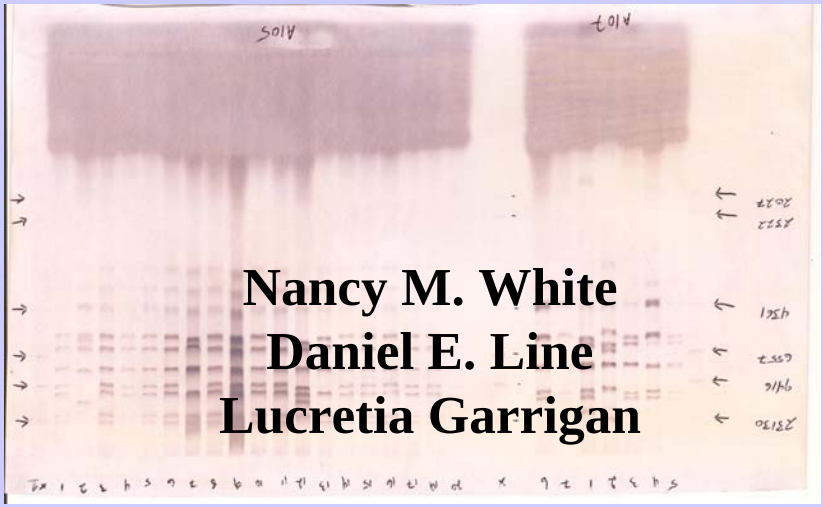
Multiple Antibiotic Resistance (MAR) and Ribotyping (RT) of Escherichia coli Isolates from Three North Carolina Impaired Watersheds



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USDA CSREES National Water Quality Conference

February 5, 2008
Sparks, Nevada

Coastal Studies Institute
University of North Carolina

Cooperators and Participants

UNC Coastal Studies Institute
NCSU CMAST
NC Division of Health, Shellfish Sanitation Program
Duke Marine Laboratory
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NOAA/NOS Center for Coastal Environmental Health and Bimolecular Research
Jumping Run Creek Watershed Citizens
Croatan National Forest
Open Grounds Farm
USDA-CSREES

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OBJECTIVES

1) Quantify fecal indicator bacterial loading rates relative to three different types of land use on the coast

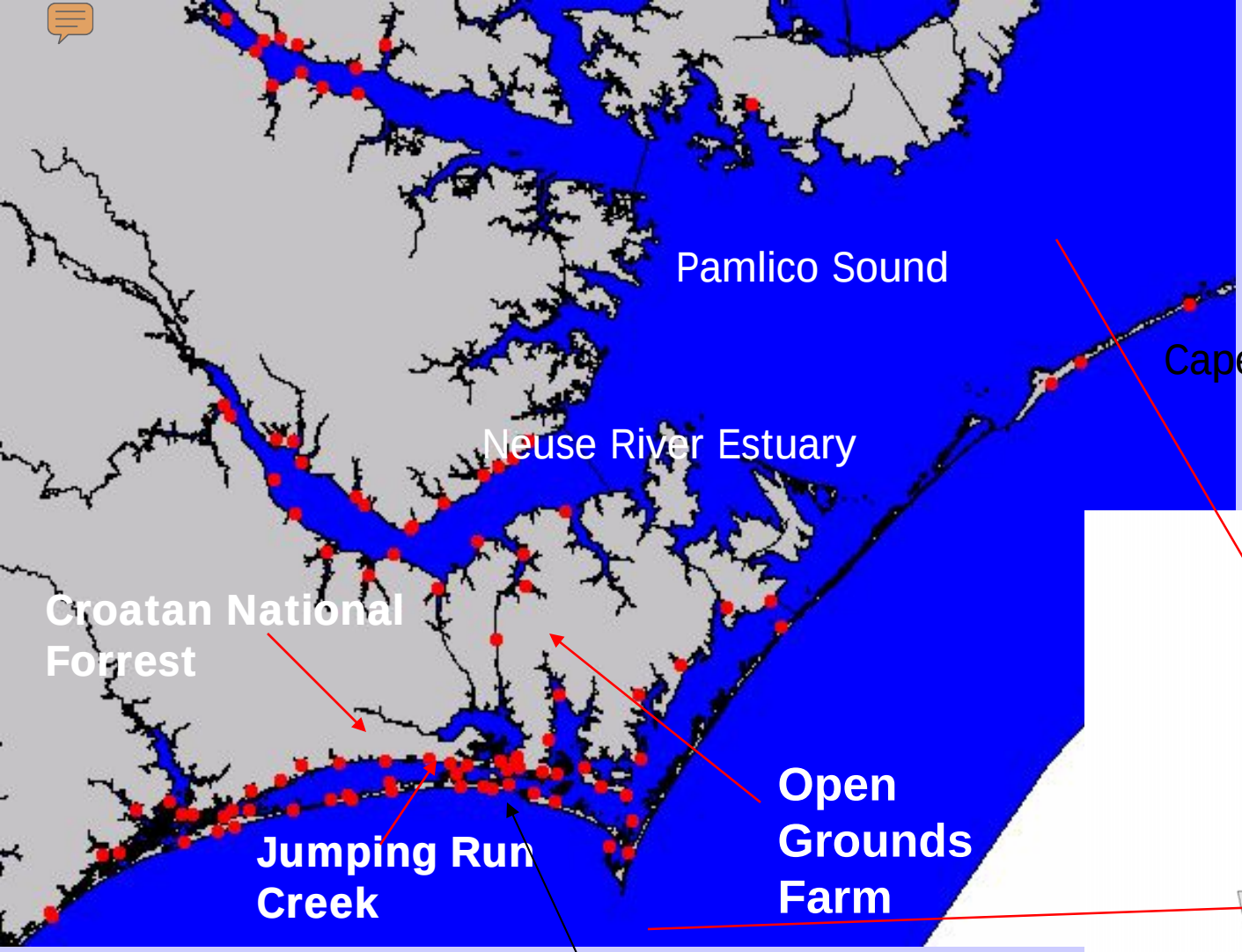
2) Apply MAR and ribotyping techniques to improve our knowledge of bacterial sources to improve management and reduce closure days for shellfish beds.

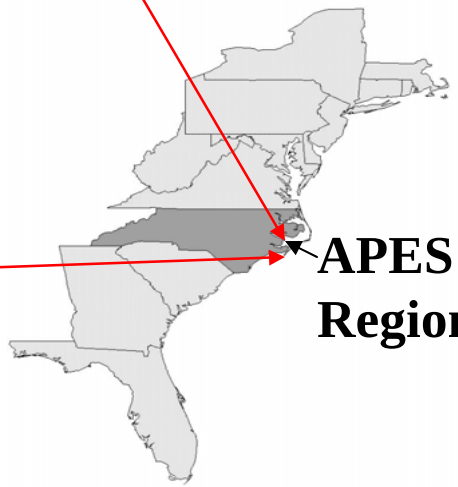
3) Provide study and education opportunities about the issues and technology.

Table 157
MANURE CHARACTERISTICS OF MISCELLANEOUS ANIMALS

Animal	Mass lb/animal/day	TS % wet wt	TS lb/animal/day	VS % TS	TN % wet wt	TN lb/animal/day	TN % TS	(TP) % wet wt	(TP) lb/animal/day	K % wet wt	K lb/animal/day	Total Coliform*	Total Streptococcus*	Ref.
Goat	—	—	—	—	—	—	3.3	—	—	—	—	—	—	418
	—	—	—	—	2.8	—	—	0.78	—	2.4	—	—	—	426
	—	—	—	—	—	—	—	0.26	—	0.25	—	—	—	427
Dog	—	—	—	—	—	—	—	—	—	—	—	0.008	500	93
	—	—	0.30	—	0.86	—	—	0.20	—	0.20	—	—	—	428
	—	—	—	—	1.9	—	—	4.3	—	0.25	—	—	—	427
	—	—	—	—	0.60	—	—	0.16	—	0.83	—	—	—	423
Mink	0.50	14.3	0.059	81	2.0	0.010	14	—	—	—	—	—	—	437
	0.17	15	0.026	79	2.4	0.0041	16	—	—	—	—	—	—	438
Bat	—	83	—	—	5.7	—	6.9	3.8	—	1.7	—	—	—	433
	—	—	—	—	6.0	—	—	3.9	—	—	—	—	—	427
Rabbit	—	—	—	—	—	—	2.0	0.58	—	1.0	—	—	—	426
	—	—	—	—	—	—	2.4	0.61	—	0.50	—	—	—	427
	—	—	—	—	0.34	—	—	0.067	—	0.22	—	—	—	428
Deer	—	40	—	—	1.1	—	2.7	0.064	—	0.088	—	—	—	176
Rat	—	—	—	—	0.23	—	—	0.034	—	0.033	—	—	—	428
Monkey	—	—	—	—	1.0	—	—	0.21	—	0.13	—	—	—	428

* Millions of organisms per gram wet weight.





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Jumping Run Creek

- **800 acres, small watershed**
- **Was: pocosin wetland; relic dunes**
- **Now: Low density residential, businesses, no agriculture**
- **Vectors: Ditches, surface runoff (increasing) direct deposit**
- **Sources: Pets, septic systems, urban wildlife**
- **Proximal to high priority closed shellfish harvesting area (Bogue Sound)**

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Open Grounds Farm (OGF)

- **Was: Pine woodlands, pocosin, wetland shrubs**
- **Now: Ditched, cleared, cultivated row crop agriculture (corn, soybeans)**
- **Proximal to South and North Rivers, high priority shellfish harvesting areas**
- **Precision farming/ water control**
- **Fecal Sources: Bobcat, bear, mice, raccoon, opossum, deer.**

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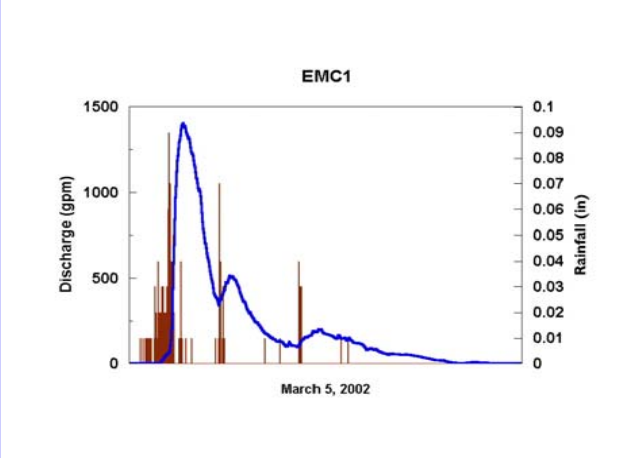
Croatan National Forest

- Pettiford Creek
- Drainage: 2500 acres
- Natural and managed pine forest and pocosin

Vectors: surface runoff, direct deposit

- Fecal sources: wildlife, horses
- Site of high priority conditionally approved shellfish harvesting areas



METHODS
Loading Assessment

Rainfall / runoff
Flow-proportional sampling
Nutrient and bacterial analysis of water samples

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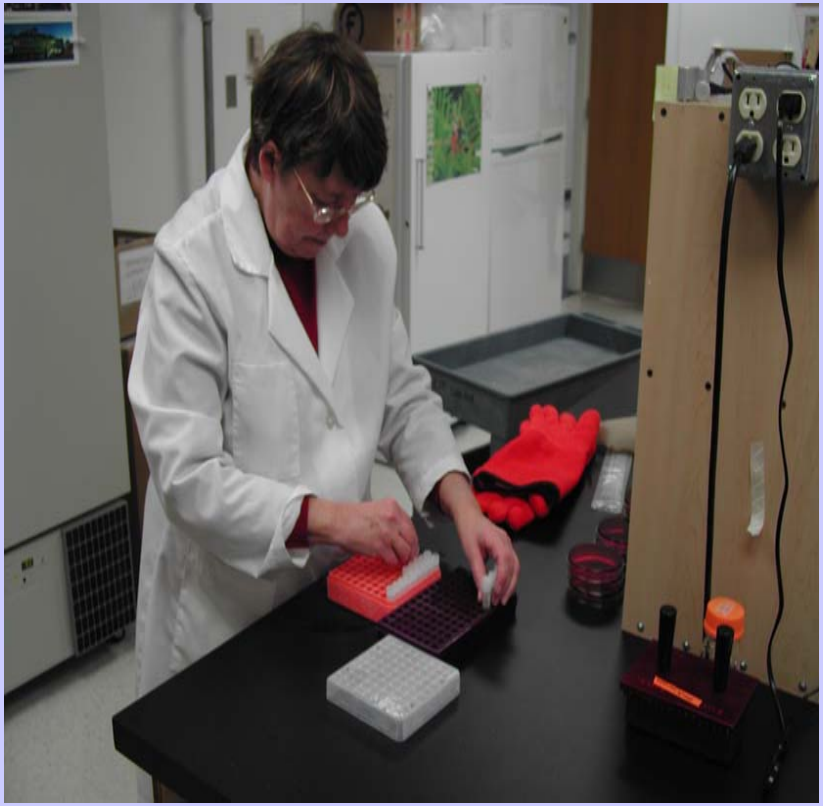

Mar Process

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

Assumption: If the e. coli exhibits resistance, then it is derived from human or pets that have been exposed to antibiotics.

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Index Development

1) Antibiotic Resistance = less than 50% reduction in colony size on the antibiotic plate compared to the control plate; and

2) Antibiotic Sensitivity = greater than or equal to 50% reduction in size compared to the control plate.

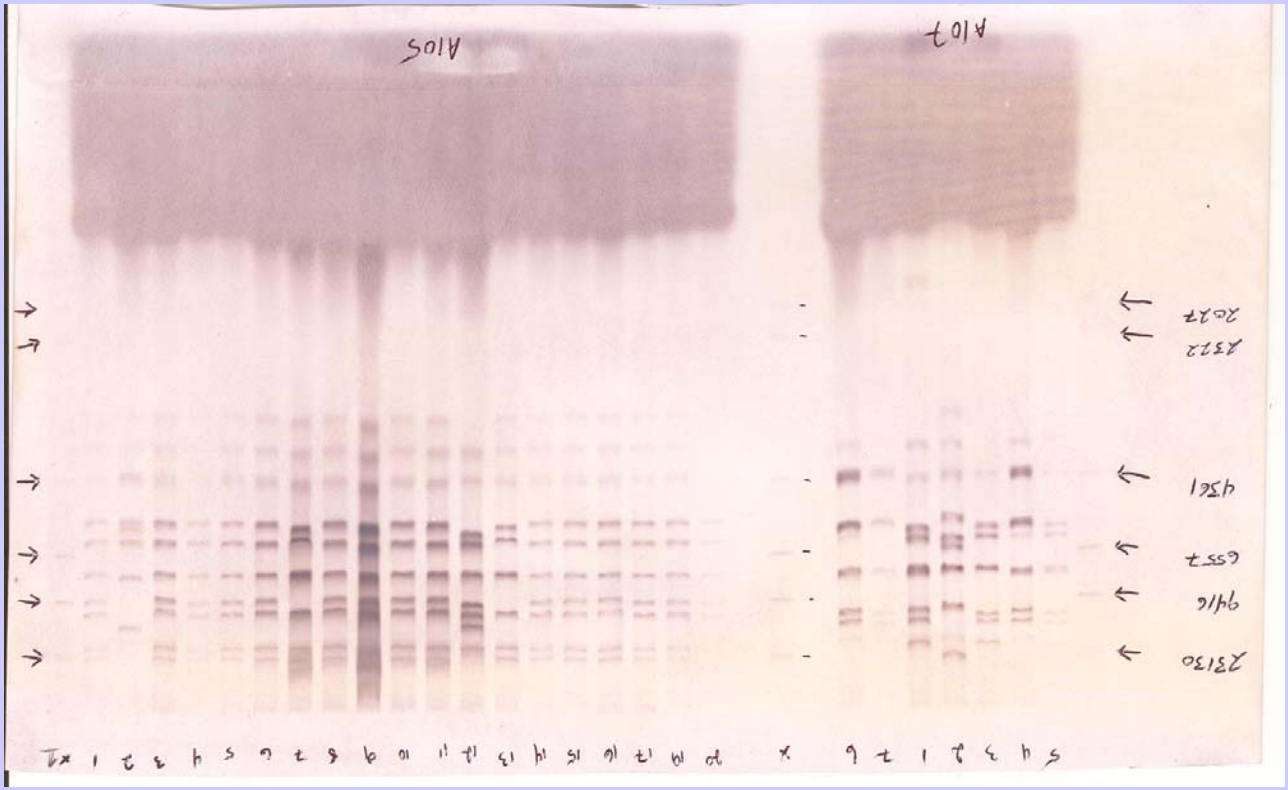
Based on these colony measurements, MAR indices were calculated for each isolate and watershed area using the approaches noted below.

MAR index (%) for an isolate = (# of antibiotics to which the isolate was resistant / total # of antibiotics tested) □ 100.

MAR index (%) for a sample site = # of antibiotics to which all the isolates were resistant / (number of antibiotics tested □ number of isolates per site) □ 100.

Ribotyping

•Tracks of DNA material are delineated and banding patterns numbered to create a numeric descriptor of each sample.
•Water samples and scat samples are compared to find matches and to potentiall identify the bacterial source in the water samples.



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Results

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

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Watershed ARA Indices

Watershed	Total Resistance %	Grab Samples %	Storm samples %
Jumping Run			
Scat Library	6	n/a	n/a
Water	12	15	11
Croatan/ Pettiford			
Scat Library	9	n/a	n/a
Water	2	1	3
Open Grounds/ South River			
Scat Library	5	n/a	n/a
Water	3	3	3

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

Species-based ARA Index

Species Resistance Index	South River	Pettiford	Jumping Run
Bear	0	3	n/a
Bobcat	3	n/a	n/a
Fox	1	n/a	0
Deer	23	12	n/a
Otter	6	n/a	4
Waterfowl	0	n/a	4
Nutria	7	n/a	n/a
Mouse	1	n/a	n/a
Racoon	n/a	1	2
Hawk	n/a	2	n/a
Horse	n/a	15	n/a
Opossum	n/a	14	30
Squirrel	n/a	n/a	0
Woodpecker	n/a	n/a	17
Cat	n/a	n/a	0
Septic	n/a	n/a	8
Dog	n/a	n/a	2
Rabbit	n/a	n/a	6
Owl	n/a	n/a	0
Bat	n/a	n/a	17

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Aggregate Results of the Replicate Test

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

	Ampicillin		Chlortetracycline		Penicillin		Streptomycin	
Number of replicates with growth	**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.

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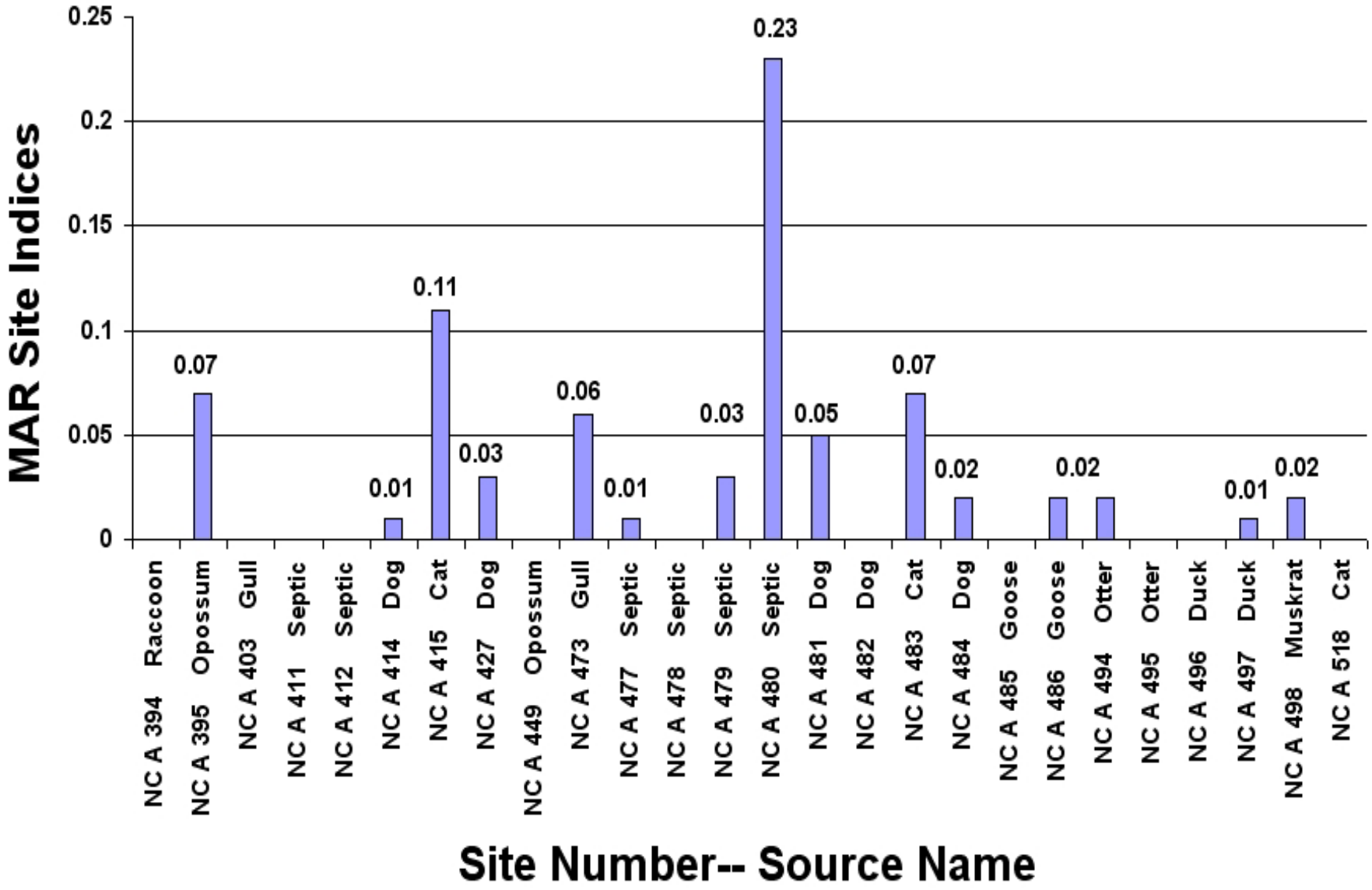
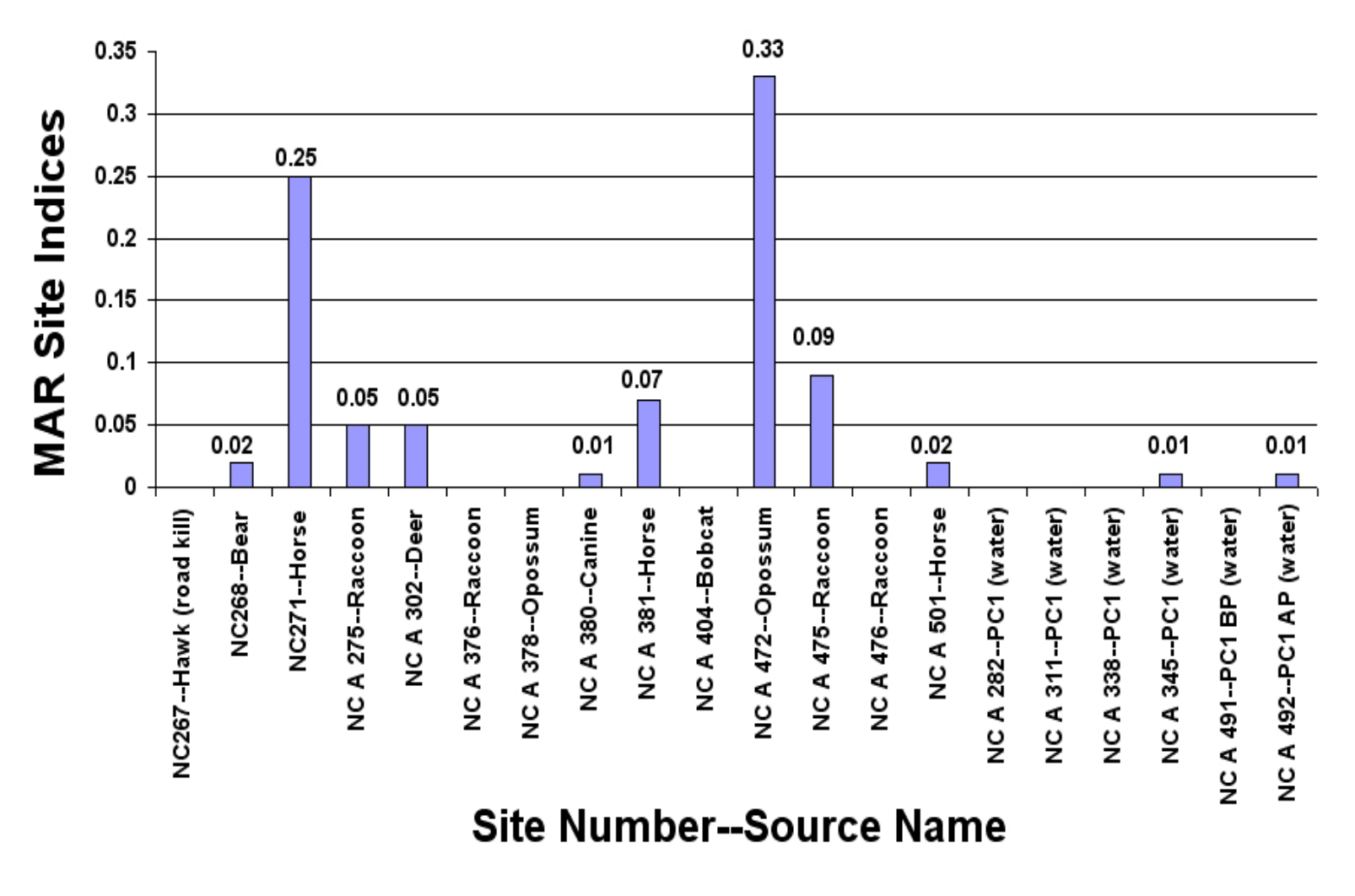
NOAA MAR Scat Indices-Jumping Run



NOAA MAR Scat Indices-Pettiford



Ribotyping Comparison of matching results between pooled and individual libraries

	# Water Isolates	No Match	Single Source Match	Multiple Matches	Total # Matched	% Matched
Pooled	221	83	57	81	138	62
Jumping Run Creek	152	73	43	36	79	52
Pettiford Creek	35	15	20	0	20	57
South River	34	14	16	4	20	59
Total # from 3 Watersheds	221	102	79	40	119	54

Summary

- **ARA and MAR 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. The utility of MAR as a field tool for source tracking can be called into question on the tacit assumption that antibiotic resistance must be acquired from the host.**

- **Research conducted by Moore et al. (2005) and Gordon (2001) on host specificity pointed out that the *E. coli* community composition can change dramatically during the transition from host to external environment; they also suggest that source-specific strains of indicator bacteria do not exist.**

- **Ribotyping is a rigorous, time-consuming process that may only be useful when pathogens are suspected.**

- **Such incredibly high levels of bacteria are loading from all land use types—even at base flows violate both recreational and shellfish water quality standards—that it would require 81-92% reduction to meet public health standards.**

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1967

1979

Conclusions

1988

1994

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