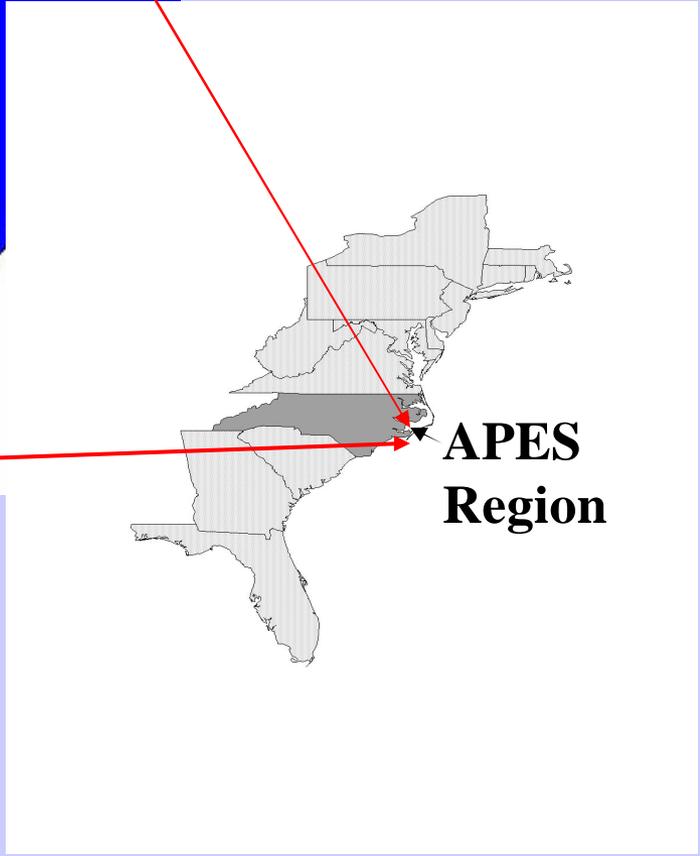
NC Clean Water Management Trust Fund

NC Wetland Restoration Program



Co-Investigator, Daniel Line



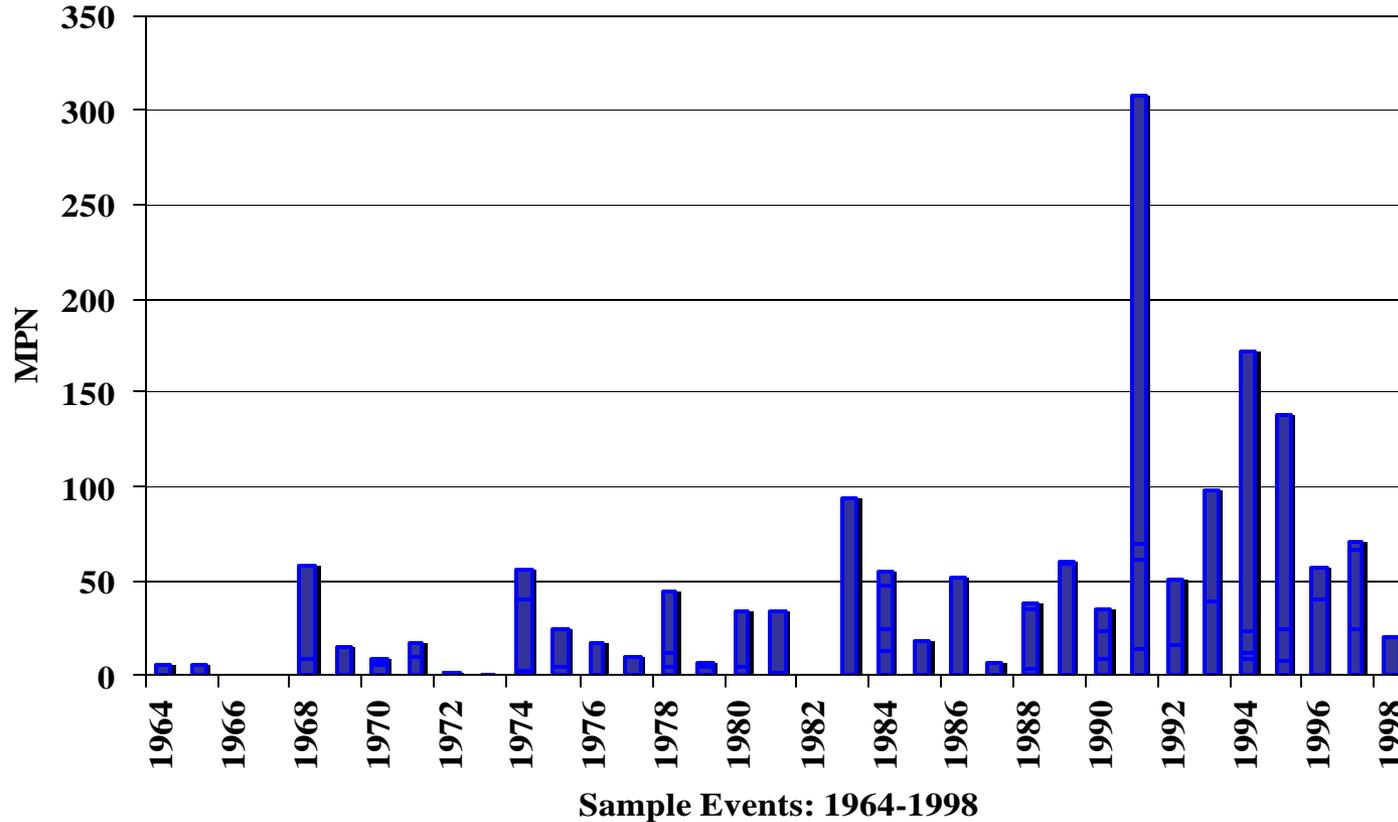


Jumping Run Creek: Was... coastal pocosion and relic dune ridges....now, *residential, light commercial*



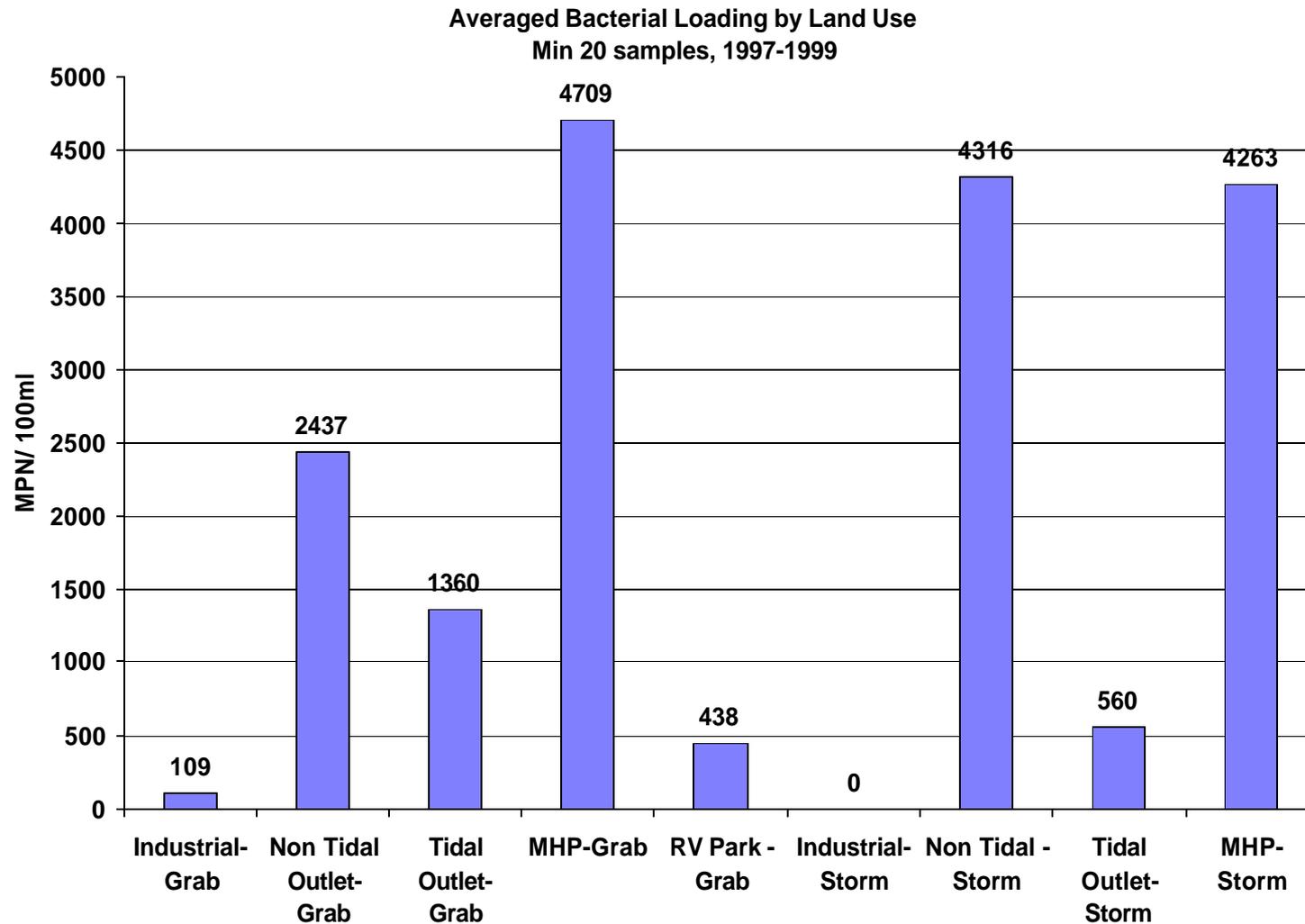
Drainage from low density watershed closes shellfish waters...in 1974!

Average Annual Bacterial Loading to Shellfish Bed



Shellfish Sanitation Bacterial Database

Where are the hotspots?



Where are the failing septic tanks????

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	325,000 sft *

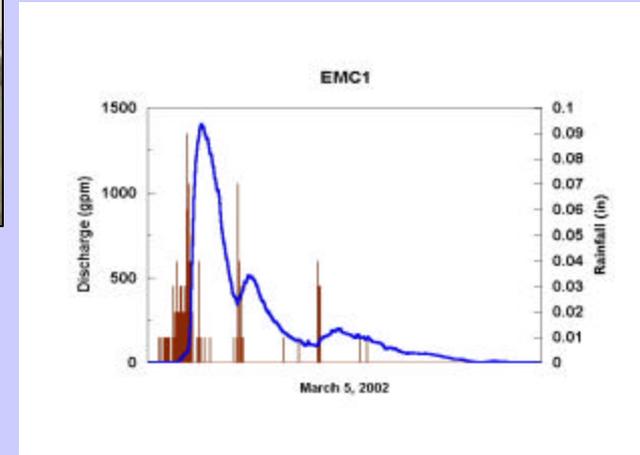
or 7.5 acres, with roads, less than 10% imperviousness.

What happened to TOC?

Assessment method is used 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



Bring on the ISCOs.....

**Rainfall / runoff assessment,
flow volumes, flow-
proportional sampling,
loading calculations.**





1967



1979

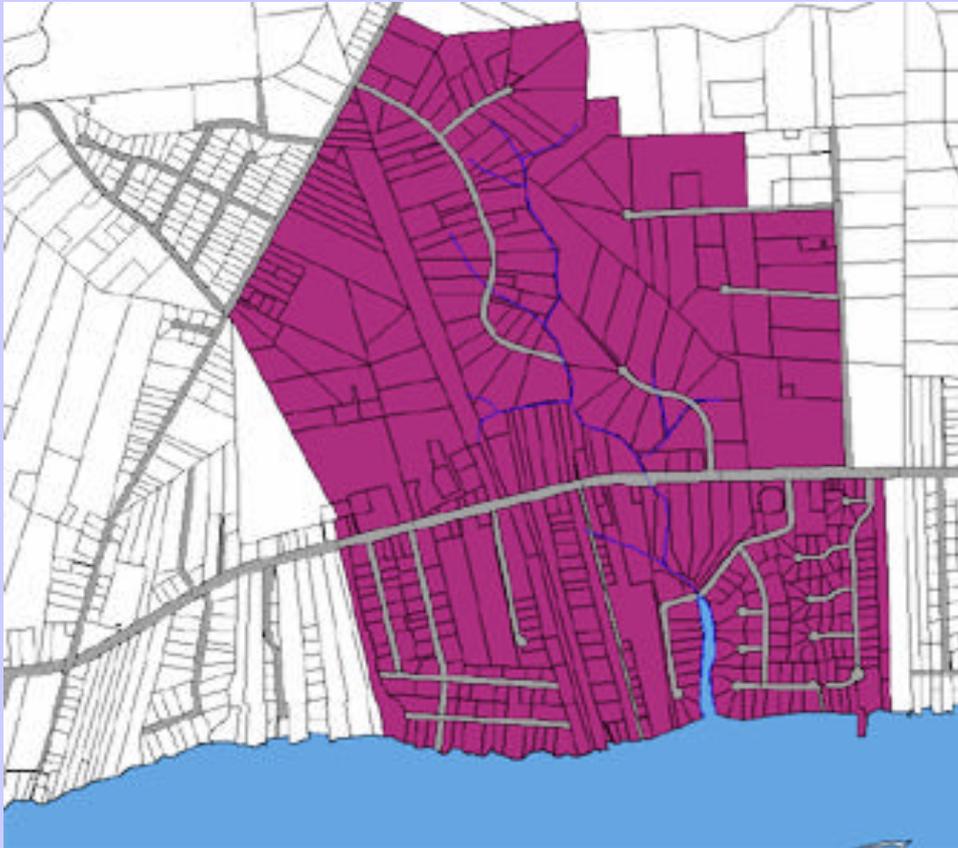
**What happened
to the land?**

1988

1994



Ranking and classification process



Vegetation:

Rank	Percent Cleared
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

Impervious Surfaces:

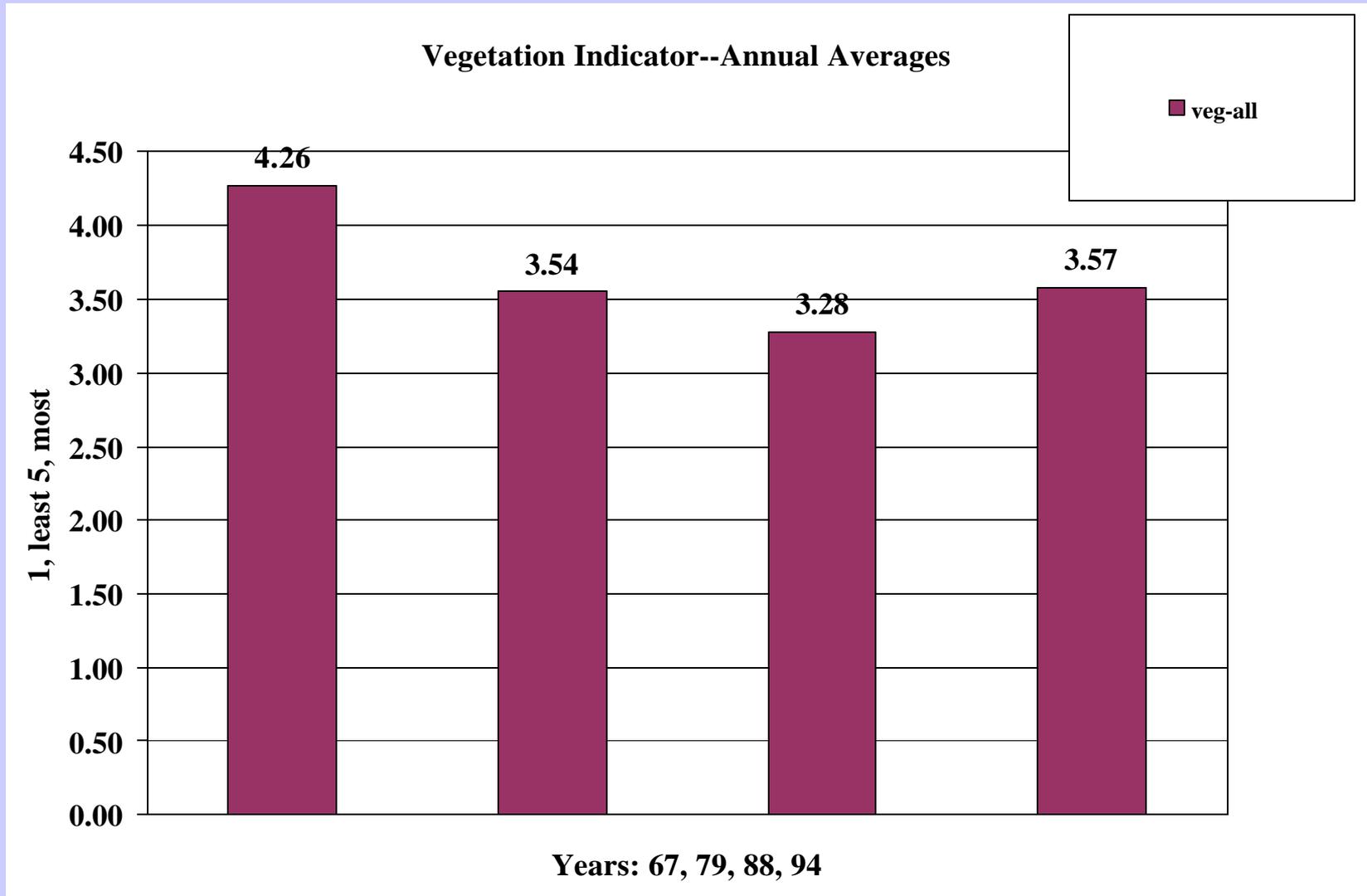
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

HydroMod

1	no ditches evident
2	1 lot-edge ditch
3	2 side ditches
4	direct discharge
5	Channelized

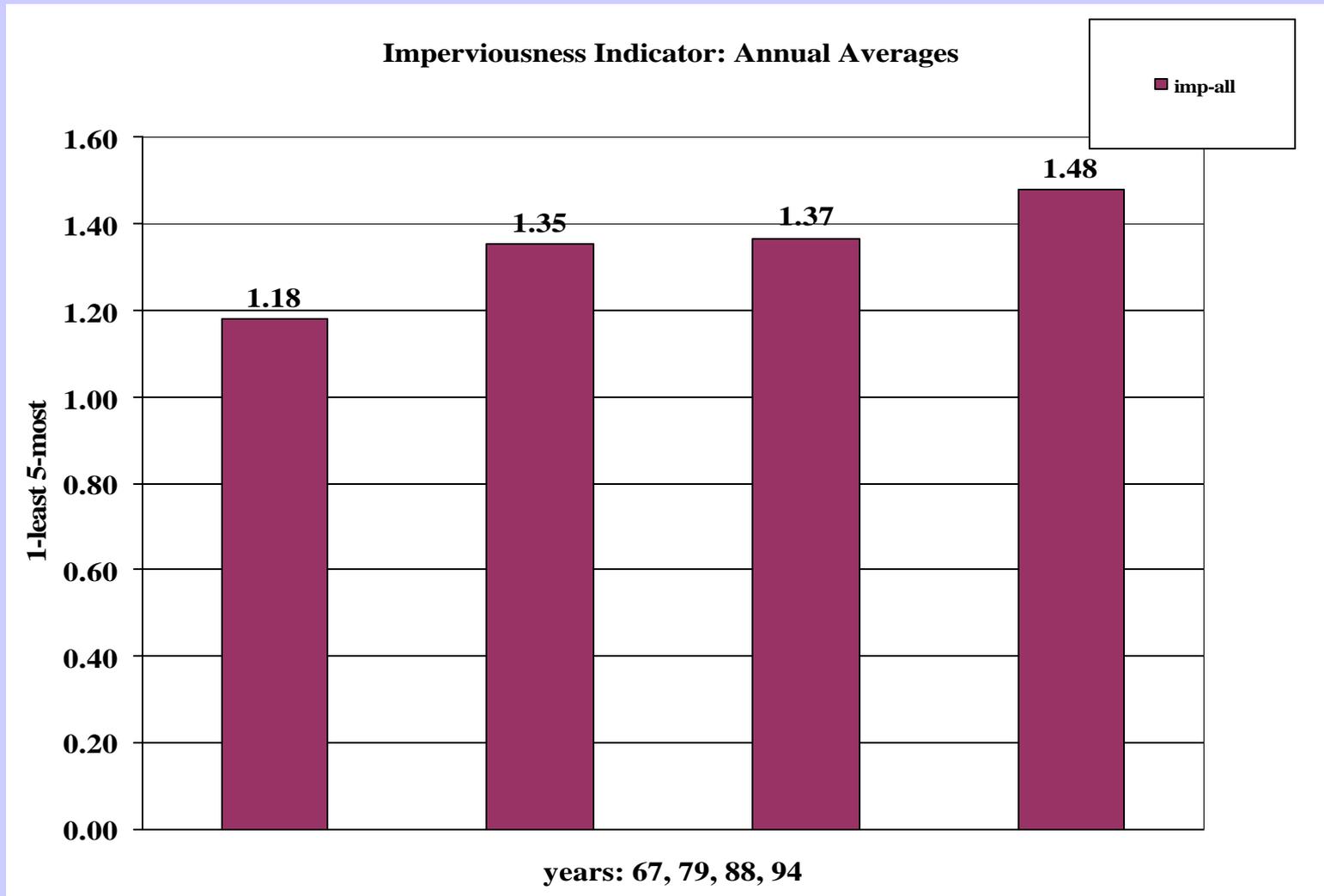
Note Cover Type – grass, forest, sand.

Classification Results Summary



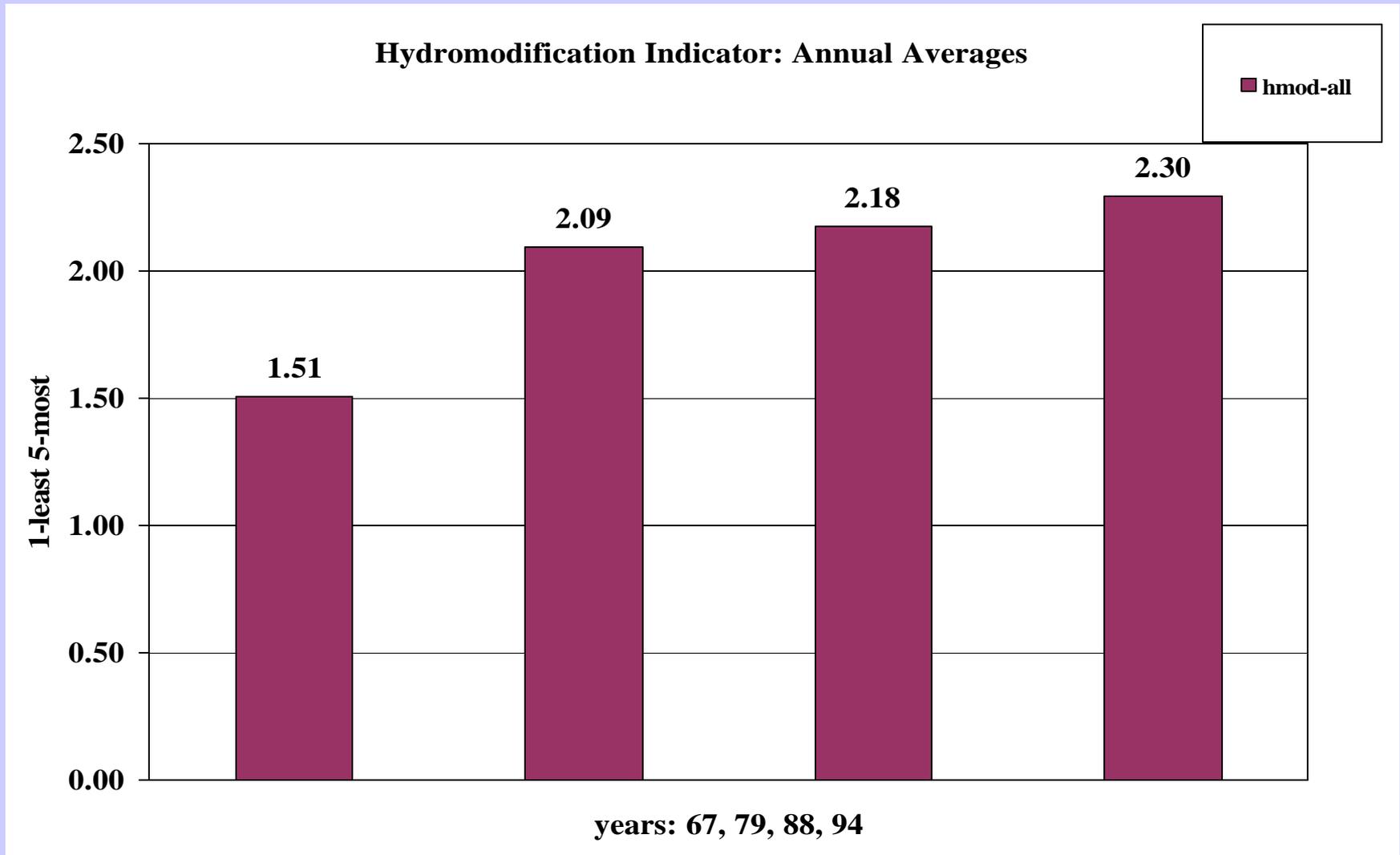
More vegetation, smaller patches...grouped near the stream and ditches....

Classification Results Summary



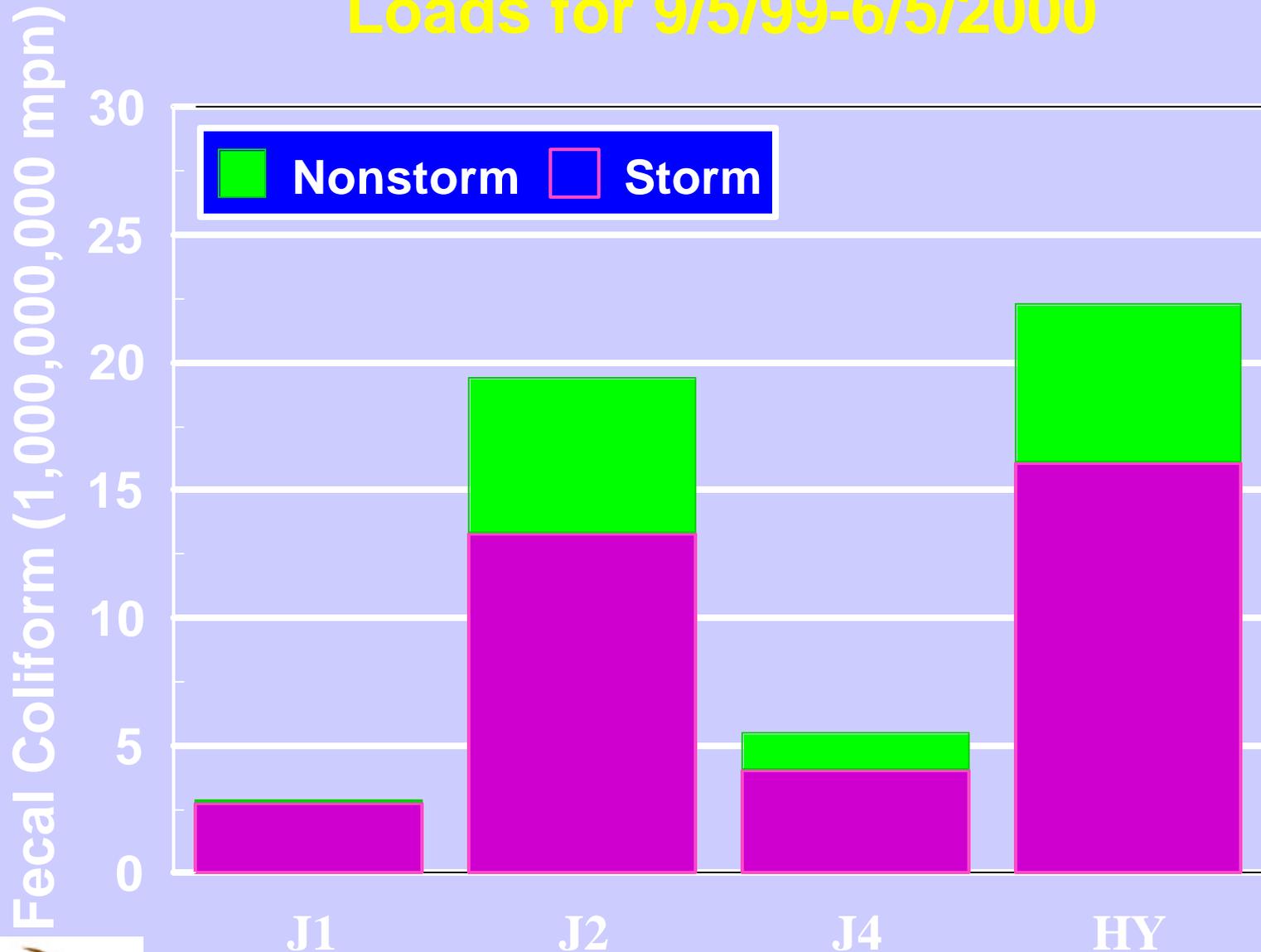
Pavement creep....

Classification Results Summary



More ditches and pipes...a complete and effective bacterial delivery system....

Loads for 9/5/99-6/5/2000



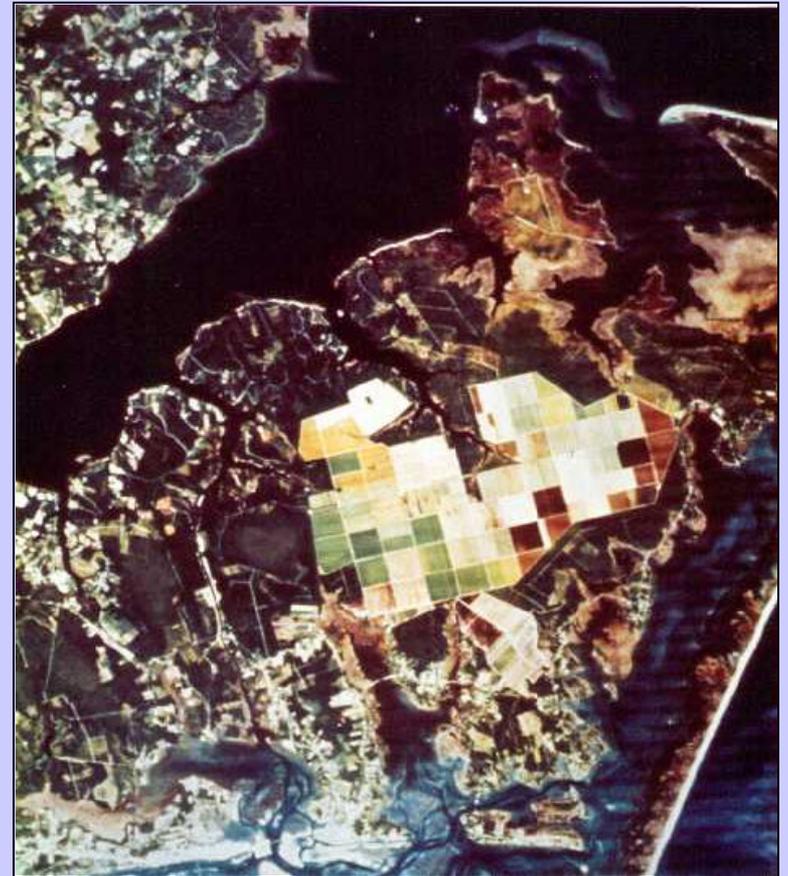
Is this normal??? Where is it all coming from???

Integrated Event and MST Monitoring

- **Added farm and forest sample sites**
- **Added two, “tried and true” MST methods—MAR and ribotyping....**

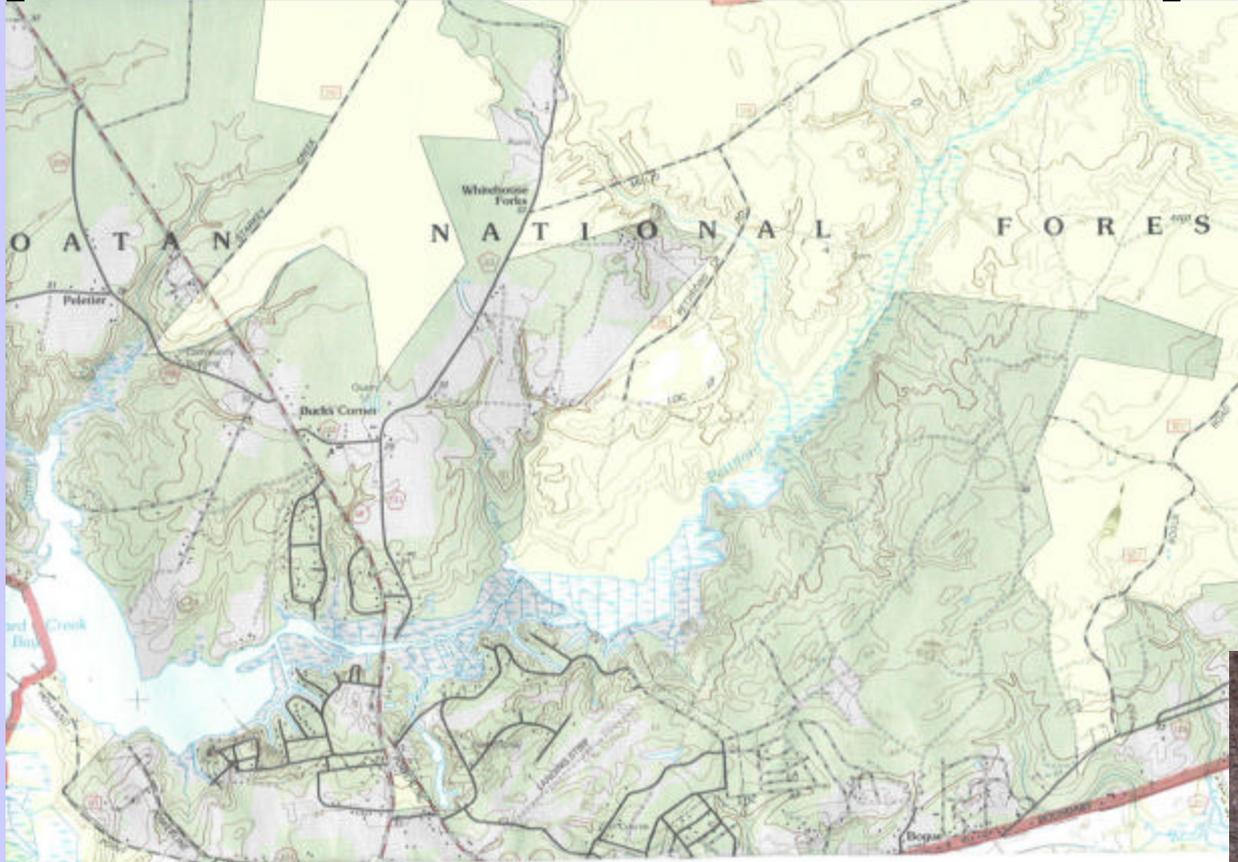
Site # 2 South River: Open Grounds Farm

Land cover: Cultivated row crop agriculture, potential bacterial sources would be bear, bobcat, rodents, birds, fox, raccoons-no septic/ human.

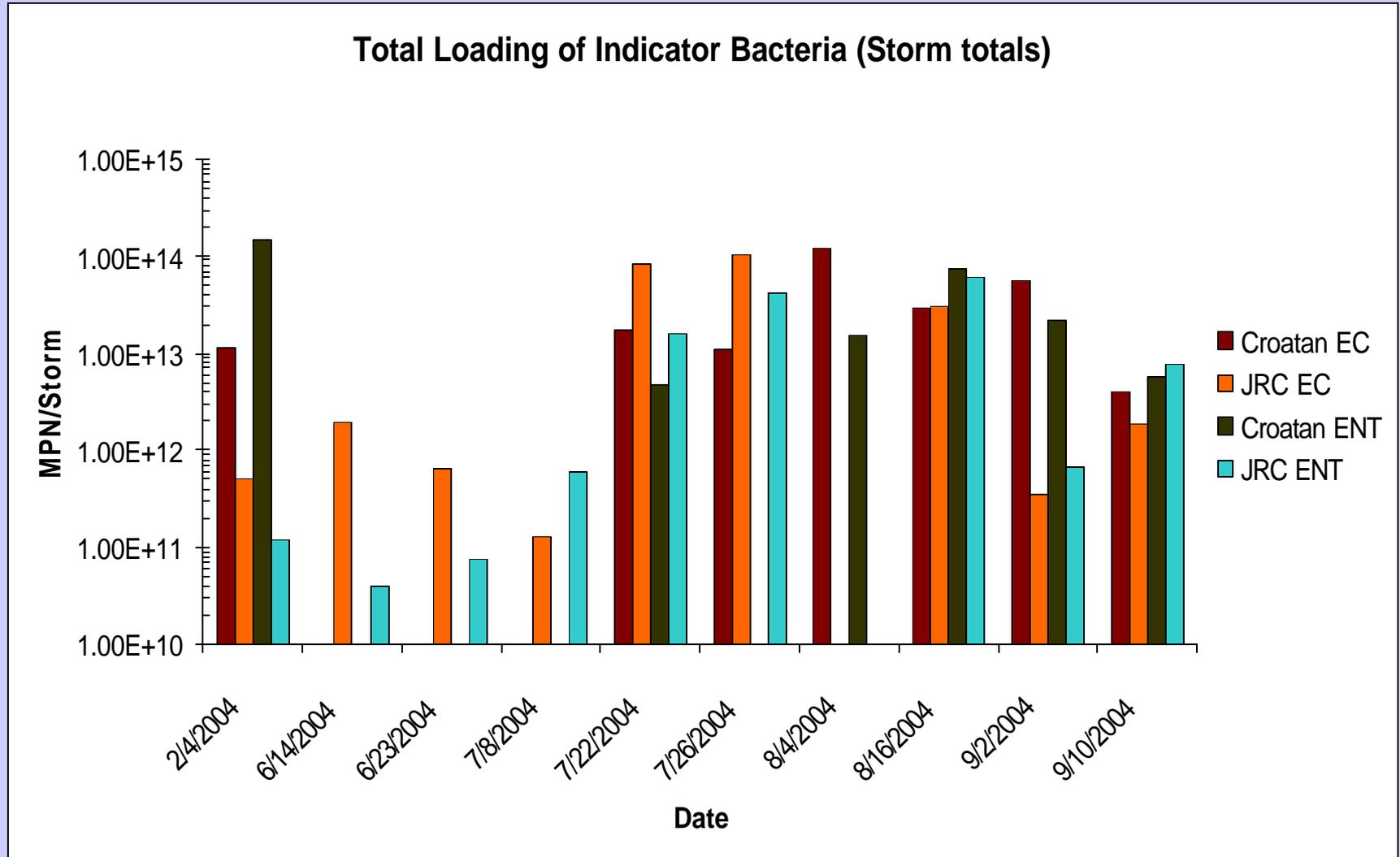


Pettiford Creek - Croatan National Forest

Land cover: Managed mixed forest of pine and pocosin, wildlife bacterial sources ... no septic.



Comparative loading of indicator bacteria



Graph courtesy Dr. Rachel Noble

Individual storms show high loading all around....

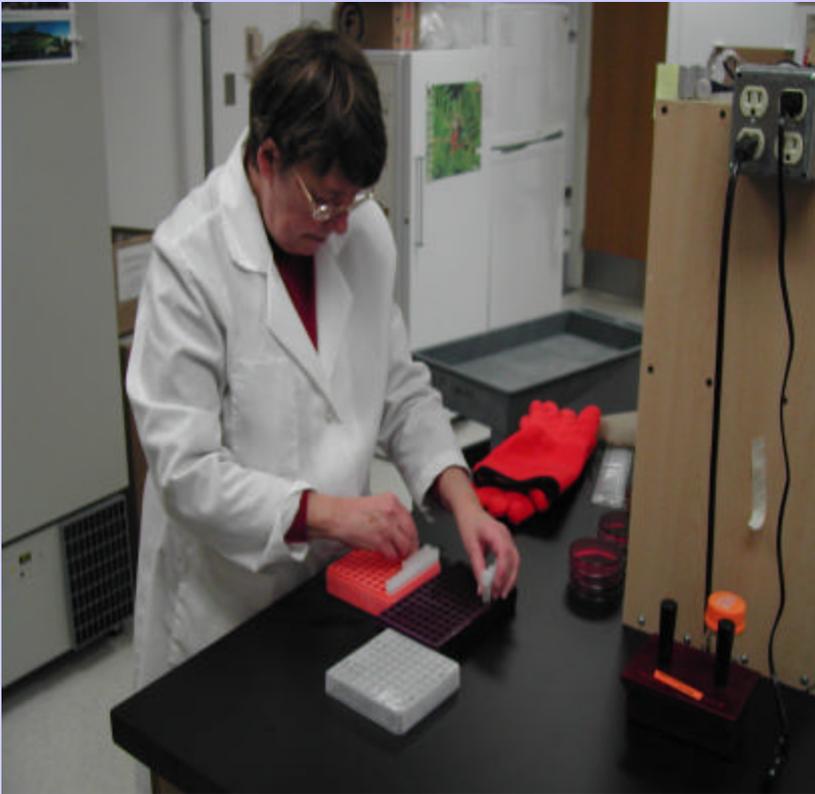
Pollutant Export from the Three Coastal Watersheds.

Date	Area	Discharge	TSS	NH ₄ -N	NO ₃ -N	PO ₄ -P	Fecal Coliform
	ac	in/yr	lb/ac-yr	lb/ac-yr	lb/ac-yr	lb/ac-yr	million mpn/ac-yr
Pettiford Creek							
10/29/02-2/24/04	2800	25.7	7.7	0.15	0.03	0.23	6,818
Jumping Run Creek							
12/13/98-2/24/04	350	30.6	209	0.32	0.47	0.16	74,820
Open Grounds							
6/10/03-9/10/04	630	21.1	206	0.22	1.26	0.97	13,487

But overall, Jumping Run wins!

Mar Process

***e. coli* isolates are developed for each sample, both library and water, and tested for multiple antibiotic resistance.**



Data were used to develop species and site indices meant to indicate risks for health threat dependant upon probable source of the pathogens...Jumping Run is higher, but what is significant?

CROATAN ALL

OF ISOLATES 95

OF TESTS 855

OF RESISTANT TESTS 19

SITE AR INDEX $19/855 = .022$

JNR ALL

OF ISOLATES 384

OF TESTS 3456

OF RESISTANT TESTS 158

SITE AR INDEX $158/3456 = .046$

OGF ALL

OF ISOLATES 153

OF TESTS 1377

OF RESISTANT TESTS 17

SITE AR INDEX $17/1377 = .012$

Kaspar et al., 1990	n/a	Developed	.09	Rural	.03
Parveen et al., 1997	Point Source	.25	NPS	.13	n/a
Webster et al., 2004	WWTP	.12	Developed	.03	Rural .01

Comparison AR index from Webster et al., 2004

At 0.046, Jumping Run not as bad as some....

Preliminary Scat Resistance Indices

Species Index	South River/ Open Grounds	Croatan	Jumping Run		Jumping Run
Bear	0 (1, 15)	3 (2, 16)		W'pecker	.17 (1,15)
Bobcat	3 (1,4)			Cat	0 (7, 30)
Fox	1 (2, 25)		0 (2, 31)	Septic	.08 (13, 94)
Deer	.23 (1,12)	.12 (1,12)		Dog	2 (11, 31)
Otter	.06 (1, 15)			Rabbit	6 (3, 15)
Waterfowl	0 (2, 27)		4 (14, 60)	Owl	0 (2, 20)
Nutria	.07 (1, 15)			Bat	.17 (1, 10)
Mouse	.01 (1,2)				
Raccoon		1 (4, 32)	2 (2, 15)		
Hawk		2 (1, 15)			
Horse		.15 (3, 31)			
Opossum		.14 (4, 21)	.30 (4, 15)		
Squirrel			0 (3,15)		

Are opossums the source???

Results of Split-Duplicate Comparison

Treatment	Number of isolates with growth	Number with growth at CMAST only	Number with growth in both labs	Number with growth at NOAA only	Percent with growth in both labs	Concordance between resistance indicators ¹
Control plates	556	0	554	2	99.6	N/A
Ampicillin	52	1	17	34	32.7	38.5
Chlortetracycline	73	22	19	32	26.0	32.7
Kanamycin monosulfate	9	1	0	8	0.0	0.0
Nalidixic acid	11	2	1	8	9.1	25.0
Neomycin sulfate	1	0	0	1	0.0	0.0
Oxytetracycline hydrochloride	66	17	18	31	27.3	34.7
Penicillin	233	52	69	112	29.6	31.5
Streptomycin sulfate	135	34	21	80	15.6	33.3
Sulfathiazole	178	134	22	22	12.4	13.6
Tetracycline hydrochloride	64	16	17	31	26.7	32.7

Same isolate. Same method. Different lab, different results.

Aggregate Results of the Replicate Test

144 antibiotic replicates per isolate, 30 isolates per drug for isolates which previously demonstrated resistance

Number of replicates with growth	Ampicillin		Chlortetracycline		Penicillin		Streptomycin	
	N	%	N	%	N	%	N	%
None	15	50	10	33	14	47	14	47
Less than 10%	0		0		7	23	4	13
10% to 90%	0		0		5	17	0	
More than 90%	0		0		0		0	
All	15	50	20	67	4	13	12	40

Same isolate. Repeated same doses. No better than coin toss.

Summary and Recommendations

- **High levels of bacterial loading are occurring from all land use types—loads even at base flow violate both recreational and shellfish water quality standards.**
- **ARA results for both the index and split-duplicate results indicate unreliability in the response patterns for e coli calling into question is usefulness as a field-based source tracking method.**
- **Good example of situation where waters are impaired (TMDL), but what management action can be taken if you cannot ID sources well enough to commit resources for mitigation? Of note is that second USDA project is finding that in more than 80% of the samples, there are positive results for salmonella for all sites including Croatan. Just reducing the volume of storm event flow would provide some relief, but given these results the problem related to pathogens may be more ubiquitous than typically thought.**

How can we fix it?

Next Steps....still trying.

- Ribotyping method assessment has standardized scanning resolution and track delineation and measurement methods are complete—the analyses of the matching rate currently being conducted.
- Dr. Nobles QPCR using *bacteriodes thetaiotamicron*
- Shea, Hyman and Gebreyes research into the antibiotic resistance, pathogen identification and salmonella resistance (with livestock farm) is underway.

See you next year!

Thank you.

