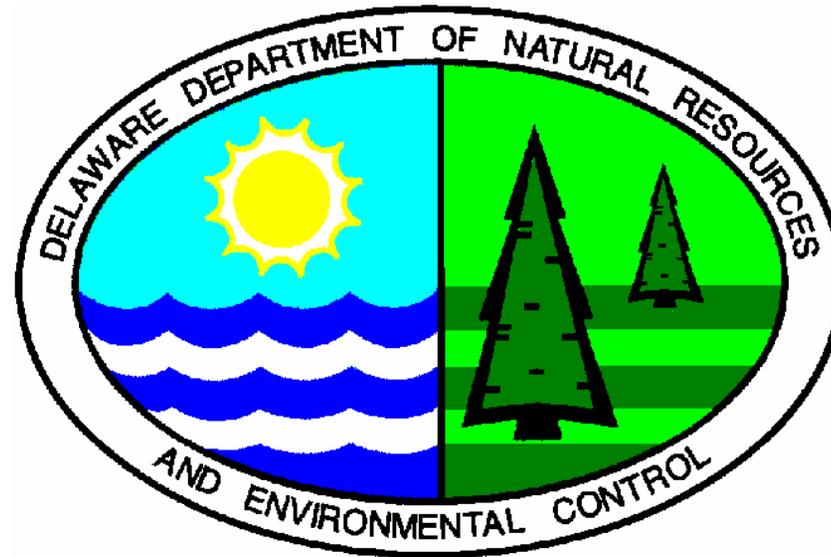


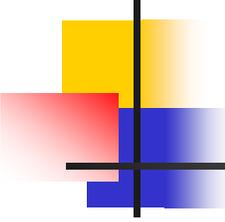
Bacteria Source Tracking



Metropolitan Washington Council of Governments

November 3, 2004

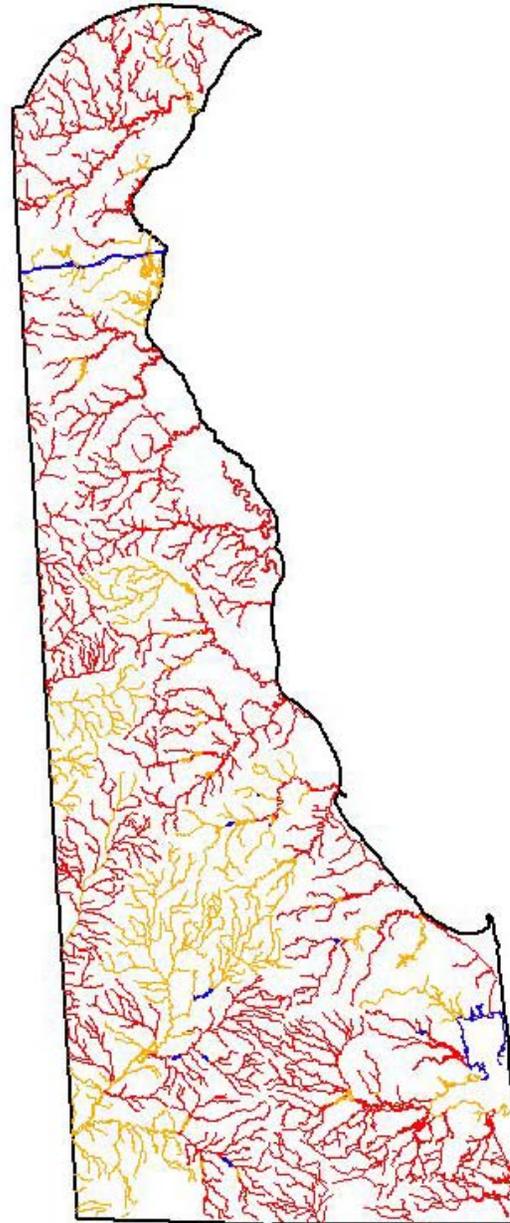
Samuel P. Myoda, Ph.D.

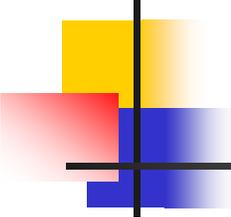


DE Waters 303 (d) Listed for Bacteria

Non supporting
(geomean & 90th > criterion)

Partially supporting
(90th > criterion > geomean)

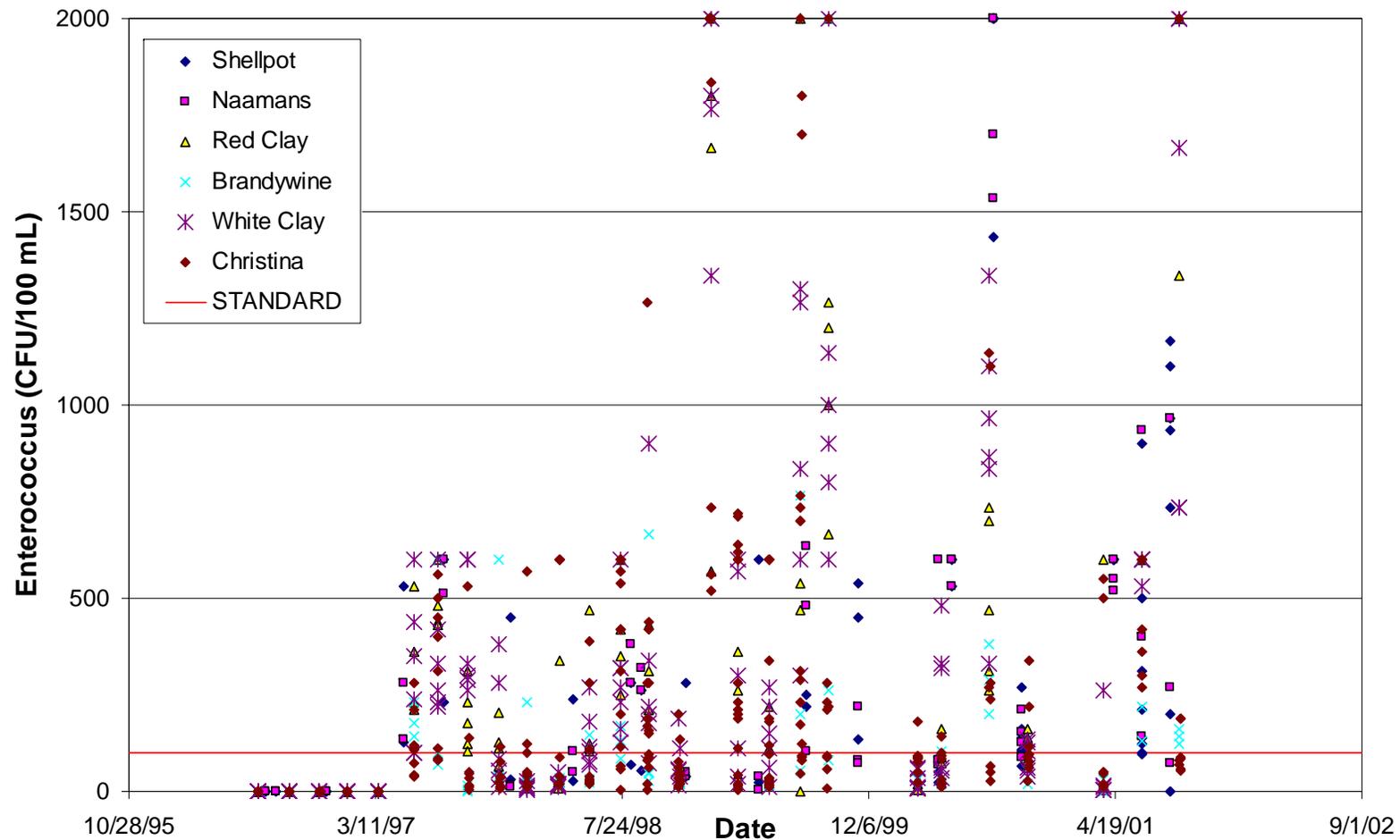


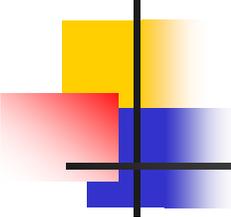


Bacteria TMDLs

- Piedmont Basin – 2004
- Chesapeake Bay & Delaware Estuary Basins – 2005
- Inland Bays, Atlantic Ocean & Delaware Bay Basins – 2006

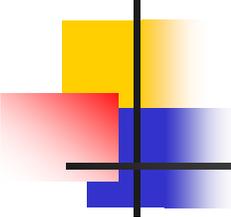
Enterococci Concentration: Piedmont Basin





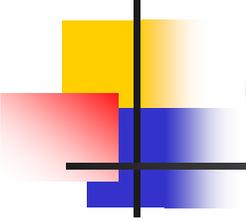
No BST Needed!





Ribotyping Steps

- Isolate target organism (known & unknown sources)
- Extract & digest DNA (PvuII & EcoR1)
- Electrophoresis
- DNA transfer from gel to membrane
- Chemiluminescent imaging
- Analysis

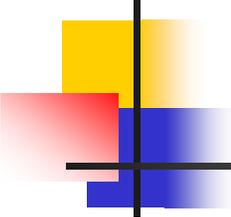


Host – Origin Database (library)

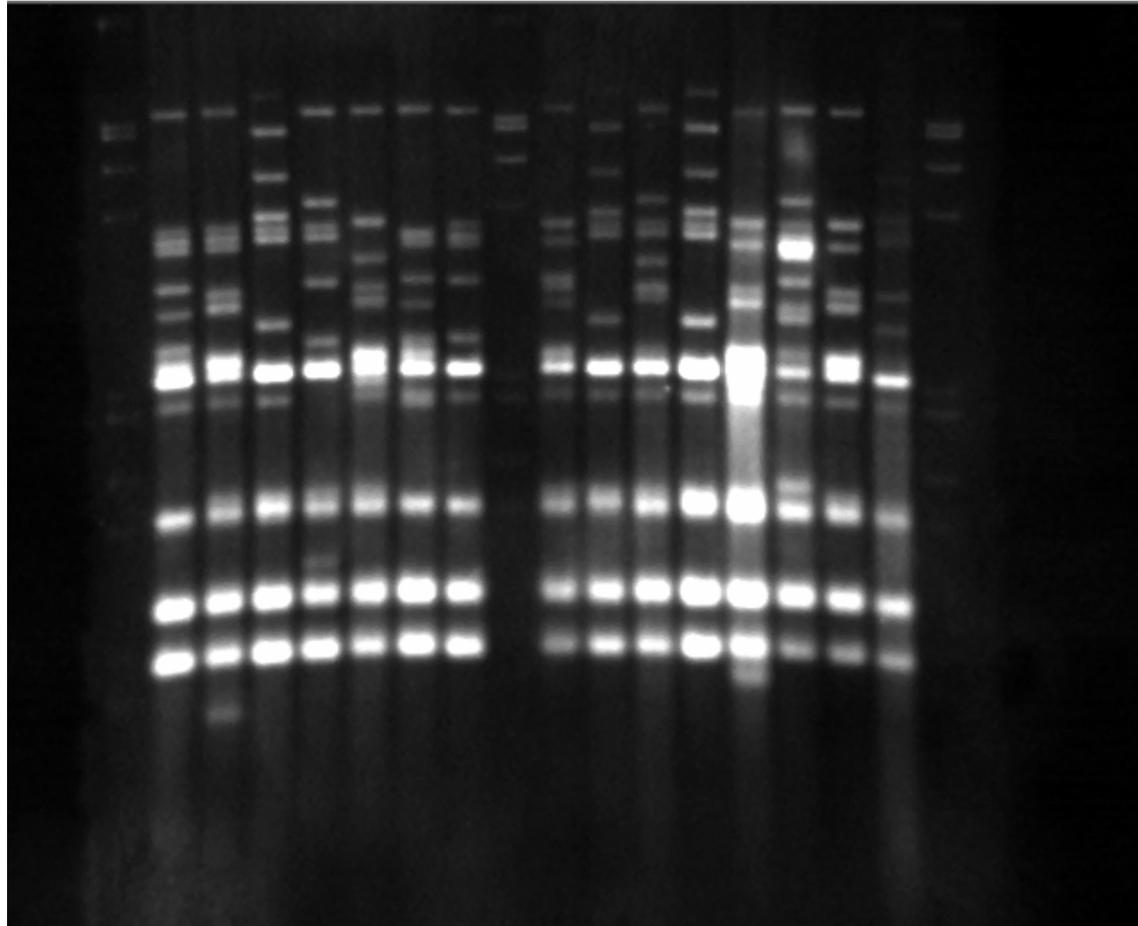
- Generate ribotype band patterns (fingerprints) from known sources, e.g. dogs, cows, birds, raccoons, etc.
- Compare unknown patterns (from environmental monitoring samples) to library patterns to determine source

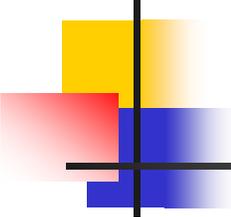
Sample Acquisition





The "fingerprint"





Two Approaches to Analysis

- Population Genetics
 - How “similar” is an unknown to a group of unknowns
- Molecular Epidemiology
 - Is the unknown an exact match with a known



BioNumerics

File Edit Database Subsets Experiments Comparison Identification Scripts

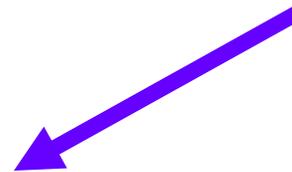
Complete database

Key	Genus	Species	Strain number	1	2	3	4	5	6
1	G@-0e104@009	STANDARD							
2	G@-0e107@002	Ambiorix	sylvestris	52441					
3	G@-0e107@003	Ambiorix	aberrans	52449					
4	G@-0e107@004	Vercingetorix	palustris	42815					
5	G@-0e107@005	Ambiorix	sylvestris	52416					
6	G@-0e107@006	Ambiorix	sp.	52415					
7	G@-0e107@007	Ambiorix	sylvestris	52414					
8	G@-0e107@008	Ambiorix	sp.	52424					
9	G@-0e107@009	STANDARD							
10	G@-0e107@010	Vercingetorix	nemorosum	42816					
11	G@-0e107@011	Ambiorix	sylvestris	52418					
12	G@-0e107@012	Ambiorix	sylvestris	52417					
13	G@-0e107@013	Ambiorix	sylvestris	52434					
14	G@-0e107@014	Ambiorix	sylvestris	52412					
15	G@-0e107@015	Vercingetorix	aquaticus	42853					
16	G@-0e107@016	Ambiorix	aberrans	52452					
17	G@-0e107@017	Vercingetorix	aquaticus	42855					
18	G@-0e108@002	Perdrix	pseudoarchaeus	25674					
19	G@-0e108@003	Perdrix	pseudoarchaeus	25671					
20	G@-0e108@004	Perdrix	pseudoarchaeus	25675					
21	G@-0e108@005	Perdrix	pseudoarchaeus	25688					
22	G@-0e108@006	Perdrix	pseudoarchaeus	25686					
23	G@-0e108@007	Perdrix	pseudoarchaeus	25672					
24	G@-0e108@008	Perdrix	sp.	25693					
25	G@-0e108@010	Ambiorix	sylvestris	52435					
26	G@-0e108@011	Perdrix	pseudoarchaeus	25673					
27	G@-0e108@012	Perdrix	sp.	53175					
28	G@-0e108@013	Ambiorix	sylvestris	52433					
29	G@-0e108@014	Ambiorix	sylvestris	52413					
30	G@-0e108@015	Perdrix	pseudoarchaeus	25681					
31	G@-0e108@016	Vercingetorix	palustris	42819					

Database: DemoBase 49 entries 6 experiments C:\Program Files\BioNumerics\data\DemoBase

Experiments: Fingerprint types (RFLP1, RFLP2), Character types (PhenoTest, FAME), Sequence types (16S rDNA), Matrix types (DNA-Hybrid)

Comparisons



Comparison settings (Fingerprint)



Similarity coefficient

Curve based:

- Pearson correlation
- Cosine coefficient

Band based:

- Jaccard
- Dice
- Jeffrey's χ
- Ochiai
- Different bands
- Fuzzy logic
- Area sensitive

Dendrogram type

- UPGMA
- Ward
- Neighbor Joining
- Single linkage
- Complete linkage

Position tolerances...

OK

Cancel

Identification

File Show

Identification library: CA

Key	sample type	s	Library units	sccwrp	ee
a10.44			human	100 ■ [1]	---
a10.45			human	100 ■ [1]	---
a10.5			gull	100 ■ [1]	---
a10.7			dog	100 ■ [1]	---
a10.50			cow	100 ■ [1]	---
a10.49			cow	93.3 ■ [1]	---
a10.43			cow	100 ■ [1]	---
c1.1	ca known	c	cow	100 ■ [1]	---

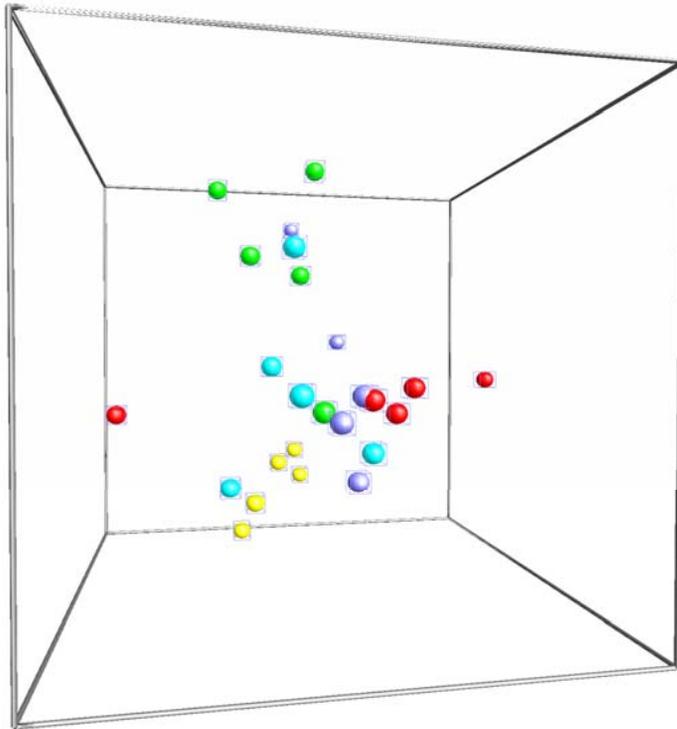
Identification

File Show

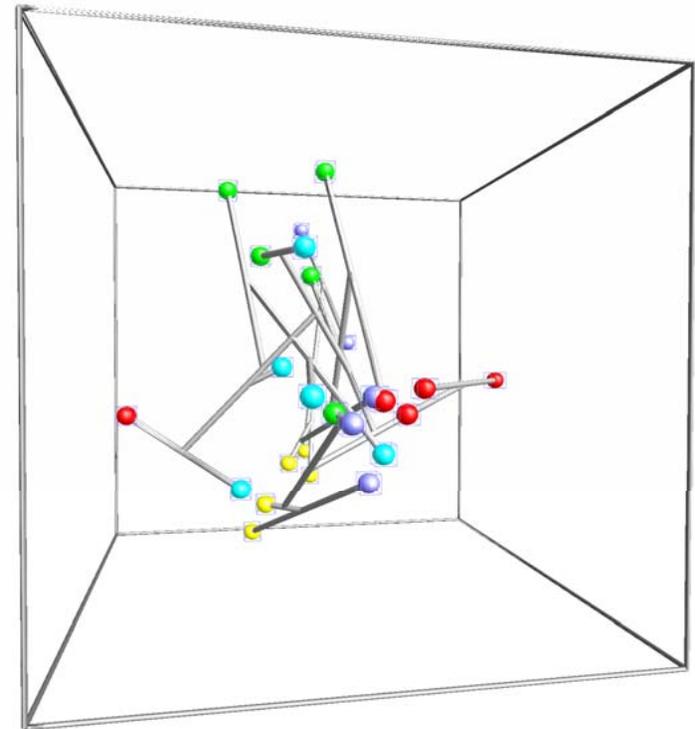
Identification library: CA

Key	sample type	s	Library units	sccwrp	ee
a10.44			human	87.8 ■ [1]	---
a10.45			gull	87.2 ■ [1]	---
a10.5			human	87.7 ■ [1]	---
a10.7			human	86.7 ■ [1]	---
a10.50			human	85.9 ■ [1]	---
a10.49			dog	71.9 ■ [1]	---
a10.43			human	82.0 ■ [1]	---
c1.1	ca known	c	human	73.9 ■ [1]	---

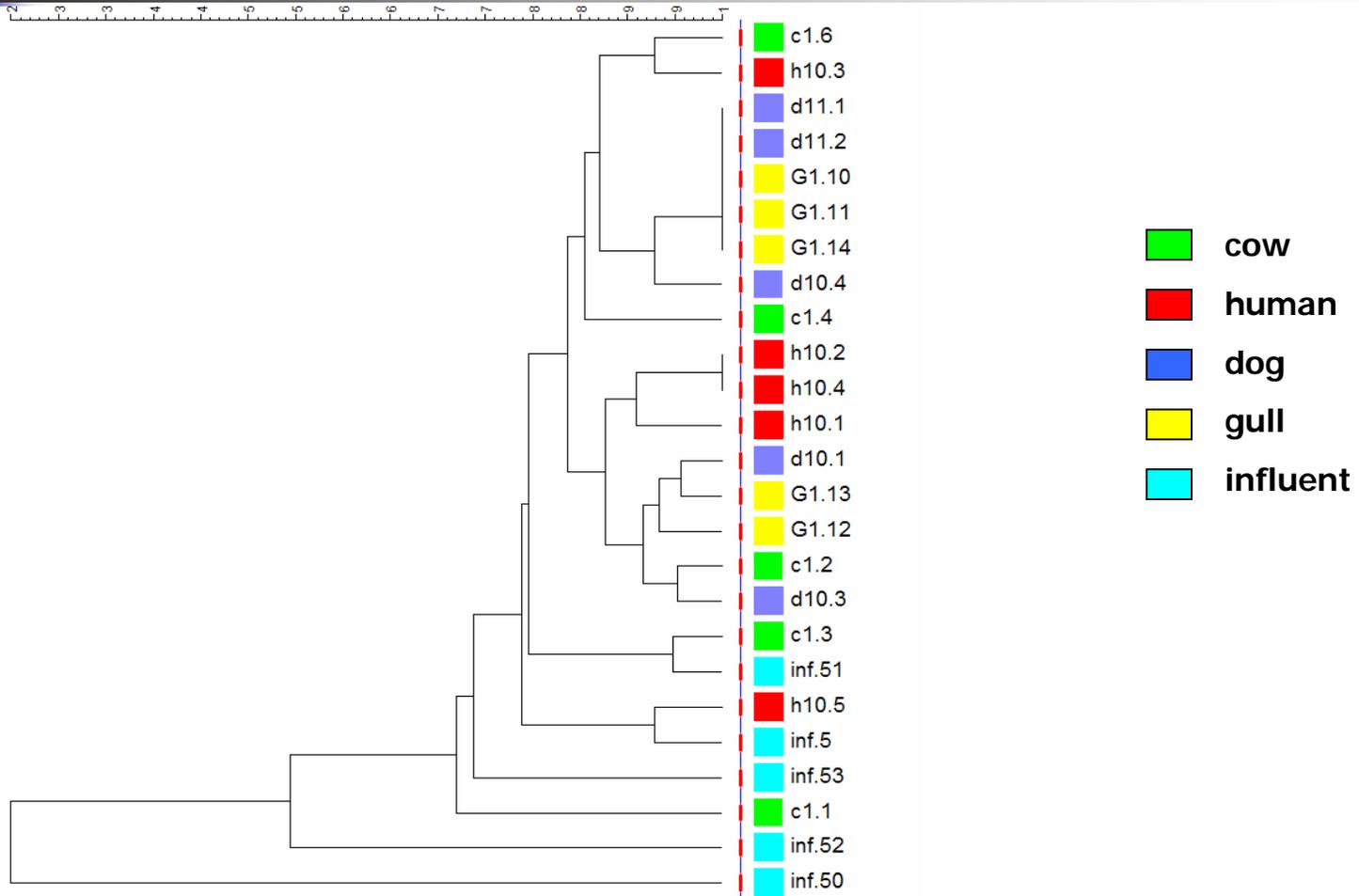
Principle Component Analysis



- cow
- human
- dog
- gull
- influent

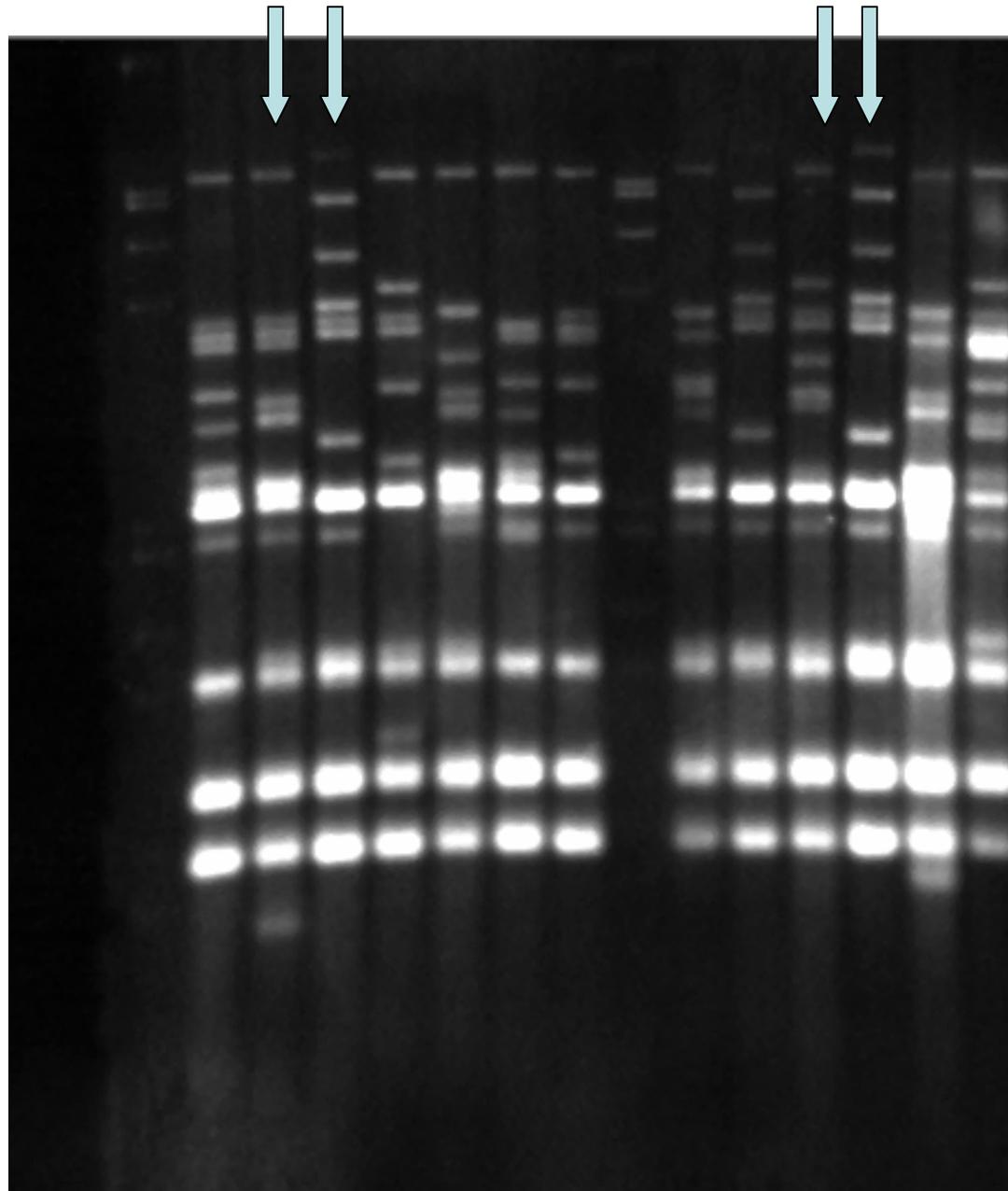


Dendrogram

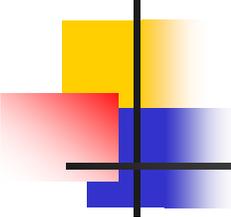




Known Pattern

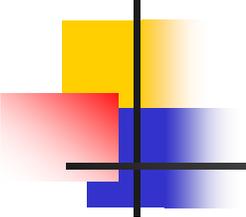


Unknown Patterns from Environmental Sampling



Key Points

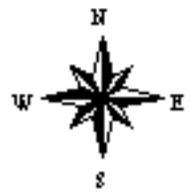
- Use the Molecular Epidemiological Approach
- Size Matters; Large Libraries are **REQUIRED**



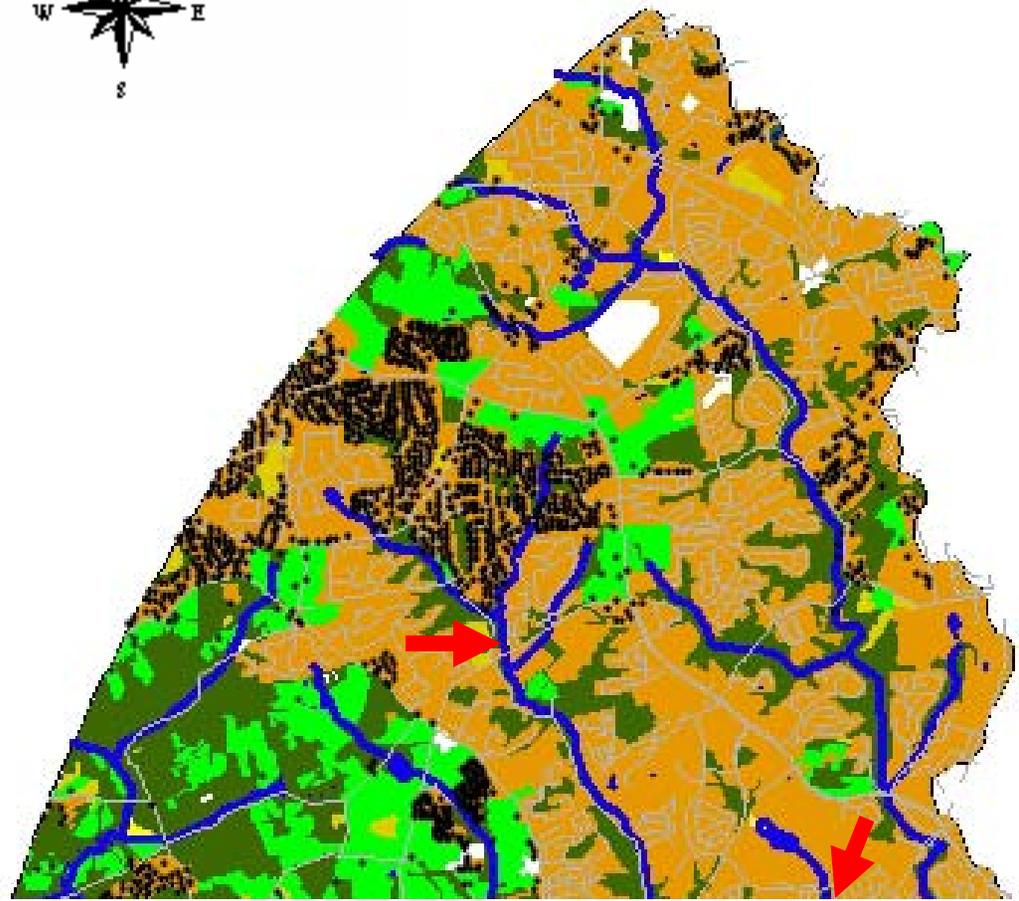
Case Studies done with the Institute of Environmental Health

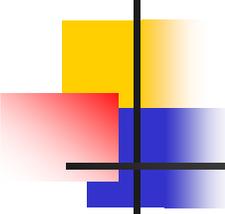
- Pike Creek
 - 11 Sampling events over 5 days
 - 2 locations
 - Study stream
 - Control stream
- Beaches
 - 8 Beaches
 - Weekly monitoring for 8 weeks

- Residential, Commercial, Industrial and Urban
- Agricultural
- Brushland
- Forest
- Water
- Wetlands
- Barren



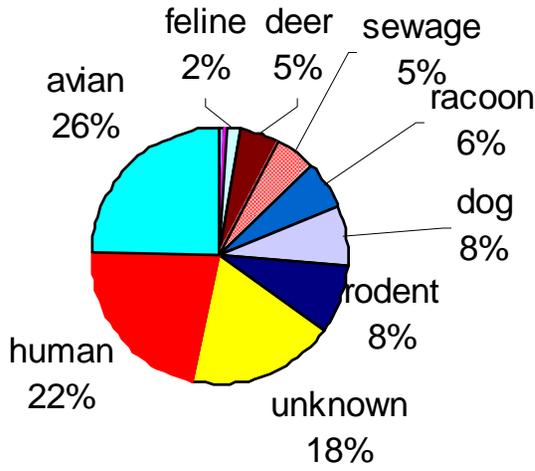
▪ Septic Systems





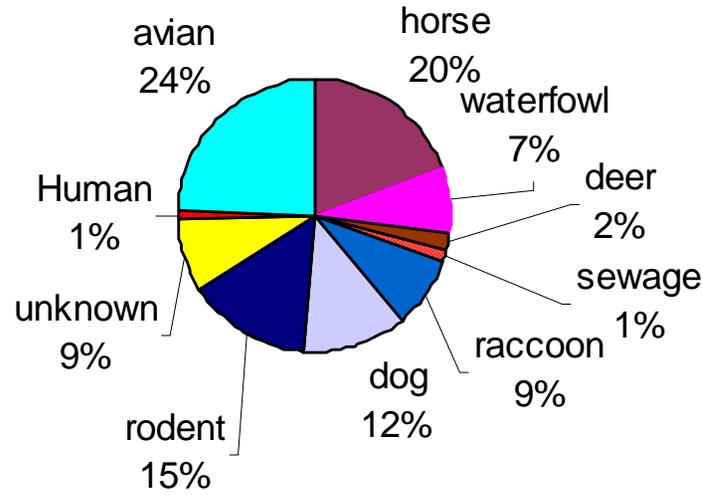
BST Results

Brookridge

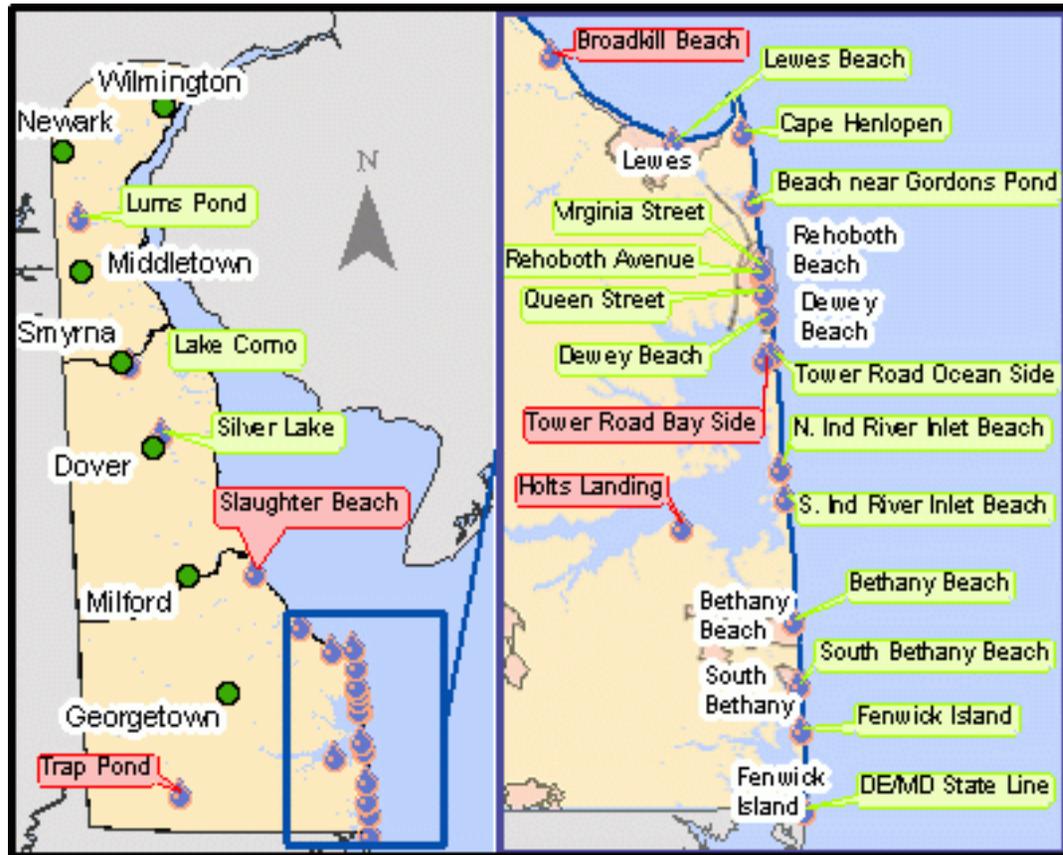


- bovine
- horse
- waterfowl
- feline
- deer
- sewage
- racoon
- dog
- rodent
- unknown
- human
- avian

Skyline

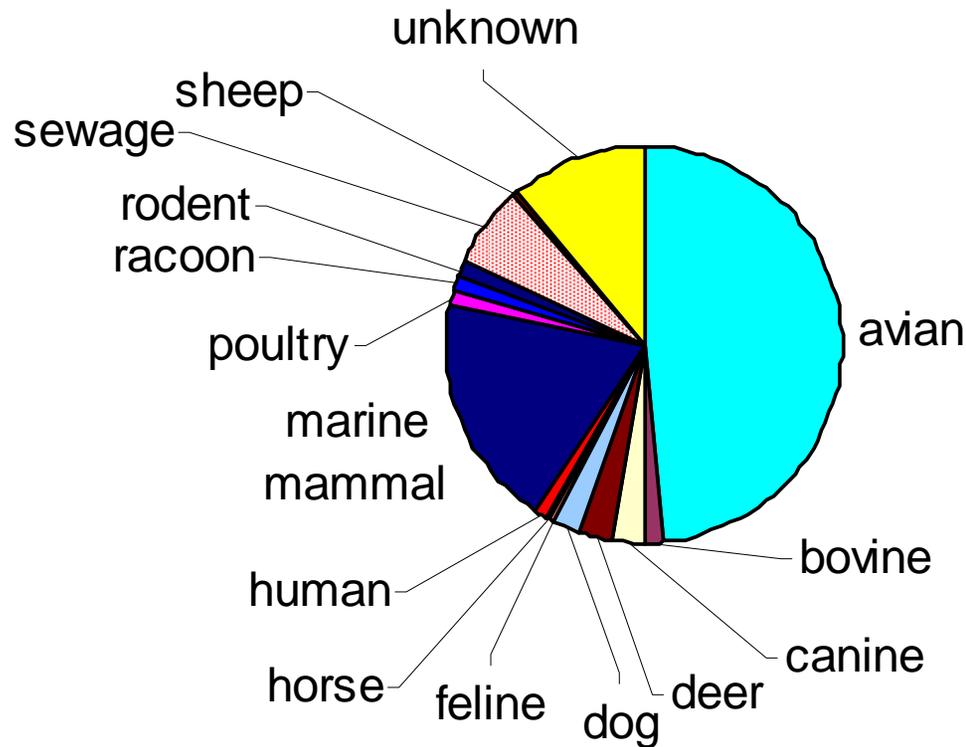


DNRREC Online



- Lake Como Guarded Beach
- Trap Pond Unguarded Beach

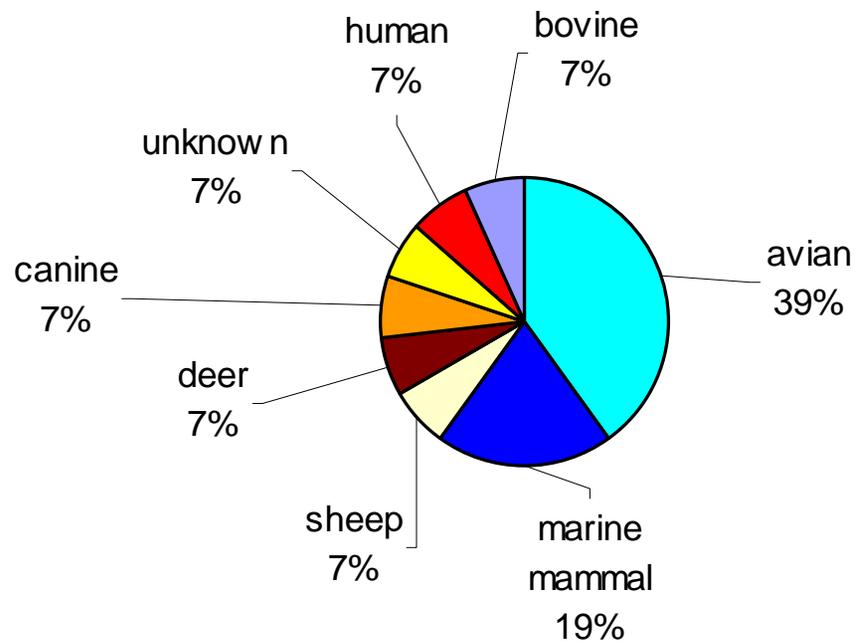
Beaches: Average BST Results



Primehook Beach

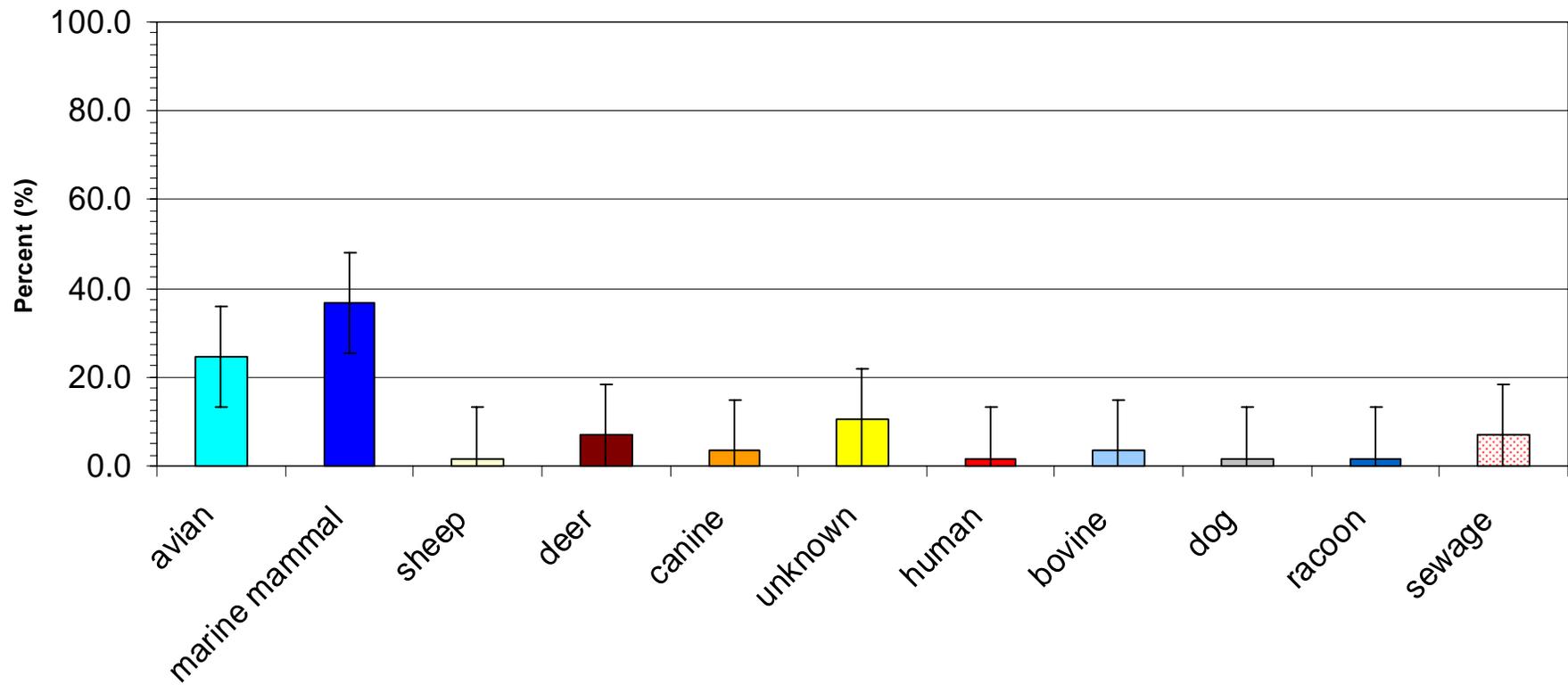
31CFU/100 mL

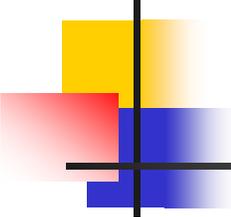
6/23/03



Prime Hook Beach

Ave conc. 44CFU/100 mL 8 grabs, 6/23-9/2





Source Tracking Adjustment Factor (STAF)

STAF =

% indicator bacteria from non-wildlife and
unknown sources

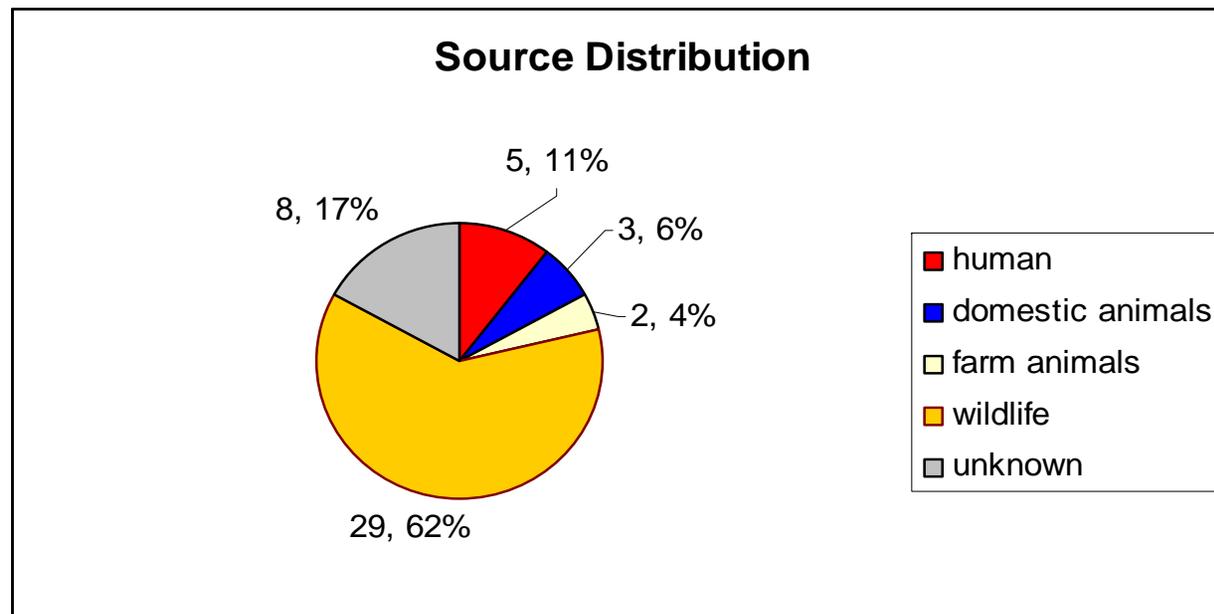
+ % standard deviation

+ % method threshold

+ % indicator bacteria from wildlife sources *

35/155 (representing risk from fecal matter
originating from wildlife)

STAF Example



- Example:
 - geometric mean = 47 CFU/100mL.
 - non-wildlife and unknown origin = $38 \pm 7\%$

- The STAF =
$$0.38 + 0.07 + 0.05 + [1-(0.38+0.07)]*35/155 = 0.62.$$

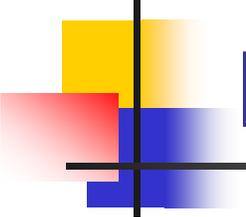
- The STAF Adjusted Count =
$$47 \text{ CFU/100mL} * 0.62 = 29 \text{ CFU/100mL}$$

Primehook Beach

Source Tracking Adjustment Factor (STAF)

- 30% non-wildlife and unknowns
- 70% wildlife

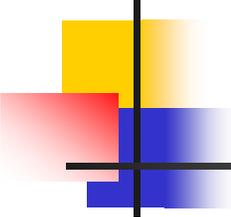
$$\begin{aligned} \text{STAF} &= 0.3 \text{ (non-wildlife \& unknown)} + \\ &\quad 0.25 \text{ (margin of safety)} \\ &= 0.55 \end{aligned}$$



Primehook Beach STAF Adjusted Enterococci Levels

	Primary Contact	Data			
		Single Sample Max (CFU/100mL)	Raw Count (CFU/100mL)	Meets Standards ?	STAF Adjusted count (CFU/100mL)
Marine	104	201	No	110*	No
		125	No	69*	Yes

*Raw Count x STAF



Thank You; Any Questions?

